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Exploration of pharmacognostic, phytochemical and antibacterial potential of *Rhoeo discolor* Hance

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Abstract
The boat lily herb *Roheo discolor* (L'Her) Hance. belonging to family- Commelinaceae. The present research work, pharmacognostic studies of this medicinal moses-in-the-cradle was attempted which included macroscopic, microscopic, physicochemical, phytochemical, studies and antibacterial potential against some bacterial strains. The physicochemical analyses were done by using WHO recommended parameters such as loss on drying, ash values (total ash, water soluble ash, acid insoluble ash, and sulphated ash) and extractive values. The qualitative phytochemical analysis revealed the presence of alkaloids, glycosides, flavonoid and tannins in maximum amount in crude as well as in various solvent extracts. The microscopic study showed the presence of various characteristics of whole plant like cuticle, epidermal adaxial green in colour, chlorenchyma, and epidermal abaxial purple in colour, rosette calcium oxalate crystals and tetracytic stomata. The antibacterial activity was carried out against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, by disc diffusion method. Among the solvent extract ethanol extract exhibited less than 16 mm zone of inhibition on bacterial strains, which was interpreted by criteria of resistance. The pharmacognostical characters enlisted in this study will help in identification of the crude drug; the standardization parameters laid down will ensure the efficacy of drug and also distinguish the drug from its adulterants. The distinguishing characters will also be helpful for the preparation of monograph of this plant.

Keywords: *Roheo discolor*, pharmacognostical study, phytochemical study, antibacterial study

1. Introduction
Herbal medicines are safe, inexpensive and have no adverse effects. They are effectively being used to treat many diseases but lack of acceptability still persists because of lack of documentation and stringent quality control. They are also prone to adulteration and substitution which puts a doubt on their efficacy. Therefore it is of almost importance to lay down proper quality control measures of herbal drugs. There are many modern methods but still most simple, reliable and easy method is pharmacognostical study. Correct identification and quality assurance of the staring material will help to maintain the reproducible quality of herbal drugs and contribute to its safety and efficacy [1]. Standardization is a system to ensure that every medicine has the correct amount of constituents and will induce its therapeutic effect [2]. Determination of ash residues and active components like saponin, alkaloids play a significant role in the standardization of the indigenous crude drugs [3]. The extract obtained after standardization may be used as a medicinal agent as such in the form of tinctures or fluid extracts. These medicinal agents contain complex mixtures of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids [4].
Phytochemicals are nonnutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself, but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals in fruits and herbs and each works differently [5]. Many plant extracts have been shown to inhibit the growth of microorganisms. These extracts consist of chemicals and are usually considered to play a role in defence reactions of plants against infections by pathogenic microorganisms [6].
Rhoeo discolor [syn. *Tradescantia spathacea* Swartz, *Rhoeo spathacea* (Swartz) Stearn] is a plant used in traditional medicine in Mexico. *R. discolor* belongs to the family Commelinaceae and is native to the Caribbean and Central America. In the Mexican southeast, it is known as "purple maguey". Its use as medicinal plant goes back to the1930s [7], and its anti-cancer activity has been reported since 1963 [8] and as recently as 2016. Furthermore, this plant contains compounds with antioxidant [9] and antimicrobial activity [10].

