Global SciTech Ocean

Current Medical and Drug Research

(Contents available at www.globalscitechocean.com)

Research article

Phytochemical, antimicrobial and antioxidant studies of the leaf extract of *Hamelia patens*

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Article history

Received : September 08, 2020
Accepted : September 22, 2020

Keywords

Hamelia patens
Phytochemical test
DPPH
Antibacterial activity

ABSTRACT

This study aimed to characterise the different extracts of the leaves of *Hamelia patens* Jacq. (Rubiaceae) and to evaluate their antibacterial and antioxidant activities in vitro. Phytochemical screening of the leaf extract revealed the presence of alkaloids, flavonoids, sterols, tannins and carotenoids. The extracts were further characterised with the help of FTIR and UV-VIS spectroscopy. The antibacterial activity was evaluated by agar well diffusion method and the zone of inhibition was measured. 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was used to evaluate the antioxidant capability of the extract. The extract was found effective in inhibiting the growth of both *E. coli* and *S. aureus* at 5 μg/mL concentration. The methanolic extract was also found to scavenge DPPH radicals. Although the extract is found effective in the present study, however, further research is required to evaluate the biological activity in higher experimental models.

INTRODUCTION

Medicinal plants are used in many modern medicines where they played a very significant role as raw material. Plants are considerably useful and economically essential. They contain numerous vital constituents that are used in the cure of many human diseases. Plants are the rich sources of ecologically developed secondary metabolites, which are likely remedies for different diseases (Audu et al., 2007). It is not surprising that the world's one-fourth population i.e. 1.42 billion-plus people are dependent on conventional or traditional medicines for the treatment of various types of ailments from ancient times. At present, there is a renewed curiosity in drugs of natural origin merely because they are contemplated as green medicine or medicines of plant origin and green medicine is without exception supposed to be safe and sound (Kadam et al., 2012). During ancient time, medicinal plants have been of age-long antidote for various human diseases because they possess components of restorative value (Adegoke et al., 2009).

Another factor which stresses this attention is the incidences of the detrimental nature of synthetic drugs which are regarded as dangerous to human beings and the surrounding environment. The major advantage of natural drugs is their easy availability, accessibility, economical and having less or nil side effects, but the drawback is that they are the victims of adulteration. The more potent the natural drug more is its demand and the chances of non-availability increases subsequently. To meet effectively the growing demand of natural drug, it is easily mixed with low-grade plant material. Adulteration is nothing but the substitution of the original plant with some another plant material unknowingly or intentionally adding any foreign substance or material to increase the weight of the product or to decrease its cost of production (Kadam et al., 2011). Proper identification and quality assurance of the raw plant or plant-based materials is an important prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and success (Tatiya et al., 2012).

MATERIAL AND METHODS

Sample collection

The fresh leaves from healthy plants of *Hamelia patens* were collected from Mahabalipuram area of

District Kanchipuram, Tamilnadu in pre-sterilized poly bags and identified from the Institute (Biswas et al., 2013).

Preparation of extracts

The collected fresh leaves were washed in running tap water twice and after that, it was surface sterilized thoroughly using distilled water. Then, the leaves were air-dried in shade at room temperature for fifteen days avoiding exposure to direct sunlight and thereafter, the dried leaves were pulverized in a mixer grinder. The fine powdered leaves were stored in an airtight container for further analysis. The leaf powder was continuously and successively extracted by Soxhlet using petroleum ether, chloroform and methanol. All extracts were dried under vacuum and performed the various chemical tests to identify the type of phytoconstituents present in it (Harborne, 1998).

Phytochemical analysis

The leaf extracts were taken separately in 5 mL of 1.5% v/v hydrochloric acid and filtered using Whatman filter paper No 1. Then these filtrates were subjected to the specific tests for phytochemicals. Alkaloids, glycosides, sterols, flavonoids, tannins, carbohydrates, proteins and carotenoids were qualitatively analysed by following the standard procedures.

Alkaloidal contents

The extract was spread on Whatman filter paper No. 1 and dried. The filter paper after spraying with ammonia was extracted with chloroform and the extract thus obtained was put into the filter paper soaked with the Dragendroff's reagent. The formation of orange-red colour indicates the presence of alkaloids.

