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# Phytochemical screening and *in vitro* antioxidant potential of methanolic extract of *Epipremnum aureum* (Linden and Andre) G. S. Bunting

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**ABSTRACT**

*In the present study, phytochemical constituents and in vitro antioxidative potential of different explants of E. aureum were evaluated. Methanol was used as solvent for extraction of E. aureum three explants (leaf, root and stem). Phytochemical screening was done by performing different chemical tests and presence of carbohydrate, protein, steroid, glycosides, alkaloids, saponins and amino acids were detected. Total phenol content and total flavonoid content was also determined using UV-VIS spectrophotometer. Antioxidant activity was analyzed for enzymes catalase, glutathione peroxidase and superoxide dismutase. Antioxidant potential and free radical scavenging activity was assessed in vitro using DPPH and FRAP methods. Observation revealed the presence of higher concentration of alkaloids, phenolics, flavonoids and terpenoids in methanol extract of leaves. Leaves showed high antioxidant activity due to catalase and peroxidase enzymes. E. aureum stem showed highest $IC_{50}$ value than leaves and aerial roots whereas leaves showed maximum free radical scavenging potential. It could be concluded that leaf fraction of Pothos (E. aureum)is rich in secondary metabolites followed by other parts and showed relatively maximum enzyme activity, antioxidant potential and free radical scavenging activity in comparison to aerial roots and stem. Thus leaf extract of E. aureum plant could serve as a good source of useful drugs.*

**Keywords:** Epipremnum aureum; Phytochemicals; Antioxidant; Scavenging activity; Phenolic compounds

## INTRODUCTION

Plants produce potent phytochemicals with strong antioxidant activities and thus represent an important source of natural antioxidants [1-4]. Phenolic compounds including flavonoids, vitamins and pigments have been identified to be accountable for antioxidant properties in majority of plants. *Epipremnum aureum* (Devil's Ivy) is popular ornamental foliage that is easily propagated through stem cuttings with minimum maintenance in suitable environment. It is a capable of removing indoor pollutants such as xylene, formaldehyde and benzene [5-7]. Aerial roots of *Pothos aurea* is regarded as a promising source for antimicrobial drugs [8, 9]. Aerial roots and leaves of *E. aureum* show great potential for antibacterial activity [10-12].

The potential of the phytochemicals have large scale pharmacological and biological activities such as antioxidant constituents (hydrolysable tannins, phenolics acid and flavonoids) of the plant materials for the care of health and protection from coronary heart diseases, cancer, anti-carcinogenic and anti-mutagenic effects [13]. Knowledge of the chemical constituent of plant is desirable for the discovery of therapeutic agents and to find the actual value of folklore remedies. Primarily, antioxidant effect is due to phenolic compounds such as phenolic acid, flavonoids and phenolic diterpenes and their mode of action for antioxidant compounds is due to its redox reaction properties which can absorb and neutralize free radicals by quenching singlet and triplet oxygen [14, 15]. Keeping in view the importance of phytochemicals, it is therefore very vital to discern the active constituents of *E. aureum* and provide a scientific data base line which may play a significant role in knowing the quantities of these phytochemicals and other constituents.

## MATERIALS AND METHODS

### 2.1. Plant material

Plant material was collected from Jaipur and authenticated as *Epipremnum aureum* (Linden and Andre) G. S. Bunting by the Botanical Survey of India, Jodhpur. It was cleaned, washed, air dried and used for the experiments.

### 2.2. Plant

Leaf, aerial root and stem of *E. aureum* (50g) were crushed using liquid nitrogen. Extraction was done with methanol thrice for three consecutive days at 28$^{o}$C (500 ml x 3). The extract was filtered twice with Whatman filter No. 1. Extract was combined and concentrated at 40$^{o}$C in water bath. Concentrated extract was kept in refrigerator at 4$^{o}$C for future use in phytochemical analysis.

### 2.3. Qualitative phytochemical analysis

Pothos explants were tested for the presence of different classes of compounds. Qualitative chemical test were used regarding the nature of phytoconstituents present in the methanolic extract of different explants [Table 1].

**Table 1. Phytochemical analysis of explants of *Epipremnum aureum***

| Test | Procedure | References |
|---|---|---|
| Alkaloids | Dragendorff's test | [16] |
| | Mayer's test | [17] |
| Saponin glycosides | Froth test | [17] |
| Anthraquinones | Modified Borntrager's test | [17] |
| Proteins/ amino acids | Ninhydrin | [17] |
| Tannins and Phenolic compounds | $FeCl_3$ Test | [16, 18] |
| sterols and terpenoids | Libermann-Buchard test | |
| Carbohydrates | Molisch's test | [17] |
| | Benedict's test | [17] |
| Flavonoids | Dil. Ammonia solution | [17] |
| | 1% Aluminium solution | [17] |

### 2.4. Determination of total phenolic content

The amount of phenol in the aqueous extract was determined by Folin-Ciocalteu reagent method with some modifications. 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of 2% solution of $Na_2CO_3$ were added to 1 ml of plant extract. The resulting mixture was incubated for 15 minutes at room temperature. The absorbance of the sample was measured at 765 nm. Gallic acid was used as standard (1 mg/ml).All the tests were performed in triplicates. The results were determined from the standard curve and were expressed as Gallic Acid Equivalent (mg/g of extracted compound) [19].