The leaves have been used in regional native cultures, consumed mostly in infusions or in direct skin contact, to treat allergic rhinitis, superficial mycosis, ulcers, as a broad-spectrum anti-inflammatory and dermatological agent, and also as a treatment for cancer [9]. These properties have been attributed to the content of bioactive molecules such as anthocyanins. The effectiveness of traditional treatments with *R. discolor* has been shared among communities, but it has never been subjected to a systematic scientific scrutiny. The leaves have been used in regional native cultures, consumed mostly in infusions or in direct skin contact, to treat allergic rhinitis, superficial mycosis, ulcers, as a broad-spectrum anti-inflammatory and dermatological agent, and also as a treatment for cancer. These properties have been attributed to the content of bioactive molecules such as anthocyanins [11]. The effectiveness of traditional treatments with *R. discolor* has been shared among communities, but it has never been subjected to a systematic scientific scrutiny; although it has proven to be antigenotoxic, antimutagenic and antioxidant, further scientific information about this plant is necessary to ensure side effects do not overcome benefits [12]. Even though the drug has many uses, it's pharmacological and Phytochemistry is very poorly explored. Hence the current exploration have been accomplished to analyze the morphological, microscopically, physicochemical and phytochemical analysis of *R. discolor* together with the purpose of contributing to the establishment of monograph.

2. Material and Methods

2.1 Collection and authentication of plant material

The leaves of *Roheo discolor* was collected from college botanical garden, Eklahare, district Nashik of Maharashtra, India within the month of June 2019 and authenticated by Dr. Shimpi, Taxonomist, head of botany department, G.E. Society's NSC Science college, Nashik Road, Nashik, India. Voucher specimen No. 156 was placed at the herbarium for future reference. One part of the leaves is conserved in Formalin (5ml): Acetic acid (5ml): 70% Alcohol (90ml) blend pertaining to histological research as well as the remaining part was shade dried, powdered and then sieved by using 20 mesh and as well, retained within an air tight container for long term use.

2.2 Macroscopic Study

The macroscopic studies were carried out using organoleptic evaluation method. The arrangement, size, shape, base, texture, margin, apex, venation pattern, colour, odour, taste of leaves were observed [13]. Macroscopic and microscopic characters were studied as described in quality control method. Photographs at different magnifications were taken by using digital camera.

2.3 Microscopic study

Microscopic was carried out by preparing thin sections leaf. The thin sections were further washed with water, stained with safranin, haematoxylin, picric acid, dil. Iodine solution and mounted in glycerine for observation and confirm its lignifications (10x, 40x) [14].

2.4 Powder Microscopy

The powder microscopy of the whole plant powder was studied using standard procedure by capturing the images of different fragments of tissues and diagnostic characteristic features were recorded [15].

2.5 Physicochemical analysis

The physicochemical parameters like loss on drying, total ash, acid-insoluble ash, water-soluble ash, sulphated ash and extractive values were determined as per WHO guidelines [13]. The solvents used were ethanol (EtoH) and water (AQ). The details of the procedure followed are as described earlier.

2.6 Preparation of extracts and preliminary phytochemical analysis

The powder material has been extracted with ethanol and water. 100g root powder was extracted with 500ml of the particular solvent by maceration at room temperature for 24 hours. After that, strained by using Whatman filter paper and then obtain the filtrate, concentrated with the roto-evaporator. Then, the extract was confronted with preliminary phytochemical screening as mentioned in standard methods. The presence of various phytoconstituents *viz*. steroids and terpenoids (Liebermann Burchard test), alkaloids (Dragendorff's test), tannins and phenolics (Ferric chloride test), flavonoids (Shinoda test), Sugars (Fehling solution test), amino acids (Ninhydrin test), etc. was detected by usual methods prescribed in standard texts [16].

2.7 Thin layer chromatography

For the TLC fingerprint the alcoholic extract and aqueous extract of herb was subjected to thin layer chromatographic analysis, to find the presence of number of chemical constituents to support the chemical test. Analytical TLC plates were prepared by pouring the silica gel G slurry on the glass plates. Drying the thin layer plates, for 30 minutes in air and then in an oven at 110 °C for another 30 minutes. For qualitative work, spot was applied in a row along one side of plate, about 2cm from edge, by using capillary tubes. The range of sample volume was controlled, spreading not more than 0.5 cm. The plate was placed in previously saturated TLC chamber with mobile phase. The chromatographic conditions were described in table 1. The Rf values are compared with standard drug and colours are recorded [17].

