Research Journal of
Phytochemistry

ISSN 1819-3471

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Phytochemistry

ISSN 1819-3471
DOI: 10.3923/rjphyto.2016.67.74

Research Article
Estimation of Total Phenolic and Total Flavonoid Content and Assessment of *in vitro* Antioxidant Activity of Extracts of *Hamelia patens* Jacq. Stems

[1]Ajaykumar Rikhabchand Surana, [1]Manoj Ramesh Kumbhare and [2]Rajendra Dayaram Wagh

[1]SMBT College of Pharmacy, Dhamangaon, Tal-Igatpuri, District Nashik, 422403 Maharashtra State, India
[2]ARA College of Pharmacy, Dhule, 424 005 Maharashtra State, India

Abstract

Background and Objective: Free radicals accumulation in the body causes many oxidative stress related diseases like arthritis, diabetes, inflammation, cancer and genotoxicity. The objectives of present study were to assess total phenolic and flavonoid content and to evaluate *in vitro* antioxidant activity of extracts of *Hamelia patens* stems. **Methodology:** Total phenolic and flavonoid content of the *H. patens* Jacq. stem extracts were determined by using the Folin-Ciocalteu method and aluminum chloride method, respectively. Antioxidant activities were performed by DPPH free radical, nitric oxide, hydrogen peroxide scavenging and metal chelating assay. **Results:** The total phenolic content of the petroleum ether extract (PHP), chloroform extract (CHP) and methanol extract (MHP) was 19.083±1.12, 30.58±1.28 and 99.25±1.39 mg gallic acid equivalent per gram of dried extract, respectively. The total flavonoid PHP, CHP and MHP was 8.47±0.67, 15.09±1.21 and 43.42±1.41 mg rutin equivalent per gram of dried extract, respectively. All extracts exhibited scavenging effect in concentration dependent manner. Chloroform extract showed good *in vitro* antioxidant activity as compared to other extracts. Antioxidant activities of the extracts were associated due to the total phenolic and flavonoid contents of the extract of *H. patens*. **Conclusion:** The present study showed that *H. patens* stems possess rich amounts of natural antioxidants and can be further explored for their possible use as a natural additive in food or in pharmaceutical products. These extracts can be regarded as a promising candidate for a plant derived antioxidant agent.

Key words: *Hamelia patens*, antioxidant, total phenolic, total flavonoids, PHP

Received: May 01, 2016 Accepted: May 20, 2016 Published: June 15, 2016

Citation: Ajaykumar Rikhabchand Surana, Manoj Ramesh Kumbhare and Rajendra Dayaram Wagh, 2016. Estimation of total phenolic and total flavonoid content and assessment of *in vitro* antioxidant activity of extracts of *Hamelia patens* Jacq. stems. Res. J. Phytochem., 10: 67-74.

Corresponding Author: Ajaykumar Rikhabchand Surana, Department of Pharmacognosy, SMBT College of Pharmacy, Nandi-Hills, Dhamangaon, Tal-Igatpuri, District Nashik, 422403 Maharashtra State, India Tel: +91-9657296551 Fax: +91-2553-282350

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The enduring increasing discovery of curative plants is owing to numerous reasons, including rising reliance in herbal drug. Allopathic medicine may alleviate a broad range of diseases although, it's soaring prices and side-effects are causing community to come back to herbal medicines which have less side effects[1].

For the majority of the developing countries, the major concern of community health is still the keen demand for crucial health care, which is miserably missing yet at the majority elementary level. This is factual in both the rapidly emerging cities and in the countryside areas. As per World Health Organization (WHO) 50% world's population do not have access to satisfactory health care services[2]. This is due to the authenticity that poor people neither have access to nor can afford the current health care services. Therefore, pioneering alternative approaches are needed to address this problem. Traditional medicinal plants offer alternative medicines with incredible opportunities. They not only provide access and affordable medicine to poor people but also they generate income, employment and foreign trade for developing countries. Many traditional medicinal herbs have been shown to have medicinal value, especially in the rural areas and that these can be used to prevent, alleviate or cure several human diseases. As per WHO more than 80% of the world's population rely either solely or largely on traditional remedies for health care[3].

