

**Original Article**

**PHYTOCHEMICAL SCREENING AND EVALUATION OF LEAF PARTS OF *HYPOESTES PHYLLOSTACHYA* ROSEA FOR *IN VITRO* ANTIOXIDANT ACTIVITIES**

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**ABSTRACT**

**Objective:** The aim of this study was focussed on phytochemical analysis, *in vitro* antioxidant activities of different solvent extracts of *Hypoestes phyllostachya* leaf.

**Methods:** The leaves were washed efficiently, shade dried and coarsely powdered. The powdered leaf material of *Hypoestes phyllostachya* was successively extracted with hexane, chloroform, methanol and water using Soxhlet apparatus. Preliminary phytochemical screenings for polyphenolics, proteins, phytosteroids, flavonoids, tannins, glycosides, alkaloids carbohydrates, polyphenolics, saponins were done by following standard procedure. *In vitro* antioxidant activities of various solvent extracts were assessed using DPPH, ABTS, Hydroxy radical, total antioxidant capacity and reducing power assay. Polyphenolics and flavonoids were also estimated.

**Results:** The results showed that most of the phytochemical compounds such as carbohydrates, proteins, steroids, fixed oils and fatty acids, flavonoids, alkaloids, saponins, tannins, polyphenolics and glycosides present in the methanolic and aqueous extract of *Hypoestes phyllostachya*. The *in vitro* antioxidant activity of *Hypoestes phyllostachya* leaf clearly showed that it has better antioxidant activity, which was confirmed by different assays.

**Conclusion:** From this work, it can be concluded that *Hypoestes phyllostachya* has the potential to be a good antioxidant but have only a lesser amount of flavonoids and polyphenolic contents. This was the first report on the pharmacological activity of the *Hypoestes phyllostachya*.

**Keywords:** Oxidative stress, Polyphenolics, Flavonoids, Inflammation, Medicinal plants, Herbal medicine

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**INTRODUCTION**

Herbal medicine contains rich varieties of free radical scavenging phytochemicals such as flavonoids, anthocyanins, carotenoids, dietary glutathione, vitamins and endogenous metabolites and they have antioxidant properties [1]. The free radicals induce oxidative damage to lipids, proteins, nucleic acids, which leads to atherosclerosis, ageing, cancer, diabetes mellitus, inflammation, AIDS and other degenerative disorders. The living body produces free radicals naturally like pollution exposure, smoke, fat-rich diet, rich sugar content, alcohol consumption, which make an individual unhealthy. Oxidative stress is defined as a "state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them. It causes dangerous effects such as peroxidation of lipids, oxidative DNA damage, but also physiologic adaptation phenomena and regulation of intracellular signal transduction.

Antioxidants are substances that inhibit oxidative stress to a target molecule [2]. Antioxidants scavenge these free radicals due to singlet oxygen quenchers and redox hydrogen donors [3]. They prevent cellular damage by reducing oxidative stress and provide a beneficial effect on human health. The free radicals formed in the body are scavenged by natural and synthetic antioxidants [4]. But the synthetic antioxidants are very toxic having side effects and are now replaced by natural ones for their safer needs [5]. As the medicinal plants contain several phytochemicals with biological activities. Phytochemicals represent a potential source of new compounds with antioxidant activity as it contains large amounts of antioxidants such as Ascorbic acid, Tocopherol, flavonoid and polyphenolic compounds.

*Hypoestes phyllostachya* Rosea is a tropical sub shrub usually called polka dot plants from Acanthaceae family. It is grown as an indoor ornamental house plant and as an accent plant in dish gardens to add colour in partially shaded areas. The plant leaves are spotted with pink and white colour with small violet flowers. The species of *hypoestes* used as folkloric medicine for a variety of diseases and

health cares as it has antimicrobial antileishmanial and antioxidants properties. The bioactive compounds isolated from the plant can be used to treat various disorders. The phytochemical investigation carried out on various *hypoestes* species reveals the presence of phytochemicals as diterpenoids, alkaloids, lignans, triterpenes etc. Chemical composition of the essential oil separated from leaf and stem of Nigerian *phyllostachya* showed the presence of volatile constituents. Based on the ethnobotanical reviews, the present work was focused on the phytochemical analysis and pharmacological properties of various solvent extracts of *Hypoestes phyllostachya* Rosea. This was the first report on the activities of *Hypoestes phyllostachya* leaves.

