

Primary and secondary metabolites identification in *Hamelia patens* leaves

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Abstract-The aim of the present study was to investigate the presence of phytochemicals in leaves extracts (aqueous, methanol, ethanol) of *Hamelia patens*, the selected medicinal plant. Ethanolic and methanolic (organic) extract showed good number of compounds compared to aqueous extracts. Phyto-chemical analysis of organic extracts of *Hamelia patens* has shown the biological compounds like protein and amino acids, carbohydrates, cardiac glycosides, tannin, phenol, alkaloids, steroids, terpenoids, flavonoides and saponin. Quinones, phlobatannins, coumarins, emodins and fatty acids were absent in both the aqueous and organic extracts.

Key words: Phytochemical, *Hamelia patens*, Organic.

Introduction

The developing countries mostly depend on traditional plants focusing towards healthcare applications [Allameh, 2002]. Medicinal plants contain phytochemical compounds that are useful as medicine in controlling of various diseases and

disorders. Plants always contain common source of medicaments, in traditional preparations or as pure active principles. Hence to identify plants or plant extracts that could be used to the drugs or that could replace some pharmaceutical preparations need to be purchased and imported [Farnsworth N Ret al, 1985]. Ancient literature such as Rigveda, Yajurveda, Atharvaveda, Charak Samhita and Sushrut Samhita also describe the use of plants for the treatment of various health problems [Samy RP et al, 2008].

Hamelia paten is a large perennial shrub or small tree of the family [Rubiaceae](#). Common names include firebush, humming bird bush, scarlet bush, and redhead [WELCH, 2003]. In Belize, this plant's name is Canaan and is also known as Guardian of the Forest. *Hamelia patens* is an ornamental plant grown almost worldwide in warm, moist areas. It is used to cure inflammation, rheumatism, headache and dysentery. [Surana and Wagh, 2015]. The plants are used in [folk medicine](#) for a range of ailments. The Firebush is used as herbal medicine to treat athlete's foot, skin lesions and insect bites and nervous shock [FENSTER, CHARLES B, 1991].

Material and methods

Collection of plant material

The leaves of *Hamelia patens* were collected from the Botanical Garden, Department of Botany, D.A.V (PG) College, Muzaffarnagar, UP, India.

Preparation of plant extracts

Preparation of sample and extraction were carried out as described by Saha *et al* (2004) with slight modifications. The fresh leaves samples were washed with distilled water and cut in to small pieces, shade- dried for 1 week followed by complete drying at 40 °C in oven. Then ground to from powder. 5 g of this dried sample from each variety was extracted separately with 50 ml of three different solvents: aqueous, ethanol and methanol for 24 h in a shaker at 50 rpm at temperature 30 °C. The extracts were filtered using Whatman filter paper and filtrates were used as an extract which was kept at 4 °C for the bioassay analyses.

Phytochemical screening (Qualitative method)

Standard methods to identify the phytochemical constituents as described by Sofowara (1993), Trease and Evans (1989), (Omoya and Akharaiyi, 2012), (Jyothiprabha and Venkatachalam, 2016). (Harborne and Williams, 2000).

Test for Carbohydrate- 2 drops of Molisch's reagent was added in 2ml of extract and shaken well. 2ml of conc. H_2SO_4 was added on the sides of the test tube. A reddish violet colour ring appeared at the junction of two layers indicated the presence of observed.

Test for Steroids- 1 ml of extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of the test tube without disturbing the contents. The upper layer turns red and sulphuric acid layer showed

yellow with green fluorescence. This indicated the presence of steroids.

Test for Proteins- 2ml of the extract were mixed with the 2ml of Burette reagent. A violet colour ring indicated the presence of peptide linkages of the molecule.

Test for Amino Acids- 2ml of the extract taken in tube and 2ml of in hydrin reagent was added into it and then kept this solution in hot water bath for 2-5 minutes. Appearance of purple colour indicated the presence of amino-acid in the sample.

Test for Alkaloids- The extract was mixed with the 6 drops of 1% HCl, development of precipitate indicated the presence of alkaloids in the sample.

Test for Flavonoides- 5ml of dilute ammonia solution were added to a portion of aqueous extract and then added conc. H_2SO_4 . A yellow coloration was observed which confirmed the presence of flavonoides and it disappeared on standing.

Test for Terpenoids- 5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3 ml of concentrated sulphuric acid. Formation of reddish-brown layer at the junction of two solutions confirmed the presence of terpenoids.

Test for Cardiac Glycosides- 5ml of extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layer with 1ml of conc. H_2SO_4 . A brown ring of the interface indicated a deoxysugar characteristic of cardenolides. A violet ring might appear below the brown ring whereas the acetic acid layer, a greenish ring might form just gradually throughout thin layer.

