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BIOEFFICACY OF *PREMNA CORYMBOSA* ROTTL. (VERBENACEAE) WITH SPECIAL REFERENCE TO ITS ANTIOXIDANT ACTIVITY

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Keywords:

Premna corymbosa, Antioxidant activity, DPPH assay, Chelating activity, Radical Scavenging Activity

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ABSTRACT: In the present investigation, the bioefficacy of an indigenous plant was investigated with special reference to its antioxidant potential. Antioxidant effects of Methanolic, Chloroform and Ethyl acetate extracts of the leaves of *Premna corymbosa* Rottl. (family Verbenaceae) were tested based on DPPH (1, 1-diphenyl-2-picryl-hydrazyl), phosphomolybdenium assay, superoxide radical scavenging activity, and metal chelating activity. Results about the present investigation indicate that the methanol extract of the *P. corymbosa* showed strong antioxidant activities than the other solvent extracts. This study has made it clear that *Premna corymbosa* is an effective antioxidant. Such extracts could be more widely used in developing countries for the prevention and treatment of cancer. These extracts may be considered as good sources for drug discovery.

INTRODUCTION: Plants develop several antioxidants that aid in antioxidant defense system, protecting plants against damage caused by active O_2 formed due to exposure to ultraviolet radiation. Certain seaweeds also function as antioxidants. Our daily diet contains vegetables, fruits, tea, wine, etc. which possess compounds rich in antioxidative properties [1]. Dietary antioxidants reduce the free radical formation as well as oxidative stress and reduce the possibility of cardiovascular diseases [2]. Several compounds such as phenolic diterpenoids-camosol, rosmanol, camosoic acid, *etc*.

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Obtained from several aromatic plants possess strong antioxidant properties. Today there are at least 120 distinct chemical substances derived from plants that are considered as important drugs currently in use in one or more countries in the world. Several of the drugs sold today are simple synthetic modifications or copies of the naturally obtained substances.

Many European countries, including Germany, regulate herbal products as drugs and pharmaceutical companies, prepare plant-based drugs simply by extracting out the active chemicals from the plants (e.g., cynarin, silymarin). Plants have evolved the ability to synthesize chemical compounds that help them defend against attack from a wide variety of predators such as insects, fungi and herbivorous mammals. By chance, some of these compounds, while being toxic to plant predators, turn out to have beneficial effects when

used to treat human diseases. Such secondary metabolites are highly varied in the structure; many are aromatic substances, most of which are phenols or their oxygen-substituted derivatives.

Premna corymbosa is a large shrub or a small tree with branches often spinous but branchlets unarmed. Leaves opposite, 5-10 cm long, broadly elliptic, entire, undulate, obtuse, very shortly acuminate Leaves pubescent when young, broadly oblong or ovate, entire or dentate.

Flowers are borne in corymbs, greenish white, somewhat viscid, with an unpleasant smell. Calyx 2-lipped or irregularly 5-toothed. Corolla twice the length of the calyx. Drupe black, globose. Seeds oblong. Fruit 4 mm long, pear-shaped. *Premna serratifolia* is used by the traditional practitioners as cardiotonic, antibiotic, anticoagulant, stomachic, carminative, hepatoprotective, antitumor *etc*. [3] The antitumor and antioxidant potentials of the ethanolic extract of *Premna integrifolia* Linn. were examined [4]. Similarly, *Premna* species have been extensively studied by various authors for their biological activities [5-9].

MATERIALS AND METHODS: General laboratory techniques recommended by Purvis *et al.,* [10] was followed for the preparation of media, inoculation, and maintenance of cultures. The leaves were carefully washed with tap water, rinsed with distilled water, and air-dried for 1 h.

Then leaves are separated & dried in room temperature for one week. Then they were ground into a powder and stored in room temperature. Direct extraction with chloroform, ethyl acetate, and methanol was done following the method described by Eloff [11]. In this method, finely ground plant material was extracted with chloroform, ethyl acetate, and methanol in the ratio of 1:10 in the conical flask in shaking condition for overnight.

