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PRELIMINARY STUDIES OF PHYTOCHEMICAL INVESTIGATION ON COASTAL MEDICINAL PLANTS OF BOLOOR, MANGALORE

Jothi Muniyandi M* and Lakshman K

Department of Biochemistry and Biotechnology, Sourashtra College, Madurai –625 004

Abstract:

The present study was aimed to screen the phytochemicals present in five different solvent extracts of Ipomoea –per caprea, Premna serratifolia and Synadenium grantii.Qualitative estimation of three coastal medicinal plants were performed to identify the constituents such as alkaloids, flavanoids, saponins, phenols, glycosides, tannins, steroids, terpenoids, phlobotannins, coumarins, carbohydrates, proteins and vitamin C by standard method.All the selected coastal medicinal plants were containing the primary phytochemicals of carbohydrates, proteins, and vitamin C. Moreover, coumarin was also present in all the selected plants extracts except ethylacetate leaves extract of Premna serratifolia. Least number of phytochemicals were present in chloroform stem extracts of Ipomoea –per caprea (7/13) and ethyl acetate leaves extract of Premna serratifolia (7/11) were shown to exhibit very weak results. Aqueous extracts of leaves and stems of Premna serratifolia (12/13) showed most number of metabolites. These studies provide the evidence that leaves of Premna serratifolia is of maximum remedial efficacy possessing mainstream of phytochemical classes of compounds and stem of Ipomoea –per caprea is has found to exhibit lowest therapeutic potential due to the lack of majority of phyto constituents.

Keywords: *Ipomoea –per caprea, Premna serratifolia, Synadenium grantii, phytochemicals, qualitative screening.*

***Corresponding author:**

M. Jothi Muniyandi,
Head & Assistant Professor,
Department of Biochemistry and Biotechnology,
Sourashtra College,
Madurai –625 004.
Email ID – joyan05@rediffmail.com

QR code

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INTRODUCTION:

Mangalore is situated on the west coast of India, and is bounded by the Arabian Sea to its west and the Western Ghats to its east. Towards the coast, the salt water tides from the sea and travels several kilometres interior through the river mouths providing congenial habitats for mangroves. Mangrove plants were grown in between the sea and land; they are used for medicinal purpose from ancient years. Mangroves are a woody plant that grows at the in tropical and sub-tropical latitudes where they exist in conditions of high salinity, extreme tides, strong winds, high temperature and muddy, anaerobic soils.

Plants are the indispensable warehouse of many chemical metabolites. They are mainly divided into two categories namely: primary and secondary metabolites. Primary metabolites needed for growth and regulation of the plants and secondary metabolites involved in their defence mechanism. They include alkaloid, terpenoid, flavanoid, saponin, phenol, glycoside, tannin, steroid, etc. Studies on secondary metabolites revealed that, these compounds have antioxidant, antibacterial, anticancer, anti-inflammatory, antitumor, antiviral and many other activities.

Ipomoea –per caprea also known as beach morning glory or goat's foot, is a commonly distributed plant in tropical and sub-tropical regions, belonging to the family Convolvulaceae. Traditionally, leaf juice of *Ipomoea –per caprea* is used as a first aid for treatment of jellyfish stings [1].

Premna serratifolia L. is an important woody, medicinal plant and has its significance in Siddha, Ayurveda, and Unnai system of medicines. It's belonging to the family of Varbenaceae and locally known as munnai [2].

Synadenium grantii is a plant of the Euphorbiaceae and a succulent shrub with milky latex, leaves, alternate, simple, fleshy and flowers-small and inconspicuous, in a small cup with a red rim of glands [3].

The present work was aimed to analyse the various phytochemicals constituents on Chloroform, Ethylaceatate, Ethanol, Methanol and Distilled water extracts of *I. per caprea*, *P.serratifolia* and *S.grantii.*

MATERIALS AND METHODS:

Collection of plant materials:

The plants were collected from Boloor costal area, Mangalore, Karnataka. The taxonomic identities of these were confirmed by Botanical Survey of India, Coimbatore and Rev.Fr.Dr.JohnBritto M.Sc., M.Phil., Ph.D. Rapinart herbarium, St. Joseph's College, Trichy. The experimental parts of plants were air dried under shade, crushed in electric grinder and powdered. The powder was used for extraction procedure and phytochemical evaluation.