Molecular oxygen is an vital component for all living organisms, where it facilitate in the process of oxidation which is a basic component of aerobic life and of our metabolism[4]. A part of the oxygen taken into living cells may be converted to several harmful Reactive Oxygen Species (ROS) and free radicals. The formed free radicals can start a chain reaction, leading to the formation of more free radicals[5]. The ROS which consist of free radicals such as superoxide anion (O_2^-) and hydroxyl ($HO^\bullet$) radicals and non-free radical species such as H_2O_2 and single oxygen ($^1O_2$) are different forms of activated oxygen. The ROS are produced by all aerobic organisms and can easily react with mainly biological molecules including lipids, lipoproteins, DNA and proteins[6]. Thus, ample generation of ROS proceed a variety of pathophysiological disorders such as diabetes, arthritis, genotoxicity, inflammation and cancer. Therefore, living organisms possess a number of protective mechanisms against the toxic effects of ROS[7]. Antioxidants regulate various oxidative reactions naturally occurring in tissues. As antioxidants have been reported to prevent oxidative damage caused by these free radical by interfering the oxidation process by reacting with free radicals, catalytic metals, chelating and also by acting as oxygen scavengers[8].

Plants contains free radical scavenging molecules, such as phenolic, terpenoids, vitamins, lignins, stilbenes, tannins, flavonoids, quinones, betalains, alkaloids, coumarins and other secondary metabolites which shows good antioxidant activity[9].

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, rheumatism, inflammation, headache, dysentery and asthma[10]. Aerial parts of *H. patens* contain oxindole alkaloids[11], flavanone glycoside[12,13] while, leaves contain ephedrine[14]. Leaves shows anti-inflammatory activity[15], anti-bacterial activity[16], antioxidant activity[17], cytotoxic activity[18], antinociceptive effects[19] and aerial parts shows wound healing activity[20]. Leaf, stem and root of *H. patens* shows anthelmintic[21] while, bark has antibacterial and antifungal properties[21,22].

From literature review it reveals that, antioxidant activity of *H. patens* is not reported. Hence, the recent trend to find naturally occurring antioxidants, this study was designed to investigate antioxidant potential and to determine total phenolic and flavonoid contents of extracts of *H. patens*.

MATERIALS AND METHODS

Plant material: *Hamelia patens* stems were collected from Nashik district in May, 2014, identified by Dr. S.G. Kotwal HOD, Department of Botany, KTHM College, Nashik and authentified by A. Benniamin, Scientist and HOD, Botanical Survey of India, Pune and herbarium specimen deposited as vou. no. FSC-1.

Chemicals: All chemicals used in the study were of analytical grade. Chemicals used in this study were DPPH obtained from Sigma-Aldrich, India, NADH and sulfanilamide obtained from Himedia, Laboratory Pvt. Ltd., India, Ascorbic acid, tocopherol, ferrozine obtained from qualigens fine chemicals, N-1-naphthylethylenediamine dihydrochloride, sodium nitroprusside, sodium nitrite, ferrous chloride Sd fine chemicals Ltd., India.

Preparation of extracts: The plant materials were air dried under shade, pulverized and successively extracted with petroleum ether (60-80°C), chloroform and methanol in

Soxhlet apparatus. The petroleum ether extract (PHP), chloroform extract (CHP) and methanol extract (MHP) obtained were dried in vacuum dryer at 40°C.

Preliminary phytochemical tests: The dried extracts subjected for various preliminary screening of phytochemicals such as glycosides (General test), alkaloids (Dragendroff's test, Mayer's reagent, Wagner's reagent, Hager's reagent), flavonoids (Shinoda and NaOH tests), saponins (foam and haemolysis tests), sterols (Liberman-Burchard and Salkowski tests), tannins (Ferric chloride test, matchstick test, gelatin test), carotenoids (Carr-price test), iridoidal glycosides (Trim-Hill test), coumarin glycosides (Ammonia and alkali test), cyanogenetic glycosides (Grignard's test), naphthaquinones (Juglone test), anthraquinones (Modified Brontager's test), proteins (Biuret test) and carbohydrates (Moilsch's test).

Total phenolic content: The phenolic content was determined according to the method described by Spanos with slight modifications[23]. The assay was determined using 1 mL of each extract stock solution (1 mg mL^{-1}) and 1 mL of each standard gallic acid solution was taken in 25 mL volumetric flask, added 10 mL of water, 1.5 mL of Folin-Ciocalteu reagent and allowed to stand for 10 min then 4 mL of sodium carbonate solution was added in each volumetric flask and volume was adjusted with distilled water. Absorbance were measured after 1 h at 765 nm by UV visible spectrophotometer against blank[24]. The total phenolic content was calculated from the calibration curve of gallic acid and the results were expressed in mg gallic acid equivalent per gram of dried extract.

