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Pharmacognostic study and development of quality parameters of *Hamelia patens* jacq. Stems

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ABSTRACT

Hamelia patens Jacq. (Rubiaceae) is an ornamental plant grown almost worldwide in warm, moist areas. Firebush is used in herbal medicine to treat athlete's foot, skin lesions and insect bites, nervous shock, inflammation, rheumatism, headache, asthma, and dysentery. Also, the plants are used in folk medicine against a range of ailments. Present study deals with to establish pharmacognostic, physiochemical and phytochemical standards of Hamelia patens stems. Microscopical evaluation of Hamelia patens young stems showed the presence of covering trichomes, epidermis and other characteristics while mature stems showed cork, phellogen, stone cells, medullary rays, xylem vessel, xylem parenchyma, phellogen fibers, cortex, coloring matter and starch grains. Results of Hamelia patens stems yielded 2.182 % w/w total ash; acid insoluble ash not more than 0.217% w/w, water soluble ash not more than 1.067 w/w, alcohol extractive value not more than 5.324 % w/w (Hot extraction), 4.283 % w/w (Cold maceration) & water extractive value not more than 8.323% w/w (Hot extraction), 6.169% w/w (Cold maceration) and 5.26 % w/w total moisture content by LOD. The Phytochemical investigation of extracts of stems of Hamelia patens shows the presence of sterols, triterpenes, alkaloids, flavonoids, tannins, glycosides, proteins and carbohydrate Pharmacognostical and phytochemical parameters determined in the present work can serve as major criteria for identity, quality and purity of a crude drug and extracts.

Key words: *Hamelia patens*, Microscopical study, physicochemical study, Phytochemical, Pharmacognostic study, Fluorescence analysis

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INTRODUCTION:

Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value [1]. Plants are used in modern medicine where they occupy a very significant place as raw material for important drugs. Plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases. Plants are rich sources of ecologically developed secondary metabolites, which are potential remedies for different ailments [2].

It is no wonder that the world's one-fourth population i.e. 1.42 billion people, are dependent on traditional medicines for the treatment of various ailments. Now-a-days there is a renewed interest in drugs of natural origin simply because they are considered as green medicine and green medicine is always supposed to be safe [3]. Another factor which emphasizes this attention is the incidences of harmful nature of synthetic drugs which are regarded as harmful to human beings and environment. The advantage of natural drugs is their easy availability, economic and less or no side effects but the disadvantage is that they are the victims of adulteration. The more effective the natural drug more is its demand and the chances of non-availability increases. To meet the growing demand, the natural drug is easily adulterated with low grade material. Adulteration or substitution is nothing but replacement of original plant with another plant material or intentionally adding any foreign substance to increase the weight or potency of the product or to decrease its cost [4]. However a key obstacle, which has hindered the acceptance of the alternative

medicines in the developed countries, is the lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as a medicine [5]. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy [6].

Hamelia patens Jacq. (Rubiaceae) is commonly known as firebush or scarlet bush. It is a large perennial shrub or small tree generally grown as ornamental plant. Also, the plants are used in folk medicine against a range of ailments. Scarlet bush is used in herbal medicine to treat athlete's foot, skin lesions and insect bites, nervous shock, inflammation, rheumatism, headache, asthma, and dysentery [7]. Aerial part of *H. patens* Oxindole Alkaloids [8], flavanone Glycoside [9,10] while leaves contain Ephedrine [11]. Leaves shows anti-inflammatory Activity [12], Anti-Bacterial activity [13], Antioxidant activity [14], Cytotoxic activity [15] and Antinociceptive Effects [16] and aerial parts shows Wound Healing Activity [17]. Leaf, stem and root of *H. patens* shows anthelmintic[18] while, bark has Antibacterial and Antifungal properties [18,19].

The process of standardization can be achieved by stepwise pharmacognostic and phytochemical studies. These studies help in identification and standardization of the plant material, till date no work done on the same. So objectives of present study to establish pharmacognostical standards and to develop physiochemical and phytochemical quality parameters of *Hamelia patens* stems.