Test for Tannins- 5ml of extract was added to few drops of 1% of lead acetate. A yellow precipitate indicated the presence of tannins.

Test for Saponins- The extract mixed with 20ml of distilled water and agitated in a graduated cylinder for 15minutes. The formation of 1cm layer of foam indicated the presence of saponins.

Test for Phlobatannins- When an aqueous extract was boiled with 1%aqueous HCl, red precipitate was deposited which was taken as evidence for the presence of phlobatinins.

Test for Fatty Acids- 0.5ml of extract was mixed with 5ml of ether. These extracts were allow it for evaporation on filter paper and dried the filter paper. The appearance of transparency on filter paper indicates the presence of fatty acids.

Test for Anthocyanins- 2ml of aqueous extract is added to 2ml of 2NHCl and ammonia. The appearance of pink-red turns blue violet indicates the presence of anthocyanins.

Test for Leucoanthocyanins- 5ml of aqueous extract was added to 5ml of isoamyl alcohol. Upper layer appeared red

in colour, this indicated the presence of leucoanthocyanins.

Test for Coumarins- 3ml of 10% NaOH was added to 2ml of aqueous extract formation of yellow colour indicated the presence of coumarins.

Test for Phenols- Take 2ml of extract to add 3ml of ethanol and a pinch of FeCl₃ to form greenish yellow colour this indicated the presence of phenols

Test for Quinones- 2ml of extract were mixed with 3ml of concentrated HCl to form green colour this indicates the presence of quinones.

Test for Emodins- 2ml of NH₄OH and 3ml of benzene were added to the extract. Appearance of red colour indicated the presence of emodins in the test solution.

Results

The phytochemicals (primary and secondary metabolites) characteristics of *Hamelia patens leaves* extract were tested and summarized in the Table below.

Table- Phytochemicals (primary and secondary metabolites) screening of aqueous and organic extracts of *Hamelia patens* leaves.

S.No.	Phytochemicals	extracts		
		aqueous	methanol	ethanol
1	Tannin	+	+	+
2	Alkaloids	+	+	+
3	Cardiac glycosides	+	+	+
4	Quinones	-	-	-
5	Steroids	+	+	+
6	Terpenoids	+	+	+

7	Flavonoids	-	+	+
8	Fatty acids	-	-	-
9	Proteins	+	+	+
10	Amino-acids	+	+	+
11	Carbohydrates	+	+	+
12	Saponin	+	+	+
13	Phlobatannin	-	-	-
14	Phenols	-	+	+
15	Anthocynin	-	+	+
16	Leucoanthocyanins	-	+	+
17	Emodins	-	-	-
18	Coumarin	-	-	-

Proteins, sugar and chlorophyll are included in primary metabolites. They are essential for growth and survival of plants. Biosynthetically derived substances from primary metabolites like flavonoides, phenolic compounds, alkaloids, steroids, tannins, saponins, terpenoid are secondary metabolites. From the results, it was found that the various phytochemical constituents like protein and amino acids, carbohydrates, cardiac glycosides, tannin, phenol, alkaloids, steroids, terpenoids, flavonoides and saponin are present in organic (methanolic and ethanolic) extracts. This phytochemical screening is more prominent in ethanolic and methanolic (organic) extract as compared to aqueous extract as bioactive compound are organic in nature and soluble in organic solvent. Quinones, phlobatannins, coumarins, emodins and fatty acids are absent in both aqueous and organic extracts. Anthocynin and leucoanthocyanins both were only found in the organic extract. The bark of *Hamelia patens* contains significant

amounts of tannins [FENSTER, CHARLES B. (1991)]. Flavonoid caused risk reduction mainly from cardiovascular diseases and cancer (Ballard & Marostica, 2019). The presence of classes of phytochemicals such as flavonoides, alkaloid, tannin showed cytotoxic effect (Chowdhury *et al.* 2017). The plants containing phenolic compounds could be useful as an antioxidant. cholesterol-lowering, as well as having cytotoxic qualities, anti-bacterial, anti-viral properties. These are credited to the presence of saponin (Bailly and Vergoten, 2020). The result suggests that the phytochemicals present in *Hamelia patens* extracts may show antimicrobial, anti-inflammatory and antioxidant properties.

Conclusions

Phytochemical screening played an important role in identifying various phytoconstituents present in plant extracts. It could be concluded that plants are a great source of phytochemicals that could be

utilized in curing various ailments. The study was only based on qualitative analysis and screening. Protein and amino acids, carbohydrates, cardiac glycosides, tannin, phenol, alkaloids, steroids, terpenoids, flavonoids and saponin were the phyto-constituents present abundantly in plants. The study provided an important basis for further investigation for the isolation and characterization of phyto-constituents from the selected plants for the development of drugs.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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