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Total phenolic compound and antioxidant properties of *Premna integrifolia* Leaf Extracts from Northern Karnataka

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Abstract

The aim of present work was to assess the total phenolic content (TPC) and antioxidant properties of ethanol, methanol & chloroform extracts of *Premna integrifolia* of Northern Karnataka, India. TPC of the Ethanol extract is 64.16±0.38 mg/g GAE followed by methanol extract is 71.75±0.28mg/g of GAE and Chloroform extract 62.46±0.59mg/g GAE. The major phenolic compounds identified using standard Gallic acid through RP-HPLC. Ethanol, methanol and chloroform extract of *Premna integrifolia* possess 1, 1-diphenyl-2-picryl hydrazl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP) as well as phasphomolbdenate activity. Among three extract, methanolic extract of *Premna integrifolia* shows good activity as compared with standard ascorbic acid. This paper represents phenolic profile and antioxidant properties of *Premna integrifolia* Leaf extract.

Keywords: *Premna integrifolia, total phenolic content, RP-HPLC, gallic acid*

Introduction:

Many drugs, which are commonly used in modern day medicines, have been derived either directly or indirectly from herbal source. In India, almost 45,000 plant species are growing naturally or being cultivated. There are so many popular Indian herbs used in traditional practices to cure various disorders of human beings. *Premna integrifolia* Lin., is an important plant belonging to the family Verbenaceae, is one of the most widespread large shrub in the forests of India, usually occurring in deciduous forests. The whole plant possesses medicinal properties useful in the treatment of cardiovascular disease, skin diseases, inflammatory diseases, arthritis, gonorrhea, rheumatism, anorexia and jaundice. It's an important Ayurvedic medicinal herb and its synonym is *Premna serrtifolia* Lin. Root forms an ingredient in well known Ayurvedic formulation "Dasamula" for variety of affections (1). It is widespread throughout Micronesia and much of the tropical Pacific and tropical Asia.

It is common all along the Indian and Andaman coasts. Infusion of the leaves is administered with pepper in cold and fever. Leaves are used to cure "weakness of limbs" and the leaves and leaf sap were used to alleviate headache (2). *Premna integrifolia* Lin., has cardio tonic (3), anti-coagulant (4), anti-arthritic (5) and anti hyperglycemic properties (6). Most of the plant parts of *Premna integrifolia* Lin have been used in the traditional system of medicine in India to treat various infectious diseases. In this juncture, the present study aims to analyse the total phenolic compound and their antioxidant potentiality from *Premna integrifolia* of Verbenaceae to understand

chemistry and active ingredients propose strategies for future utilization.

Materials and methods

Plant collection

Premna integrifolia leaves were collected from the campus of Basaveshwar Science College, Bagalkot, India in the month of June 2014. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder. The powder was stored in airtight containers at -20 °C for further use

Crude extraction

About 100 g dried leaves were coarsely powdered and subjected to successive extraction by Soxhlet extractor. The extraction was done with different solvents in their increasing order of polarity such as chloroform, methanol and ethanol. Each time the plant material was dried and later extracted with other solvents. All the extracts were concentrated by rotary vacuum evaporator and evaporated to dryness.

Estimation of total phenolic content

The total phenolic content of the *Premna integrifolia* leaf extract was estimated by using Folin Ciocalteu method of Singleton *et al.* with slight modification (7). Gallic acid was used as the reference standard. A volume of 0.5 ml of plant extract was mixed with 2ml of the Folin-Ciocalteu reagent (10 fold) and was neutralized with 4 ml of sodium carbonate solution (8%w/v). The reaction mixture was incubated at room temperature for 30 min for color development. The absorbance of the resulting color was measured at 765 nm using UV-vis spectrophotometer. The total phenolic contents were estimated from the linear equation of standard curve prepared with Gallic acid. The content of total phenolic compounds expressed as mg/g Gallic acid equivalent (GAE).

Identification and quantification of the phenolic compounds by RP-HPLC

Shimadzu LC-VP HPLC system (Kyoto, Japan) consisting of LC-10ADVP pump, SIL-HTc auto sampler, CTO 10 ASvp column oven and a DGU-14A degasser was used for setting the reverse-phase liquid chromatographic conditions. Inertsil ODS-C18 (150 mm length × 4.6 mm inner diameter, 5µ particle diameter) analytical column from Phenomenex Inc. (Torrance, CA, USA) was used. Column oven temperature was 30°C, UV detector was used, separation mode was isocratic, mobile phase was acetonitrile-methanol (70:30 v/v) and flow rate was 1 ml/min. Total chromatographic run time was 30 min and injection volume was 1 µl. Plant extract and gallic acid standard were separately run on chromatographic column. Based on the retention period of gallic acid standard and its corresponding peak in the crude extract chromatogram identification and quantification of the same was achieved. Spiking the standard gallic acid solution with the leaf extract *Premna integrifolia* was done to confirm presence of gallic acid in the extract.