The extract was filtered through the Whatman no. 1 filter paper in a separate container. The process was repeated 3 times and the same plant material but using fresh solvent. The solvent was removed by placing the extracts in the distillation unit in the respective temperature. The extracted residues were weighed and re-dissolved in different solvents to yield 10 mg/mL solutions ready for further analysis.

Antioxidant Assays:

DPPH Assay (2, 2-diphenyl-1-picrylhydrazyl): The Radical Scavenging Activity of different extracts was determined by the method proposed by Chang [12] but with a small modification. The decrease of the absorption at 517nm of the DPPH solution after the addition of the antioxidant was measured in a cuvette containing 2960µl of 0.1mM ethanolic DPPH solution was mixed with 40µl of 20 to 200 µg/mL of plant extract and vortexed thoroughly. The setup was left at dark in room temperature and the absorption was monitored after 20minutes. Ascorbic acid and Butylated hydroxytoluene (BHT) were used as references. The ability of the plant extract to scavenge DPPH radical was calculated by the following equation:

% DPPH Radical Scavenging Activity (% RSA) = Abs. control – Abs. sample × 100 / Abs. Control

Where, Abs. control is the absorbance of DPPH radical and ethanol; Abs. sample is the absorbance of DPPH radical + plant extract. Measurements were performed in triplicates. Absorbance values were corrected for radicals decay using blank solutions. The contents in the test tube were incubated for 20 mins at room temperature in dark condition. The optical density was read at 517nm. % of DPPH radical scavenging activity is found by the following equation:

% DPPH Activity = [(OD of control – OD of sample) / OD of control] × 100

Phosphomolymbdenium Assay: The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation [13]. An aliquot of 100µl of sample solution was combined with 1ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate) in a 4ml vial. The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported (Ascorbic acid equivalent antioxidant activity) are mean values expressed as g of ascorbic acid equivalents / 100g extract. Superoxide anion radical scavenging activity was studied [14]. Hydroxyl radical scavenging activity of methanol extract of *P. corymbosa* on hydroxyl radical was measured. The chelating of ferrous ions by methanol extract of *P. corymbosa* was estimated [15].

RESULTS AND DISCUSSION:

DPPH Assay: (2, 2-diphenyl-1-picrylhydrazyl): After the addition of an antioxidant, a decrease in absorption was measured at 517 nm. A significant decrease in O.D. value was obtained. This indicated the radical scavenging activity of the plant extract. The ethanolic, chloroform, and methanol extracts were subjected to DPPH assay. The decrease in absorbance was noted for all the three extracts. When compared, the methanolic extract of the plant showed more radical scavenging activity. More than 60% of radical scavenging activity was observed even at very low concentration of the extract used. Also, the results confirm that *P. corymbosa* has a very good antioxidant property **Table 1. Fig. 1** shows the combined % RSA of ethanol, chloroform and Methanol extracts. The methanol extract shows increased radical scavenging activity when compared with others.

TABLE 1: DPPH ASSAY OF *PREMNA CORYBOSA*

Conc. (µg/mL)	O. D. Methanol	O. D. Chloroform	O.D. Ethyl Acetate	% RSA Methanol	% RSA Chloroform	% RSA Ethyl Acetate
Control	0.765	0.584	0.251	--	--	--
20	0.302	0.543	0.826	60.7	7.0	41.2
40	0.150	0.529	0.485	80.4	9.4	42.9
60	0.141	0.515	0.472	81.6	11.8	52.5
80	0.138	0.497	0.392	82.0	14.9	54.5
100	0.130	0.461	0.376	83.0	21.1	56.5
120	0.127	0.459	0.359	83.4	21.4	65.5
140	0.124	0.447	0.285	83.8	23.5	66
160	0.113	0.425	0.281	85.2	27.2	66.3
180	0.115	0.402	0.278	85.0	31.2	67.1
200	0.106	0.396	0.272	86.1	32.2	69.6