**MATERIALS AND METHODS**

**Collection and extraction of plant material**

The leaves of *Hypoestes phyllostachya* Rosea was collected from the rural area of Wayanad district. The leaves are washed thoroughly, shade dried and coarsely powdered. The powdered leaf material of *Hypoestes phyllostachya* Rosea was successively extracted with hexane, chloroform, methanol and water using soxhlet apparatus and is stored for further *in vitro* assays.

**Phytochemical screening of various solvent extracts of the leaf sample**

Phytochemical analysis was done to analyze the presence of phytochemicals found in different solvent extracts of *Hypoestes phyllostachya* leaf [6, 7].

***In vitro* antioxidant activities of various solvent extracts of leaf**

**DPPH radical scavenging activity**

1,1-Diphenyl-2-picryl hydrazyl (DPPH) is a free radical for measuring antioxidant activity. The reaction mixture include 2.8 ml 100µM DPPH in methanol and was added with 0.2 ml leaf extract at different concentrations. The mixture was incubated for 30 min and

the optical density was taken at 517 nm. Ascorbic acid is used as a reference standard and methanol without sample along with DPPH was taken as control [8].

$$\% \text{ of Scavenging} = (A \text{ control} - A \text{ Test}) / A \text{ control} \times 100.$$

#### Hydroxyl radical scavenging assay

The assay is based on the capacity of phytochemicals to compete with salicylic acid for hydroxy radicals. The mixture include 1 ml 1.5 mmol FeSO<sub>4</sub> added with 0.7 ml 6 mmol hydrogen peroxide, 0.3 ml 20 mmol sodium salicylate and 1 ml of different concentrations of three solvent extracts of the leaf material. The mixture is then incubated for 1 hour at 37 °C and the optical density of the hydroxylated salicylate complex was measured at 562 nm. The standard used for the assay is ascorbic acid [9].

Total antioxidant capacity (Phospho molybdenum assay)

The principle behind assay is the reduction of Mo(VI) to Mo(V) by the leaf extract and forms a green coloured phosphate Mo(V) complex at acidic pH. 0.3 ml leaf extract was added with 3 ml reagent solution which contains 0.6 M Sulfuric acid, 28 mmol Sodium phosphate 4 mmol Ammonium molybdate. Incubate the mixture for 90 min at 95 °C and sample was cooled. The optical density was taken at 695 nm [10].

#### Reducing power assay

Different concentrations of leaf extract (0.2–1 mg/ml) dissolved in methanol was added with 2.5 ml 0.2 M phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. Incubated the mixture for 20 min at 50 °C and add 2.5 ml 10% TCA. Then centrifuge mixture at 3000 rpm for 15 min. 0.5 ml of the upper layer is added with 2.5 ml of distilled water and add 0.5 ml 0.1% ferric chloride. Read the absorbance at 700 nm. An increased absorbance value show high antioxidant potential [11].

Estimation of total polyphenolics

The reaction mixture contain 50 µl of sample, 3 ml water, 0.25 ml FC reagent and 0.75 ml 20 % Na<sub>2</sub>CO<sub>3</sub>. The total volume make up to 5 ml

using water. Mixed well and incubated the mixture at 50 °C for 2 h. Read the absorbance at 765 nm using spectrophotometer [12]. Here gallic acid was used as the standard. The concentration of total polyphenolic content was obtained from gallic acid standard curve.

Estimation of total flavanoid content

In this method Quercetin is used as standard. Here quercetin in methanol is diluted to different concentrations. The diluted quercetin (0.5 ml) were added to 1.5 ml methanol, 0.1 ml 10% aluminum chloride, 0.1 ml 1M potassium acetate and 2.8 ml of distilled water. Incubated the mixture for 30 min. Optical density was determined at 415 nm. 10% aluminum chloride replaced by distilled water was taken as blank. Similarly, 0.5 ml of different solvent extracts of leaf material reacts with aluminum chloride for estimating flavanoid content. The results were expressed as mg Quercetin equivalents/g sample [13].

## RESULTS AND DISCUSSION

### Phytochemical screening of different solvent extracts of the leaf material

The hexane, chloroform and methanolic and aqueous leaf extract of *Hypoestes phyllostachya* were screened for the presence of phytochemical compounds. The qualitative analysis of methanolic and aqueous leaf extract showed the presence of carbohydrates, proteins, steroids, fixed oils and fatty acids, flavonoids, alkaloids, saponins, tannins and polyphenolics. The hexane extract of the leaf contains only carbohydrates and proteins whereas the chloroform extract contains carbohydrates, proteins and saponins. When related to other solvent extracts, the aqueous and methanolic leaf extract had rich content of phytochemicals with higher precipitation. The results of the analysis were represented in table 1. Even though the phytochemical constituents are reported to have many therapeutic and biological properties, *Hypoestes phyllostachya* is expected to have certain medicinal uses [14-16]. From this phytochemical screening, the results showed that methanolic and aqueous extract of *Hypoestes phyllostachya* contains rich phytochemical constituents, which may be due to high polar index of water and methanol solvent than the other solvents [17].