Determination of antioxidant activity by using *in-vitro* methods

Ferric ion reducing antioxidant power (FRAP) assay

Ferric ions reducing power was measured according to the method of Oyaizu with a slightest modification (8). Methanol, ethanol and chloroform extract of *Premna integrifolia* in different concentrations ranging from 100µl to 500µl were mixed with 2.5ml of 20m mol/l phosphate buffer and 2.5 ml (1% w/v) potassium ferricyanide and then the mixture was incubated at 50°C for 30 min. Afterwards, 2.5 ml of (10% w/v) trichloroacetic acid and 0.5 ml of (0.1% w/v) ferric chloride were added to the mixture, which was kept aside for 10 min. Finally, the absorbance was measured at 700 nm. Ascorbic acid was used as positive reference standard.

Phosphomolybdenum (PM) assay

Total antioxidant activity was estimated by PM assay using standard procedure of Prieto *et al.* (9). Methanol, ethanol and chloroform extract of *Premna integrifolia* in different concentration ranging from 100µl to 500µl were added to each test tube individually containing 3ml of distilled water and 1 ml of molybdate reagent solution. These tubes were kept incubated at 95 °C for 90 min. After incubation, these tubes were normalized to room temperature for 20–30 min and the absorbance of the reaction mixture was measured at 695 nm .Ascorbic acid was used as reference standard.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability assay

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component .This method is based on the reduction of DPPH in methanol solution in the presence of hydrogen donating antioxidant due to the formation of the non radical form DPPH-H (10). The free radical scavenging activity of all the extract was evaluated by 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) according to the previously reported method (11). Briefly ,an 0.1mm solution of this solution was added to 3ml of the solution of all extract in methanol at different concentration (62.5,125,250,500&1000µg/ml).The mixture were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance were measured at517nm using a UV-VIS

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spectrophotometer. Ascorbic acid was used as reference. Lower values of the reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following all the tests were performed in triplicates and the results were averaged.

Result and discussion

Ethanol, methanol & chloroform extract of *P integrifolia* was fractionated by solxelet extraction with solvents of increasing polarity. It is well known that phenolic compounds are considered to be major contributors to the antioxidant capacity of plants (12). Therefore, in the present study, the total phenolic content (TPC) of these fractions was evaluated. Extraction yield of the different solvent fractions obtained from *P integrifolia*. Methanol extract exhibited the highest amount of phenolic content among the extracts, i.e. 71.75±0.28 mg/g GAE followed by ethanol extract 67.16 + 0.07 mg/g GAE & 62.46 ± 0.59mg/g GAE in chloroform extract. The total phenolic contents of the samples, as obtained from the calibration curve (y = 0.012x + 0.021, R2 = 0.982, x is the absorbance; y is the concentration of Gallic acid solution, 1 g/ml) and TPC varied among the solvent system, various parts and species analyze.

RP-HPLC analysis of phenolic compounds

Phenolic compounds are secondary metabolites in plants which play an important role in human health and nutrition. Moreover, some of them present in natural products have higher antioxidant activities than those of synthetic antioxidants (13). Methanol extract of *P. integrifolia* showed the highest total phenolic content, hence considered for RP HPLC analysis. The major peaks were identified by comparison with authentic standards (Fig. 1D). The major phenolic compounds identified in the leaves of *Premna integrifolia* is gallic acid present in highest concentration in all three extract (Fig: 1 A, B & C). Gallic acid has several pharmacological properties and recently it shows strong anticancer efficacy against human prostate cancer cells (14) .

Antioxidant activity:

FRAP Assay

The FRAP assay was originally developed by Benzie and Strain (1996) (15), to measure reducing power in plasma, but the assay subsequently has also been adapted and used for the assay of antioxidants in botanicals. The reaction measures reduction of ferric 2, 4, 6-tripyridyl-s-triazine (TPTZ) to a colored product. This method is used to determine reducing the capacity of extracts as well as standard. Absorption is directly proportional to reducing potential. Higher absorbance indicates the high reducing capacity of the antioxidants (16). In the present work, methanol extract shows higher antioxidant activity than the ethanol extract (Figure2) which was comparable to the standard ascorbic acid.

