ejpmr, 2020,7(2), 452-458

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

PRELIMINARY PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF *HYGROPHILA SCHULLI*

Manisha U. Mishra[1]*, Nitin H. Indurwade[2], Shubham R. Ukey[3], Rahul Chaurasia[4], Sanket S. Murmurwar[5]

[1]Associate Professor, Manoharbhai Patel Institute of B.Pharmacy Gondia, Maharashtra, India.
[2]Professor and Principal, Manoharbhai Patel Institute of B.Pharmacy Gondia, Maharashtra, India.
[3]Student, Final Year B.Pharm, Manoharbhai Patel Institute of B.Pharmacy Gondia, Maharashtra, India.
[4]Lecturer, Manoharbhai Patel Institute of Diploma Pharmacy Gondia, Maharashtra, India.
[5]Student, Final Year B.Pharm, Manoharbhai Patel Institute of B.Pharmacy Gondia, Maharashtra, India.

***Corresponding Author: Manisha U. Mishra**

Associate Professor, Manoharbhai Patel Institute of B.Pharmacy Gondia, Maharashtra, India.

Article Received on 14/12/2019 **Article Revised on 04/01/2020** **Article Accepted on 25/01/2020**

ABSTRACT

Hygrophila schulli is an important plant described in Indian Ayurvedic literature. The main objective of the present study was to carry out preliminary phytochemical screening and evaluation of antibacterial activity of *H.schulli* collected from local areas of Gondia city, Maharashtra State, India. The plant material was identified and fresh leaves were collected. The dried leaf powder was extracted by hydro-alcoholic solvent extraction method using Soxhlet apparatus. The extract was qualitatively analyzed for presence of active phytochemicals prior to study of the antibacterial activity and minimum inhibitory concentration. The extracts containing different concentrations of *H.schulli* were investigated against clinically effective bacterial pathogens such as *Staphylococcus aureus, Bacillus subtilis and Escheria coli*. Streptomycin powder was used as a standard drug for analyzing antibacterial activity. The highest inhibition zone of 20 mm was observed against *Bacillus subtilis* with plant extract in the concentration of 100µg/ml. The results revealed that the hydroalcoholic extract of *H.schulli* possesses antibacterial potential as reported in earlier investigations and have the potential in the treatment of bacterial infections. Further the studies were extended to formulate a cream containing hydroalcoholic extract of *H.schulli* for topical application and its evaluation. It was found that the hydroalcoholic extract of *H.schulli* can be formulated as cream and used for topical application.

KEYWORD: *Hygrophila schulli,* Hydroalcoholic extraction, Leaf extract, Antibacterial activity, Minimum inhibitory concentration, Cream formulation.

INTRODUCTION

Traditional medicine comprises medical aspects of traditional knowledge that developed over generations within various societies before the era of modern medicine. Traditional medicine is described as "the sum total of the knowledge, skills, and practice based on the theories, beliefs, and experiences indigenous or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness".[1]

Hygrophila schulli (Family Acanthaceae) commonly called as Gokula in Hindi, is a sub shrubs, stigose with sharp thorns. It grows widely throughout India, Sri Lanka, Myanmar, Indochina, Malaya. Leaves in whorls of 8, unequal, 6-10 x 2-4 cm, linear-lanceolate, apex acute, base connate, margins dentate and wavy, larger pair, sessile. Thorns 2-3 cm long, auxiliary. Flowers in axillary whorls; bracts and bracteoles leafy, 1.2 cm long; calyx lobes 4, larger lobe 1 cm long; corolla pink, 1.5 cm long, bilipped, lobes obtuse, ciliate. Capsule 1 cm long; seeds orbicular and 3 mm across.[2]

In Indian Ayurvedic system of medicine it is described as Ikshura, Ikshugandha and Kokilaksha. It is used for the treatment of various conditions such as Premeham (Diabetes) and athisaram (dysentery). Leaves are used traditionally in/as diuretics, jaundice, antibacterial, dropsy, rheumatism, anasaraca, diseases of urino-genital tract, sweet, sour, bitter tonic, aphrodisiac, hypnotic, diarrhea, dysentery, anti-inflammatory, eye diseases, anemia, anuria, gleets, cough, demulscent, stomachic, lumbago, arthritis, gastric disorder and leucorrhea.[3]

The main objective of the present study was to carry out preliminary phytochemical screening and evaluation of antibacterial activity of *H.schulli* collected from local areas of Gondia city, Maharashtra State, India and formulate and evaluated the extract as cream for topical application.

