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Research Paper

Evaluation of The Phytochemical and Antioxidant Composition of *Hamelia Patens* Jacq. Stem And Bark

Snehal Navnath Alat[1*]

[1]PES Modern College of Pharmacy, Moshi, Pune Maharashtra, India

Corresponding Author: *Snehal Navnath Alat

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ABSTRACT

The *Hamelia patens* Jacq. is used in traditional medicine for the treatment of wounds and inflammatory processes. The purpose of the present work was to analyze the Phytochemical screening of the bark and stem extract revealing the presence of alkaloids, flavonoids, sterols, tannins and carotenoids composition and the antioxidant activity, thus this study showed that these plant parts could be used as an important source for the production of drugs of definite action.

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1. INTRODUCTION

Hamelia patens Jacq., commonly referred to as "Firebush," is a medium-sized shrub that belongs to the family Rubiaceae. This species is indigenous to central and southern Florida, as well as the West Indies, and Central America, and extends to South America, reaching as far as Paraguay and Bolivia. In traditional practices such as Ayurveda and Siddha medicine, various parts of this plant are recognized for their medicinal properties. Folk medicine utilizes these plants to address a range of ailments, including athlete's foot, skin lesions, insect bites, nervous shock, inflammation, rheumatism, headaches, asthma, and dysentery (CSIR, 1959). The Ayurvedic system, which consists of traditional medicine, has acted as a foundation for alternative therapies, new pharmaceutical products, and healthcare solutions. This system offers insights into the extensive variety of plants that exhibit various properties, including immunostimulant, tonic, neurostimulator, anti-aging, antibacterial, antiviral, anti-rheumatic, anticancer, and adaptogenic effects (Agarwal SS., Singh VK 1999). Natural medicinal herbs have been utilized in various forms across different cultures and civilizations worldwide (Diallo *et al*, 1999). Hamelia patens is recognized for its application in herbal medicine. Therefore, this study was conducted to scientifically validate its properties through

the assessment of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and antioxidant activity.

2. MATERIALS & METHODS

Collection and Authentication

During various seasons, the plant specimen used in this study was collected from the Madha region in the Solapur district of Maharashtra, India. For precise botanical identification, care was taken to guarantee that the plant materials were gathered during the flowering and fruiting stages. The Flora of the Presidency of Bombay (Cooke, 1958), the Flora of Maharashtra State (Monocotyledons) (Sharma *et al.*, 1996), and the Flora of Solapur District (Gaikwad and Garad, 2016) were used to confirm the identification.

Preparation of sample extract

Sample extract preparation involved chopping the stem and bark of *H. patens* into tiny pieces and then drying them in an incubator set at 50°C. A spice mill was used to grind the dried samples into a fine powder. Additionally, ethanol and water were used to extract all of the powdered samples. 50 ml of the appropriate solvent was mixed with roughly 10 g of leaf powder, and the mixture was shaken for 12 hours at 100 rpm and 30 °C in an orbital shaker incubator. To get rid of debris, all of the extracts were centrifuged for 15 minutes at 5000 rpm. The supernatant was utilized for antioxidant (phosphomolybdenum) and phytochemical (TPC, TFC) purposes.

3. Qualitative Phytochemical Analysis

The examination of phytochemical compounds in different solvent extracts (aqueous, ethanol, methanol, and acetone) from the bark and stem of H. patens was conducted qualitatively following established methods (Baghel and Sudip, 2017).

1. Alkaloid Test (Dragendroff's Test)

Dilute hydrochloric acid was mixed with each extract, shaken thoroughly, and then filtered. A few drops of Dragendroff's reagent were subsequently added; the appearance of a red precipitate indicates the presence of alkaloids.

2. Carbohydrate Test (Fehling's Test):

To 1 ml of each extract, 1 ml of Fehling's A solution and 1 ml of Fehling's B solution were added to a test tube and heated in a water bath for 10 minutes. A red precipitate forms, indicating the presence of carbohydrates.

3. Test for Saponins (Froth test)

Each of the extracts was diluted with distilled water and shaken in a graduated cylinder for 15 minutes. The formation of a layer of stable foam indicates the presence of saponins.

4. Steroid Test (Salkowski's test)

Each extract was combined with chloroform, and then sulfuric acid was gradually added to the chloroform layer along the side of the test tube. The appearance of a red color signifies the presence of steroids.

5. Glycoside Test (Keller-Killiani Test)

2 ml of each extract, 3 ml of glacial acetic acid, and a single drop of 5% ferric chloride solution were introduced into a test tube. Concentrated sulfuric acid, measuring 0.5 ml, was then carefully added along the side of the test tube. The development of a blue color in the acetic acid layer indicates the presence of glycosides.

