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Evaluation of aqueous and methanolic extract of leaves of *Epipremnum aureum* for radical scavenging activity by DPPH Method, total phenolic content, reducing capacity assay and FRAP assay

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Abstract
Objective: Evaluation of aqueous and methanolic extracts of *Epipremnum aureum* leaves for antioxidant, total phenolic contents, total reduction capacity and FRAP assay.
Materials and Methods: The Antioxidant evaluation of the aqueous and methanolic extracts of *Epipremnum aureum* leaves were carried out by using DPPH radical scavenging activity assay, total reduction capacity assay and FRAP assay.
Results: The results of the DPPH radical scavenging activity indicate a concentration dependent antioxidant activity of *Epipremnum aureum* is comparable with standard ascorbic acid and IC_{50} was found to be 100 µg/ml. The total phenolic content was determined and found to be 852.379 mg/ml GAE for aqueous extract and 559.522 mg/ml GAE. The FRAP values of aqueous and methanolic extracts were found to be 1.932 and 1.716 respectively which are comparable to standard ascorbic acid.
Conclusion: Our findings provide evidence that the crude methanolic and aqueous extract of *Epipremnum aureum* is a potential source of natural antioxidants, and this justified its uses in folkloric medicines

Keywords: *Epipremnum aureum,* DPPH radical scavenging activity, antioxidant activity and FRAP values.

Introduction

Asia has abundant species of medicinal and aromatic plants and traditional medicines has practiced in Asia since ancient times. India has made use of medicinal plants to cure ailments of thousands of years. According to WHO the goal of health for all can't be achieved without herbal medicines, while the demand for herbal medicine is growing in developing countries, there are indications that consumers in developed countries are becoming disillusioned with modern healthcare and are seeking alternatives in traditional medicine. As much as 70% of India's population used traditional medicine [1]. There are about 45,000 plant species are found in the Indian subcontinent of these, about 3500 species of both higher and lower plant groups are of medicinal value. Of around 500 medicinal plants species used by the Ayurvedic industry, around 80% are procured from wild areas, mostly notified as forest land [2]. Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. A rich heritage of knowledge to preventive and curative medicine was available in ancient scholastic included in atharvaveda, Charaka, sushruta etc. Over 505 of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry. Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness [3].

The evaluation of the antioxidant activity of plant may also necessary because of their nutraceuticals effects. Antioxidants are compounds that delay or inhibit the oxidation of lipids or other molecules by inhibition initiation or propagation of oxidizing chain reaction and hence pharmacological effects on neurological disorders on the basis of *in vitro* observation [4].

Antioxidants have the ability of protecting organisms from damage caused by free radical-induced oxidative stress [5]. Presently, the probable toxicity of synthetic antioxidants has been condemned. It is strongly believed that regular consumption of plant-derived phytochemicals may drift the balance toward an adequate antioxidant status [6]. Thus, in recent years, interest on natural antioxidants, especially of plant origin, has increased manifolds [7].

Epipremnum aureum plant belongs to Araceae family. It is commonly known as money plant. It has yellow, green heart shaped leaves which gives it ornamental value. Height of the plant is depends on the support on which it grows and it grows with fast rate in slightly alkaline salt

and shade region. It is easily available plant which removes pollutants such as formaldehyde, xylene and benzene.

The aim of present work is to prepare extract of leaves of *Epipremnum aureum* by using Soxhlet extraction method. To determine the total phenolic content in the leaves extracts as GAE (Gallic Acid Equivalent), screening for the radical scavenging activity by using the DPPH method, determination of reducing capacity of the leaves extracts by using the Total Reduction Capacity Assay and to check for iron reducing capacity of the leaves extracts using the FRAP Assay.

Materials and Methods

The leaves of *Epipremnum aureum* were collected from the campus of Haffkine Institute for Training, Research and Testing, Parel. The identification of the leaves of *Epipremnum aureum* was done by Dr. S. Y. Jadhav, Head of Botany Dept, YC Mahavidyalay, Waranangar, Affiliated to Shivaji University, Kolhapur.