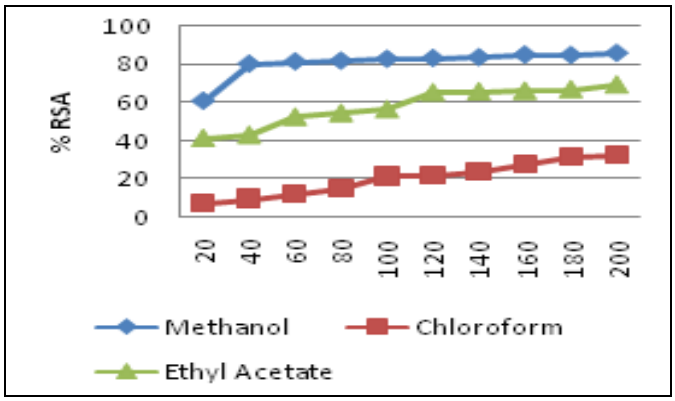

FIG. 1: DPPH ASSAY OF *PREMNA CORYBOSA*

Table 2 shows the combined effect of the methanolic extract of *P. corymbosa* against Phosphomolybdenium Assay (PMA), Superoxide Radical Scavenging Activity (SOD), and Metal Chelating Activity (MCA). The extract has scavenged the free radicals in all the three assays. The % RSA was more for the test when compared to the standard and the control for Phosphomolybdenium Assay. This shows the reduction of phosphomolybdic acid to phosphomolybdenum blue complex by sodium sulfide. The obtained phosphomolybdenum blue complex was oxidized by the addition of nitrite, and this caused a reduction in the intensity of the blue color. The methanolic extract of *P.corymbosa* has reacted with the hydrogen peroxide produced in Super Oxide Radical Scavenging Activity and has eliminated it, which reflects in the O.D. Value. Thus, it eliminates the free radical and makes it unavailable, which can lead to cellular damage and apoptosis.

TABLE 2: PHOSPHOMOLYBDENIUM ASSAY, SUPER-OXIDE RADICAL SCAVENGING ACTIVITY & METAL CHELATING ACTIVITY OF *PREMNA CORYBOSA*

Sample	O. D. value		
	PMA	SOD	MCA
Control	0.315	0.305	0.263
Standard	o.252	--	0.028
Test	0.217	0.010	0.37

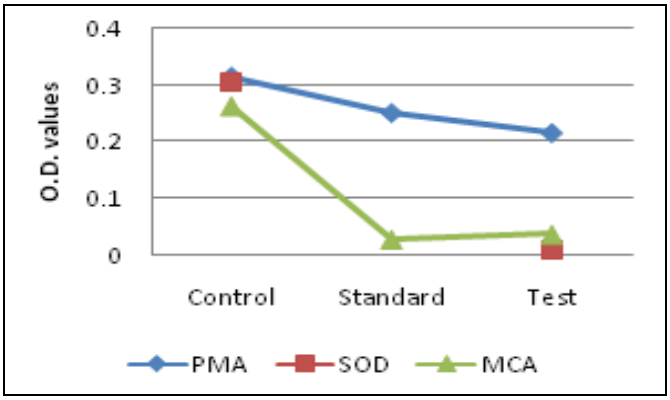

FIG. 2: PHOSPHOMOLYBDENIUM ASSAY, SUPEROXIDE RADICAL SCAVENGING ACTIVITY & METAL CHELATING ACTIVITY OF *PREMNA CORYBOSA*

The antioxidants present in plant extract forms a coordinate complex with the metal ions (chelating activity) and inhibit the transfer of electrons. Thus oxidation reaction is arrested, and no free radicals are produced. The results obtained shows that the ferrous ions have been chelated by the methanolic extract of the plant. Thus, a color change has occurred when compared to the control and a decrease in O.D. Value. Similarly, **Fig. 2** shows the free radical scavenging activity of the plant extract when compared to the control and standard.