### 2.5. Determination of total flavonoid content

Aluminium chloride colorimetric method was used with some modifications to determine flavonoid content. 1 ml of sample plant extract was mixed with 3ml of methanol, 0.2 ml of 10% Aluminium chloride, 0.2 ml of 1 M Potassium acetate and 5.6 ml of distilled water and remains at room temperature for 30 minutes. The absorbance was measured at 420 nm. Quercetin was used as standard (1mg/ml). All the tests were performed in triplicates. Flavonoid contents were determined from the standard curve and were expressed as Quercetin Equivalent (mg/g of extracted compound) [19].

### 2.6. Antioxidant enzyme assay

#### *2.6.1. Catalase Assay*

The enzyme extract was prepared by crushing 0.5 g plant tissue in 50 mM Potassium phosphate buffer (pH 7.0). Supernatant was collected by centrifugation at 18000 g for 30 min at 4$^{o}$C. Catalase (CAT, E.C. 1.11.1.6) activity was determined by measuring the rate of $H_2O_2$ disappearance at 240 nm using spectrophotometer. The reaction mixture (2 ml) contained 50 mM Potassium phosphate (pH 7.0), 80 µl enzyme extract, 0.5 µl $H_2O_2$ and the initial linear rate of decrease in absorbance at 240 nm was used to calculate the enzyme activity [20].

#### *2.6.2. Peroxidase Assay*

The enzyme extract was prepared by crushing 1 g plant tissue in 60 mM Potassium phosphate buffer (pH 6.1). Supernatant was collected by centrifugation at 18000 g for 15 min at 4$^{o}$C. Guaiacol peroxidase (GPX, EC 1.11.1.7) activity was measured by spectrophotometer [21]. The reaction mixture (2 ml) contained 50 mM potassium phosphate (pH 7.0), 0.3 mM $H_2O_2$ and 5mM guaiacol. The reaction was started by the addition of 80µl enzyme extract. The formation of tetraguaiacol was measured at 470 nm.

#### *2.6.3. Superoxide Dismutase Assay*

The enzyme extract was prepared by crushing 0.2 g plant tissue in 25 mM Sodium phosphate buffer (pH 7.8).

Supernatant was collected by centrifugation at 17000 g for 15 min at $4^0$C. Activity of superoxide dismutase (SOD, E.C. 1.15.1.1) was assayed by measuring the inhibition of photo-reduction of Nitro-Blue Tetrazolium (NBT) [22]. The reaction mixture (2 ml) contained 50 mM Potassium phosphate buffer (pH 7.8), 10 mM L- Methionine, 1.17 mM Riboflavin, 56 mM NBT and 100 µl protein extract and the absorbance of purple formazon was measured at 560 nm. One unit of SOD activity was defined as the exact volume of enzyme causing half maximal inhibition of the NBT reduction under the assay condition.

**2.7. Antioxidation activity**

***2.7.1. DPPH Free radical scavenging activity***

The method first developed by Williams et al (1995) was employed with slight modifications [23]. The principle of the assay is based on the color change of the DPPH solution from purple to yellow as the radical is quenched by the

antioxidant. Briefly, 100 $\mu$L of methanol extract of plant material was added to 1.9mL of 3mM of DPPH in methanol up to completing 2mL. The free radical scavenging capacity using the free DPPH radical was evaluated by measuring the decrease of absorbance at 517 nm every 2min until the reaction reached its state. Additional dilution was needed if the DPPH value measured was over the linear range of the standard curve. The inhibition activity I(%) was calculated as

$$\text{Inhibition activity (\%)} = 100\times (A_O - A1)/A_O$$

Where, $A_O$ is the absorbance of the control sample and $A_1$ is the absorbance of the test compound. Extract concentration providing 50% inhibition ($IC_{50}$) was calculated from the graph plotting inhibition percentage against extract concentration [24].