Glycosides

Test 1

The leaf extract (5 mg) was added to 5 ml of dilute sulphuric acid followed by heating on a water bath and filtered. Then, neutralized the acidic extract with 5% NaOH solution and 0.1 mL Fehling's solution A and Fehling's solution B were added to it and boiled for 2 minutes.

Test 2

The leaf extract (5 mg) was mixed with 5 mL of water by indirectly warming on a water bath and 5% NaOH solution was added to it. Thereafter, 0.1 mL Fehling's solution 1 and Fehling's solution 2 were added to the mixture and heated for 2 minutes (Gokhale et al., 2014).

The red precipitate thus formed was compared in Test 1 and Test 2. Test 1 containing more precipitate indicates the presence of glycosides while similar appearance in Test 1 and Test 2 indicates the absence of glycosides.

Sterols

The leaf extract (2 mg) was taken in 2 mL of chloroform and 2 mL of concentrated sulphuric acid through the side of the test tube and shaken for some time. The formation of red colour in the chloroform layer indicated the presence of sterols.

Flavonoids

The leaf extract (2 mg) was dissolved in 5 mL 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 colours indicate the presence of flavonoids (Ali et al., 2018).

Tannins

A matchstick was dipped to the methanolic solution of the extract and after drying, dipped it to HCl and dry again. This stick was taken near the flame. The flame burns with magenta colour indicates the presence of tannins (Kokate, 1986).

Carbohydrates

To the leaf extract (2 mg), few drops of Molisch's reagent were added and shaken it well followed by the addition of sulphuric acid through the wall of the test tube. The appearance of the violet ring at the meeting point of two liquid indicates presences of carbohydrate (Aquino et al., 1990).

Proteins

To the 5 mg of leaf extract, 2 ml of alcohol-water was added and shaken vigorously. Thereafter, 2 mL of the Biuret reagent was added to it. The colour changed to violet indicates the presence of protein.

Carotenoids

The leaf extract was treated with antimony trichloride. The appearance of blue colour indicates the presence of carotenoids (Borges et al., 1979).

Characterization of plant extract

The characterization of the extract was carried out by UV-VIS spectroscopy and Fourier Transform Infrared Spectroscopy. The extract was analysed by the instrument SV 210 UV double beam, ELICO, India at a resolution of 1 nm. The FTIR analysis was

carried out under transmission mode (400–4000 cm^{-1}) at a resolution of 4 cm^{-1}

Antibacterial activity

The antibacterial activity of the methanolic leaf extract from *Hamelia patens* was evaluated by agar well plate method by punching holes of 2.5 mm diameter using well puncher. The agar well diffusion method was performed in Mueller Hinton Agar (MHA) plates (Rukenya et al., 2014). The test organisms were inoculated in Nutrient broth and incubated overnight at 37 °C to adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.5×10^8 CFU/ml. MHA plate was lawn cultured with standardized microbial culture broth. The leaf extracts of different concentrations i.e. 5, 10, 15 and 20 mg/mL were prepared using dimethyl sulfoxide (DMSO). The wells were bored in the inoculated media with the help of sterile cork-borer. Each well was filled with 50 µL extract. Amikacin (5 µg/mL) and DMSO were used as positive and negative controls, respectively. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 24 hours at 37 °C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of leaf extracts. The zone of inhibition was observed and measured in mm using a zone measuring scale.

Antioxidant activity

The antioxidant activity of methanolic extract of the leaves against DPPH substrate was carried out. The effect on DPPH radicals was estimated according to the procedure described by Von et al. (1997). Two mL of 6×10^{-5} M methanolic solution of DPPH was added to 50 µL of a methanolic solution (10 mg/mL) of the sample and the absorbance measurement was commenced immediately. The decreasing of absorbance at 515 nm was continuously recorded in a spectrophotometer for 16 min. The scavenging effect (decreased absorbance) was plotted against the time and the percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16 min. All determinations were performed in duplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994).

$$IP = [(AC(0) - AA(t) / AC(0)] \times 100$$

Where, AC(0) is the absorbance of the control at t = 0 min; and AA (t) is the absorbance of the antioxidants (ascorbic acid) at t = 16 min.

RESULT AND DISCUSSION

Phytochemical analysis

The leaf extract showed the presence of alkaloids, sterols, proteins, flavonoids, tannins, carbohydrates and carotenoids (Table 1). In the petroleum ether fraction, only sterols were detected.