2.8 Antibacterial Assay

2.8.1 Test Microorganisms

The test organisms used in this experiment includes two Gram positive bacteria strains (*Staphylococcus aureus*, *Bacillus subtilis)* and one gram negative bacterial strains namely (*Escherichia coli*). The cultures were obtained from National Collection of Industrial Microorganism (NCIM) Pune, India. The cultures of these bacteria were grown in nutrient broth at 37 °C and maintained nutrient agar slants < 12 °C. All the cultures procured from Dr. Sir M. S. Gosavi College of pharmaceutical education and research, Nashik, Maharashtra, India.

2.9 Culture Media

Nutrient broth (NB), Nutrient media, Sabouraud Maltose Agar media were procured by Hi Media Laboratories Ltd., India.

2.10 Agar diffusion Method

All the experimentation was done in aseptic area under laminar airflow cabinet. The agar diffusion method 10 was adopted for the study. Broth cultures of the test isolates (0.1 ml) containing 1.0 X 105 CFU/ml of organism was introduced into a sterile petri dish and 15 ml of molten nutrient agar were added. The content was thoroughly mixed and then allowed to solidify. The extracts were dissolved in DMSO and10, 000

µg/ml, 30,000 µg/ml and 50,000µg/ml. Cephalexin 250 (5 µg/ml) was used as standard for antibacterial activity. Holes were bored in the plates, using a standard sterile cork borer of 8 mm diameters and equal volumes of the plant extracts (1000 µl) were transferred into the wells with the aid of micropipette. The experiments were carried out in triplicate. The plates were kept for 1hr for pre-diffusion and incubated at 37 °C/24hr (plates containing bacterial cultures). At the end of incubation, zone of inhibition was measured in all the plates [18].

3. Results and Discussion

3.1 Macroscopic character of *Roheo dicolor* (Hance.)

The leaves are crowded, large, upright, elongate, broadly linear-lanceolate, sheathed at the base, up to 30-40 cm x 4.0-7.5 cm, the upper surface green, the lower surface rich reddish-purple, the tips acute, the margins entire, the leaf base sheathing the stem, both the surface glabrous; petioles cylindrical, about 2.0- 7.0 mm long.

Inflorescence: Axillary, short:, bracts subsessile, boat-shaped, 10.2 cm long, glabrous.

Flowers: Bracteate, 5-13 mm x 3-6 mm, bracteolate, 2-5 cm x 3-7 cm, white, pedicellate, 2.0 cm long, often showy, borne in pairs of boat-shaped bracts or in cymes subtended by spathe-like or involucre-like, complete, bisexual, actinomorphic, hypogynous. It bears capsule shape fruits constining numerous number of seeds which are angled shape and endospermic in nature.

Fig 1: Morphological character of *Roheo discolor* (Hance)

3.2 Microscopy of *Roheo disolor* (Hance.) leaves

In surface view, the cuticles are present and trichomes are absent, the epidermal cells of both surfaces are parenchymatous and thin-walled, the anticlinal walls of both surfaces are straight. Stomata are abundant on the lower surfaces but fewer from the upper one. The upper surfaces are present small intercellular space. Stomata present are tetracytic, oval in outline. The guard cells are reniform with abundant chloroplast. In Transverse section, the cuticle is thin, present on both surfaces. The buliform cells or motor cells are present on lower epidermis cells and barrel shaped in upper epidermis cells, compactly arranged, sunkened stoma in the lower epidermis of the leaves. Lower epidermis contain purple layered cuticle. The mesophyll cells are made up of parenchymatous cells, which are not differentiated into palisade and spongy cells, about 12-30 layers in thickness and rounded to polygonal shaped, compactly arranged intercellular spaces absent the upper side and small intercellular space present the lower one; calcium oxalate crystal (Prism) tetragonal system present in the mesophyll cells. The vascular bundles of the lateral veins embedded in the mesophyll, collateral type. Each bundle is surrounded by a compact layer of thin-walled parenchymatous bundle sheath, distinct from the neighbouring cells. The xylem towards the upper side and the phloem towards the lower side. The xylem tissue consists of vessels, tracheids, fibres, fibres-tracheids and xylem parenchyma. The phloem tissue consists of sieve tube elements, and companion cells.