***2.7.2. Ferric ion ($Fe^{3+}$) Reducing Antioxidant Power Assay (FRAP)***

The procedure of FRAP assay was according to Benzie and Strain [25]. The principle of this method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, colored form in presence of antioxidants. Briefly, the FRAP reagent contained 2.5mL of 10mmol/L TPTZ (2,4,6-tripyridyl-striazine, Sigma) solution in 40mmol/L HCl plus 2.5mL of 20mmol/L FeCl3 and 25mL of 0.3mol/L acetate buffer, pH 3.6, and was prepared freshly and warmed at $37^O$C. Aliquots of 40 µL sample supernatant were mixed with 0.2 mL distilled water and 1.8 mL FRAP reagent. The absorbance of reaction mixture at 593 nm was measured by spectrophotometer after incubation at $37^O$C for 10 min. The 1mmol/L FeSO4 was used as the standard solution. The final result was expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1mmol/L FeSO4. Adequate dilution was needed if the FRAP value measured was over the linear range of standard curve [24].

**2.8 Statistical analysis**

The experiments were conducted in triplicates. The data were represented as Mean ± S.E. and One-way analysis of variance (ANOVA) was performed by Tukey's Multiple Range test using software SPSS (version 16.0, SPSS Inc.). Significant differences between means were determined and $p<0.05$ was regarded as a significant value.

## RESULTS AND DISCUSSION

The preliminary phytochemical screening of the of the methanol extract of leaves, aerial root and stem of *E. aureum* showed the presence of various secondary metabolites of which alkaloids, flavonoids, terpenoids, saponin glycosides, tannin and phenolic compounds were the most prominent [Table 2]. While, aerial roots of *Pothos aurea* intertwined on *Areca catechu* showed alkaloid, tannin, glycoside, anthraquinone and phenol in ethanol extract. Aerial roots of *Pothos aurea* intertwined on *Lawsonia intermis* showed alkaloid, flavonoid, tannin, glycoside, saponin and anthraquinone in ethanol extract [8]. Similarly, phytochemical screening of leaves revealed the presence of alkaloids, flavonoids, tannins, terpenoids and anthraquinones in the methanolic extract [26]. The phenolics compounds possess biological properties such as anti-ageing, anti- carcinogenic, anti-inflammatory, cardiovascular protection and improvement of endothelial function as inhibition of angiogenesis and cell proliferation activities [27]. Presence of tannic acid was found more in leaves and it is reported to be an effective antioxidant and radical scavenging activity [28]. The hot and cold methanolic extracts showed presence of tannins, phenolic compounds, alkaloids, proteins, reducing sugars, triterpenoids and saponins [29].

**Table 2. Preliminary phytochemical analysis of the explants extract of *Epipremnum aureum***

| Test | Method | Leaf | Stem | Aerial Root |
|---|---|---|---|---|
| Alkaloids | Draggendorff's test | +++ | ++ | + |
| | Mayer's test | +++ | ++ | + |
| Saponin glycosides | Froth test | ++ | ++ | ++ |
| Anthraquinones | Anthraquinones test | + | + | + |
| Proteins/ amino acids | Ninhydrin | +++ | +++ | +++ |
| Tannins and Phenolics | $FeCl_3$ Test | ++ | - | + |
| Sterols and terpenoids | Libermann-Buchard test | ++ | + | + |
| Carbohydrates | Molisch's test | ++ | + | + |

| | | | | |
|---|---|---|---|---|
| Flavonoids | Benedict's test | ++ | + | + |
| | By dil. Ammonia | ++ | + | + |
| | By 1% aluminium | ++ | + | + |

*+++ Maximum presence of the compound, ++ Moderate, +Least presence, -Absence of the compound*

Antioxidant property was measured by using methanol extract of all three explants (leaf, root and stem). Several reports have shown a close relationship between total phenolic content and high antioxidant activity [30]. Total phenolic contents obtained were 8.284 mg/g of the leaves extract and total flavonoid contents obtained were 51.156 mg/gm of the leaves extract for the plants *E. aureum* [Table 3]. Some researchers have investigated the relationship between antioxidant activity and polyphenol content. Polyphenol compounds are reported to be a good source of natural antioxidants [31]. Plant phenols represent one of the major groups of compounds acting as primary antioxidants or free radical terminators. Thus, it was reasonable to determine their total amount in the selected plant extracts [32].

**Table 3. Total phenolic and flavonoid content in leaves of *Epipremnum aureum***

| Plant | Total Phenolic Content | Total Flavonoid Content |
|---|---|---|
| *Epipremnum aureum* leaf | 8.284 mg/g GAE | 51.156 mg/g QE |

In addition to the phenolic compounds as antioxidants, plants have array of enzymes such as peroxidase, superoxide dismutase and catalase also do away with reactive oxygen species produced during natural biochemicals activities as well as stress. Free radicals and reactive oxygen species are byproducts of numerous physiological and biochemical processes. Antioxidative assay for catalase, peroxidase and superoxide dismutase was calculated for the leaves of *Epipremnum aureum* and peroxidase activity was found maximum with 53.66µmol/min/ml of activity. Catalase activity was 33.34µmol/min/ml and least SOD activity was observed [Table 4].

