*ejpmr, 2016,3(10), 173-178.*

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

# PRELIMINARY SCIENTIFIC APPRAISAL OF THE POTENTIAL MEDICINAL PLANT *PREMNA LATIFOLIA* ROXB. VAR. *MOLLISSIMA*

**Kavya A.[1] and Jayanthi G.[2]***

[1]Research and Development Centre, Bharathiar University, Coimbatore, Tamil Nadu.
[2]Department of Botany, Vellalar College for Women, Erode-12, Tamil Nadu.

***Correspondence for Author: Jayanthi G.**

Department of Botany, Vellalar College for Women, Erode-12, Tamil Nadu.

**Article Received on 27/07/2016** **Article Revised on 17/08/2016** **Article Accepted on 07/09/2016**

## ABSTRACT

Medicinal plants are rich sources of bionutrients or bioactive phytochemicals. Studies carried out during the past two decads have shown that these bionutrients have an important role in preventing chronic diseases like cancer, diabetes and coronary heart diseases. The major classes of phytochemicals with disease preventing functions are dietary fibre, antioxidants, anticancerous, immunity potentiating agents and neuropharmacological agents. Hence, the successive extracts namely petroleum ether, benzene, chloroform, ethanol and water were used to evaluate the bionutrients of the promising medicinal plant *Premna latifolia* var. *mollissima* and presented in this paper.

**KEYWORDS:** Bionutrients, Glycosides, alkaloids, *Premna latifolia* var. *mollissima*, Verbenaceae.

## INTRODUCTION

The plant chemicals that protect plant cells from environmental hazards such as pollution, stress, draught, UV exposure and pathogenic attack are called as bionutrients or phytochemicals.[1-2] More than 4,000 phytochemicals have been cataloged and about 150 phytochemicals have been studied in detail.[3] These bionutrients (phytoconstituents) are classified on the basis of their protective function, physical and chemical characteristics.[4] They accumulate in different parts of the plants such as in roots, leaves, flowers, fruits and seeds.[5] They are of two types namely primary and secondary metabolites based on their role in plant metabolism. Primary constituents include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlorophyll etc. and secondary constituents are the remaining phytoconstituents such as alkaloids, terpenes, flavonoids, ligans, steroids, saponins, phenolics, flavonoids and glucosides.[6] *Premna* is a promising genus which is used to treat diseases like rheumatism, asthma, dropsy, cough, obesity, boils and cancer. *Premna* genus belongs to the family Verbenaceae which is widely distributed in tropical and subtropical regions of Asia, Africa, Australia, and the Pacific Island[7] and *Premna latifolia* var. *mollissima* is endemic to the peninsula.[8] *Premna latifolia* var. *mollissima* is a large straggling shrub reaching up to a height of 25 ft. and has cordate leaves with shortly acuminate apex.

## MATERIALS AND METHODS

### Collection and identification of the study plant sample

The aerial parts of *Premna latifolia* var. *mollissima* were collected from the coastal areas of Kandalloor village, Alappuzha District, Kerala State. The plant material was identified and authenticated taxonomically with the help of the local flora[9] and Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu, India. The herbarium specimen number in BSI is 'BSI/SRC/5/23/2012-13/Tech.401'.

### Preparation of the extracts

The air dried and coarsely powdered plant material was extracted successively with the help of a Soxhlet apparatus by using different solvents in the increasing order of polarity [Petroleum ether (60-80$^{o}$C), Benzene (60$^{o}$C), Chloroform (60$^{o}$C) Ethanol (78$^{o}$C) and water (100$^{o}$C)]. The extracts obtained were condensed and stored for future purposes.

### Qualitative phytochemical analysis

Phytochemical screening of different successive solvent extracts were carried out by following the standard methods.[10] Carbohydrates, proteins and aminoacids, alkaloids, anthroquinones, flavonoids, glycosides, phenols and tannins, saponins, steroids and sterols, triterpenoids and volatile oil were qualitatively analyzed.

### Tests for carbohydrates

a) Fehling's test: Five ml of Fehling's solution was added to 2 ml of each extract separately and boiled

in a water bath. The formation of yellow or red precipitate indicates the presence of reducing sugars.

b) Iodine test: Two ml of dilute iodine solution was added to each extract. The appearance of blue colour indicates the presence of starch.