MATERIALS AND METHODS

2.1 Pharmacognostic Study

2.1.1. Collection, identification and drying of plant material:

The stems of ***Hamelia patens*** were collected from Nashik district, identified by Dr. S.G.Kotwal HOD, Dept. of Botany, K.T.H.M. College, Nashik and authentified by A. Benniamin, Scientist and HOD, Botanical Survey of India. The herbarium of the plant specimen has been deposited at B.S.I.Pune, the voucher specimen No.FSC-1.

It was air dried in shade avoiding exposure to direct sunlight and then pulverized in grinder. The stem of powder (# 60-80) was used for standardization.

2.1.2. Macroscopic evaluation

Organoleptic evaluation refers to evaluation of the plant material by color, odour, taste, fracture, size and shape. The organoleptic characters of the samples were evaluated based on the textual methods [20].

2.1.3. Microscopy evaluation

Free hand section of the materials were taken using a Microtome, suitably stained and subjected to microscopic observations. Photomicrographs were taken using compound Microscope by using digital camera [21, 22].

2.2. Physiochemical investigation

Standardization of stem powder was done to evaluate the quality and purity of the drug. Various Standardization parameters like loss on drying (Moisture content), extractive values (Water soluble and alcohol soluble) and ash values (Total ash, acid insoluble ash and water soluble ash) were calculated as per WHO guidelines [21, 23, 24].

2.3. Florescence analysis

Florescence analysis of powder of *H. patens* stem were carried out under visible light and ultra violet light (254nm and 365nm), after treatment with various chemical and organic reagents [25].

2.4. Preliminary Phytochemical investigation

2.4.1. Extraction Methodology

The shade dried stems of *H.patens* were pulverized in grinder. The stem of powder (# 60-80) was continuous successively extracted by Soxhlet extraction method by using Petroleum ether (60-80^{0}C), Chloroform and Methanol. All extract is dried under vacuum and perform following chemical test to identify type of phytoconstitents [26].

2.4.2. Phytochemical Test

A) Test for Alkaloids

Few mg of each extract was taken separately in 5ml of 1.5%v/v Hydrochloric acid and filtered. These filtrates were then subjected to following test.

Dragendroff's Test
The extract was spread on Whatmann filter paper and dried. The test filter paper after basification with ammonia was extracted with chloroform and chloroform extract was applied on filter paper impregnated with the Dragendroff's reagent. Development of orange-red colour indicates the presence of alkaloids.

Mayer's reagent:
The extract was treated with Mayer's reagent. Development of Cream colour indicates the presence of alkaloids.

Wagner's Reagent:
The extract was treated with Wagner's reagent. Development of Reddish yellow colour indicates the presence of alkaloids.

Hager's Reagent:
The extract was treated with Hager's reagent. Development of Yellow colour precipitate indicates the presence of alkaloids [27].

B) Test for Glycosides
General test:
Test A: To 5 mg of extract add 5 ml of dil sulphuric acid by warming on water bath, filter it. Then neutralise the acidic extract with 5% NaOH solution. Add 0.1 ml Fehling's solution A and Fehling's solution B and heat on a water bath for 2 minutes.

Test B: To 5 mg of extract add 5 ml of water by warming on water bath, filter it. Then add same volume of water as used in Test Aof 5% NaOH solution. Add 0.1 ml Fehling's solution A and Fehling's solution B and heat on a water bath for 2 minutes.

Compare the red precipitate formed in test A and Test B. If Test A contains more precipitate then presences of Glycoside. If Same in test A and Test B absent of glycoside [28].

C) Test for Naphthoquinones:
Juglone Test:
To the 2mg extract add 2ml solvent ether and equal volume of ammonical layer appearance of reddish pink colour indicates presence of naphthaquinones.

D) Test for anthraquinones
Brontagers test: To the 2 mg extract add dil. HCl. Boil, Cool and add 2ml solvent ether. Separate the ethereal layer and add strong Ammonia solution, appearance of pink colour to ammonical layer indicates presence of anthraquinones.

Modified Brontagers test: To the 2 mg extract add dil. HCl and 2-3 drops of $Fecl_3$. Boil, Cool and add 2ml solvent ether. Separate the ethereal layer and add strong Ammonia solution, appearance of pink colour to ammonical layer indicates presence of anthraquinones [21].