DPPH solution (0.135mM) using methanol as the solvent, Ascorbic acid (1mg/mL), Folin Ciocalteu Reagent, Gallic Acid (5mg/mL), Sodium Carbonate Solution (20%), Phosphate buffer (50 mM, pH 7.0), Potassium ferricyanide [K_3 Fe$(CN)_6$] solution (1%), Trichloro acetic acid solution [TCA] (10%), Ferric chloride [$FeCl_3$] solution (0.1%), FRAP reagent procured from Unique Biologicals and Chemicals, Kolhapur.

Preparation of leaf extracts

The leaf extracts of the plant under study were obtained using the Soxhlet Extraction Method. A Soxhlet Extractor was used for this purpose. A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. The soxhlet extraction of leaves was carried out by using following procedure;

20 grams of washed and dried leaves were taken and placed in a thimble made up from thick filter paper, which was loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor was placed onto a round bottom flask containing the extraction solvent i.e. distilled water. The Soxhlet was then equipped with a condenser. The solvent was heated at 90 ^oC to reflux: As the solvent vapour travelled up a distillation arm, into a condenser, the condensed vapours dripped back down into the chamber housing the solid material. The chamber containing the solid material slowly filled up with warm solvent. When the Soxhlet chamber was almost full, the chamber was automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle was allowed to repeat 18 times. After extraction the solvent was removed, by means of a rotary evaporator. The extracted compound was collected. The non-soluble portion of the extracted solid in the thimble was discarded. The aqueous extract was then used for further investigation. Similarly methanolic extract was also obtained by using Soxhlet extraction method.

DPPH radical scavenging assay

The free radical scavenging capacity of the extracts was determined using DPPH [8]. Calibration curve was prepared by adding 0, 1, 2, 5, 8 and 10 mL of the ascorbic acid stock solution into 100 mL volumetric flasks, and then dilute to volume with water. These solutions will have concentrations of 0, 10, 20, 50, 80 and 100 µg/L ascorbic acid, and the effective range of the assay. From each calibration solution, sample or blank, 200µL was pipetted into separate cuvettes, and to each 2mL of DPPH solution was added. Solutions was kept at room temperature, in dark for 30 mins and the absorbance of each solution was determined at 515 nm against the blank (the "0 mL" solution) and percent inhibition vs. concentration graph was plotted.

Total phenolic content determination

Total phenolic content was determined using the Folin-Ciocalteu (FC) reagent method [9] with slight modification. The standard used for this assay is gallic acid and the results are reported at Gallic Acid Equivalent (GAE) because the phenols present in plants contain mostly other phenols, and only small amounts of gallic acid. Therefore the presence of gallic acid indicates the presence of wide range of phenols in the sample. Calibration curve was prepared, by adding 0, 1, 2, 3, 5 and 10 mL of the gallic acid stock solution into 100 mL volumetric flasks, and then dilute to volume with water. These solutions will have phenol concentrations of 0, 50, 100, 150, 250, and 500 µg/L gallic acid, the effective range of the assay. From each calibration solution, sample, blank, 20 µL was pipetted into separate cuvettes, and to each 1.58 mL water was added, and then 100 µL of the Folin- Ciocalteu reagent was added and mixed. After 5 min, 300 µL of the sodium carbonate solution was added and mixed. Solution was kept at 40 °C for 30 mins and the absorbance of each solution was determined at 765 nm against the blank (the "0 mL" solution) and absorbance vs. concentration graph was plotted. Results were reported as Gallic Acid Equivalent (GAE).