PM ASSAY

PM assay is calorimetric quantitative method which measures the reduction of Phosphate-Mo (VI) to Phosphate-Mo (V) by the sample and subsequent formation of a bluish green colored Phosphate-Mo (V) complex at acidic pH. It helps to investigate the reduction rate among antioxidant and molybdenum ligand. The PM method is routinely applied in the laboratory to evaluate the total antioxidant capacity of plant extracts. Absorbance is directly proportional to the antioxidant activity and gives reducing potential of plant extracts (17). In the present study methanol extract exhibited higher absorbance than the ethanol extract. (Figure 3)

DPPH radical-scavenging activity:

Free radicals are known to induce oxidative damage in biomolecules and play an important role in aging, cardiovascular diseases, cancer, impaired immune system, and inflammatory diseases (18). In this assay, the radical-scavenging activity of the all three extracts of *Premna integrifolia* was tested using the DPPH free radical, which has the advantage of being unaffected by certain side reactions, such as metal ion chelateion and enzyme inhibition (19) . The scavenging activity of the extracts tested was compared to those of ascorbic acid, used as positive control, and the DPPH radical-scavenging activity of the samples increased at sample concentrations ranging from 62.5 to 1000µg/ml. As shown in (Table1) methanol fraction shows the highest potent DPPH radical-scavenging activity, followed by ethanol fraction and chloroform extract. Significant difference was observed among antioxidant activities of evaluated extracts and methanol extract came out as a superior total antioxidant capacity, with significant higher results in all performed assays.

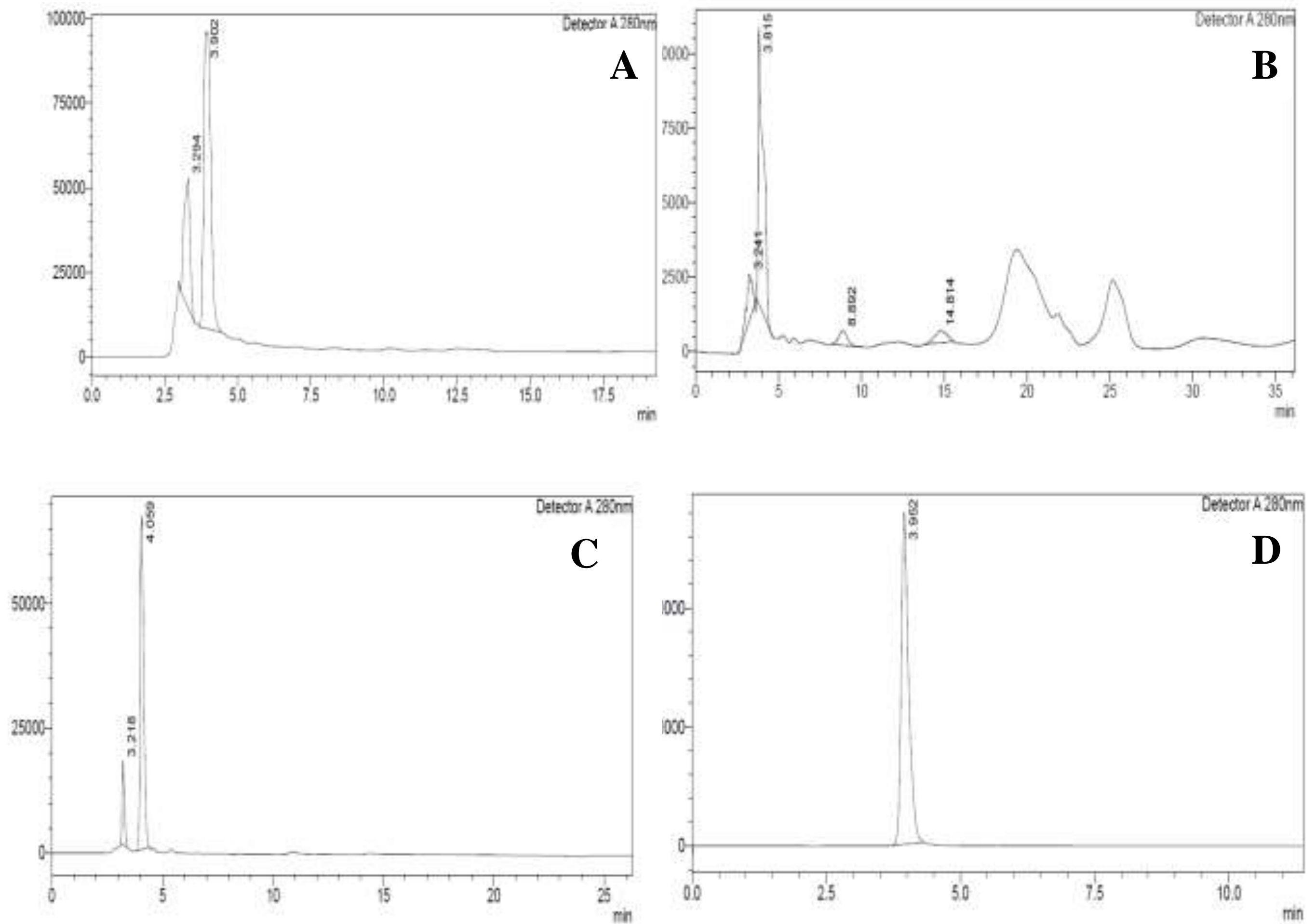

Figure 1: RP-HPLC Chromatogram of *Premna integrifolia* A. Methanol extract B. Ethanol extract C. Chloroform extract D. Standard Gallic acid