Total flavonoid content: The flavonoids content was determined according to the method described by Kumaran and Karunakaran with slight modifications[25]. Total flavonoid content was determined using 0.50 mL of each extract stock solution (1 mg mL^{-1}) and each dilution of standard rutin solution (10-100 µg mL^{-1}) taken separately in test tubes. To each test tube 1.50 mL methanol, 0.10 mL aluminium chloride solution, 0.10 mL potassium acetate solution and 2.80 mL distilled water were added and shaked. Sample blank for all extract and all the dilution of standard rutin and were prepared in similar manner by replacing aluminium chloride solution with distilled water. All the prepared solutions were filtered through Whatmann filter paper No. 1 before measuring their absorbance. Absorbance was measured at 510 nm against the suitable blank. From a calibration curve of rutin, the total flavonoid content was calculated and the result was expressed in mg rutin equivalent per gram dry weight extract.

Antioxidant method

Nitric oxide scavenging assay: Aqueous sodium nitroprusside solution at physiological pH, instinctively produce nitric oxide. This nitric oxide reacts with oxygen to produce nitrite ions, which determined by Griess Illosvoy reaction. Griess Illosvoy reagent was slightly modified instead of 1-naphthylamine (5%) naphthylethylenediamine dihydrochloride (0.1% w/v) was used. The nitric oxide scavengers compete with oxygen and reduce the production of nitric oxide[26]. The reaction mixture contained 2 mL of 10 mM sodium nitroprusside, 0.5 mL of phosphate buffer in saline (pH 7.4, 0.01 M) and 0.5 mL of extract was incubated for 150 min at 25°C. After 150 min 0.5 mL of the reaction mixture was pipetted and mixed with 1 mL of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization reaction. Then, 1 mL of 0.1% naphthylethylenediamine dihydrochloride was added and allowed to stand for 30 min in dark place[27]. The absorbance of the pink coloured chromophore was measured at 540 nm by UV visible spectrophotometer against the blank solutions. Percentage inhibition was calculated by using following formula:

$$\text{Percentage inhibition} = \frac{(A_{control} - A_{test})}{A_{control}} \times 100$$

where, $A_{control}$ is the absorbance of the control and A_{test} is the absorbance in the presence of the sample of extract and standard. Plotted the graph percentage inhibition vs concentration and calculated the IC_{50}.

Hydrogen peroxide scavenging assay: The capacity of the extracts to scavenge H_2O_2 was determined according to Halliwell *et al.*[28]. A solution of 40mM hydrogen peroxide was prepared in phosphate buffer solution (pH 7.4). Extracts (25-125 µg mL^{-1}) in methanol were added to a test tube contained 0.6 mL hydrogen peroxide solution. The concentration of hydrogen peroxide wasdetermined by absorbance taken at 230 nm using UV visible spectrophotometer after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. Percentage inhibition and IC_{50} calculated similar as given in nitric oxide scavenging activity[29].

DPPH free radical scavenging assay: The DPPH scavenging potential of different extracts of *H. patens* was measured based on scavenging ability of stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals. The method modified by Brand-Williams was employed to perform the free radical scavenging activity[30]. Freshly prepared 2 mL DPPH solution (33 mg L^{-1}) was mixed with 2 mL of different dilution of *H. patens* extracts. The reaction mixture was stand for 15 min at room temperature in dark place. Absorbance of the resultant mixture was recorded at 517 nm using UV visible spectrophotometer[26]. The percentage of DPPH scavenging by the extracts and standard compounds were calculated as given in nitric oxide scavenging activity.

Metal chelating assay: The chelating activity of the *H. patens* extracts toward ferrous ions was studied by the method of Dinis *et al.*[31] with minor modification. Different dilutions of each extracts in methanol (25-125 µg mL^{-1}) were prepared from the dried extracts. Take 1 mL of each dilution of extracts, 0.1 mL of $FeCl_2$ (2 mM) was added and the reaction was initiated by the addition 0.2 mL of ferrozine (5 mM), the mixture was shaken vigourously for 10 sec. The mixture was stand at room temperature for 10 min and the absorbance was determined at 562 nm. Methanol without test sample was used as a control and methanol without ferrozine mixture was used as a sample blank. The EDTA was used as a standard for the assay[32].