MATERIALS AND METHODS

The solvent system used for extraction process was of analytical grade and procured from S.D.Fine chemicals Mumbai. Streptomycin powder was purchased from medical stores. Nutrient agar and nutrient broth was purchased from Hi Media Laboratories Mumbai India.

Collection of Plant Material

The plant material of *H.schulli* were collected from clean local areas of Gondia city Maharashtra India and identified with the help of authentic herbarium species at Department of Botany, D.B.Science College Gondia and the voucher specimens of the samples were deposited. The collected plant material was washed leaves were plucked and dried under shade. The dried leaves were pulverized to coarse powder. The powder was then stored in airtight bottles for further use.

PREPARATION OF EXTRACT

The 350g coarsely dried powder of the leaves of *Hygrophilia schulli* was extracted by reflux extraction method in Soxhlet apparatus with 500ml of ethanol - water mixture in a ratio of 80:20 at 45-50°C for 5h. At the end, the extract was filtered with Whatman filter paper and concentrated by evaporation on water bath to form semisolid greenish gummy crude extract. The crude extract was further fractionated with petroleum ether (100 ml: 100 ml) for 4-5hr to get highly concentrated extract. The extract was then transferred in porcelain dish and evaporated at 50°C to give its concentrated form.[4]

PRELIMINARY PHYTOCHEMICAL SCREENING OF EXTRACT[5]

Test of sterol

a) Salkowaski reaction

Few mg of the residue of extract was taken in 2 ml of chloroform and 2 ml of concentrated sulphuric acid was added from the side of the test tube. The test tube was shaken for few minutes. The development of red color in the chloroform layer indicates the presence of sterols.

b) Liebermann's test

To a few mg of the residue in a test tube, few ml of acetic anhydride was added and gently heated. The contents of test tube were cooled. Few drops of concentrated sulphuric acid were added from the side of the test tube. Formation of blue color gives the evidence of presence of sterols.

c) Liebermann-Burchard reaction

Few mg of residue was dissolve in chloroform and few ml of acetic anhydride were added to it, followed by concentrated sulphuric acid from the side of tube. A transient color development from red to blue and finally green indicates the presence of sterols.

Test for alkaloids

Few mg of residue of extracts was taken separately in 5 ml 1.5 % v/v hydrochloric acid and filtered. These filtrates were then used for testing alkaloids.

a) Dragendorff's reagent

The Dragendorff's reagent was sprayed on Whatman filter paper no. 41 and the paper was dried. The test filtrate after basification with dilute ammonia was extracted with chloroform and the chloroform extract was applied on the filter paper, impregnated with Dragendorff's reagent, an orange red colour on the paper indicates the presence of alkaloids. An orange red color on the paper indicates the presence of alkaloids.

b) Mayer's reagent (Potassium mercuric iodide reagent)

The Mayer's reagent was prepared as follows:

1.36gm of mercuric chloride was dissolved in 60 ml distilled water. Both the solution were mixed and diluted to 100 ml with distilled water. To a little of the test filtrate, taken in a watch glass, a few drops of the above reagent were added. Formation of cream colored precipitate indicate presence of alkaloids.

c) Mayer's reagent (Iodine-potassium iodide)

1.27gm of iodine and 2gm of potassium iodide were dissolved in 5 ml of water and the solution was diluted to 100 ml of water. When few drops of these reagents were added to test filtrate, formation of brown precipitate indicates the presence of alkaloids.

d) Wagner reagent

A saturated aqueous solution of picric acid was employed for this test. The test filtrate was treated with this reagent. An orange yellow precipitate indicates the presence of alkaloids.

Test for saponins

a) Foam test

A few mg of test residue was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. If a stable, characteristics honeycomb like froth is obtained then the saponins are present.

b) Haemolysis test

A little of test residue was dissolved in normal saline in such a way that 5 ml of the solution represented 1gm of the crude drug. In a series of 5 test tubes, doses of 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 ml were added and volume was made up to 1 ml in each case with normal saline. 1 ml of dilute blood (0.5 ml of rabbit's blood diluted to 25 ml with normal saline) was added to each tube and changes observed. If haemolysis of blood occurs, the saponins are present.

