International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 774-780 www.ijprajournal.com ISSN: 2249-7781

Phytochemical Screening and Antioxidant Potential on the Inflorescence of Hedyotis Purpurascens Hook. F (Rubiaceae) – An Endemic Species from Southern Western Ghats

Betty T*, Malini RP, Vasini V, Sumathi P
Department of Botany, Kongunadu Arts and Science College, Coimbatore, India.

Submitted: 25-03-2024 Accepted: 05-04-2024

ABSTRACT: The aim of this study is to highlight the antioxidant capacity and phytochemical screening of Hedyotis purpurascens Hook. F (Rubiaceae), an endemic species found in the Southern Western Ghats, in its inflorescence.The extractive yield percentage of the solvent systems was examined and the highest yield percentage was found in ethanol (5%). Qualitativeanalysis of the inflorescence evidenced that theflavonoids were found abundantly in all the three solvent systems.Alkaloids, tannins, phenols, saponin, glycosides, fixed oils and fats and coumarin were also observed.Gums and mucilage were considerably found in the hexane extract. The ethanolic inflorescence extract revealedsubstantialamount of flavonoids, phenols and tannin in case of quantitative analysis. Invitro antioxidant analysis exhibited an excellent IC_{50}value which is highly appreciable when compared with the standard Rutin and BHA in case of DPPH radical scavenging activity. Subsequently in nitric oxide radical scavenging activity as well as ABTS radical scavenging activity, the ethanolic extract of H.purpurascens inflorescence accords significant values when compared with the other extracts.

Keywords: Phytochemical screening, antioxidant, flavonoids, Hedyotis purpurascens.

I. INTRODUCTION

Plants are fascinating living organisms found all around us. Plants have deepconnection with human culture and civilization since ancient times. Plants produce chemical compounds, many of which have medicinal properties. Since ancient times humans depends on medicinal plants to treat various ailments and diseases (Ayaz et al., 2017). From traditional herbal remedies used by indigenous cultures to modern pharmaceuticals derived from plant compounds, the healing properties of plants have played a crucial role in healthcare.

Medicinal plants contain a diverse range of bioactive compounds, including alkaloids, flavonoids, terpenoids and polyphenols, which possess pharmacological effects. These compounds can target specific biological pathways in the human body, such as reducing inflammation, fighting infections or alleviating pain (Ullah et al., 2020; Arulselvanet al., 2016).

In recent years, there has been a resurgence of interest in natural remedies and herbal medicine, driven by a growing awareness of the limitations and side effects of conventional pharmaceuticals(AhlamMushtaqetal., 2014). Additionally, the exploration of plant biodiversity in remote regions has led to the discovery of novel compounds with promising medicinal properties.

However, it's essential to approach the use of medicinal plants with caution and respect for traditional knowledge systems. While plants offer a rich source of potential medicines, their efficacy and safety can vary widely, and proper dosage and administration are crucial to avoid adverse effects. In this present study an attempt has been made to document the secondary metabolic compounds and antioxidant potential on the inflorescence of H. purpurascens Hook. F.

II. MATERIALS AND METHODS

2.1. Collection and authentication of plant materials:

The inflorescence of the plant H. purpurascenswas collected from the Maharajamettu of Megamalai hills, Theni, Tamilnadu during the month of March 2023.The flowers are purple, borne in many-flowered Corymb like clusters a branch ends. (Fig:1)

The authenticity of the selected plant materials were duly identified and confirmed (vide no: BSI/SRC/5/23/2017/tech/745) by comparison with reference specimens preserved at Botanical

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 774-780 www.ijprajournal.com ISSN: 2249-7781

Survey of India, Southern Region Centre, Coimbatore.

Fig: 1Hedyotis purpurascens – Inflorescence

2.2. Preparation of crude plant extract:

Fresh and healthy inflorescence of H. purpurascens were harvested, shade dried and coarsely powdered for extraction.