Chelating activity was expressed as IC_{50}, the concentration that chelates 50% of Fe^{2+} were calculated as follows:

$$\text{Chelating activity (\%)} = \frac{(A_{control}-A_{test})}{A_{control}} \times 100$$

RESULTS AND DISCUSSION

Phytochemical investigations: The preliminary phytochemical investigations was studied to explore its type of chemical component, the study reveals the presence sterols and triterpenoids in pet-ether extract and 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.

Total phenolic content: The phenolic compounds are main source of antioxidants and free radical scavengers hence, there should be a close correlation between the phenolic content and antioxidant activity. It was reasonable to determine the total phenolic content in the plant extract. The total phenolic content of extracts were determined from regression equations for the calibration curves of gallic acid ($y = 0.004x$, $R^2 = 0.992$). The TP content of the PHP, CHP and MHP are listed in Table 1.

Total flavonoid content: Total flavonoids content of the crude extract of *H. patens* was determined using the aluminium chloride colorimetric method. The aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavonols and flavones[33]. For building the calibration curve, rutin is used as standard materials. The TFP of extracts were determined from regression equations for the calibration curve of rutin ($y = 0.007x$, $R^2 = 0.996$). The FP content of the PHP, CHP and MHP are listed in Table 1.

Antioxidant activity

Nitric oxide scavenging activity: Nitric Oxide (NO) is an important pleiotropic mediator generated by endothelial cells, macrophages, neurons, etc. Nitric oxide is involved in the regulation of various physiological processes such as smooth muscle relaxant, neuronal signaling, regulation of cell mediated toxicity and inhibition of platelet aggregation. Excess concentration of NO is associated with many diseases[34,35]. These compounds are responsible for changing the structural and functional behavior of several cellular components. Incubation of sodium nitroprusside solutions at 25°C for 150 min resulted in linear time dependent nitrite production which is reduced by the extracts of *H. patens*. In order to evaluate the antioxidant potency through NO scavenging by extracts, the concentraction NO was monitored. The NO scavenging capacity is determined by the decrease in the absorbance at 550 nm, induced by antioxidants[36]. Figure 1a shows the comparative NO scavenging activity of the extract. Nitric oxide inhibitors have been shown to have beneficial effects on some aspect of inflammation and tissue damage seen in inflammatory diseases[37]. Extracts of *H. patens* significantly inhibited nitric oxide in a concentration-dependent manner. Petroleum ether,

Table 1: Total phenolic and flavonoid content of different extracts of *H. patens*

Sample	Total phenolics (mg gallic acid equivalent per gram of dried extract)	Total flavonoid (mg rutin equivalent per gram of dried extract)
PHP	19.083±1.12	8.47±0.67
CHP	30.580±1.28	15.09±1.21
MHP	99.250±1.39	43.42±1.41

Values represent Mean±Standard Deviation (n = 3), PHP: Petroleum ether extract, CHP: Chloroform extract and MHP: Methanol extract