Test For Glycoside

a) Borntrager's Test

The extract was boiled with dilute sulphuric acid, filtered and to the filtrate toluene or ether was added and shaken well. The organic layer was separated to which ammonia was added slowly. The ammoniacal layer shows pink to red color due to presence of anthraquinone glycoside.

b) Modified Borntrager's Test
A little extract is shaken with 10 ml of ferric chloride solution mixed with 5 ml hydrochloric acid and heated on water bath for 10 minute, after filtration and cooling, the filtrate is extracted with 10 ml carbon tetrachloride. The organic layer is separated, washed with 5 ml of dilute ammonia, development of rose-pink to cherry-red color indicate the presence of anthraquinone glycosides.

c) Keller-killiani test
The test consists of boiling about 1gm of extract with 10 ml 70% alcohol for 2-3 min. The extract was filtered. To the filtrate was added, 5 ml water and 0.5 ml strong solution of lead acetate were, shaken well and filtered. The clear filtrate was treated with equal volume of chloroform and evaporated to yield the extractive. The extractive was dissolved in glacial acetic acid and after cooling, 2 drop of ferric chloride solution was added to it. These contents were transferred to a test tube containing 2 ml concentrated Sulphuric acid. A reddish brown layer acquiring bluish-green color after standing indicate the presence of cardiac glycosides.

d) Legal test
The extract is dissolved in pyridine, sodium nitroprusside solution was added to it and made alkaline, pink or red color shows presence of cardiac glycoside.

Test for tannins
The test residue of extract was taken separately in water, warmed and filtered. Test was carried out with the filtrate using following reagents.

a) Ferric chloride reagent: A 5 % w/v solution few drops of conc. hydrochloric acid of ferric chloride in 90 % alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. If dark green or deep blue color is obtained, indicate the presence of tannins.

b) Lead acetate test
10 % w/v solution of basic lead acetate in distilled water was added to the test filtrate. If precipitate is obtained, indicates the presence of tannins.

c) Potassium dichromate test
If on addition of a solution of potassium dichromate to test filtrate, dark color is developed, tannins are present.

d) Bromine water test
Bromine solution was added to the test filtrate. If decolorization of bromine water occurs tannins are present.

Test for flavonoids (Shinoda test)
A small quantity of test residue was dissolved in 5 ml ethanol (95 % v/v) and treated with few drops of concentrated hydrochloric acid and 0.5 gm of magnesium metal. The pink, crimson or magenta red color is developed within a minute or two if flavonoids are present.

Test for sugars
a) Molisch's test
The Molisch's reagent was prepared by dissolving 10gm of α- naphthol in 100 ml of 95 % alcohol.

A few mg of test residue was taken in test tube containing 0.5 ml of water, and it was mixed with 2 drops of Molisch's reagent. To this solution, was added one ml of concentrated Sulphuric acid from the side of the inclined test tube, so that acid formed a layer beneath the aqueous solution without mixing with it. If a red brown ring appears at the common surface liquid, sugars are present.

b) Barford's test
This reagent was prepared by dissolving 13.3 gm of crystalline neutral copper acetate in 200 ml of 1 % acetic acid solution.

The test residue dissolved in water, heated with little of the reagent. If a red precipitate of cuprous oxide is formed within 2 minutes monosaccharides are present.

c) Fehling's solution test
The two solutions were mixed in equal volume immediately before use. A little of the test residue was dissolved in water and a few ml of a Fehling's solution was added to it. This mixture was then warmed. If a red precipitate of cuprous oxide is obtained, reducing sugars are present.

TEST FOR PROTEINS
a) Biuret test
A few mg of the residue was taken in water and 1 ml of 4 % solution of copper sulfate was added to it. Violet or pink color is formed if proteins are present.

b) Xanthoproteic test
A little residue was taken with 2 ml of water and 0.5 ml concentrated nitric acid was added to it. Yellow color is obtained if proteins are present.

Test for amino acid
a) Ninhydrin test
The ninhydrin reagent is 0.1 % w/v solution of ninhydrin in n-butanol. A little of this reagent was added to the test extract. A violet or purple color is developed if amino acid is present.