50g of coarsely powdered plant samples were exhaustively extracted with hexane, diethyl ether and ethanol using Soxhlet apparatus.

The sample 50g is transferred to a 33 mm × 100 mm cellulose thimble and placed after inthe extraction chamber of a 200 mL capacity Soxhlet apparatus. The cellulose thimble wasclogged with cotton in order to avoid transfer of sample particles to the distillation flask. TheSoxhlet apparatus, fitted with a condenser, was placed on a 500 mL distillation flask containing200 mL of solvent and 3 boiling glass regulator. Samples were thus extracted under reflux withhexane, diethyl ether and ethanolduring 3 hours (5–6 cycles/hr). After theextraction, the major solvent was eliminated. The extracts wereconcentrated to dryness under reduced pressure using rotary vacuum evaporator (Supervae R-185, India), lyophilized (4KBTXL-75; Vir Tis Benchtop K, New York, USA) to remove tracesof water molecules and the lyophilized powders were stored at -20°C for further studies.

The percentage yield (recovery) of evaporated plant extracts were calculated as follows:

Yield (%) =[Extract + container (g)] – [Empty container (g)] / Sample weight (g)×100

2.3. Qualitative phytochemical analysis

Preliminary qualitative phytochemical analysis of the hexane, diethyl ether and ethanolextracts of H. purpurascensinflorescence were determined using standardprocedures as described by Sofowora (1990), Trease and Evans (1989), Harbone (1973).

2.3.1. Test for Alkaloids

Dragendroff's test

To 2mg of the plant extracts 5ml of distilled water was added2ml hydrochloric acid wasadded until an acid reaction occurs. To this 1ml of dragendroff's reagent was added. Formation of orange red precipitate indicates the presence of alkaloids.

2.3.2. Test for Flavonoids

Shinoda's test

2mg of plant extracts was diluted in isopropyl alcohol; add 3 drops of concentratedhydrochloric acid and 1ml absolute ethanol. Formation of red color indicates the presence of flavonoids. In case of no coloration, a small piece of metallic magnesium was added. Formationof red, orange or pink color indicates the presence of flavones and flavanols.

2.3.3. Test for steroids

Liebermann-Burchard's test

1ml of anhydrous acetic acid was added to2mg of crude extracts, heated till boiling andcooled. 3 drops of concentrated sulphuric acid was added along the sides of test tubes. After5minutes formation of blue-green color middle layer indicate the presence of steroids.

2.3.4. Test for saponins

Froth test

1ml of distilled water was added to10drops of crude plant extracts dissolved and boiledand distilled water in a test tube shaken vigorously to froth and then allowed to cool the testtubes. Saponin was measured by measured by observing the height of froth: froth3mm (poor), froth6mm (moderate) and froth up to 1cm (Good).

2.3.5. Test for phenolics compound

Lead acetate test

To 2mg of crude plant diluted with 5ml of distilled water add 3ml of 10% lead acetatesolution. The occurrence of white precipitates indicates the presence of phenols.

2.3.6. Test for coumarins

NaOH test (Harbone, 1988)

3ml of 10%sodium hydroxide was added to3ml of extracts and placed in boiling waterbath. Formation of yellow color indicates the presence of coumarins.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 774-780 www.ijprajournal.com ISSN: 2249-7781

2.3.7. Test for terpenoids
Salkowski's test

In 2ml of each crude extracts, 2ml of chloroform was added and about 3ml ofconcentrated sulphuric acid was added carefully to form a layer. Occurrence of reddish browncoloration of the interface indicates the presence of terpenoids.

2.3.8. Test for carbohydrates
Molisch's test

About 3ml of extract was added with 1ml of Molisch's reagent and 3 drops ofconcentrated sulphuric acid. Red or violet ring appear in the interface of the two layers indicatingthe presence of carbohydrates.

2.3.9. Test for Glycosides
Keller-Kilani test

An aliquot of 4ml glacial acetic acid with 3 drops of 2.0%ferric chloride in 3 ml of crudeplant extracts and with 1ml concentrated sulphuric acid was added and observed. Appearance ofviolet-green ring below brown ring, in the acetic acid layer showed the entity of glycosides.