Extraction:

The powdered leaves and stems of the plants were soaked in solvents like chloroform, ethyl acetate, methanol, ethanol and water in the ratio of 1:2 and kept at room temperature for 2 days. The filtrate obtained was then used for phytochemical analysis.

Phytochemical analysis:

Each extract of the three plants were investigated for the presence of various phytochemicals such as alkaloids, flavonoids, saponins, phenols, glycosides, tannins, steroids, terpenoids, phlobotannins, coumarins, carbohydrates, proteins and vitamin C [4-8].

Test for Alkaloids: Hager's test -2ml of plant extract was treated with few drops of Hager's reagent (1% picric acid.) Formation of yellow precipitate would show a positive result for the presence of alkaloids.

Test for Saponins: Foam test- 2ml of plant extracts was treated with five milliliter distilled water and shaken vigorously for 2min. If the appearance of foam persists for at least 15min, it confirms the presence of saponins.

Test for Total phenols: Ferric chloride test - 2ml plant extracts was treated with five milliliter of distilled water and few drops of 5% ferric chloride were added. Bluish black indicated the presence of phenolic compounds.

Test for Glycosides: Liebermann's test - 2ml of test solution was mixed with 2ml of chloroform and 2ml of acetic acid. The mixture was cooled by in ice. Carefully, Conc. H_2SO_4 was added. A colour from violet to blue to green indicated the presence of glycosides.

Test for tannins: Lead acetate test - 2ml extract was treated with few drops of 1% lead acetate. If yellowish precipitate appears, then it contains tannins.

Test for flavonoids: Alkaline reagent test – 2ml of extract when treated with few drops sodium hydroxide solution, shows an increase in the intensity of yellow colour which would become colorless on addition of few drops of dilute hydrochloric acid, this indicates the presence of flavonoids.

Test for steroids: Salkowski test - 2ml of extract in a test tube was treated with 2ml acetic anhydride acid, 1ml chloroform followed by 0.5 ml sulphuric acid. If

the test solution shows colour change from violet to blue to green, it denotes the presence of steroids.

Test for terpenoids: 2ml of extract was treated with 2ml of acetic acid followed by 1ml sulphuric acid which might result in blue green ring formation. This shows the presence of terpenoid.

Test Phlobotannins: Precipitate test - The formation of red precipitate deposition by boiling 2ml of extract with 2ml 1% hydrochloric acid shows the presence of phlobatanins.

Test for coumarins: NaoH test - 2ml of extract was treated with 3ml of 10% sodium hydroxide in a test tube. If the solution turns to yellow colour, then it contains coumarins.

Test for Quinones: Add of 2ml plant extracts was treated with 5ml hydrochloric acid, which would result in yellow colored precipitate denoting the presence of quinones.

Test for carbohydrates: Molisch's test- 2ml of test solution was taken in a tube and treated with 2drops of ethanolic solution of α-naphthol (5%). Carefully one ml of concentration sulphuric acid was run down the slides of the tube, without mixing. A violet coloured ring appeared at the junction of the two liquid in the positive test.

Test for free amino acids: Ninhydrin test- 2ml of the test solution when boiled with 2ml of 0.2% of ninhydrin solution, violet colour appeared signifying the presence of amino acids.

Test for proteins: Biuret test-2ml of the test solution was treated with 10% of sodium hydroxide solution and few drops of 0.1% copper sulphate solution and observed for the formation of violet/pink colour.

Test for vitamin C: DHPH test - 2ml of the test solution was treated with Dinitrophenyl hydrazine dissolved in Conc. H_2SO_4. The formation of yellow precipitate indicates the presence of Vitamin C.

RESULTS:

The presence of various phytochemical constituents such as alkaloids, flavonoids, saponins, phenols, glycosides, tannins, steroids, terpenoids, phlobotannins, coumarins, carbohydrates, proteins and vitamin C in five different solvent extracts of leaves and stem of *P.serratifolia* and *I. per caprea* and leaves of *S.grantii* has been reported in Table 1-5 respectively.