Antibacterial activity
The antibacterial activity testing was carried out by disc diffusion method. The plant extract was tested against the selected bacterial strains. The selected bacterial cultures were mixed with Mueller Hinton agar and evenly spread in Petri plates. The sterile discs (5mm in diameter) were impregnated with 0.1 ml of (1 mg/ml) extract solution and placed in the inoculated agar. The

plates were thus incubated at 32-37^0 c for 48 hours. After incubation the plates were observed for zones of inhibition. The inhibition zones were measured with the help of scale in mm. One plate was prepared as control using Streptomycin having 0.1 ml of (1 mg/ml) of pure solvent Dimethylsulfoxide (DMSO).[6,7]

MINIMUM INHIBITORY CONCENTRATION (MIC)

MIC by turbidity method

Prepare nutrient broth test tubes and label. In first tube (UT), inoculums is not added which is used for checking the sterility of medium and as a negative control. Other all test tubes, inoculums (three to four drops) is added to reach the final concentration of microorganism is 10^6 cell/ml. In all test tubes, test antimicrobial compound is added about 0.1 to 1.0 ml except un-inoculated (negative control) and control (positive) tube. The positive control tube is used to check the suitability of the medium for growth of the test microorganism and the viability of the inoculums. Adjust the final volume (10 ml) in all test tubes by using sterile water. All test tubes are properly shaken and then incubated at 37^{0}c for two days.[8]

PREPARATION OF CREAM

The antimicrobial o/w emulsion based cream was prepared containing varying quantities of *H.schulli* extracts by melting together stearic acid, stearyl alcohol and bees wax on water bath at 75°C. Potassium hydroxide, methy paraben and propyl paraben were added to purified water and heated to 75°C. The molten phase was then added to aqueous phase by stirring with glass rod until congealed. The extract was added and mixed thoroughly.[9,10]

Table No.1: Composition of optimized formulations using O/W emulsion type cream.

Ingredients	Formulations					
	F1	F2	F3	F4	F5	STD
Stearic acid	2.6	2.6	2.6	2.6	2.6	2.6
Strearyl alcohol	0.2	0.2	0.2	0.2	0.2	0.2
Bees wax	0.2	0.2	0.2	0.2	0.2	0.2
Potassium hydroxide	0.18	0.18	0.18	0.18	0.18	0.18
H.schulli extract	10	12	14	16	18	--
Streptomycin	--	--	--	--	--	0.5
Methyl paraben	0.025	0.025	0.025	0.025	0.025	0.025
Propyl paraben	0.015	0.015	0.015	0.015	0.015	0.015
Purified water	q.s	q.s	q.s	q.s	q.s	q.s

EVALUATION OF FORMULATED ANTIBACTERIAL CREAM[11]

The formulated creams were evaluated for appearance, pH, viscosity, spreadability, stability testing, antimicrobial activity and skin irritation study using Guinea pigs.

Organoleptic properties

The appearance of the cream was judged by its colour, pearlness and roughness.

Homogeneity

The formulations were tested for the homogeneity by visual appearance and by touch.

pH and viscosity

The pH of each formulated cream was measured using a previously calibrated digital pH meter (Elico, Model no. LI-610). Accurately weighed 5 g of the sample was dispersed in 45 ml. of water. The pH of the suspension was determined at27°C using digital pH meter. Viscosity was determined using Brookfield digital viscometer (Model no LVDV- I - Prime) with spindle no 62.The sample temperature was controlled at 25±1°C before each measurement. The determinations were carried out in triplicate and the average of three readings were recorded.

Spreadability

Spreadability of formulations was determined with a modified apparatus consisting of wooden block provided with two glass slides. Lower slide was fixed on wooden block and upper slide with one end tied to weight pan. Anointment equivalent to 2.5 g was placed between two slides and 1000 g weight was placed over it for5 minutes to press the sample to a uniform thickness. Weight of 80 g was added to pan. The time (in seconds) required to separate the two slides was taken as a measure of spreadability. Shorter time interval to cover the distance of 7.5cm indicates better spreadability.

The spreadability (S) can be calculated using the formula,

$s=m\times L\div T$

Where, S – Spreadability, m- Weight tied to upper glass slide, L- Length moved on a glass slide, T- Time taken. The determinations were carried out in triplicate and the average of three readings were recorded.