2.3.10. Test for proteins
Ninhydin test

Plant extracts 3ml and 3 drops of 5% lead acetate solution was mixed and placed inboiling water bath for 10 min. Appearance of blue or purple color indicates the presence ofprotein.

2.3.11. Test for oils and fats
Saponification test (Kokate 1999)

2mg of plant extracts was treated with 0.5N alcoholic potassium hydroxide and 1 drop of phenolphthalein separately and heated on boiling water bath for 1-2 hours. Formation of soap orneutralization indicates the presence of fixed oils and fats.

2.3.12. Test for Gums and Mucilage's (Whistler and Be Miller 1993)

5ml various extracts diluted in distilled water were added separately to 25ml of absolutealcohol with constant stirring. White or cloudy precipitate indicates the occurrence of gums andmucilages.

2.3.13. Test for Tannins
$Fecl_3$test (Trease and Evans, 1989)

Crude extracts of 0.1mg was boiled with 4 ml of distilled water and filtered.2 ml of 5%ferric chloride was added slowly. Resulting in dark blue or greenish black color indicates thepresence of tannins.

2.3.14. Test for Amino acid

1 ml of the extract was added with Ninhydrin reagent. Appearance of purple colour shows the presence of amino acid.

2.4. Quantitative phytochemical analysis
2.4.1. Estimation of total flavonoids

The flavonoids content of the samples was determined by the gravimetric method described by (Harborne, 1998). 5 g of each powdered sample was placed into a conical flaskand 50 ml of water and 2 ml of ethyl acetate solution were added. The solution was allowed toboil for 30 min. The boiled mixture was allowed to cool and filtered through Whatman filterpaper (No.1). About 10 ml of ethyl acetate extract which contained flavonoids was recovered,while the aqueous layer was discarded. A pre-weighed Whatman filter paper was used to filterthe second (ethyl acetate) layer. The residue was then placed in an oven to dry at 60^{o}C. It wascooled in desiccators and weighed.

2.4.2. Estimation of total phenolic content

The total phenolic content was estimated using Folin ciocalteau reagent by Sidduraju and Becker (2003). 20µg of the extract was taken and made up to 1ml using distilled water. To that 500µl of Folin-Phenol reagent (1:1) was added and 2.5ml of sodium carbonate was added. The mixture was shaken well and incubated in dark for 40 mins to develop colour. The absorbance was measured at 725 nm. A calibration curve of Gallic acid was constructed and linearity was obtained in the range of 10-50 µg/ml. The total phenol content in the plant extracts were expressed as mg of Gallic acid equivalent (mg GAE/g extract) by using the standard curve.

2.4.3. Estimation of tannins content

Tannin content was estimated by the method of Siddhurajuet al., (2007). 500 µl of the extract were taken in the test tube separately and treated with 100mg of polyvinyl polypyrrolidone and 500 µL of distilled water. This solution was incubated at 4°C for 4 h. Then the sample was centrifuged at 5000rpm for 5 mins and 20 µL of the supernatant was taken. The phenolic content of the supernatant was measured at 725nm and expressed as the content of the free phenolics on a dry matter

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 774-780 www.ijprajournal.com ISSN: 2249-7781

basis. From the above results, the tannins content of the extract were calculated as follows:
Tannins (mg GAE/g extract) = Total phenolics (mg GAE/g) - Free phenolics (mg GAE/g).