**Tests for proteins and amino acids**

a) Biuret test:1 ml of each extract, equal volume of 40% sodium hydroxide solution and 2 drops of 1% copper sulphate were added separately. The appearance of violet colour indicates the presence of proteins.

b) Ninhydrin test: 5 ml of each extract, 2 drops of freshly prepared 0.2% ninhydrin reagent was added separately and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

**Tests for alkaloids**

a) Dragendroff's reagent: To 1 ml of each extract, 1 ml of Dragendroff's reagent was added. The appearance of orange red precipitate indicates the presence of alkaloids.

b) Wagner's reagent: To 1 ml of each extract, a few drops of Wagner's reagent was added and the formation of a reddish brown precipitate indicates the presence of alkaloids.

a) Mayer's reagent: To 1 ml of each extract, 2 ml of Mayer's reagent was added. Appearance of dull white precipitate indicates the presence of alkaloids.

**Borndragers test for anthroquinones**

a) 5 ml of each extract was added with 10 ml of benzene. The mixture was shaken and the appearance of a pink, red or violet colour in the lower phase indicates the presence of free anthroquinones.

b) For combined anthroquinones, 5ml of each extract was boiled with 10 ml of aqueous sulphuric acid and filtered while hot. The filtrate was shaked with 5 ml of benzene and the organic layer was separated. To half of its own volume, 10 per cent ammonia solution was added. A pink, red or violet colour in the ammonia phase (lower layer) indicates the presence of anthroquinone derivatives in the extract.

**Tests for flavonoids**

a) Shinoda test: To 1 ml of each extract, magnesium turnings and 1-2 drops of concentrated hydrochloric acid were added. Formation of pink colour indicates the presence of flavonoids.

b) To 1 ml of each extract, 1 ml of neutral ferric chloride was added. Appearance of brown colour confirms the presence of flavonoids.

c) To 0.5 ml of each extract, few drops of lead acetate solution were added. Yellow coloured precipitate indicates the presence of flavonoids.

**Tests for glycosides**

a) Legal test: Each extract was dissolved in pyridine and freshly prepared sodium nitro prusside solution was added. The formation of pink to red colour indicates the presence of glycosides.

b) Keller killani test: Each extract was added with acetic acid containing traces of ferric chloride and transferred to a test tube containing sulphuric acid. Formation of a reddish brown colour, at the junction, which gradually turned to blue, confirms the presence of glycosides.

**Tests for tannins and phenolic compounds**

a) To 1 ml of each extract, few ml of 5% neutral ferric chloride was added. The development of a dark bluish black colour indicates the presence of tannins.

b) To 1 ml of each extract, few ml of gelatin solution was added. The formation of a white precipitate reveals the presence of tannins and phenolic compounds.

c) A small quantity of each extract was dissolved in 0.5 ml of 20% sulphuric acid solution followed by addition of few drops of aqueous sodium hydroxide solution; it turns blue in the presence of phenols.

**Test for saponins (Foam test)**

a) About 1 ml of alcoholic extract was diluted with 20 ml of distilled water and was shaken in a graduated cylinder for 15 min. The formation of 1 cm layer of foam indicates the presence of saponins.

b) 5 ml of each extract was taken in a test tube and few drops of 5% sodium bicarbonate solution were added. The mixture was shaken vigorously and kept for 3 min. Formation of honey comb like froth shows the presence of saponins.

**Tests for steroids and sterols**

a) Salkowski's test: Each extract was dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterol compounds, in the extract.

b) Libermann - Burchard's test: Each extract was dissolved separately in 2 ml of chloroform to which 10 drops of acetic anhydride and 5 drops of conc. sulphuric acid were added and mixed. The change of red colour through blue to green indicates the presence of steroids.

**Test for terpenoids**

a) Libermann - Burchard's test: The extracts were dissolved in 2 ml of chloroform and 10 drops of acetic anhydride and 5 drops of concentrated sulphuric acid were added. Appearance of red to violet colour indicates the presence of terpenoids.

**Test for volatile oil**

A drop of concentrated extracts was pressed in-between two filter papers and kept undisturbed. Oil stains on the paper indicate the presence of oils and fats.

**Quantitative phytochemical studies**

**Determination of total phenolics and tannins.[11-12]**

Ten microlitre aliquots of the extracts (10 mg / 2 ml) were taken in test tubes separately and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteau phenol reagent and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as tannic acid equivalents.