Table 1: Phytochemical analysis of *Hypoestes phyllostachya* leaf

Phytochemicals	<i>Hypoestes phyllostachya</i> leaf			
	Aqueous extract	Hexane extract	Chloroform extract	Methanol extract
Carbohydrates	+++	+++	+++	+++
Proteins	+++	+++	+++	+++
Steroids	+++	-	-	+++
Fixed oils and fatty acids	+++	-	-	+++
Volatile oils	-	-	-	-
Alkaloids	+++	-	-	+++
Flavanoids	+++	-	-	+++
Saponins	+++	-	+++	+++
Tannins	+++	-	-	+++
Poly phenolics	+++	-	-	+++
Terpenoids	-	-	-	-
Glycosides	-	-	-	-

### In vitro antioxidant activities of different solvent extracts of leaf material

#### DPPH radical scavenging activity

In this assay, the percentage of scavenging of hexane extract is 31.28±0.67 at a maximum concentration of 2500µg/ml whereas the chloroform extract showed inhibition of 39.09±1.22. But the methanolic extract of the leaf showed the highest significant radical scavenging effect of 64.29±0.89 on DPPH radicals at 2500 µg/ml. In the present study, there was increased scavenging property of the DPPH radicals with increased concentration of various extracts of *Hypoestes phyllostachya*. This may specify an increased ability to provide hydrogen ions resulting in a lighter solution which is proportional to electrons gained. Therefore, it may be suggested that the methanolic extract of *Hypoestes phyllostachya*, has DPPH scavenging activity, by decreasing the DPPH radical to hydrazine due to its hydrogen ion-donating ability.

#### Hydroxy radical scavenging assay

In this assay, scavenging of hydroxy radicals increases with increased concentrations of the *Hypoestes phyllostachya* leaf extract. Here hexane extract of the leaf showed a percentage inhibition of 37±1.5 whereas chloroform extract shows 62±3.2 % inhibition at 2500 µg/ml. The methanolic extract of the leaf showed the highest percentage of inhibition of 72.22±1.3 at 2500 µg/ml. The hydroxyl radicals are highly active of the reactive oxygen species, which cause severe injury in adjacent biomolecules or they cause oxidative stress to nucleic acids, proteins and lipids. The hydroxyl scavenging activity of the methanolic extract of *Hypoestes phyllostachya* was determined by its capacity to compete with salicylic acid for •OH radicals in the •OH generating system. Hence the methanolic extract can be regarded as a better scavenger of hydroxy radicals.

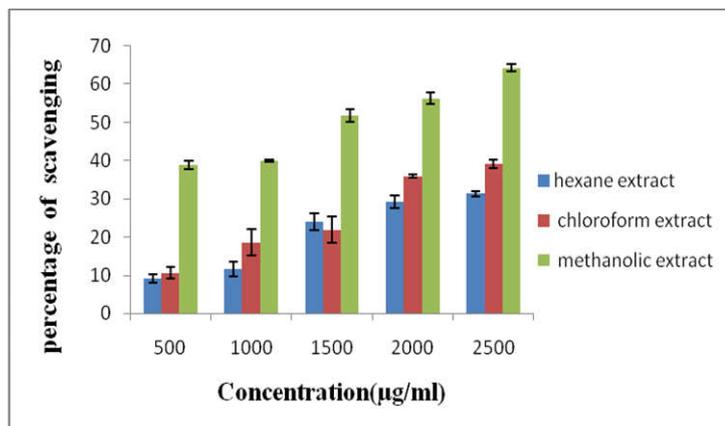


Fig. 1: DPPH radical scavenging activity of various solvent extracts of *Hypoestes phyllostachya*