Skin irritation study

Guinea pigs (400-500) g of either sex were used for skin irritation study. The animals were kept under standard condition, maintained on standard animal feed and had free access to water. Hair was shaved from back of guinea pigs and area of 4 cm^2 was marked on both the sides. One side served as a control and other served as test. Cream was applied (0.5g/animal) twice a day for 7

days. The site was observed for any sensitivity and reaction if any, was graded 0,1,2,3 for no reaction, slight patchy erythema, slight but confluent or moderate but patchy erythema and severe erythema with or without edema, respectively

RESULTS AND DISCUSSION

PRELIMINARY PHYTOCHEMICAL SCREENING OF *H. SCHULLI*

The various phyto-chemicals present in the extract and the observation and results for preliminary phyto-chemical screening of *H. Schulli* are given in Table No.2.

Table No. 2: Preliminary phytochemical screening of *H. schulli.*

Sr. No.	Plant Constituents	Test/Reagent	Ethanol Extract
1	Sterols	Salkowaski	+
		Liebermann's	+
		Libermann-Burchard	+
2	Alkaloids	Dragendorff's	+
		Hager's	+
		Mayer's	+
		Wagner's	+
3	Saponins	Foam test	-
4	Glycoside	Keller-killiani test	+
		Legal test	+
		Kedde's test	+
		Bortrager's test	-
		Modified Borntrager's Test	+
5	Tannins	Ferric Chloride	+
		Lead acetate	+
		Pot. Dichromate	+
6	Flavonoids	Shinoda test	-
7	Carbohydrates	Molisch	+
		Fehlings	+
		Barfoed's	+
8	Amino acid	Ninhydrine	+
9	Protein	Millon's	+

Present = (+) Absent = (-)

Preliminary phytochemical screening indicated the presence of Sterols, Alkaloids, Glycoside, Tannins, Carbohydrates, Amino acids and Proteins.

ANTIBACTERIAL ACTIVITY

Observation and result of antibacterial activity are given in Table 3.

Table No. 3: Antibacterial result of *H. Schulli*.

Sr. No.	Test Culture	Positive control 0.1 ml for (1 mg/ml)	Plant extract 0.1 ml for (1mg/ml)	Zone of inhibition in (mm)		
				DMSO Solvent	Positive Control (mm)	Plant extract
1	*S. aureus*	*Streptomycin*	Ethanol extract	-	24	20
2	*E. coli*	*Streptomycin*	Ethanol extract	-	23	20
3	*B. subtilis*	*Streptomycin*	Ethanol extract	-	25	22

The ethanolic extract showed good antibacterial activity against *Bacillus subtillus* with 2.5 and 2.0mm for positive control and extract repectively and mild to moderate antibacterial activity against *Staphylococcus aureus and Escherichia coli* with 2.4 cm and 2.3cm for positive control and plant extract respectively.

Table No. 4: Minimum Inhibitory Concentration Of *H.Schulli.*

Sr.no.	Microorganism		Concentration (µg/ml)										MIC (µg/ml)
			50	100	150	200	250	300	350	400	450	500	
1	*H.schulli*	*S.aureus*	+	-	-	-	-	-	-	-	-	-	100
		E.coli	++	+	-	-	-	-	-	-	-	-	150
		B. subtilis	+	+	-	-	-	-	-	-	-	-	150

Turbidity: Present = (+) Absent = (-)

Table No. 5: Observation TLC Of *H.Schulli.*

Sr.No	Solvent system	UV Light	No.of component	*H.schulli* Rf value
1	Methanol :Water(8:1)	366 nm	1	0.79
2	Ethyl alcohol:Acetic acid(5:5)	366nm	1	0.93
3	Ethyl acetate :Chloroform(9:1)	366 nm	1	0.08

EVALUATION OF CREAM CONTAINING EXTRACT FOR ANTIBACTERIAL ACTIVITY OF *H. Schulli*

Table No. 6: Color and Appearance.

Test	Result
Colour	White
Appearance	Semisolid cream

Table No. 7: Homogeneity.

All developed cream were tested for homogeneity by visual inspection after the cream is have been set in the container**.**

Sr.No.	Batch	Result
1	F1	Homogeneous
2	F2	Homogeneous
3	F3	Homogeneous
4	F4	Homogeneous
5	F5	Homogeneous

Table No. 8: pH of the formulation.

The pH of cream was determined by digital pH meter 1gm of cream was dissolved in 50 ml distilled water and the pH was measured**.**

Sr. No.	Batch	pH
1	F1	**6.51**
2	F2	**6.49**
3	F3	**6.34**
4	F4	**6.44**
5	F5	**6.52**

Table No. 09: Spreadability of formulated creams.