Using the same extract the tannins were estimated after treatment with Poly Vinyl Poly Pyrrolidone (PVPP).100mg of PVPP was weighed into a 100 x 12 mm test tube and to this 1 ml distilled water and then 1 ml of the sample extract was added. The content was vortexed and kept in the test tube at 4°C for 4 Hrs. Then the sample was centrifuged (3000 rpm for 10 min at room temperature) and the supernatant was collected. This supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was measured and expressed as the content of non-tannin phenolics on a dry matter basis. From the above results, the tannin content of the sample was calculated as follows:

Tannin (%) = Total phenolics (%) - Non-tannin phenolics (%)

**Determination of total flavonoid contents**

0.5ml aliquot of appropriately (10mg / 2ml) diluted sample solution was mixed with 2ml of distilled water and subsequently with 0.15ml of 5% $NaNO_2$ solution. After 6 minutes, 0.15ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min and then 2ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5ml, and then the mixture was thoroughly mixed and allowed to stand for another 15min. Absorbance of the mixture was determined at 510nm versus water blank. The results were expressed as rout in equivalent.

**Mineral studies[13]**

The macro and micro nutrient analysis were carried out using Atomic Absorption Spectrophotometer (Model ECIL AAS 4127).

**Estimation of Vitamins**

Vitamin C (Citric acid), Vitamin $B_1$ (Thiamine), Vitamin $B_2$ (Riboflavin), Vitamin $B_3$ (Niacin), Vitamin $B_6$ (Pyridoxine) and Vitamin $B_{12}$ (Cyanocobalamin) contents were determined using standard methods.[14]

**RESULTS AND DISCUSSION**

The results of the present study reveals that the ethanolic extract taken from the aerial parts of *Premna latifolia* var. *mollissima* showed maximum number of components such as terpenoids, alkaloids and phenolics which are having antimicrobial activity and flavonoids possessing wound healing activity.[15] Previous studies reported that the stem bark of *Premna latifolia* showed the presence of iridoid glucosides and geniposidic acid.[16,17] Another reports revealed the presence of carbohydrates, proteins, phenols, oils, fats, terpenoids, steroids, saponins, flavonoids, alkaloids and tannins.[16,18] Phytochemical analysis on the bark of *Premna integrifolia* revealed the presence of alkaloids, tannins and phenolic compounds in petroleum ether, chloroform and ethyl acetate.[19] **Table: 1** showed the result of qualitative screening of bionutrients from the successive solvent extracts of our study plant. Almost all tested phytoconstituents shows the positive results when treated with respective reagents. The positive results are expressed as '+ve' and negative as '-ve' symbols. The positive symbol represents the presence and negative symbol for the absence of phytoconstituents. The successive extracts namely petroleum ether, benzene, chloroform, ethanol and water gives positive results for flavonoids in Shinoda test, phenol and tannins in lead acetate test, steroids and sterols in Salkowshi's test, the terpenoid by Libermann-burchard's test and volatile oils by spot test. Alkaloids were present in all extracts except petroleum ether. Except water, all remaining extracts showed positive results for glycosides.

In contrast to our study another report revealed the usage of methanol and chloroform extracts to study the phytochemical constituents of leaves of *Eclipta alba*, *Aphanamixis polystachya* and bark of *Premna integrifolia*.[19] A preliminary phytochemical analysis revealed the presence of alkaloids, tannins, flavonoids and glycoside in both extracts of the above plants which resembles our result. Similar studies are carried out in *Premna esculenta*[20] and *Premna latifolia* root.[21] The root and leaves of *Premna obtusifolia* has been reported to contain an alkaloid premnazole, which is a proven anti-inflammatory agent.[22] Leaves also contain flavonoids and sterols.[23] The leaves of *Premna latifolia* is composed of flavone glycosides and premnalin.[24]

The gums and mucilage were totally absent in all tested extracts. In the Fehling's test for detecting carbohydrates showed positive results for chloroform, ethanol and water extracts. But Benedict's reagent showed positive response in benzene, ethanol and water. Ethanol and water extracts showed positive results against foam test for saponins but the remaining extracts did not show any positive results. In Honeycomb test for saponins all the extracts exhibited negative results which indicate the absence of saponins. Quantitative estimation of bionutrients from the ethanolic extract of the study plant is given in **Table: 2.** The results are expressed in mg/100g powder. The results showed phytosteroids and glycosides in high level (11.33mg) followed by phenols (8.44), alkaloids (7.55), flavonoids (6.76), terpenoids (4.65), aminoacids (2.454) and anthraquinones (1.645). Saponins and fattyacids present only in trace quantity.