Table 1: Qualitative phytochemical analysis of different solvents leaves extracts of *Ipomoea –per caprea*

Phytochemical	Chloroform	Ethyl acetate	Ethanol	Methanol	Water
Alkaloids	+	-	+	+	+
Saponins	+	+	-	-	-
Phenols	-	-	-	-	+
Glycosides	+	+	+	+	+
Tannins	-	-	+	+	+
Flavonoids	+	-	-	-	-
Steroids	+	+	+	+	-
Terpenoids	+	+	+	+	-
Phlobotanins	+	-	+	+	+
Coumarins	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Proteins	+	+	+	+	+
Vitamin C	+	+	+	+	+

+ - Present, - -Absent

Table 1 represents the phytochemical screening for five different solvents extract of leaves of *I. per caprea*. The leaves extract of *I. per caprea* when treated with chloroform revealed the presence of alkaloids, saponin, glycosides, flavonoids, steroid, terpenoid, phlobotanins, coumarin, carbohydrates, proteins, and vitamin C. The presence of saponin, glycosides, steroid, terpenoid, coumarin, carbohydrates, proteins, and vitamin C was also confirmed in Ethyl acetate extract. Further, Ethanol extract indicated the presence of alkaloids, glycosides, tannin, steroid, terpenoid, phlobotanins, coumarin, carbohydrates, proteins, and vitamin C. Methanol extract indicated the presence of alkaloids, glycosides, tannin, steroid, terpenoid, phlobotannin, coumarin, carbohydrates, proteins, and vitamin C. The distilled water leaf extracts indicated the presence of all other phytochemical under study except saponin, flavonoid, steroid and terpenoid.

Table 2: Qualitative phytochemical analysis of different solvents stem extract of *Ipomoea –per caprea*

Phytochemical	Chloroform	Ethyl acetate	Ethanol	Methanol	Water
Alkaloids	-	-	+	+	+
Saponins	-	-	-	+	-
Phenols	-	-	-	-	+
Glycosides	+	+	+	+	+
Tannins	-	-	+	+	+
Flavonoids	+	-	-	-	-
Steroids	+	+	-	-	+
Terpenoids	-	+	+	-	+
Phlobotanins	-	+	-	-	+
Coumarins	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Proteins	+	+	+	+	+
Vitamin C	+	+	+	+	+

+ - Present, - -Absent

The different solvent extracts of stem of *I. per caprea* table 2 showed diverse phytoprofiles with reference to the solvents. Out of five extracts distilled water extract demonstrated the maximum occurrence of phytoconstituents (11/13) such as alkaloids, phenols, glycosides, tannins, steroids, terpenoids, phlobotannins, coumarins, carbohydrates, proteins, and vitamin C and absence of saponins and flavonoids were observed. In the case of chloroform extract glycosides, flavonoids, steroids, coumarin and carbohydrates, proteins, and vitamin C. Ethyl acetate extract showed the presence of glycosides, steroids, terpenoids, phlobotannins, coumarin and carbohydrates, proteins, and vitamins C. Followed by ethanol extract indicated the presence of alkaloids, glycosides, tannins, terpenoids, coumarin and carbohydrates, proteins, and vitamin C and absence of saponins, phenols, flavonoids, steroids and phlobotannins, whereas methanol extract showed the presence of alkaloids, saponins, glycosides, tannins, coumarin and carbohydrates, proteins, and vitamin C.

Table 3: Qualitative phytochemical analysis of different solvents leaves extract of *Synadenium grantii*

Phytochemical	Chloroform	Ethyl acetate	Ethanol	Methanol	Water
Alkaloids	+	+	+	+	+
Saponins	+	-	+	-	-
Phenols	-	-	-	-	+
Glycosides	+	+	+	-	-
Tannins	-	-	+	+	+
Flavonoids	+	+	-	+	-
Steroids	+	+	+	+	+
Terpenoids	+	+	+	+	+
Phlobotanins	+	-	+	-	-
Coumarins	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Proteins	+	+	+	+	+
Vitamin C	+	+	+	+	+