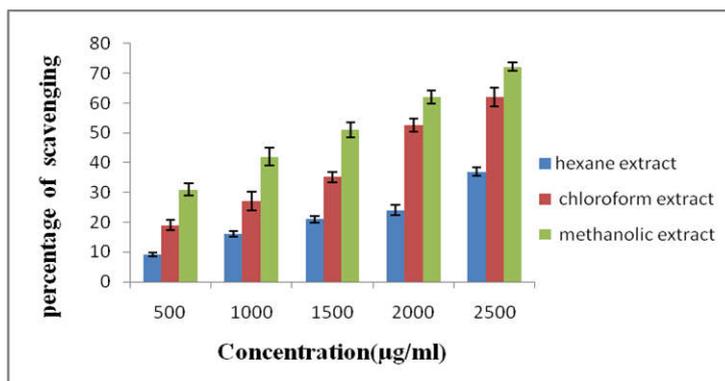


Fig. 2: Hydroxy radical scavenging activity of *Hypoestes phyllostachya*

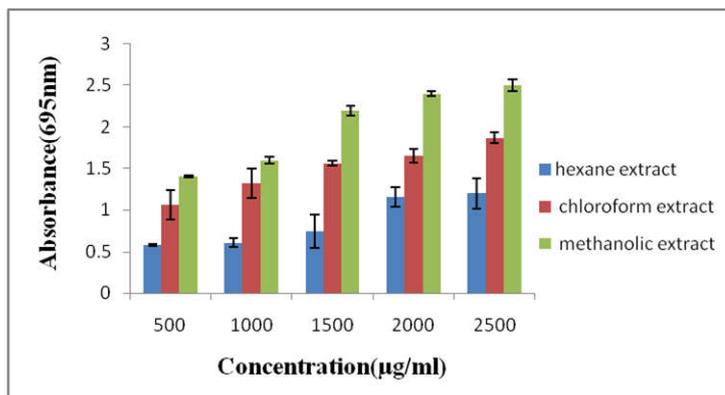


Fig. 3: Total antioxidant capacity of various solvent extracts of *Hypoestes phyllostachya*

#### Total antioxidant capacity (Phospho molybdenum assay)

The total antioxidant activity of the plant extracts was determined by the Phospho molybdenum assay. Here the methanolic extracts of *Hypoestes phyllostachya* leaf showed increased total antioxidant capacity with increasing concentration when compared to chloroform and hexane extracts of the sample. 500µg/ml of the methanolic extract of *Hypoestes phyllostachya* leaf was equivalent to 284 µg/ml of the ascorbic acid standard. When related to the hexane and chloroform extracts, the methanolic extract showed higher antioxidant capacity. It is a method which evaluates the reduction of Phosphate-Mo (VI) to Phosphate Mo(V) by the methanolic extract

and presence of a bluish-green coloured Phosphate Mo(V) complex. Increase in optical density indicates the higher antioxidant activity of *Hypoestes phyllostachya* leaf extract.

#### Reducing power assay

In reducing power assay, the different extracts of *Hypoestes phyllostachya* leaf showed reducing power ability. Her 2500µg/ml of the methanolic extract of *Hypoestes phyllostachya* was found to be equivalent to 46 µg/ml of ascorbic acid standard. From these results, it can be substantiated as the absorbance of leaf extracts increases, the reductive ability increases. The methanolic leaf

extract has natural antioxidants to donate electrons and reduce of Fe+3 to Fe+2 ions. The reducing ability of extract depends on the presence of reductones which has antioxidant activity by breaking the free radical chain and donate a hydrogen atom. Hence the reducing power leads to the termination of the radical chain

reactions that may otherwise be very damaging. The presence of antioxidant reductants in the methanolic extract of *Hypoestes phyllostachya* of causes the reduction of the Fe<sub>3</sub>+complex to ferrous form, which indicates the significant reducing power of the plant extract.

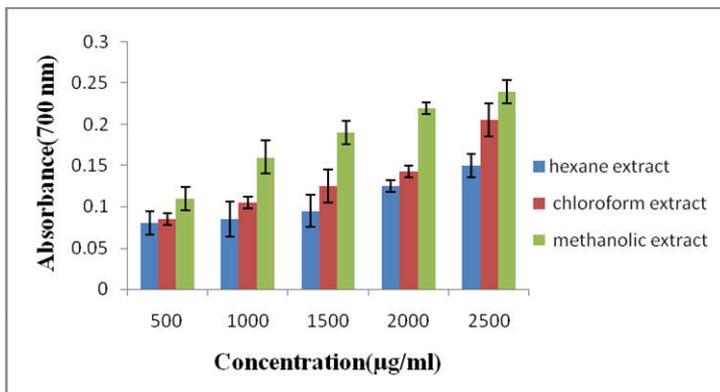


Fig. 4: Reducing power assay of various solvent extracts of *Hypoestes phyllostachya*