UV-VIS spectral studies

UV-VIS spectroscopy deals with the changes in electronic energy levels inside the molecule arising attributable to the transfer of electrons from bonding or non-bonding orbital. It ordinarily provides the data concerning electron systems, conjugated unsaturation, aromatic compounds and conjugated non-bonding lepton systems. Typically, the transitions take place from HOMO (highest occupied molecular orbital) to LUMO (lowest unoccupied molecular orbital). LUMO is 's' orbital that corresponds to σ bond. The next higher orbital is 'p' orbital, that has individual try of electrons and is non-bonding in nature; type π-bonds. The unoccupied bonding orbitals (π* and σ*) are the best-occupied energy levels. The absorption and wavelength for different extracts are shown in Table 2.

Table 1. Phytochemical analysis of different leaf extracts

Phytochemicals	Extracts		
	Petroleum ether	Chloroform	Methanol
Alkaloids	-	+	+
Sterols	+	+	+
Flavonoids	-	-	+
Glycosides	-	-	-
Tannins	-	-	+
Carbohydrates	-	-	+
Proteins	-	-	+
Carotenoids	-	-	-

(+) = Presence and (-) = Absent

Table 2. Wavelength and absorption values in UV-VIS spectroscopy

Extracts	Wavelength (nm)	Absorbance
Methanol	320	0.4
Chloroform	286	0.5
Petroleum ether	322	0.8

Fourier Transform Infrared (FTIR) studies

The biomolecules present in the *Hamelia patens* leaf extract were identified by IR spectrum. Perkin Elmer Spectrum RXI FTIR instrument was operated under transmission mode (400–4000 cm^{-1}) at a resolution of 4.0 cm^{-1}. Prominent absorption peaks were obtained at 495, 843, 1253, 1525, 1630, 1725, 2250, 3400 and 3756 cm^{-1} (Fig. 1). In this case, a quantum confinement effect, size effect, dipolar interactions, interfacial effects, surface amorphousness, high internal stress, etc. play some important roles in vibrational frequencies. In this study, the broad absorption peaks at 3756 and 3400 cm^{-1} were attributed to the presence of hydrogen-bonded water molecule. The bands at 1253 and 1525 cm^{-1} correspond to γ-NO, vibrations, which may be present because of inadequate decomposition of nitrates. The band at 1630 cm^{-1} was assigned to the deformation vibration of water molecules. But, due to particle size effects, the assignment of vibrational peaks at 495 and 843 cm^{-1} could not be identified. The wave-numbers correspond to symmetric stretching and anti-symmetric stretching in some places and also symmetric bending and anti-symmetric bending in rest of the surface. The evolution of new bands and shift in IR acting mode were due to surface amorphousness.