+ - Present, - -Absent

Table 3 shows the qualitative phytochemical analysis of various stem extract of *S.grantii*. The leaves of *S.grantii* when treated with chloroform revealed the presence of alkaloids, saponins, glycosides, flavonoids, steroids, terpenoids, phlobotannins, coumarins, carbohydrates, proteins, and vitamin C. The presence of alkaloids, glycosides, tannins, steroids, terpenoids, coumarins, carbohydrates, proteins, and vitamin C was also confirmed in ethyl acetate extract. The ethanolic extract was found to contain alkaloids, saponins, glycosides, tannins, steroids, terpenoids, phlobotanins, coumarin carbohydrates, proteins, and vitamin C. Further, the methanol extract indicated the presence of alkaloids, tannins, flavonoids, steroids, terpenoids, coumarin carbohydrates, proteins, and vitamin C. The aqueous extract of *S.grantii* reveals the presence of alkaloids, phenols, tannins, steroids, terpenoids, coumarin carbohydrates, proteins, and vitamin C.

Table 4: Qualitative phytochemical analysis of different solvent leaf extracts of *Premna serratifolia*

Phytochemical	Chloroform	Ethyl acetate	Ethanol	Methanol	Water
Alkaloids	-	-	+	+	+
Saponins	-	-	-	-	+
Phenols	+	+	+	-	-
Glycosides	+	+	+	+	+
Tannins	-	-	+	+	+
Flavonoids	+	-	-	-	+
Steroids	+	+	+	+	+
Terpenoids	+	+	+	+	+
Phlobotanins	+	-	+	+	+
Coumarins	+	-	+	+	+
Carbohydrates	+	+	+	+	+
Proteins	+	+	+	+	+
Vitamin C	+	+	+	+	+

+ - Present, - -Absent

The preliminary phytochemical screening revealed the leaves different solvents extract of *P.serratifolia* give as shown in the Table 4. The chloroform extracts showed the presence of phenols, glycosides, flavonoids, steroids, terpenoids, phlobotanins, coumarin, carbohydrates, proteins and vitamin C. The ethyl acetate extract was found to contain phenols, glycosides, steroids, terpenoids, carbohydrates, proteins and vitamin C. The presence of ethanol extract was alkaloids, phenols, glycosides, tannins, steroids, terpenoids, phlobotannins, coumarins, carbohydrates, proteins and vitamin C. Further, methanol extract indicated the presence of alkaloids, glycosides, tannins, steroids, terpenoids, phlobotannins, coumarins, carbohydrates, proteins and vitamin C. Followed by aqueous leaves extract of *P.serratifolia* which showed the presence of alkaloids, saponins, glycosides, tannins, flavonoids, steroids, terpenoids, phlobotanins, coumarins, carbohydrates, proteins and vitamin C.

Table 5: Qualitative phytochemical analysis of different solvent extracts of stem of *Premna serratifolia*

Phytochemical	Chloroform	Ethyl acetate	Ethanol	Methanol	Water
Alkaloids	-	-	+	-	+
Saponins	+	+	-	+	-
Phenols	-	-	-	+	+
Glycosides	+	+	+	+	+
Tannins	-	-	+	+	+
Flavonoids	+	+	-	-	+
Steroids	+	+	+	+	+
Terpenoids	+	+	+	+	+
Phlobotanins	-	-	-	-	+
Coumarins	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Proteins	+	+	+	+	+
Vitamin C	+	+	+	+	+

+ - Present, - -Absent

Out of thirteen phytochemicals screened glycosides, Steroids, Terpenoids, Coumarin, Carbohydrates, Proteins and Vitamin C were present commonly all the five extracts of stem of *P.serratifolia* as shown in the Table 5. The chloroform extract of *P.serratifolia* revealed the presence of saponins, glycosides, flavonoids, steroids, terpenoids, coumarins, carbohydrates, proteins and vitamin C. Similarly the presence of ethyl acetate found to contain extract was saponins, glycosides, flavonoids, steroids, terpenoids, coumarins, carbohydrates, proteins and vitamin C. The occurrence of alkaloids, glycosides, tannins, steroids,

terpenoids, saponins, glycosides, flavonoids, steroids, terpenoids, coumarins, carbohydrates, proteins and vitamin C were found in ethanol extract. The methanol leaves extract showed the presence of saponin, phenol, glycosides, tannins, steroids, terpenoids, saponins, glycosides, flavonoids, steroids, terpenoids, coumarins, carbohydrates, proteins and vitamin C. Finally the distilled water extract stems *P.serratifolia* revealed the presence of alkaloids, phenols, glycosides, tannins, flavonoids, steroids, phlobotannins, coumarins, carbohydrates, proteins and vitamin C.