Total reduction capacity assay

This method is based on the chemical reduction of ferricyanide (Fe^{3+}) complex to the ferrous (Fe^{2+}) form [10]. In this assay, the yellow color of the test solution changes to various shades of green and blue, that exhibits a broad light absorption with a maximum at 700 nm, depending on the reducing power of each compound. Calibration curve was prepared by adding 0, 1, 2, 5, 8 and 10 mL of the ascorbic acid stock solution into 100 mL volumetric flasks and then dilute to volume with water. These solutions will have concentrations of 0, 100, 200, 500, 800 and 1000 µg/L ascorbic acid, the effective range of the assay. From each calibration solution sample or blank, 1mL was pipetted into separate tubes, and to it 2.5mL of phosphate buffer and then 2.5 mL of potassium ferricyanide solution was added. Solutions wwre kept at 50 ^oC for 20 mins. Then 2.5 mL of trichloroacetic acid was added and solution was centrifuged for 10 mins at 3000 rpm. supernatant was collected. 1.25mL of the supernatant was taken into separate cuvettes and to each 1.25 mL distilled water and then 0.25 mL of ferric chloride solution was added. The absorbance of each solution was determined at 700 nm against the blank (the "0 mL" solution) and absorbance vs. concentration graph was plotted.

FRAP assay

Total antioxidant activity is measured by ferric reducing antioxidant power (FRAP) Assay. At low pH, reduction of ferric tripyridyl triazine (Fe^{3+} TPTZ) complex to ferrous form

(which has an intense blue colour) can be monitored by measuring the change in absorption at 593nm. The reaction is non-specific, in that any half reaction that has lower redox potential, under reaction conditions, than that of ferric ferrous half reaction, will drive the ferrous (Fe^{3+} to Fe^{2+}) ion formation. The change in absorbance is therefore, directly related to the combined or "total" reducing power of the electron donating antioxidants present in the reaction mixture [11].

Calibration curve was prepared by adding 0, 1, 2, 5, 8 and 10 mL of the ascorbic acid stock solution into 100 mL volumetric flasks, and then dilute to volume with water. These solutions will have concentrations of 0, 100, 200, 500, 800 and 1000 µg/L ascorbic acid, the effective range of the assay. From each calibration solution, sample or blank, 100µL was pipetted into separate cuvettes and to 3 mL of FRAP reagent was added and mixed well. The absorbance of each solution was determined at 593 nm against the blank (the "0 mL" solution) at 0 min. The solutions were kept at 37 ºC for 4 mins and again the absorbance of each solution was determined at 593nm against the blank (the "0 mL" solution). FRAP value of the sample was calculated.

Results

Plant Yield

The yield of the *Epipremnum aureum* methanolic leaf extract was 3.33% and aqueous 7.11%.

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the extract revealed the presence of various bioactive components, of which flavonoids and tannins were the most prominent, and the result of the phytochemical test has been summarized in Table 1. Reports are also available where the phenolic compounds and flavonoids are found to be associated with the antioxidative activity in biological systems [12, 13].

Table 1: Preliminary phytochemical screening of the *Epipremnum aureum*

Sr. No	Chemical compounds	Aqueous extract	Methanolic extract
1.	Saponins	+	+
2.	Steroids	-	-
3.	Alkaloids	+	+
4.	Tannins	-	+
5.	Carbohydrates	+	+
6.	Flavonoid	+	+
7.	Anthraquinone	-	-
8.	Glycosides	+	+
9.	Reducing sugars	+	+

- = Compound not detected; + = compound detected

DPPH radical scavenging assay

In this present study, the antioxidant activity of the aqueous and methanol extracts of the *Epipremnum aureum* leaves was investigated using the DPPH scavenging assay, reducing power of the extract and by determining the total antioxidant capacity of the extract. All these have proven the effectiveness of the aqueous and methanol leaf extract of *Epipremnum aureum* compared with the reference standard antioxidant ascorbic acid. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. Comparison of the antioxidant activity of the extract and ascorbic acid is shown in Figure 1. The methanol extract of *Epipremnum aureum* exhibited a significant dose-dependent inhibition of DPPH activity, with a 50% inhibition (IC50) at a concentration of 100 µg/ ml as compared with the standard ascorbic acid.