6. Tannin Test (Ferric Chloride Assay)

Approximately 2 ml of the filtered extract was placed in a test tube, followed by the addition of 2 ml of a 5% ferric chloride solution. The formation of a blue-black precipitate signifies the presence of tannins.

7. Test for Terpenoids (Salkowski's Test)

To each of the extracts, 2 ml of chloroform was added. To it, 3 ml of concentrated sulfuric acid was carefully added along the sides of the test tube to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids.

8. **Flavonoid Detection Using Lead Acetate**

A few drops of lead acetate solution were introduced to each extract. The formation of a yellow precipitate signifies the presence of flavonoids.

9. **Test for Phenolic Compounds (Diluted Iodine Solution)**

A few drops of diluted iodine solution were added to 2-3 milliliters of extract. The emergence of a temporary red color indicates the existence of phenolic compounds.

10. Ninhydrin Test for Amino Acids

Three milliliters of the extracts were heated for ten minutes in a water bath alongside three drops of 5% Ninhydrin solution. The development of a blue color signifies the presence of amino acids.

4. Quantitative phytochemical analysis of different Stem and bark extracts.

I. Total Phenolic Content (TPC):

The Folin-Ciocalteu method was employed to ascertain the TPC of the extracts **(Singleton and Rossi, 1965).** Thus, 1 ml of Folin-Ciocalteu reagent was mixed with 200 µl of extract (mg ml) and thoroughly combined. After a 5-minute incubation period, 0. 8 ml of 7. 5% w/v sodium carbonate was introduced, and the mixture was allowed to sit for 60 minutes at room temperature. The absorbance was measured at 765 nm using the UV-vis spectrophotometer. A calibration curve was created with gallic acid as the standard (20-200 µg ml). TPC was expressed as gallic acid equivalent per 100 grams (GAE g^1) of the extract.

II. Total Flavonoid content:

The aluminum chloride colorimetric method was utilized to assess TFC (Sakanaka *et al.*, 2005). In a test tube, 200 µl (mg ml) of plant stem and bark extract or 50–250 µgml of rutin standard solution were added. The volume was adjusted to 1. 25 ml with distilled water, followed by the addition of 75 µl of a 5% (w/v) sodium nitrite solution. The mixture was incubated at room temperature for five minutes after the addition of 150 µl of a 10% (w/v) aluminum trichloride solution after six minutes. Once 0. 5 ml of a 1M sodium hydroxide solution was included, the absorbance at 510 nm was recorded. The overall flavonoid content was expressed as rutin equivalent per 100 grams (RE g^1) of extract.

III. Antioxidant Activity

Prieto *et al.* (1999) used the phosphomolybdenum technique to evaluate the total antioxidant activity of all solvent extracts. One milliliter of the reagent (0.6M sulfuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate) was combined with an aliquot of plant extract. After being sealed, the vials were incubated for 90 minutes at 95 °C in a water bath. Absorbance was measured at 695 nm against a blank after the samples had cooled. Plotting a calibration curve was done using a standard ascorbic acid solution (20–120 µg ml). The ascorbic acid equivalent per 100 grams (AAE g^1) of extract was used to express the total antioxidant activity.