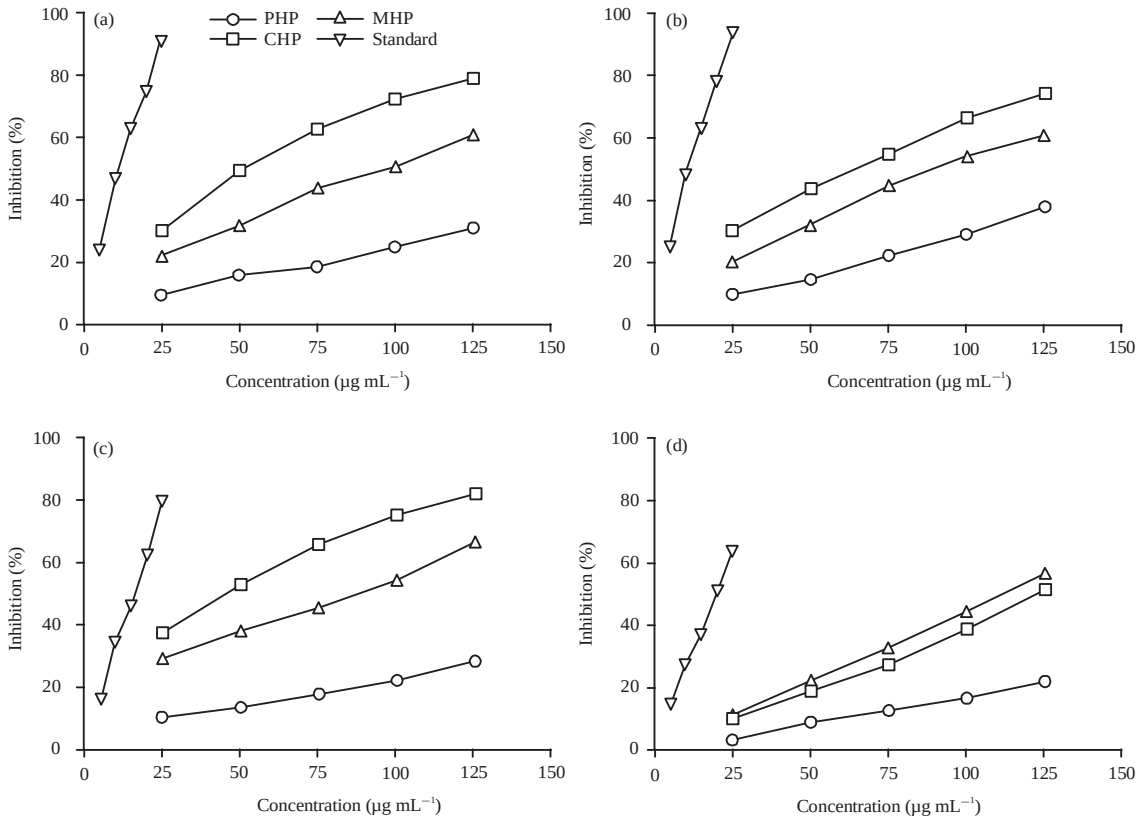

Fig. 1(a-d): Antioxidant activity of *Hamelia patens* extracts at different concentrations. Each value represents mean (n = 3), (a) Nitric oxide free radical scavenging activity, (b) Hydrogen peroxide scavenging activity, (c) DPPH free radical scavenging and (d) Ferrous ion chelating activity

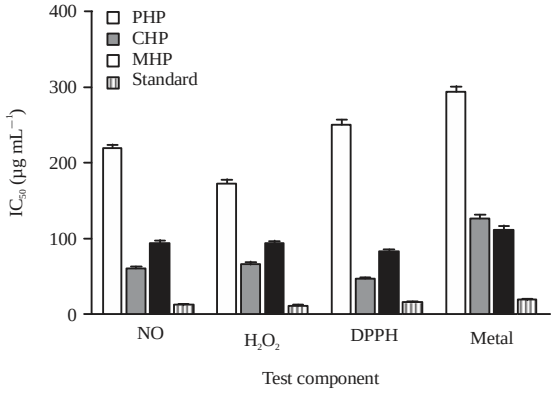

Fig. 2: Antioxidant profile (IC_{50} values) of different extract of *H. patens* for nitric oxide free radical scavenging activity, hydrogen peroxide scavenging activity, DPPH free radical scavenging and ferrous ion chelating activity

chloroform, methanol extracts of *Hamelia patens* stems and ascorbic acid were found to be scavenger of nitric oxide free radical with an IC_{50} of 219.97, 61.33, 94.57 and 12.88 µg mL^{-1}, respectively. Chloroform extract was found to be good scavenger of Nitric oxide free radical than methanol extract and Petroleum ether extract. Comparison of IC_{50} value of extracts and standards are given in Fig. 2. The result indicated that the extract contain compounds able to inhibit nitric oxide free radical.

Hydrogen peroxide scavenging assay: Scavenging of H_2O_2 by extracts may be attributed to their phenolics and flavonoids which can donate electrons to H_2O_2, thus neutralizing it to water[38]. The ability of the extracts to effectively scavenge hydrogen peroxide, determined according to the method of Gulcin, where they are compared with that of tocopherol as standard. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner (Fig. 1b). Petroleum ether, chloroform, methanol extracts of *Hamelia patens* stem and tocopherol were found to be scavenger of H_2O_2 with an IC_{50} of 172.54, 66.09, 93.51 and 11.58 mg mL^{-1}, respectively. Chloroform extract was found to be good scavenger of H_2O_2 than methanol extract and petroleum ether extract. Although, hydrogen peroxide itself is not very reactive

but it produces hydroxyl radicals in the cell and cause cytotoxicity. Thus, removing H_2O_2 is very important throughout food systems[39].