Estimation of total polyphenolics

The total phenolic content was found to be 10 mg Gallic acid equivalents present per gram *Hypoestes phyllostachya*. The results were obtained from the calibration curve of gallic acid standard. Among the different phytochemicals, Polyphenolics are widespread in the plant kingdom as part of our daily diet and are considered as

natural antioxidants. Poly phenolics have gained great attention due to their radical scavenging, anti-mutagenic, anti-carcinogenic and anti-inflammatory properties. The antioxidant property of phenolics is due to their redox activities which make it as hydrogen donors, metal chelators, reducing agents and as well as singlet oxygen quenchers. In this study, there is an only lesser amount of polyphenolics present per gram of the methanolic extract of *Hypoestes phyllostachya*.

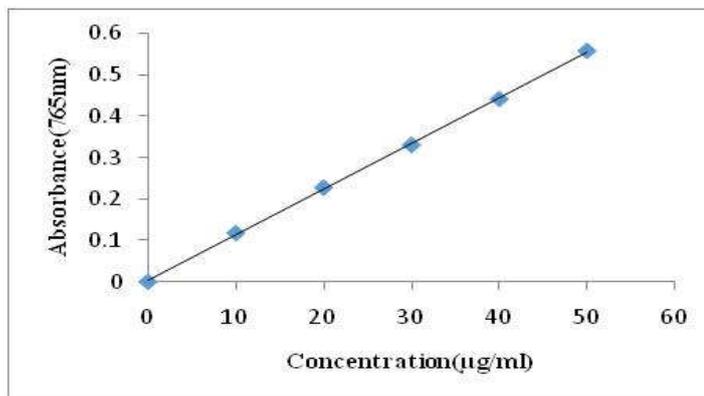


Fig. 5: Determination of total polyphenolic content-Calibration curve of gallic acid

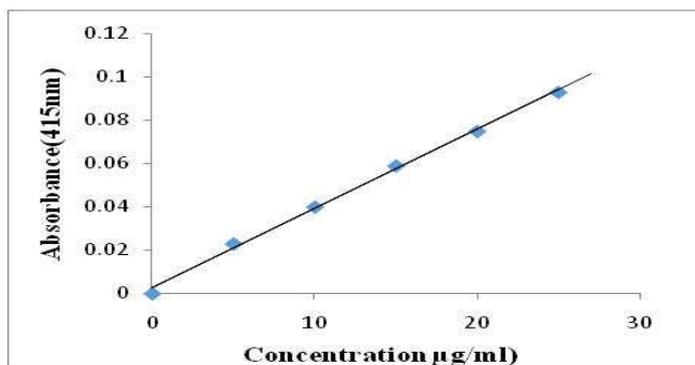


Fig. 6: Determination of total flavanoid-calibration curve of quercetin

#### Estimation of total flavanoid content

The flavonoid of methanol extract was found to be 13 mg quercetin equivalents per gram of the leaf material. The results were obtained from the standard plot of quercetin. Flavonoids are plant secondary metabolites, the antioxidant property of which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids have both *in vitro* and *in vivo* antioxidant activity [19]. As this is the first report on the antioxidant activity of *Hypoestes phyllostachya*, the flavonoid contents present in the plant leaf is very less.

#### CONCLUSION

In this study, *Hypoestes phyllostachya* leaf was tested to analyse the presence of phytochemicals and their antioxidant activities. Successive extraction of the leaf was done using Soxhlet apparatus and the extract was partitioned using hexane, chloroform and methanol. The analysis of different solvent extracts of the leaf material showed the presence of several phytochemicals. The antioxidant activity was measured by DPPH, ABTS, Hydroxy radical, Total antioxidant capacity and reducing power assays. The flavonoid and polyphenolics present in the leaf sample were also estimated. The phytochemical analysis of methanolic and aqueous leaf extract showed the presence of carbohydrates, proteins, steroids, fixed oils and fatty acids, flavonoids, alkaloids, saponins, tannins and polyphenolics, which are the perfect sources of antioxidants. The findings of these studies suggested that *Hypoestes phyllostachya* leaf could have a major role as a therapeutic agent in reducing oxidative stress-related disorders. But there is lesser amounts of polyphenolics and flavonoids present in the leaf sample. The work regarding the isolation of active compounds responsible for antioxidant capacity will be carried out for the future.

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#### AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

#### CONFLICTS OF INTERESTS

The authors declare that there was no conflicting interest

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