Hydroxyl Radical Scavenging Activity: Table 3 and **Fig. 3** shows the hydroxyl radical scavenging activity of the methanolic extract of *P. corymbosa* leaves. As the concentration of the sample increases, the % RSA also increases. 100 µL of the sample can scavenge more than 60% of hydroxyl radicals.

TABLE 3: HYDROXYL RADICAL SCAVENGING ACTIVITY OF *PREMNA CORYBOSA*

Conc. (µL)	O. D.	% RSA
Control	0.248	--
25	0.160	35.5
50	0.138	44.4
75	0.107	56.9
100	0.078	68.6

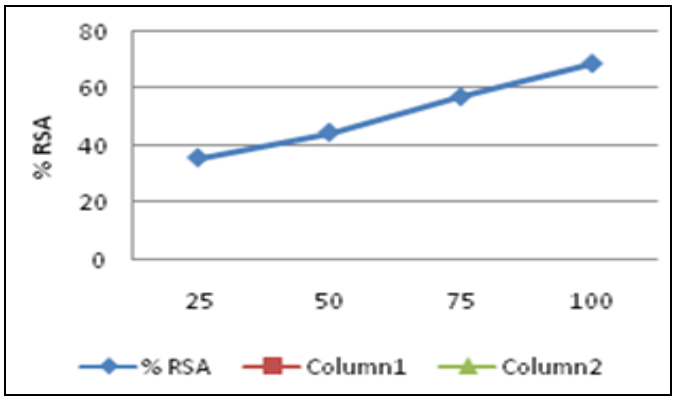

FIG. 3: HYDROXYL RADICAL SCAVENGING ACTIVITY OF *PREMNA CORYBOSA*

Nature has provided an excellent storehouse of remedies to cure all the ailments of mankind. In ancient days, almost all the medicines used were from natural sources, particularly from plants. Plants continue to be an important source of new drugs even today. The importance of botanical, chemical, and pharmacological evaluation of plant-derived agents used in the treatment of human ailments has been increasingly recognized in the last decades. Herbal remedies are widely used for the treatment and prevention of various diseases and often contain a highly active multitude of chemical compounds. Modern research is now focusing greater attention on the generation of scientific validation of herbal drugs based on their folklore claim. In this modern era, a large Indian population still relies on the traditional system of medicine, which is mostly plant-based.

Plants have played a significant role in the treatment of cancer and infectious diseases for the last four decades. Natural products have been rediscovered as important tools for drug development despite advances in combinatorial chemistry. This study has made it clear that *Premna corymbosa* might provide effective anti oxidant and anti-cancer therapeutic. Such extracts should be more widely used in developing countries for the prevention and treatment of dangerous diseases like cancer. These extracts should be considered as good sources for drug discovery.

On the other hand, without more supporting evidence, the notion that the antioxidant capacity of plant-based foods can directly determine the therapeutic utility of these foods in cancer treatment should be observed with some caution. The exact mechanism behind the observed antitumor effects of *Premna corymbosa* is still under exploration. Many studies suggest that there are additional functions responsible for the antitumor activity produced by this family of plants that go beyond their antioxidant capacity [16].

Extensive molecular and metabolic studies are needed to identify these other pathways. Even though studying whole foods provides a more normal way for the provision of food components and phytochemicals, it is still much simpler than the real human diet. This present investigation provides first-hand report on the selected plant species.

CONCLUSION: The screening of plant extract using the DPPH free radical method proved to be effective for the selection of plants that could have antioxidant activity. The present study revealed that the leaves of *P. corymbosa* are an effective potential source of natural antioxidants. Further, more detailed studies on the chemical composition

of the extracts, as well as studies with other models, such as lipid peroxidation and *in vivo* assays may lead to the development of a potential drug from *P. corymbosa.*

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CONFLICT OF INTEREST: Nil

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