DPPH radical-scavenging activity: The model of scavenging the stable DPPH radical is a widely used method to perform the free radical scavenging ability of test components[40]. The DPPH is a nitrogen-centered a stable free radical with a maximum absorption at 517 nm. The DPPH can readily undergo scavenging by an antioxidant by hydrogen or electron-donation. The DPPH changes from violet to yellow upon reduction. Substances which are able to perform this reaction can be considered as antioxidants[41]. Extracts of *H. patens* significantly shows radical-scavenging activities in a concentration-dependent manner (Fig. 1c). Petroleum ether, chloroform, methanol extracts of *Hamelia patens* stems and tocopherol were found to be scavenger of DPPH radical with an IC_{50} of 250.58, 46.03, 83.44 and 16.07 mg mL^{-1}, respectively. Chloroform extract was found to be good scavenger of DPPH radical than methanol extract and petroleum ether extract.

Metal chelating activity: Ferrous ions are one of the most effective prooxidants they interact with hydrogen peroxide in biological systems can lead to formation of highly reactive hydroxyl radicals. Ferrozine is a ferroin compound and forms complex with ferrous ions. In the presence of chelating agents, complex (magenta colored) formation is interrupted and as a result, absorbance of the complex is decreased. Thus, the chelating effect of the coexisting chelator can be determined by measuring the rate of color reduction[42]. The formation of the ferrozine-ferrous complex is interrupted by the *H. patens* extract, indicating ferrous chelating activity with concentration dependent manner (Fig. 1d). Chelating agents that forms bonds with a metal are effective as secondary antioxidants because they reduce the redox potential and thereby stabilize the oxidized form of the metal ion. Petroleum ether, chloroform, methanol extracts of *Hamelia patens* stems and EDTA were found to be ferrous ion chelating activity with an IC_{50} of 294.12, 126.90, 112.36, 19.63 µg mL^{-1}, respectively. Chloroform extract was found to be good scavenger of DPPH radical than methanol extract and petroleum ether extract.

Phytochemicals have been of enormous awareness as a supply of natural antioxidants used for health support, food safeguarding, food flavoring and cosmetics as they are safer than synthetics[43].

The antioxidant activities of various extracts of *Hamelia patens* stems are in accordance with their amount of phenolic contents. At present there has been an improved interest globally to identify antioxidant compounds from plant sources which are pharmacologically potent and have small or no side effects for use in protective medicine and the food industry. Increasing acquaintance in antioxidant phytoconstituents and including them in daily uses and diet can give enough support to human body to fight those diseases. Phytochemical analysis reveals the presence of tannins, flavonoids, steroids and alkaloids. This study affirms the *in vitro* antioxidant potential of crude methanol, chloroform and petroleum ether extracts of the *Hamelia patens* stems, with results comparable to those of the standard compounds such as ascorbic acid. This assay is very useful and inexpensive way of assessing the bioactivity of plant extracts. These extracts can be regarded as a promising candidate for a plant derived antioxidant agent.

CONCLUSION

This study affirmed that *H. patens* extracts is a potential source of antioxidant and could be used in preservative in food, medicine and other non-food material as a natural antioxidant. Further phytochemical analysis is required to isolate the phytoconstitents from the *H. patens* that shows pharmacological activity related to diseases resulting from oxidative stress.

ACKNOWLEDGMENT

The authors are grateful to Principal, SMBT College of Pharmacy for providing necessary facilities and support.

REFERENCES

1. Kala, C.P., 2005. Current status of medicinal plants used by traditional *Vaidyas* in Uttaranchal state of India. Ethnobot. Res. Applied, 3: 267-278.
2. Kumbhare, M.R., T. Sivakumar, P.B. Udavant, A.S. Dhake and A.R. Surana, 2012. *In vitro* antioxidant activity, phytochemical screening, cytotoxicity and total phenolic content in extracts of *Caesalpinia pulcherrima* (Caesalpiniaceae) pods. Pak. J. Biol. Sci., 15: 325-332.
3. Smith-Hall, C., H.O. Larsen and M. Pouliot, 2012. People, plants and health: A conceptual framework for assessing changes in medicinal plant consumption. J. Ethnobiol. Ethnomed., Vol. 8. 10.1186/1746-4269-8-43.
4. Aiyegoro, O.A. and A.I. Okoh, 2010. Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. BMC Complement. Altern. Med. 10.1186/1472-6882-10-21.
5. Al-Mamun, M., K. Yamaki, T. Masumizu, Y. Nakai, K. Saito, H. Sano and Y. Tamura, 2007. Superoxide anion radical scavenging activities of herbs and pastures in northern Japan determined using electron spin resonance spectrometry. Int. J. Biol. Sci., 3: 349-355.