E)Test For Iridoidal Glycosides
Trim –Hill test
Extract was treated with 5 ml of aqueous 1% HCl for 3-6 hours. 0.1 ml was decanted and transfer in another test tube containing 1 mL Trim Hill reagent (10 ml of acetic acid, 1ml of 0.2 % Copper sulphate, and 0.5 ml Conc. Hydrochloric acid) and it was heated over a flame, blue colour devlops indicates presence of iridoids.

F) Test for Coumarin Glycosides
With ammonia
Extract solution was added on paper impregnated with ammonia developed fluorescence indicates the presence of coumarins.

With alkali solution
Extract wa dissolved in alcohol and made alkaline showed blue or green fluorescence indicates the presence of coumarins [28].

G) Test for Cyanogenetic Glycosides
Grignard's test
The extract was transferred in flask stoppered with strips of sodium picrate paper. Care was taken paper not to touch the inner side of the test tube. The content was warmed for 30 min. The sodium picrate paper turned to red colour indicates the presence of cyanogenetic glycosides.

H) Test for Sterols
Salkowaski Test:
Few mg of extract was taken in 2.0 ml of chloroform and 2.0ml of concentrated sulphuric acid was added from the side of test tube. It was shaken for few min. The development of red colour in the chloroform layer indicated the presence of sterols.

Liebermann Test:
To few mg of extract in a test tube, few ml of acetic anhydride was added and heated gently. Few drops of concentrated sulphuric acid was added to it appearance of blue colour gave evidence for presence of sterols.

I) Test for Triterpenoids
Libermann-Burchard Test
Few mg of extract was dissolved in chloroform and few drops of acetic anhydride was added to it followed by concentrated sulphuric acid from side of test tube. Developments of reddish brown ring at the junction indicated the presence of triterpenoids.

J) Test for Flavanoids
Shinoda test
A small quantity of the extract was dissolved in 5ml of ethanol (95%v/v) and treated with few drops of concentrated Hydrochloric acid and 0.5 g of Magnesium turnings. Pink, magenta and crimson colour indicates presence of flavonoids[28].

K) Test for Tannins
With $FeCl_3$: Blue colour – Hydrolysable tannins
Brownish green–Condensed tannins

Matchstick Test
Dip a Matchstick to the methanolic solution of the extract and after drying dip the stick again in HCl. Dry it and take the stick near the flame. The flame burns with magenda colour was the indication of presence of tannins.

L) Tests for Carotenoids
Carr-price reaction
Extract when treated with Antimony trichloride give blue colour due to presence of carotenoids.

M) Test for proteins
Biuret test: To the 5mg of extract add 2 ml of alcohol/ water shake it. Add 2 ml of Biuret reagent; violet colour indicates presences of protein.

N) Test for carbohydrates
Molisch's Test: To the 2 mg extract add Molisch's reagent, shake and from the side of test tube add H_2SO_4. Appearance of violet ring at the junction of two liquid indicates presences of carbohydrate

RESULT AND DISCUSSION

3.1. PHARMACOGNOSTIC STUDY

3.1.1. Macroscopic evaluation

Macroscopic identity of herbal materials is based on shape, size, colour, fracture characteristics and appearance of the cut surface (Figure 1). However, since these characteristics are judged subjectively and substitutes or adulterants may closely resemble the genuine material, it is often necessary to substantiate the findings by microscopy and/or physicochemical analysis.
1 Color: Outer surface: Greenish brown Inner surface : Yellowish Node: Reddish brown
2 Odour: None
3 Taste: Astringent
4 Size: Distance between node-6.00-12.00cm, Diameter: 0.5to 1.2cm