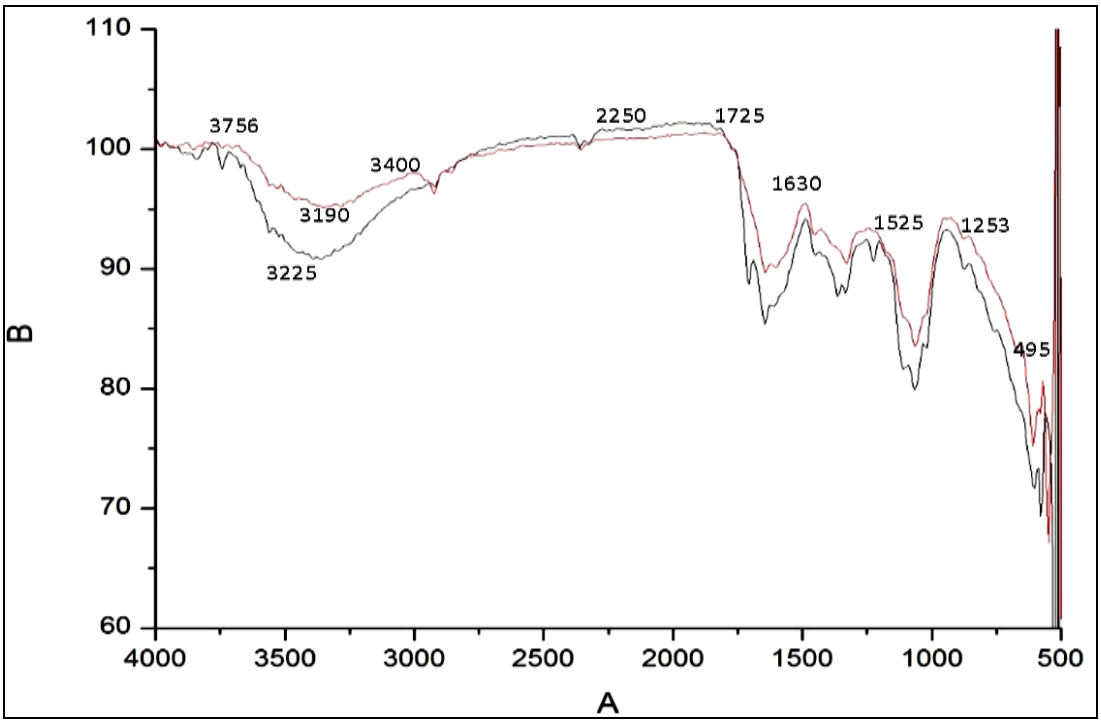

Fig. 1. FTIR spectrum of the leaf extract of *Hamelia patens*

Antibacterial activity

In the present study, the antibacterial activity of *Hamelia patens* was analysed with the methanolic leaf extract. According to the well plate assay, the methanol extract showed a minimum inhibition of 9.5 mm at 5 µg/mL and the maximum zone of inhibition by 13 mm at 20 µg/mL against *E. coli*. Similar results were obtained against *S. aureus*. Zone of inhibition was measured by HiMedia scale in mm and summarised in Table 3.

The phytochemical and antimicrobial studies of *Hamelia patens* were also conducted earlier (Murugan et al., 2012). Camporese et al. (2003) also evaluated the antibacterial activity of hexane, chloroform, and methanol leaf extracts of *H. patens* against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Enterococcus faecalis,* amongst which only hexane extract was found effective against *E. coli*. Okoye (2016) studied the antimicrobial activity of the plant leaf extracts (ethanol, methanol, petroleum ether, and aqueous extract) against *E. coli, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhiand, S. aureus* and antifungal activity against *C. albicans* and *A. niger*.

Table 3. Zone of inhibition (mm) of methanolic leaf extracts from *Hamelia patens*

Pathogen	Zone of inhibition (mm)				
	Extract (5 µg/mL)	Extract (10 µg/mL)	Extract (15 µg/mL)	Extract (20 µg/mL)	Amikacin (5 µg/mL)
E. Coli	9.5	10.4	11.6	13	10.5
S. aureus	9.2	11.5	12.0	14	12.0