6. Viturro, C., A. Molina and G. Schmeda-Hirschmann, 1999. Free radical scavengers from *Mutisia friesiana* (Asteraceae) and *Sanicula graveolens* (Apiaceae). Phytother. Res., 13: 422-424.
7. Halliwell, B., 2006. Oxidative stress and neurodegeneration: Where are we now? J. Neurochem., 97: 1634-1658.
8. Kumbhare, M.R., V. Guleha and T. Sivakumar, 2012. Estimation of total phenolic content, cytotoxicity and *in-vitro* antioxidant activity of stem bark of *Moringa oleifera*. Asian Pac. J. Trop. Dis., 2: 144-150.
9. Cai, Y., M. Sun and H. Corke, 2003. Antioxidant activity of betalains from plants of the Amaranthaceae. J. Agric. Food Chem., 51: 2288-2294.
10. CSIR., 1959. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products, Volume 5. 1st Edn., Council of Scientific Industrial Research, New Delhi, India.
11. Borges, J., M.T. Manresa, J.L. Martin Ramon, C. Pascual and A. Rumbero, 1979. Two new oxindole alkaloids isolated from *Hamelia patens* Jacq. Tetrahedron Lett., 20: 3197-3200.
12. Aquino, R., M.L. Ciavatta, F. de Simone and C. Pizza, 1990. A flavanone glycoside from *Hamelia patens*. Phytochemistry, 29: 2359-2360.
13. Suarez, A., B. Diaz, S. Tillett, E. Valdivieso and R. Compagnone, 2008. Leishmanicidal activity of alkaloids from *Hamelia patens*. Ciencia, 16: 148-155.
14. Chaudhuri, P.K. and R.S. Thakur, 1991. *Hamelia patens*: A new source of ephedrine. Planta Medica, 57: 199-199.
15. Sosa, S., M.J. Balick, R. Arvigo, R.G. Esposito, C. Pizza, G. Altinier and A. Tubaro, 2002. Screening of the topical anti-inflammatory activity of some Central American plants. J. Ethnopharmacol., 8: 211-215.
16. Camporese, A., M.J. Balick, R. Arvigo, R.G. Esposito, N. Morsellino, F. de Simone and A. Tubaro, 2003. Screening of anti-bacterial activity of medicinal plants from Belize (Central America). J. Ethnopharmacol., 87: 103-107.
17. Ramos, A., A. Visozo, J. Piloto, A. Garcıa, C.A. Rodrıguez and R. Rivero, 2003. Screening of antimutagenicity via antioxidant activity in Cuban medicinal plants. J. Ethnopharmacol., 87: 241-246.
18. Mena-Rejon, G., E. Caamal-Fuentes, Z. Cantillo-Ciau, R. Cedillo-Rivera, J. Flores-Guido and R. Moo-Puc, 2009. *In vitro* cytotoxic activity of nine plants used in Mayan traditional medicine. J. Ethnopharmacol., 121: 462-465.
19. Alonso-Castro A.J., S. Balleza-Ramos, A. Hernandez-Morales, J.R. Zapata-Morales, M.M. Gonzalez-Chavez and C. Carranza-Alvarez, 2015. Toxicity and antinociceptive effects of *Hamelia patens*. Revista Brasileira Farmacognosia, 25: 170-176.
20. Gomez-Beloz, A., J.C. Rucinski, M.J. Balick and C. Tipton, 2003. Double incision wound healing bioassay using *Hamelia patens* from El Salvador. J. Ethnopharmacol., 88: 169-173.
21. Khandelwal, S., P. Sharma, T. Singh and R. Vijayvergia, 2012. Anthelmintic and antimicrobial activity of *Hamelia patens* Jacq. (Rubiaceae). Int. J. Nat. Prod. Res., 1: 54-56.
22. Abubacker, M.N., C. Sathya and R. Prabakaran, 2013. *In vitro* antifungal potentials of *Hamelia patens* Jacq. (Rubiaceae) aqueous extracts of leaves, flowers and fruits. Biosci. Biotechnol. Res. Asia, 10: 699-704.
23. Spanos, G.A. and R.E. Wrolstad, 1990. Influence of processing and storage on the phenolic composition of Thompson Seedless grape juice. J. Agric. Food Chem., 38: 1565-1571.
24. Alothman, M., R. Bhat and A.A. Karim, 2009. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chem., 115: 785-788.
25. Kumaran, A. and R.J. Karunakaran, 2007. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT-Food Sci. Technol., 40: 344-352.
26. Marcocci, L., L. Packer, M.T. Droy-Lefaix, A. Sekaki and M. Gardes-Albert, 1994. Antioxidant action of *Ginkgo biloba* extract EGb 761. Methods Enzymol., 234: 462-475.
27. Banerjee, S., A. Chanda, A. Ghoshal, R. Debnath, S. Chakraborty, R. Saha and A. Das, 2011. Nitric oxide scavenging activity study of ethanolic extracts of *Ixora coccinea* from two different areas of Kolkata. Asian J. Exp. Biol. Sci., 2: 595-599.
28. Halliwell, B., J.M.C. Gutteridge and O.I. Aruoma, 1987. The deoxyribose method: A simple test-tube assay for determination of rate constants for reactions of hydroxyl radicals. Anal. Biochem., 165: 215-219.
29. Hossain, M.A. and M.D. Shah, 2015. A study on the total phenols content and antioxidant activity of essential oil and different solvent extracts of endemic plant *Merremia borneensis*. Arab. J. Chem., 8: 66-71.
30. Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol., 28: 25-30.
31. Dinis, T.C.P., V.M.C. Madeira and L.M. Almeida, 1994. Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys., 315: 161-169.
32. Robu, S., A.C. Aprotosoaie, A. Miron, O. Cioanca, U. Stanescu and M. Hancianu, 2012. *In vitro* antioxidant activity of ethanolic extracts from some *Lavandula* species cultivated in Romania. Farmacia, 60: 394-401.
33. Chang, C.C., M.H. Yang, H.M. Wen and J.C. Chern, 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal., 10: 178-182.
34. Ialenti, A., S. Moncada and M. Di Rosa, 1993. Modulation of adjuvant arthritis by endogenous nitric oxide. Br. J. Pharmacol., 110: 701-706.