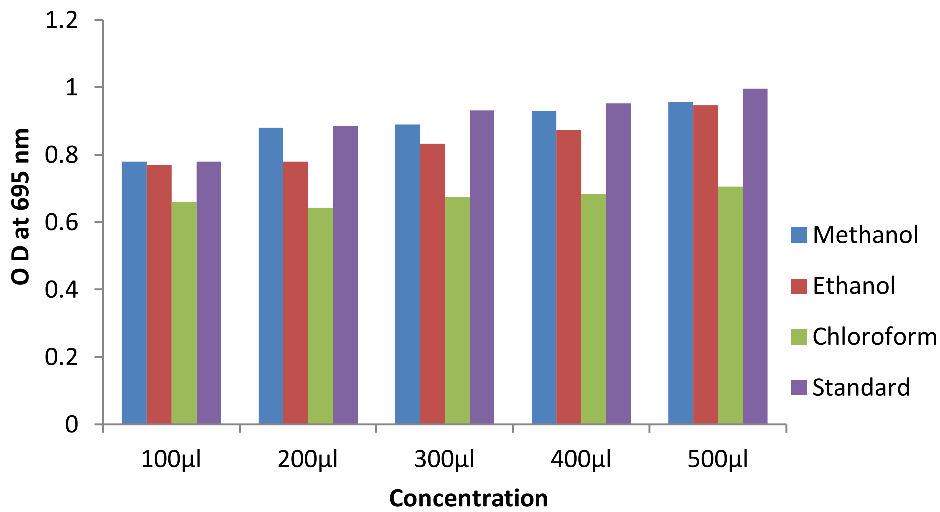

Figure 3: Phospho molybdate Assay

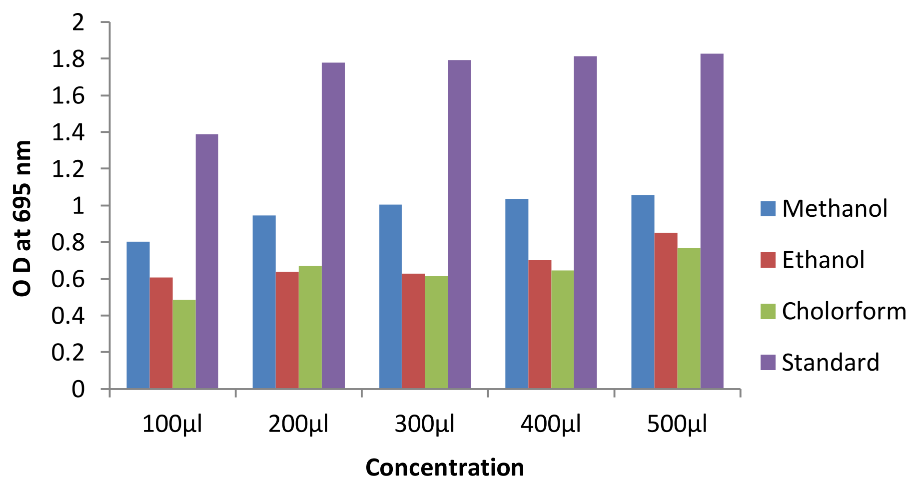

Figure 2: FRAP assay

Table 1: Percentage inhibition of DPPH free radical of *Premna integrifolia* extracts.

%.Concentration Inhibition

Concentration	Inhibition (in %)			
	Methanol Extract	Ethanol Extract	Chloroform Extract	Standard
62.5µg/ml	62.99±1.21	45.93±1.01	27.55±2.01	95.9±1.31
125µg/ml	78.47±0.98	63.51±1.35	64.04±1.45	96.85±1.12
250µg/ml	88.45±0.85	75.85±0.85	66.66±0.68	97.11±0.24
500µg/ml	90.81±1.21	80.83±1.24	79.52±0.58	97.63±0.35
1000µg/ml	92.12±0.96	83.20±1.58	83.20±0.96	98.95±0.86

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Conflict of interest statement

We declare that we have no conflict of interest.

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