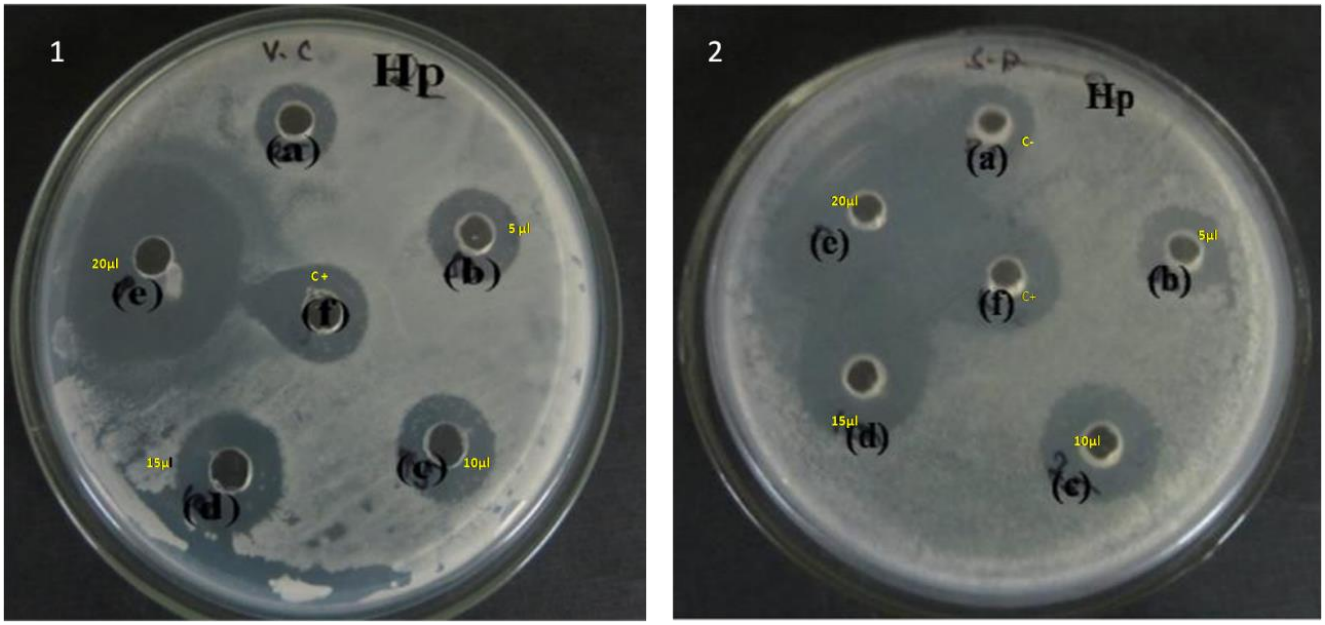

Fig. 2. Petri dishes showing the inhibitory activity of methanol extract against *S. aureus* (1) and *E. coli* (2)

Antioxidant activity

The scavenging activity of the methanolic leaf extract of *Hamelia patens* was evaluated using different concentrations (Fig. 3). When the leaf extract was subjected to DPPH activity, enhanced antioxidant activity was observed. The test sample showed a reduction in absorbance level by a noticeable colour change which was quite visible. This colour change indicated that the leaf extract does have good antioxidant activity.

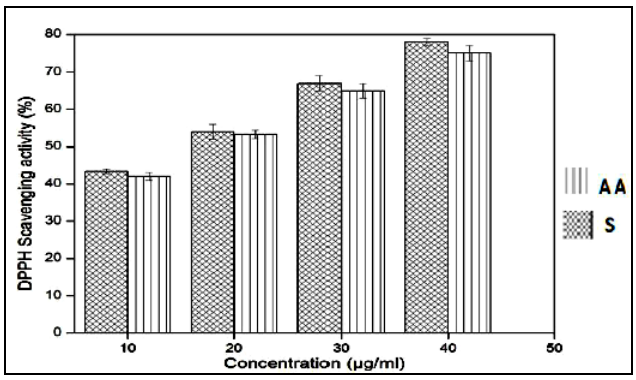

Fig. 3. Antioxidant activity of leaf extract. AA = ascorbic acid; S = sample of methanol extract

The present results suggest that the extracts are good free radical scavengers and likely have the capacity to obstruct lipid peroxidation. The methanol leaf extract was found to be a better scavenger of DPPH radicals than the chloroform and petroleum ether extracts due to the presence of phenolic contents (Ajaykumar et al., 2016). The results were compared with ascorbic acid.

The free radical chain reaction is one of the widely accepted methods as the main mechanism of lipid peroxidation (Asnaashari et al., 2016). To break the free radical chain reaction, the radical scavenging molecules could react and bring out peroxide products. DPPH radical scavenging assay is one of the most delicate and easy spectrophotometric methods for antioxidant activity of the plant extracts (Reza et al., 2019).

CONCLUSION

There is an increasing interest in the use of medicinal plants for the prevention and treatment of many diseases worldwide. The general population perceives herbal medicines as safe because of their natural origin, efficacy and their long use in traditional medicine. The present study suggested that the plant extract has potential in treating various diseases and it can be used as a candidate in drug discovery. The present findings also suggested that the extract of *Hamelia patens* can be used as an easily accessible source for natural bioactive compounds with antibacterial and antioxidant property. However, further analysis is needed to isolate the bioactive constituents from the plant and their biological activity in higher models.

ACKNOWLEDGMENT

The authors thank the management of PRIST University for providing facilities to do the project.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCE

Adegoke A, Adebayo Tayo A, Bukola C (2009). Antibacterial activity and phytochemical analysis of leaf extracts of *Lasienthera africanum*. African Journal of Biotechnology, 8, 77-80.

Ajaykumar RS, Manoj RK, Rajendra DW (2016). Estimation of total phenolic and total flavonoid content and assessment of in vitro antioxidant activity of extracts of *Hamelia patens* jacq. stems. Research Journal of Phytochemistry, 10, 67-74.

Ali S, Khan MR, Irfanullah, Sajid M, Zahra Z (2018). Phytochemical investigation and antimicrobial appraisal of Parrotiopsis jacquemontiana (Decne) Rehder. BMC Complement Alternative Medicine, 18, 43.