35. Ross, R., 1993. The pathogenesis of atherosclerosis: A perspective for the 1990s. Nature, 362: 801-809.
36. Awala, S.I. and V.O. Oyetayo, 2016. Assessment of free radical scavenging potentials of extracts of *Trametes lactinea* collected from Akure. Res. J. Phytochem., 10: 10-20.
37. Kelm, M.A., M.G. Nair, G.M. Strasburg and D.L. DeWitt, 2000. Antioxidant and cyclooxygenase inhibitory phenolic compounds from *Ocimum sanctum* Linn. Phytomedicine, 7: 7-13.
38. Su, W., P. Li, L. Huo, C. Wu, N. Guo and L. Liu, 2011. Phenolic content and antioxidant activity of *Phymatopteris hastata*. J. Serbian Chem. Soc., 76: 1485-1496.
39. Surana, A.R., A.N. Aher and S.C. Pal, 2013. *In vitro* and *in vivo* antioxidant activity of *Ixora coccinea*. J. Med. Plants Res., 7: 3071-3075.
40. Ebrahimzadeh, M.A., F. Pourmorad and S. Hafezi, 2008. Antioxidant activities of Iranian corn silk. Turk. J. Biol., 32: 43-49.
41. Dehpour, A.A., M.A. Ebrahimzadeh, N.S. Fazel and N.S. Mohammad, 2009. Antioxidant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition. Grasas Aceites, 60: 405-412.
42. Sudan, R., M. Bhagat, S. Gupta, J. Sing and A. Koul, 2014. Iron (FeII) chelation, ferric reducing antioxidant power and immune modulating potential of *Arisaema jacquemontii* (Himalayan Cobra Lily). BioMed. Res. Int. 10.1155/2014/179865
43. Khasawneh, M.A., H.M. Elwy, N.M. Fawzi, A.A. Hamza, A.R. Chevidenkandy and A.H. Hassan, 2011. Antioxidant activity, lipoxygenase inhibitory effect and polyphenolic compounds from *Calotropis procera* (Ait.) R. Br. Res. J. Phytochem., 5: 80-88.