2.5. Determination of in vitro antioxidant activity

2.5.1. DPPH radical scavenging assay

The hydrogen donating capacity was assessed by using stable DPPHmethod (Blois,1958).Briefly, a solution of 0.1mM DPPH was prepared using methanol. The sample (50-250µg/mL) was mixed with 5.0mL of DPPH solution. Reaction mixture was shaken incubatedat 30ºc for 20 minutes and the absorbance was at measured at 517nm. Results were comparedwith the activity of rutin, quercetin, BHA and BHT. Discoloration of the samplewas calculated using the formula:
DPPH radicals scavenging activity (%) = [(control OD –sample OD)/control OD] ×100
Anti- oxidant activity of the extract were expressed as IC_{50}, the values were calculatedfrom the linear regression of the percentage anti oxidant activity versus concentration of theextracts (Ebrehimabadi et al., 2010). A lower IC_{50}, values indicate greater anti-oxidant activity.

2.5.2. Trolox equivalent anti- oxidant capacity (TEAC) assay

Antioxidant activity was performed using an improved $ABTS^{\cdot+}$ method proposed bySiddhuraju and Manian (2007). The ABTS radical cation was generated by a reactionof 7mM $ABTS^{\cdot+}$and 2.45 mM potassium per sulfate and the mixture was incubated for 12-16hours at room temperature in dark. Prior to assay, the solution was diluted in ethanol (about 1:89v/v) and equilibrated to obtain an absorbance of 0.700± 0.02 at 734 nm. 10µL/ml of sample wasadded to 1.0 ml of diluted $ABTS^{\cdot+}$ Solution. After 30 min of incubation, absorbance was read at734 nm. Trolox was used as a reference material.

2.5.3. Nitric oxide scavenging activity

Nitric oxide scavenging activity was determined according to the method suggested by Sreejayan and Rao (1997). Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacted with oxygen to produce nitrite ions, which can be estimated using Griess reagent. Scavengers of nitric-oxide act against oxygen, leading to reduced production of nitrite ions. In brief, 3.0 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with different concentrations of the extract and incubated at 25°C for 150 min. 0.5 mL of the incubated solution was removed and diluted with 0.5 mL of Griess reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% N-Inaphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1 naphthyl ethylene diamine dihydrochloride was measured at 546 nm and percentage scavenging activity was measured with reference to standards. IC_{50}, an inhibitory concentration was estimated from the % inhibition plot.

III. RESULTS AND DISCUSSION

The present study was made to investigate the phytochemical constituents and the antioxidant capacity of the plant H. purpurascens. The qualitative analysis of the plant has been carried out and it reveals the presence of both primary and secondary metabolites. The primary metabolites such as protein and amino acids are found to be present moderately; whereas carbohydrates found to be in medium quality. While analysing the secondary metabolites flavonoids were found to be present abundantly in all the three solvent systems when comparing to the other secondary metabolites. Steroids and terpenoids were absent in both hexane and diethyl ethyl ether and present in ethanolic extract. Alkaloids, tannins, phenols, saponin, glycosides, fixed oils and fats and coumarin is present in all the three solvent systems.

Gums and mucilage were abundantly found in the hexane and completely absent in the other two solvent systems. (Table:1). The extractive yield percentage of the solvent systems were examined and the highest yield percentage was found in ethanol of 5% followed by hexane with 4.4% and diethyl ether with 1.5% (Table:2; Fig:2).

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 774-780 www.ijprajournal.com ISSN: 2249-7781

Table:1 Qualitative analysis of H.purpurascens inflorescence

Constituents	Hexane	Diethyl Ether	Ethanol
Alkaloids	++	++	++
Tannins	++	++	+++
Phenols	+	+	++
Flavonoids	+++	+++	+++
Saponin	+	++	++
Terpenoids	-	-	+
Glycosides	++	+	++
Steroids	-	-	++
Gums and Mucilage	+++	-	-
Fixed oils and fats	+	+	++
Coumarin	++	++	+++
Carbohydrates	++	++	++
Proteins	+	+	+
Aminoacids	+	+	++

Note: Maximum present (+++); Medium present (++); Moderate present (+); Absent (-)