Aquino R, Ciavatta ML, De Simone F, Pizza C (1990). Furostanol oligosides from *Tamus communis.* Phytochemistry, 29, 2359–2360.

Asnaashari M, Farhoosh R, Farahmandfar R (2016). Prediction of oxidation parameters of purified Kilka fish oil including gallic acid and methyl gallate by adaptive neuro-fuzzy inference system (ANFIS) and artificial neural network. Journal of the Science of Food and Agriculture, 96, 4594-4602.

Audu SA, Ilyas M, Kaita MA (2007). A review on therapeutic uses of *Pedalium murex.* Life Science Journal, 4, 75-79.

Borges J, Manresa MT, Martín Ramón JL, Pascual C, Rumbero A (1979). Two new oxindole alkaloids isolated from *Hamelia patens* Jacq. Tetrahedron Letters, 20, 3197–3200.

Camporese A, Balick MJ, Arvigo R, Esposito RG, Morsellino N, De Simone F, Tubaro A (2003). Screening of anti-bacterial activity of medicinal plants from Belize (Central America). Journal of Ethnopharmacology, 87, 103-107.

Gokhale SB, Kokate CK, Purohit AP (2014). A Text book of Pharmacognosy, 50th Ed. Nirali Prakashan, Pune, pp. A22-A27.

Harborne JB (1998). Methods of extraction and isolation. In: Phytochemical methods, 3rd Ed. Chapman and Hall, London, pp. 60-66.

Kadam PV, Deoda RS, Shivatare RS, Yadav KN, Patil MJ (2012). Pharmacognostic, phytochemical and physiochemical studies of *Mimusops Elengi* Linn stem bark (Sapotaceae). Der Pharmacia Lettre, 4, 607-613.

Kadam PV, Yadav KN, Narappanawar NS, Shivatare RS, Bhusnar HU, Patil MJ, (2011). Pharmacognostic and phytochemical studies on roots of *Agave americana* (Agavaceae). Pharmacognosy Journal, 3, 19-24.

Biswas B, Kimberly R, Fredrick M, Dwayne D, Anand Y (2013). Antimicrobial activities of leaf extracts of guava (Psidium guajava L.) on two gram-negative and gram-positive bacteria. International Journal of Microbiology, 2013, 746165.

Kokate CK (1986). Practical Pharmacognosy, 1st Ed. Vallabh Prakashan, New Delhi, pp. 15-30.

Murugan M, Mohan VR, Thamodharan V (2012). Phytochemical screening and antibacterial activity of Gymnema sylvestre (Retz) R. Br ex. Schultes and Morinda pubescens J.E. Smith var. pubescens. Journal of Applied Pharmaceutical Science, 2(2), 73-76.

Okoye EL (2016). Antimicrobial activity of the crude extracts of *Hamelia patens* on some selected clinical samples. Journal of Complementary and Alternative Medical Research. Online available at SSRN: https://ssrn.com/abstract=3448907.

Reza F, Reza EK, Maryam A, Dina S, Tahmineh B (2019). Bioactive compounds, antioxidant and antimicrobial activities of *Arum maculatum* leaves extracts as affected by various solvents and extraction methods. Food Science and Nutrition, 7, 465–475.

Rukenya ZM, Mbaria JM, Mbaabu MP, Kiama SG, Okindo RO (2014). Antimicrobial activity of aqueous and methanol extract of naturally growing and cultivated *Aloe turkanensis*. The Journal of Phytopharmacology, 3, 343-347.

Tatiya A, Surana S, Bhavsar S, Patil D, Patil D (2012). Pharmacognostic study and development of quality parameters of *Hamelia patens* Jacq. stems. Asian Pacific Journal of Tropical Disease, 2, 50-55.

Von GA, Joubert E, Hansmann CF (1997). Comparison of the antioxidant activity of aspalathin with that of other plant phenols of roibos tea (*Aspalathus linearis*), x-tocopherol, BHT, and BHA. Journal of Agriculture and Food Chemistry, 45, 632-638.

Yen GC, Pin DD (1994). Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen species. Journal of Agriculture and Food Chemistry, 42, 629–632.

How to cite this article?

Megala S, Venkat Kumar G, Shalini RV, Manjamadha VP (2020). Phytochemical, antimicrobial and antioxidant studies of the leaf extract of Hamelia patens. Current Medical and Drug Research, 4 (2), Article ID 206.

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