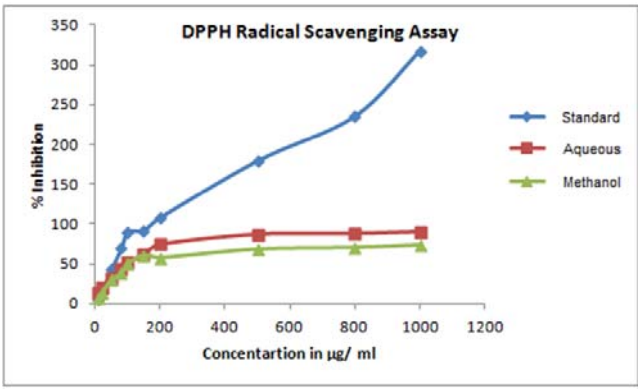

Fig 1: DPPH Radical Scavenging assay of Standard (Ascorbic acid), Aqueous and Methanolic Extract of *Epipremnum aureum*

Determination of the Total Phenolic Content

Phenolic compounds may contribute directly to the antioxidative action. The total phenolic content was 571.428 mg/ml for aqueous extract and for methanol 842.857 mg/ml, gallic acid equivalent per 10 mg plant extract. Results are summarised in Table 2.

Table 2: Total phenolic content of the *Epipremnum aureum*

Concentration of extract in mg/ml	GAE of Aqueous extract in mg/ml	GAE of methanol extract in mg/ml
1	571.428	857.142
2	535.71	857.14
10	571.428	842.857
Average	559.522	852.379

Total reduction capacity assay

The reductive capabilities of the plant extract compared with ascorbic acid have been depicted in Figure 2. The reducing power of the extract of *Epipremnum aureum* leaves was found to be remarkable, which increased gradually with a rise in the concentration. As illustrated in Figure 2, Fe3+ was transformed to Fe2+ in the presence of the extract and the reference compound ascorbic acid to measure the reductive capability. At 500 µg/ml, the absorbance of aqueous and methanol extract was 0.55 and 0.32 respectively and ascorbic acid was 0.77, respectively.

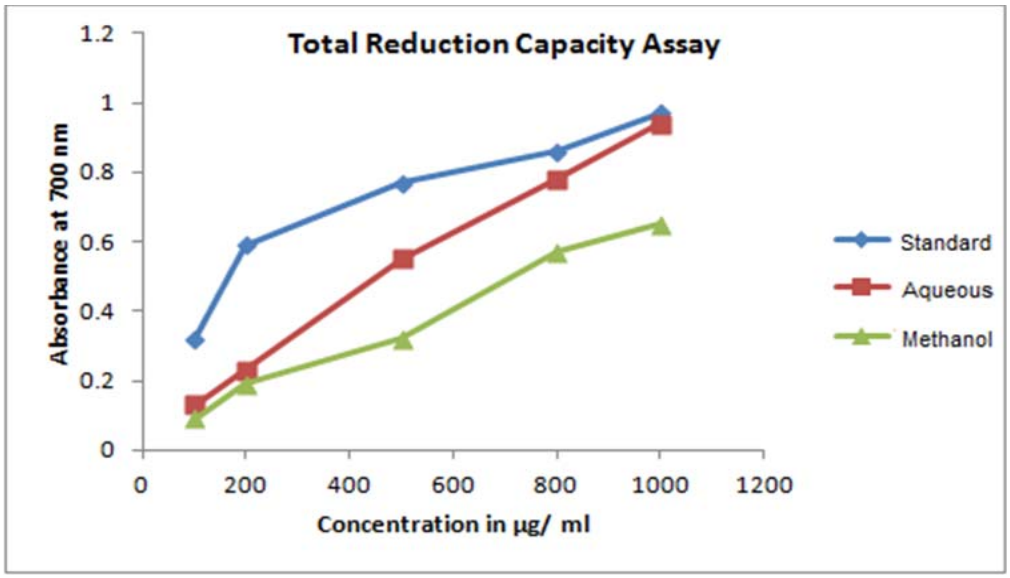

Fig 2: Total Reduction Capacity of Standard, Aqueous and Methanolic Extract of *Epipremnum aureum*

FRAP assay

FRAP Value = (Change in absorbance of sample from 0 to 4 mins/Change in absorbance of standard from 0 to 4 mins) ×FRAP value of standard i.e. 2.