Sr.No.	Batch	Spreadability
1	F1	**Easily spread**
2	F2	**Easily spread**
3	F3	**Easily spread**
4	F4	**Easily spread**
5	F5	**Easily spread**

Table No. 10: Viscosity of formulated creams.

Batch No	Viscocity (cps)				
	10rpm	20rpm	30rpm	40rpm	50rpm
F1	**1520**	**6035**	**7050**	**9596**	**16010**
F2	**1590**	**6120**	**7100**	**10200**	**16295**
F3	**1640**	**6500**	**8600**	**12920**	**17100**
F4	**1785**	**8300**	**10400**	**14200**	**17600**
F5	**2000**	**9500**	**12300**	**15600**	**18400**

Table No.11: Antibacterial activity of formulated creams.

Sr. No	Batch	Test culture	Positive control (1mg/ml)	Zone of inhibition (mm)	
				Positive control	Formulated cream
1	**F3**	*S.aureus*	**Streptomycin**	**25**	**24**
		E.coli	**Streptomycin**	**24**	**26**
		B. subtilis	**Streptomycin**	**24**	**25**
2	**F4**	*S.aureus*	**Streptomycin**	**23**	**25**
		E.coli	**Streptomycin**	**24**	**24**
		B. subtilis	**Streptomycin**	**26**	**24**

CONCLUSION

H.schulli are potential source of many chemical constituents and widely used for many health problems. This plants also provide pharmacological properties that have been reported previously. The evaluation of antimicrobial activities of both plants would plays a significant role for the findings of their more chemical entities and their bioactivities. From these results the antimicrobial activity of *H.schulli* leaves extracts was shown effective and efficient result compare with standard against bacterial pathogens used.

Topical formulation include oils, ointments, paste and cream out of which cream are getting more popular now a days because they are most stable and also can provide control release than any other semi-solid preparation. The vanishing cream provide better absorption characteristics and hence the bioavailability of the drug.

Among the two formulations prepared, on the basis of pharmaceutical parameter cream formulation loaded with herbal extract give the considerable results. Hence these herbal cream formulation was utilized for further pharmaceuticals studies.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Nitin H. Indurwade Principal, Manoharbhai Patel Institute of Pharmacy (B.Pharm), Gondia for providing the facilities for completion of this research work and Dr. Mahesh V. Kawale, Department of Botany, D.B. Science College, Gondia for providing necessary bacterial cultures that have been very helpful for conducting antibacterial activity.

REFERENCES

1. Purohit AP, Kokate CK, Gokhale SB. *Pharmacognosy*. 51st ed. Nirali Prakashan; 2015.
2. Chandran RP, Manju S, Vysakhi M V. In vitro Antimicrobial Activities of Hygrophila schulli (Buch . -Ham) Leaf and Root Extracts Against Clinically Important Human Pathogens. 2013; 6(2): 421-428.
3. Rangari V. *Pharmacognosy and Phytochemistry*. 2nd ed. Nashik: Career Publications; 2008.
4. Sufian A, Haque MR. Cytotoxic , thrombolytic , membrane stabilizing and anti-oxidant activities of Hygrophila schulli. 2015: 692-696. doi:10.3329/bjp.v10i3.23718.
5. Khandelwal, K.R and Sethi V. *Practical Pharmacognosy Technique and Experiments*. 26th ed. Pune: Nirali Prakashan; 2016.
6. Kokare C. *Pharmaceutical Microbiology*. 10th ed. Pune: Nirali Prakashan
7. Length F. Evaluation of antibacterial activities of Barleria Prionitis. 2015; 9(30): 1840-1848. doi:10.5897/AJMR2015.7545.
8. Pa R, Mathew L. Antimicrobial activity of leaf extracts of Justicia adhatoda L . in comparison with vasicine. *Asian Pac J Trop Biomed*., 2012; 2(3): S1556-S1560. doi:10.1016/S2221-1691(12)60452-3
9. Mehta R. *Pharmaceutics-II*. 4th ed. Vallabh Prakashan; 2015.
10. Lachman, L, Kanig, J.L and Lieberman H. *Theory and Practice of Industrial Pharmacy*. 3rd ed. Mumbai: Varghese Publishing House; 2008.