Table:2 Extractive yield percentage of H.purpurascens

Extract	Yield Percentage (%)
Hexane	4.4
Diethyl ether	1.5
Ethanol	5

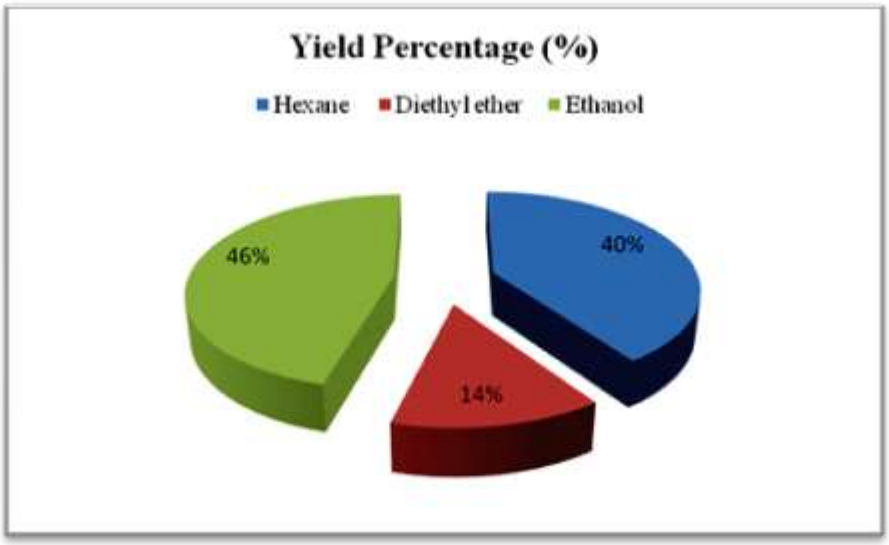

Fig:2 Yield percentage of H .purpurascens

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 774-780 www.ijprajournal.com ISSN: 2249-7781

The quantitative analysis of the inflorescence was carried out and it is found out that flavonoids were found high in case of ethanolic extract; similarly the ethanolic inflorescence extract shows the high quantity of phenols and tannin. (Table:3)

Table:3 Total phenolics, tannins and flavonoid content of H.purpurascens

Solvents	Total Phenols	Tannins	Total Flavonoids
	mg GAE/g	mg GAE/g	mg RE/g
Hexane	1.34±0.2	1.06±0.2	21.32±0.1
Di Ethyl ether	1.40±0.1	1.02±0.2	25.08±0.1
Ethanol	5.81±0.1	2.12±1.1	41.55±0.1

Invitro antioxidant activity

The invitro antioxidant activity of the plant part was determined and it has been observed that the ethanolic inflorescence extract exhibits an excellent IC_{50}value of 12.1±1.2 which is highly appreciable when compared with the standard Rutin and BHA that shows the values of 15.8±0.2 and 21.4±0.2 in case of DPPH radical scavenging activity.

Subsequently in nitric oxide radical scavenging activity the ethanolic extract of H.purpurascens inflorescence accords a significant lowest IC_{50} value of 95±1.4.

Similarly in ABTS radical scavenging activity also the ethanolic inflorescence extract registers a prominent value of 9561±1.7 when compared with the other two extracts (Table:4).

Table:4 Invitro antioxidant radical scavenging activity of H. purpurascens

SOLVENTS	DPPH	Nitric Oxide	ABTS
	IC_{50} µg/mL	IC_{50} µg/mL	
Hexane	35.6±1.5	166±1.7	2341±1.1
Diethyl Ether	24.8±0.6	118±2.1	6452±0.2
Ethanol	12.1±1.2	95±1.4	9561±1.7
Rutin	15.8±0.2	18.8±0.1	_
BHA	21.4±0.2	26.3±0.3	_

IV. CONCLUSION

Plants are not only vital for sustaining life on Earth but also hold immense potential as a source of medicines to alleviate human suffering and promote health and well-being. Through continued research and collaboration between traditional knowledge holders, scientists, and healthcare professionals, we can harness the healing power of plants to improve healthcare globally. From the above observations it has been found out that the inflorescence of the plant H.purpurasens has revealed the presence of flavonoids and the ethanolic extract of the plant part also registers a basic antioxidant property. Flavonoids have been reported to possess many useful properties, including anti-inflammatory, oestrogenic, enzyme inhibition, anti-microbial, anti-allergic, vascular and cytotoxic anti-tumour activity (Dias et al., 2021).Therefore, it is concluded that the inflorescence of this plant possesses therapeutic properties and can potentially be utilized for treating various ailments. Ultimately, further research on this botanical component may lead to the identification of novel drugs, facilitating the treatment of diverse medical conditions.