5 Shape: Cylindrical
6 Fracture: Short fracture to bark and wood both

Figure 1: Whole plant and Stems of *Hamelia patens*

3.1.2. Microscopy evaluation

Microscopy evaluation

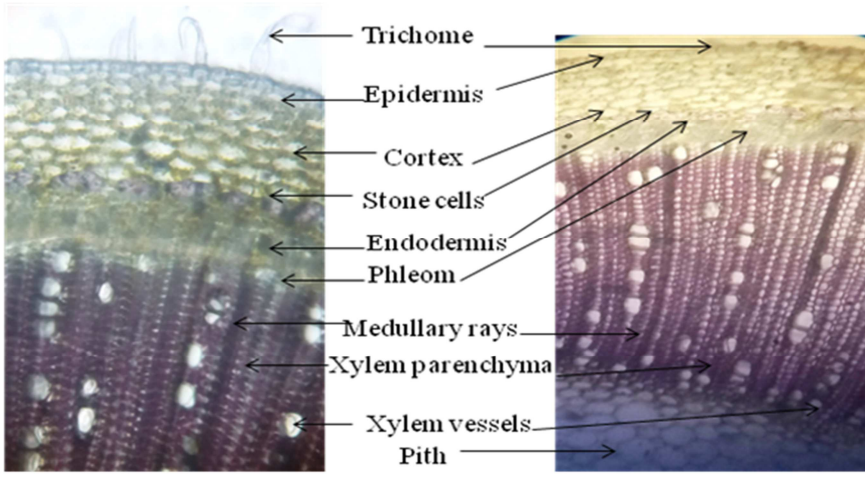

Figure 2: T.S. of Young stem of *Hamelia patens* after stain with phloglucinol: HCl(1:1)

Epidermis: Single layers of straight wall cubical cells covered with cuticle. Many multicellular, uniseriate covering tapering or hooked shaped trichomes are present.

Cortex 12-15 layers parenchyma contains starch grains.
Single layer more thick walled Stone cells.

Endodermis is distinct, not contain starch grains.
Secondary phloem consist of phloem parenchyma
Uniseriate or biseriate Medullary rays narrow at inner side while wider at start
Xylem parenchyma and xylem vessels are present in between medullary rays
Pith also present (Figure 2)

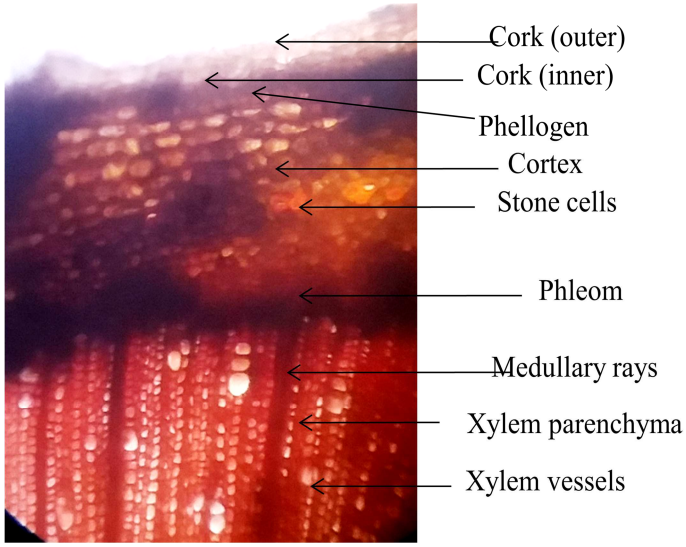

Figure 3: T.S. of mature stems of *Hamelia patens* after stain with phloglucinol: HCl(1:1)

Cork: few layers of polygonal tabular thin walled cells.
Phellogen and phelloderm are not separated.
Cortex 12-15 layers parenchyma contains starch garains.
Single layer Stone cells
Secondary phloem consist of phloem parenchyma
Uniseriate or biseriate Medullary rays narrow at inner side while wider at start
Xylem parenchyma and xylem vessels are present in between medullary rays
Pith also present (Figure 3) [24, 21]

3.2. Physiochemical investigation

Loss on drying is the loss in weight in % w/w resulting from water and volatile matter of any kind that can be driven off under specified conditions. Moisture triggers the enzymatic activity or facilitates growth of microbes which leads to its deterioration.

Total ash is designed to measure the total amount of material produced after complete incineration of the ground drug at about 450° C to remove all the carbons. The ash of any organic material is composed of their non-volatile inorganic components (metallic salts and silica). This value varies within wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs. More direct contamination, such as by sand or earth, is immediately detected by the ash value. Acid insoluble ash is the residue obtained after extracting the total ash with hydrochloric acid.