**Table:3** shows the mineral contents of the powder of the study plant. The values are given as mg/100g in dry weight of the powder. Among the tested microelements the percentage of sodium was high (124.5) followed by calcium (114.5) and magnesium (34.6). Microelements like Iron and Zinc present only in minimum quantity (6.45 and 4.36). The result of the estimation of vitamins in the plant powder of the study plant is given in the **Table: 4.** Among the vitamins available in the powder sample, vitamin $B_1$ was high (17.34) followed by vitamin $B_3$ (11.43), vitamin $B_6$ (10.34) and vitamin $B_2$(2.64). Only traces of vitamin $B_{12}$ (0.291) and vitamin C (0.445) were noted.

## CONCLUSION

The present study suggests that the study plant *Premna latifolia* var. *mollissima* have maximum number of bionutrients in the ethanolic extract, therefore the ethanolic extract can be further analysed for the activities against many pathogenic diseases as well as may be helpful in future for preventing or slowing down the progress of the disease.

**Table: 1. Qualitative phytochemical screening of the ethanol extract of the study plant *Premna latifolia* var. *mollissima.***

| S. No | Bionutrients | Test applied Or Reagents used | Presence / Absence | | | | |
|---|---|---|---|---|---|---|---|
| | | | Petroleum ether | Benzene | Chloroform | Ethanol | Water |
| 1. | Alkaloids | Wagner's test | - | + | + | + | + |
| 2. | Flavonoids | Lead acetate test | + | + | - | + | + |
| | | Shinoda test | + | + | + | + | + |
| 3. | Saponins | Honey comb test | - | - | - | - | - |
| | | Foam test | - | - | - | - | + |
| 4. | Phenols & Tannins | Lead acetate test | + | + | + | + | + |
| | | Ferric chloride test | - | - | - | + | - |
| | | Sodium hydroxide test | - | - | - | + | - |
| 5. | Carbohydrates | Fehling's test | - | - | + | + | + |
| | | Benedict's test | - | + | - | + | + |
| 6. | Protein & Aminoacids | Biuret test | + | - | - | + | + |
| | | Ninhydrin test | - | - | - | - | + |
| 7. | Glycosides | Glycoside test | + | + | + | + | - |
| 8. | Steroids & Sterols | Salkowshi's test | + | + | + | + | + |
| 9. | Triterpenoid | Libermann - Burchard's test | + | + | + | + | + |
| 10. | Anthroquinones | Borndragers test | + | + | - | + | - |
| 11. | Gums and mucilage | Alcoholic precipitation | - | - | - | - | - |
| 12. | Volatile oils | Spot test | + | + | + | + | + |

Note: '(+)' Present, '(-)' Absent.

**Table: 2. Quantitative analysis of total Bionutrients in the ethanolic extract**

| S. No. | Bionutrients | Mg/100g powder |
|---|---|---|
| 1. | Alkaloids | 7.55 |
| 2. | Steroids | 11.33 |
| 3. | Phenols | 8.44 |
| 4. | Flavonoids | 6.76 |
| 5. | Glycosides | 11.33 |
| 6. | Aminoacids | 2.454 |
| 7. | Fattyacids | 0.645 |
| 8. | Terpenoids | 4.65 |
| 9. | Saponins | 0.334 |
| 10. | Anthraquinones | 1.645 |

**Table: 3. Estimation of minerals in the powder of the study plant**

| S. No. | Minerals | Values (mg/100g) dry weight |
|---|---|---|
| **Macroelements** | | |
| 1. | Calcium | 114.5 |
| 2. | Magnesium | 34.6 |
| 3. | Sodium | 124.5 |

| **Microelements** | | |
|---|---|---|
| 2. | Iron | 6.45 |
| 4. | Zinc | 4.36 |

**Table: 4. Estimation of Vitamins in the plant powder of *P.latifolia* var. *mollissima***

| S. No | Vitamins | Values in mg/100g |
|---|---|---|
| 1. | Vitamin $B_1$ | 17.34 |
| 2. | Vitamin $B_2$ | 2.64 |
| 3. | Vitamin $B_3$ | 11.43 |
| 4. | Vitamin $B_6$ | 10.34 |
| 5. | Vitamin $B_{12}$ | 0.291 |
| 6. | Vitamin C | 0.445 |