Results are summarised in Table 3.

Table 3: Determination of FRAP value for *Epipremnum aureum*

Sample Concentration (µg/mL)	FRAP Value for methanol extract	FRAP Value for aqueous extract
100	1.5	2
200	1.65	2
500	1.75	1.88
800	1.82	1.92
1000	1.86	1.86
Average	1.716	1.932

Discussion

During oxidative stress and exposure to radiation, excessive free radicals are produced that are known to cause damage to the biomolecules [14, 15]. Antioxidant and radioprotection studies indicate that *Epipremnum aureum* should possess the ability of either inhibiting free radical formation or itself be a free radical scavenger.

The free radical scavenging capacity of aqueous and methanol extract of leaves of *Epipremnum aureum* was tested by its ability to bleach the stable DPPH radical. This assay provided information on the reactivity of the test compound with a stable free radical, since its odd electron DPPH gives strong absorption band at 517 nm in visible spectroscopy (deep violet colour). As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes and the resulting decolourization is stoichiometric with respect to the number of electrons taken up. It showed excellent antiradical activity by inhibiting DPPH radical with an IC_{50} value of 100 µg/ml for aqueous and methanol extract. The scavenging activity was comparable to that observed with ascorbic acid.

It is found that phenolic compounds contribute directly to antioxidant action. Therefore total phenolic content of aqueous and methanol extracts of *E. aureum* was determined by using FC method. GAE of aqueous extract and methanol extract was found to be 852.379 mg/ml and 559.522mg/ml respectively. The assay used for the determination of total phenolics content employs Folin and Ciocalteu's phenol reagent which response depending on the chemical structure of phenolics (i.e. the higher the number of functional –OH group the higher the total phenolics content). Total soluble phenolic compounds in the extracts were measured and expressed as gallic acid equivalents on comparison it is observed that GAE of extracts and standards such as ascorbate were comparable. Results are reported as Gallic Acid Equivalent because gallic acid is least phenol present in extracts. As phenols are hydrophilic compounds, phenolic content of aqueous extract is higher than methanol extract.

In TRC assay it is observed that as concentration of sample increases absorbance also increases. Higher absorbance for the reaction mixture indicated greater reducing power. As compared with standards both aqueous and methanol extract of plant shows comparable antioxidant activity.

FRAP assay, depends upon the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant at low pH. (Fe (II)-TPTZ has an intensive blue colour and can be monitored at 593 nm. Change in absorbance is directly related to antioxidant present in reaction mixture. FRAP value of sample was calculated and it was found to be 1.88 for aqueous extract and 1.80 for methanolic extract. FRAP value of sample and standard were comparable.

Conclusion

Evaluation of antioxidant activity of *Epipremnum aureum* was successfully carried out. In DPPH radical scavenging activity, IC_{50} for aqueous extract and methanolic extract was found to be 100µg/ml. Total phenolic content for aqueous extract and methanolic extract of *Epipremnum aureum* was found to be 852.379 and 559.552 mg/ml respectively. The total phenolic content for aqueous extract of *Epipremnum aureum* was found to be more than that of methanolic extract. In Total Reduction Capacity Assay it was observed that as concentration of

sample increases absorbance also increases and increase in absorbance was comparable with standard (Ascorbic acid). FRAP value for aqueous and methanolic extract was determined and it was found to be 1.932 for aqueous extract and 1.716 for methanolic extract. The FRAP value was more for aqueous extract than that of methanolic. As plant extract is not pure compound, to study partial presence of active compound in total extract and effect of these compounds in various activities further investigations are needed for phytochemical screening and structural elucidation of compounds in plant extract.

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