REFERENCES

[1]. Ayaz M, Sadiq A, Junaid M, Ullah F, Ovais M, Ullah I. (2019). Flavonoids as Prospective Neuroprotectants and Their Therapeutic Propensity in Aging Associated Neurological Disorders. Front. Aging Neurosci. 11, 155.

[2]. Arulselvan, P., Fard, M. T., Tan, W. S., Gothai, S., Fakurazi, S., Norhaizan, M. E., & Kumar, S. S. (2016). Role of Antioxidants and Natural Products in Inflammation. Oxidative medicine and cellular longevity, 2016, 5276130.

[3]. Ullah, A., Munir, S., Badshah, S. L., Khan, N., Ghani, L., Poulson, B. G., Emwas, A. H., & Jaremko, M. (2020). Important Flavonoids and Their Role as a Therapeutic Agent. Molecules (Basel, Switzerland), 25(22), 5243.

[4]. Blois, M.S. (1958) Antioxidant Determinations by the Use of a Stable Free Radical. Nature, 181, 1199-1200.

[5]. Dias, M. C., Pinto, D. C. G. A., & Silva, A. M. S. (2021). Plant Flavonoids: Chemical Characteristics and Biological Activity. Molecules (Basel, Switzerland), 26(17), 5377.

[6]. Ebrahimabadi A, and HaghirEbrahimabadi E, and Djafari-Bidgoli Z, and Jookar F, and Mazoochi A, and Batooli H. (2010). Composition and antioxidant and antimicrobial activity of the essential oil and extracts of Stachys inflata Benth from Iran. Food Chemistry. 119. 452-458.

[7]. Harborne JB. (1973). Phytochemical methods: a guide to modern techniques of plant analysis. London: Chapman and Hall Publishers.

[8]. Harborne, J. B. (1998). Textbook of Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. 5th Edition, Chapman and Hall Ltd, London, 21-72.

[9]. Kokate C.K. (1999). Practical Pharmacognosy. New Delhi: Vallabh Prakashan; p. 107-21.

[10]. Mushtaq A, Akbar S, Zargar M A, Wali A. F, Malik A. H, Dar M. Y, Hamid R, and Ganai B. A. (2014). Phytochemical screening, physicochemical properties, acute toxicity testing and screening of hypoglycaemic activity of extracts of Eremurus himalaicus baker in normoglycaemic Wistar strain albino rats. BioMedresearch international, 867547.

[11]. Siddhuraju P, and Becker K. (2003). Antioxidant Properties of Various Solvent Extracts of Total Phenolic Constituents from Three Different Agroclimatic Origins of Drumstick Tree (Moringa oleifera Lam.) Leaves. Journal of Agricultural and Food Chemistry, 51, 2144-2155.

[12]. Siddhuraju P, and Manian S. (2007). The antioxidant activity and free radical-scavenging capacity of dietary phenolic extracts from horse gram (Macrotyloma uniflorum (Lam.) Verdc.) seeds, 950-958.

[13]. Sofowara A. (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp. 191-289.

[14]. Sreejayan and Rao M N A. (1997). Nitric Oxide Scavenging by Curcuminoids. Journal of Pharmacy and Pharmacology. 49: 105-107.

[15]. Trease G E and Evans W C. (1989). Textbookof pharmacognosy. 14. London: W.B. Sanders.

[16]. Whistler R L and Be Miller J N. (1993).Academic Press, New York, In Industrial Gums, 2, 360-367