**REFERENCES**


1. Gibson EL, Wardel J, Watts CJ. Fruit and Vegetable Consumption, Nutritional Knowledge and Beliefs in Mothers and Children. Appetite, 1998; 31: 205-228.
2. Mathai K. Nutrition in the Adult Years. In Krause's Food, Nutrition and Diet Therapy, 10th ed.,ed. L.K. Mahan and S. Escott-Stump, 2000; 271: 274-25.
3. Mamta S, Jyoti S, Rajeev N, Dharmendra S, Abhishek G. Phytochemistry of Medicinal Plants. Journal of Pharmacognosy and Phytochemistry, 2013; 1(6): 168-182.
4. Meagher E, Thomson C. Vitamin and Mineral Therapy. In Medical Nutrition and Disease, 2nd ed., G. Morrison and L. Hark, Malden, Massachusetts: Blackwell Science Inc.1999; 33-58.
5. Costa MA, Zia ZQ, Davin LB, Lewis NG. Chapter Four: Toward Engineering the Metabolic Pathways of Cancer- Preventing Ligans in Cereal Grains and Other Crops. In Recent Advances in Phytochemistry, Phytochemicals in Human Health Protection, Nutrition and Plant Defence, ed, J.T. Romeo, New York, 1999; 33: 67-87.
6. Hahn NI. Phytostergens Nature's Cure for What Ails Us? A Look at the Research. Journal of the American Dietetic Association. 1998; 98: 974-976.
7. Harley RM, Atkins S, Budantsev PD. The families and genera of vascular plants: Flowering Plant Dicotyledons. In: K. Kubitzki, editor, Labiatae. Germani: Springer-Verlag, 2004; 267-268.
8. Vijaya Sankar R. Floristic and ethnobotanical inventories of Tiruvannamalai District, Tamil Nadu. Ph.D. Thesis, 2006.Manonmaniam Sundaranar University, Thirunelveli.
9. Gamble JS, Fischer CEC. Flora of the Presidency of Madras. London: Ad Lord and Sons Limited, 1956; I-III.
10. Kokate CK. Practical Pharmacognosy, Vallabh Prakashan, New Delhi, India, 1995.
11. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of Drumstick tree (Moringa oleifera Lam.) leaves. Agri.and Food Chem, 2003; 51: 2144-2155.
12. Siddhuraju P, Manian S. The antioxidant activity and free radical scavenging capacity of dietary phenolic extracts from horse gram (Macrotyloma uniflorum (Lam.) verde.) seeds. Food Chem, 2007; 105: 950-958.
13. Tandon HLS. Methods of analysis of soils, plants, water and fertilizers. Fertilizers development and Consultation Organization, New Delhi, India, 1993; 144: 152.
14. Lawrence Evans. DSN Dietary supplements, Non-Botanicals, Pharmacopeial Forum, 2009; 28(5): 1545.
15. Shenoy C, Patil MB, Kumar R, Patil S. Preliminary phytochemical investigation and wound healing activity of Allium cepa Linn.(Liliaceae). Int. J. Pharm Pharm Sci, 2009; 2: 167-175.
16. Khare CP. Indian Medicinal Plants. An illustrated Dictionary. Ist edition. Springer- Verlag. Heidelberg, 2007.
17. Dutta S, Dey P, Choudhuri T. Quantification and correlation of the bioactive chemicals of Chroton bonpladinum leaves of sub Himalayan region of West Bengal. Asian J Pharm Clin Res, 2013; 6: 142-147.
18. Singh N, Rajinia PS. Free radical scavenging activity of an aqueous extract of potato peel. Food chem, 2004; 82: 593-597.
19. Ripa FA, Nahar L, Abul Fazal, Hajera Khatun. Antibacterial and Phytochemical evaluation of three medicinal plants of Bangladesh. International journal of pharmaceutical sciences and research, 2012; 3(3): 788-792.
20. Qais Nazmul, Mahmud, Zobaer A, Karim Md R, Bachar, Sitesh C. Anti-nociceptive, Anti-inflammatory and Sedating Activities of Leaf Extracts of *Premna esculenta* (Roxb). Journal of Pharmacy Research, 2011; 4(10): 3463-3465.
21. Sai Krushna Padhy, Srinivas K, Iswori Prasad Padhy, Jnyana Ranjan Panda. Hepatoprotective effect of *Premna latifolia* Roxb. Roots on carbon tetrachloride induced hepatotoxicity in rats. Journal of Advanced pharmaceutical Research, 2013; 4(2): 46-51.
22. Barik BR. Premnazole, an isoxazole alkaloid of *Premna integrifolia* L. and *Gmelina arborea* L. with anti-inflammatory activity. Fitoterapia, 1976; 639(4): 395.
23. Vadivu R, Suresh JA, Girinath K, Kannan BP, Vimala R, Kumar SNM. Evaluation of hepatoprotective and in-vitro cytotoxic activity of

leaves of *Premna serratifolia* Linn. J. Sci. Res, 2009; 1: 145-152.
24. Khare CP. Indian medicinal plants-An illustrated dictionary. Choukhambha Publication, 2007; 516.