**Table 4: Antioxidation assay for various enzymes of *Epipremnum aureum***

| Plant | Assay | Activity (µmol/min/ml) |
|---|---|---|
| *Epipremnum aureum* leaf extract | Catalase | 33.3±4.792 |
| | Peroxidase | 53.66 ±5.789 |
| | Superoxide Dismutase | 3.33 ±0.833 |

Antioxidant activities of *E. aureum* determined by the free radical scavenging activity (DPPH) assay method indicated a steady increase in the scavenging activity of free radicals in all extracts from 322 to 1011 µg/mL [Figure 1]. It was observed that the ability of test materials to scavenge DPPH was assessed on the basis of their $IC_{50}$ values, defined above as the concentration of test material to decrease the absorbance or concentration at 515 nm of DPPH solution to half of its initial value. It can be seen that stem shows higher $IC_{50}$ value (1011 µg/mL) than leaves (322 µg/mL) and aerial roots (549.33 µg/mL). This result can be attributed to the higher phenolics and flavonoid compounds in the leaves and aerial root. The higher DPPH radical scavenging activity is associated with a lower $IC_{50}$ value. FRAP values of leaves, aerial root and stem is summarized in Figure 2. The reducing ability of different parts of Pothos is expressed, respectively in $FeSO_4$ equivalent; they ranged from 0.546 to 1.886 mM $FeSO_4$ [Figure 2]. The decreasing order efficiency in FRAP system is leaves>aerial root>stem. These results agreed with the DPPH values. An earlier study has been done using acetone, ethanol and aqueous extracts of the aerial roots of *Pothos aurea* intertwined over *Lawsonia inermis* and *Areca catechu*. Percentage inhibition using DPPH assay showed highest antioxidant activity of acetone extract aerial roots intertwined over *L. inermis.* The aqueous extract of *P. aurea* showed least inhibition against DPPH. Aerial roots climbed over *L. inermis* showed better reducing power than aerial roots climed over *A. catechu* [33-36]. Chloroform, acetone and ethanol extract of leaves of *E. aureum* is proven effective against free radicals. Ethanol extract was found to possess highest antioxidant potential compared to chloroform and acetone [37-40].

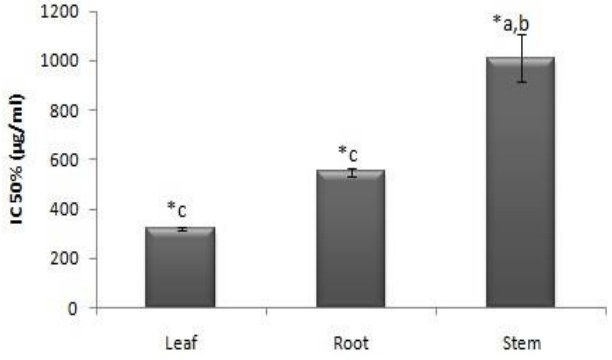


**Figure 1.** DPPH assay of various parts of *Epipremnum aureum*
**The mean difference is significant at the 0.05 level*

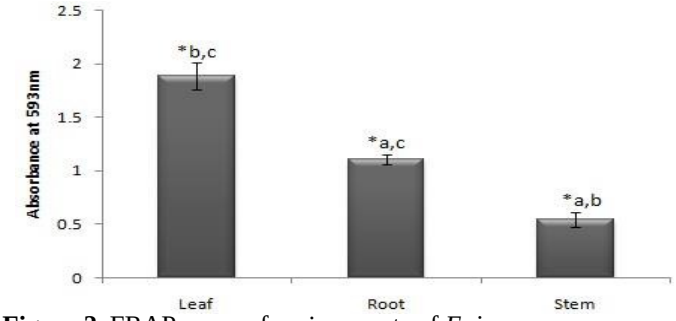


**Figure 2.** FRAP assay of various parts of *Epipremnum aureum*
**The mean difference is significant at the 0.05 level*

The high antioxidant activity of the methanol extract of leaves may be attributed to the phytochemical compounds present in the extract. Flavonoids are very good antioxidants and presence of high flavonoids in the leaves extract may be the cause for the extract having more antioxidant activity compared to aerial root and stem.

## CONCLUSION

The usage of this ornamental plant should be promoted as an alternative for synthetic chemicals as it is easily available, grown and maintained, accessible and affordable. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. The high antioxidant potential, free radical scavenging activity and antioxidative enzymes of *Epipremnum aureum* should be utilized to develop new drug candidates for antioxidant therapy.

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