5. RESULTS & DISCUSSIONS

Table 1: Phytochemical screening of *H. patens* Jacq. (Stem and Bark) extracts

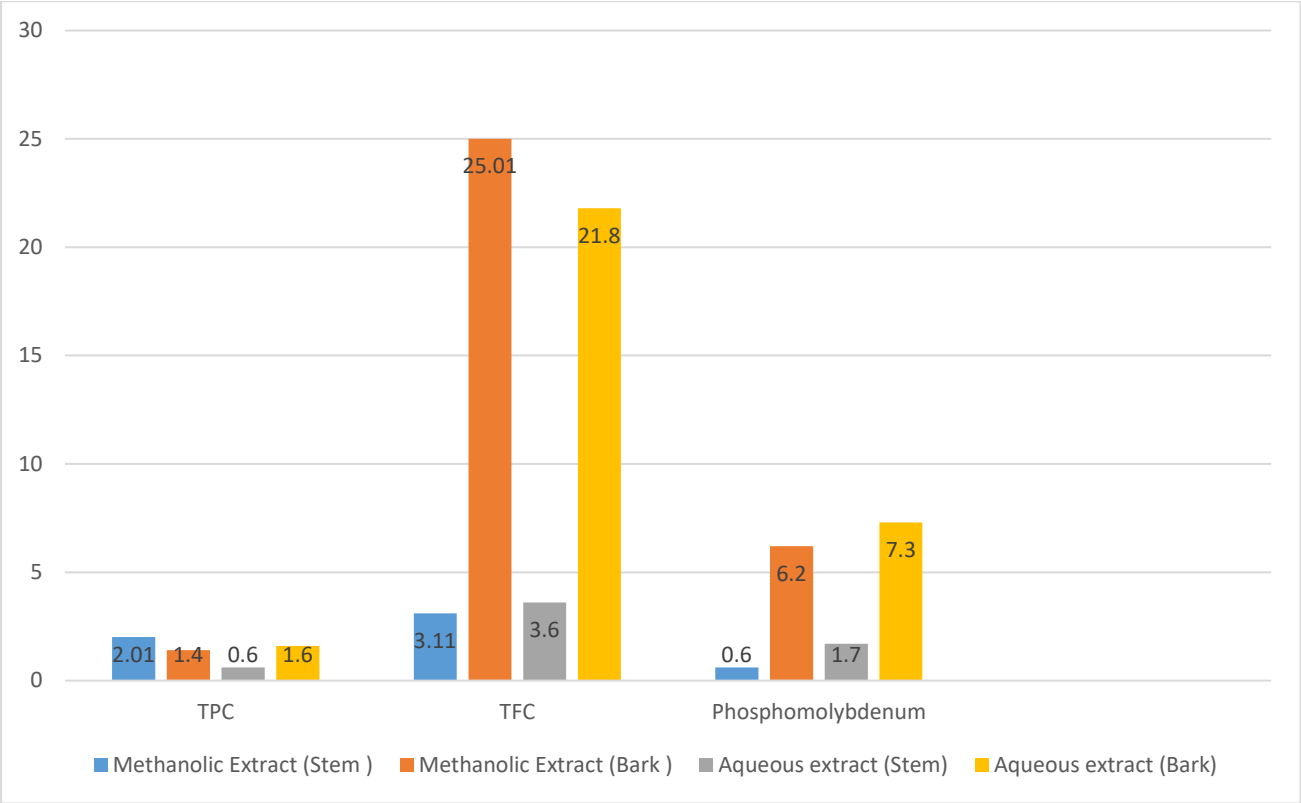
Sr. No.	Phytochemical test for	*H. patens* Jacq. (Stem and Bark) extracts			
		Aqueous	Ethanol	Methanol	Acetone
1.	Alkaloids	+++	+++	+++	+++
2.	Carbohydrates	+++	+++	- - -	- - -
3.	Saponins	+++	+++	+++	+++
4.	Steroids	- - -	- - -	- - -	- - -
5.	Glycosides	- - -	- - -	- - -	- - -
6.	Tannins	- - -	+++	+++	+++
7.	Terpenoids	- - -	+++	+++	- - -
8.	Flavonoids	+++	+++	+++	+++
9.	Phenols	- - -	+++	+++	- - -
10.	Amino acids	- - -	+++	+++	- - -

Phytochemical components of diverse solvent extracts from the stem and bark of *H. patens* Jacq. were identified, and the findings were summarized in Table No. 1. The detailed examination of all four solvent extracts of *H. patens* stem revealed the existence of alkaloids, saponins, tannins, and flavonoids. In comparison, glycosides and steroids were absent in all four extracts. The methanolic and ethanolic extracts indicated the presence of tannins, phenols, terpenoids, and amino acids, whereas water extracts did not contain tannins, terpenoids, or phenols. Carbohydrates were detected in both ethanol and water extracts. Of the four different extracts, the ethanol extract of both plant parts exhibited the highest number of phytochemical constituents, followed by the methanol extracts, which may be attributed to differences like the solvents employed for extraction.

Table 2: TPC, TFC, and Antioxidant properties of *H. patens*

Phytochemical Analysis	Methanolic Extract		Aqueous extract	
	Stem	Bark	Stem	Bark
TPC g/100g (Gallic Acid equivalent)	2.01 (±) 0.01	1.4(±) 0.03	0.6(±) 0.21	1.6(±) 0.04
TFC g/100g (Rutin equivalent)	3.11(±) 0.11	25.01(±) 0.14	3.6(±) 0.23	21.8 (±) 0.19
Phosphomolybdenum g/100g (Ascorbic acid equivalent)	0.6(±) 0.12	6.2(±) 0.11	1.7(±) 0.18	7.3 (±) 0.15

Table 3: Graphical comparison of bark and stem for TPC, TFC, and Antioxidant properties of *H. patens*:



Tables no. 2 and 3 demonstrated that the phytochemical analysis of the methanolic extract of the stem exhibited the highest total phenolic content and total flavonoid content. The methanolic extract of bark demonstrated the highest level of antioxidant activity. The phytochemical evaluation indicated that stem bark showed considerable antioxidant potential. Due to its high TPC, TFC, and antioxidants, it is essential to investigate the critical secondary metabolites from various plant parts. It is necessary to explore different phytochemicals in this uncommon medicinal plant. Initial analysis of bark and stem revealed a range of phytoconstituents that can additionally serve as a prominent source of antioxidant, anti-inflammatory, antidiabetic, antibacterial, antifungal, and dietary nutrients.

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