Extractives values represent the amount of active constituents in given amount of medicinal plant material when extracted with solvents [20,24]. As mentioned in different official books (I.P.1996, B.P.1980, B.H.P.1990 etc.), the determination of water soluble and alcohol soluble extractives, is used as means of evaluating crude drugs which are not readily estimated by other means. The results of the physicochemical parameters of *H. patens* Stems are given in Table 1

Table1: Physicochemical Parameters of *H. patens* stem powder

Physicochemical Parameters	% w/w
Loss on drying	5.66
Total ash	2.182
Acid insoluble ash value	0.217
Water soluble ash value	1.067
Alcohol extractive value: Cold maceration	4.283
Alcohol extractive value: Hot extraction	5.324
Water extractive value: Cold maceration	6.169
Water extractive value: Hot extraction	8.323

3.3. Fluorescence analysis

The fluorescence analysis helps to identify the drug with reagent shows specific fluorescent colours, and also to find out the fluorescent impurities. Thus the study of fluorescence analysis can be used as a diagnostic tool for testing adulteration [30]. The results of the fluorescence analysis are given in Table 2.

Table 2: Fluorescence Analysis of *H.patens* stem powder

Sample +Reagent	Visible light	Short wavelength UV_{254nm}	Long wavelength UV_{365nm}
Powder	Reddish Brown	Reddish Brown	Brown
Powder+50%NaOH (aq)	Brown	Blue	Yellow
Powder +Ammonia	Brown	Blue	Yellow
Powder+ Picric acid	Yellowish brown	Yellowish brown	Dark brown
Powder+ 10%HCL	Light brown	Brown	Yellowish brown
Powder+ 10%H2SO4	Light brown	Yellowish brown	Yellowish brown
Powder+ Conc HCL	Brown	Brown	Dark Brown
Powder+ Conc H2SO4	Blackish Brown	Black	Dark Brown
Powder+ Conc HNO3	Yellowish brown	Yellowish green	Dark brown
Powder+ 10% NaOH	Brown	Brown	Yellowish green
Powder+ Water	Cream	Yellow	Yellowish green
Powder+ Methanol	Yellow	Blue	Brown
Powder+ Pet. Ether	Yellow	Yellow	yellow
Powder+ $CHCl_3$	Yellow	Yellow	Reddish brown

3.4. Phytochemical investigation

Extraction : The extraction was carried out by continuous hot extraction method using Soxhlet extractor till all constituents removed. The end of completion of extraction was indicated by no colour with iodine chamber when spot on TLC plate [31]. Percentage and colour of extract are given in Table 3

Table 3 : Extractive values after continuous extraction of *Hamelia patens* stems

Extract	Colour of Extract	% (w/w)of Extract obtained
Petroleum ether	Yellowish green	1.13
Chloroform	Dark brown	2.67
Methanol	Dark Brown	4.26

3.5. Preliminary Phytochemical Investigation

Pet-ether extract showed the presence of sterols and triterpenoids. Chloroform extract showed the presence of alkaloids, glycoside, sterols and triterpenoids. Methanol extract showed the presence of alkaloids, sterols, glycosides, flavonoids, tannins, proteins and carbohydrate (Table 4).

Table 4: Observation table of preliminary phytochemical test of extracts of *Hamelia patens*

Sr No.	TEST	Extracts		
		Pet. Ether	Chloroform	Methanol
1.	Test for Alkaloids	–	+	+
2.	Test for Glycosides	–	+	+
3.	Test for Naphthoquinones	–	–	–
4.	Test for anthraquinones	–	–	–
5.	Test For Iridoidal Glycosides	–	–	–
6.	Test for Coumarin Glycosides	–	–	–
7.	Test for Cyanogenetic Glycosides	–	–	–
8.	Test for Sterols	+	+	+
9.	Test for Triterpenoids	+	+	–
10.	Test for Flavanoids	–	–	+
11.	Test for Tannins	–	–	+
12.	Tests for Carotenoids	–	–	–
13.	Test for Proteins	–	–	+
14.	Test for Carbohydrates	–	–	+

CONCLUSION

Ascertaing pharmacognostic and physiochemical standards are an integral part correct identity and quality of a crude drug. Before any drug can be included in the pharmacopeia, these standards must be established. The majority of the information on the identity, purity and quality of the plant material can be obtained from its macroscopy, microscopy and physiochemical parameters. Pharmacognostical and phytochemical parameters of *H.patens* determined in the present work can serve as major criteria for identity, quality and purity of a crude drug and its extracts.

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