DISCUSSION:

Plant derived materials has gained as a great interest nowadays. Plants are richest bio resource for traditional systems such as medicines, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [9, 10]. The pharmacological activities of a plant are associated with the type and nature of secondary metabolites present in them. The need for phytochemical screening has become imperative, since many plants accumulate biologically active substances in various parts and tissues. In this study, three coastal medicinal plants were used for screening phytochemicals. Five different solvents were chosen for extracting bioactive substance from these medicinal plants (Tables 1-5) and phytochemical examinations were carried out for all the solvent extracts as per the standard methods. During extraction, the selected solvents diffuse into the solid plant material and solubilize compounds with similar polarity [10]. Phytochemical screening of those plants revealed the possible presence of phenols, terpenoids, alkaloids, flavonoids and glycosides. Among the phytochemicals coumarin, carbohydrates, proteins and vitamins are abundant in three medicinal plants and were found to present in all five solvent extracts. Least number of phytochemicals was present in chloroform stem extract of *I. per caprea* (7/13) and ethyl acetate leaves extract of *P.serratifolia* (7/11) were showed to exhibit very weak results (Table 2& 4). Aqueous extracts of leaves and stems of *P.serratifolia* (12/13) showed most number of metabolites (Table 4 & 5). Water is a good solvent system for extraction of secondary metabolites, the present result has also proved that the presence of metabolites such as alkaloids, flavonoids, saponins, phenols, glycosides, tannins, steroids, terpenoids, phlobotannins, coumarins, carbohydrates, proteins and vitamin C.

The plant has potential source of alkaloids and have been reported to possess antioxidant effect, thus reduce nitrate generation which is useful for protein synthesis, suppresses transfer of sucrose from stomach to small intestine, diminishing the support of glucose to the helminthes, acts on CNS causing paralysis [11]. Flavonoid belonging to the group of polyphenolic compounds and are typically had known for health promoting properties such as antioxidant, antimicrobial, antiinflammation and anticancer properties. (12). According to the research, Saponin has antidiarrhoeal, anticancer and anthelmintic activities. Also, it possesses membrane permeabilizing properties [11]. Phenolic compounds are aromatic secondary metabolites that impact colour, flavour and reduce risk of heart and cardiovascular diseases. Most of them have antioxidant properties [13, 14]. The action of glycosides starts by inhibiting Na+/K+ pump which then increases the level of calcium ion, so more ca+ ion would be available for contraction of heart muscles which would recover cardiac output and reduce the distension of heart [12, 15]. Tannins work by precipitating the microbial protein; they make nutritional protein unavailable for them and have antibacterial, antitumour and anticancer activities [16]. Steroids help in regulating the immune response [17] and play a vital role in sex hormone synthesis. Terpenoids are known to possess antimicrobial, anticancer, antifungal, antiparasitic, antiviral, anti allergenic, antispasmodic, antihyperglycemic, anti-inflammatory, immunomodulatory and insecticidal properties [18, 19]. Phlobotannin have been reported to possess astringent properties [20]. Coumarin has an effective anti hemorrhagic, antifungicidal and antitumour activities [21]. Carbohydrates, proteins and vitamins have vital role in plant growth and metabolic actions. Carbohydrates are stimulated as the body strength and hence are valuable as dietary supplements. Proteins are large group of macromolecules and act as antibiotic and antimicrobial agents [22, 23].

CONCLUSION:

In conclusion, the selected three coastal medicinal plants species study consist of many useful phytochemical compounds having significant biological properties. The presence and absence of the phytoconstituents in the three coastal medicinal plants depends on the solvent medium used for extraction and the physiological property of individual taxa. These plant species studied here can be used as a potential source of useful drugs. It also justifies the folklore medicinal use and the claims about the therapeutic values of this plant as a curative agent.

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