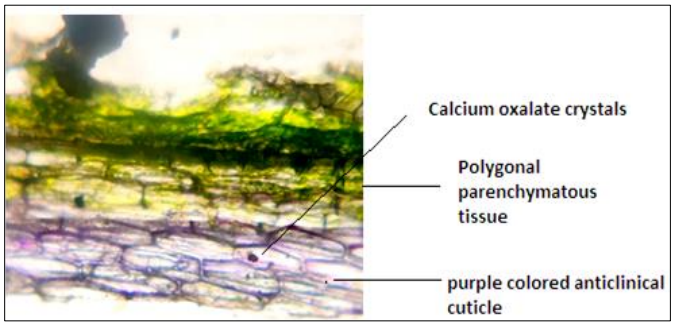
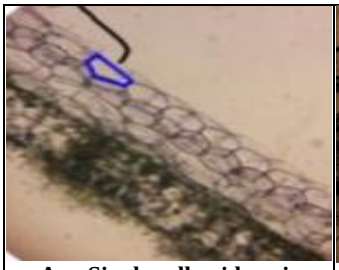
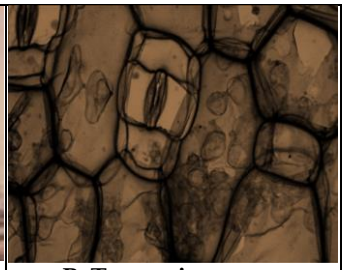


Fig 2: LS of lower epidermis

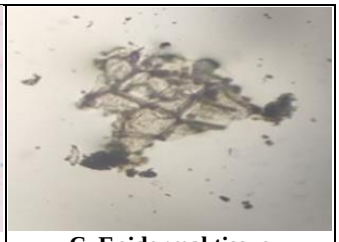
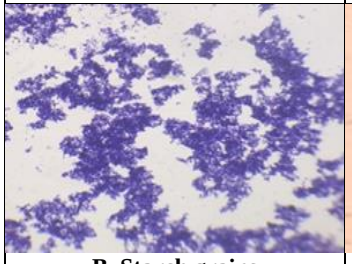
Table 1: Shows transverse section of *Rhoeo discolor* leaf

	
A. Single cell epidermis	**B. Tetracytic stomata**
	
C. Presence of starch grains	**D. Plasmolysis**

3.3 Powder characteristics

Leaf powder evidenced the presence of multilayered rapheal bundles, lignified xylem and single layered polygonal epidermal tissue, some fragments of xylem vessels, tracheids, starch grains, fragments of chloroplast, pink colored plasmolysis or vacuoles and lignified fibres.

Table 2: Shows powder characteristics of *R. discolor* leaf

	
A. Fibres	**C. Epidermal tissue**
	
B. Starch grains	**D. Tracheids**

3.4 Physicochemical analysis

In physical constant study, the ash values, extractive values, moisture content of leaves were determined. The total ash, acid insoluble ash, water soluble ash and sulphated ash values were found to be 18.37± 0.04% w/w, 4.64 ± 0.02% w/w, 9.91± 0.02% w/w and 5.56± 0.04 % w/w respectively. However, 24.± 0.24% w/w alcohol soluble and 22.56 ± 0.34% w/w water soluble extractives were observed. The moisture content of leaf powder was nearly 3.51 ± 0.01% w/w.

3.5 Phytochemical investigation: Preliminary phytochemical analysis revealed the presence of flavonoids, terpenoids, tannins, saponins, steroids, carbohydrates, phenolic compounds, carbohydrates and proteins (Table 3)

Table 3: Preliminary phytochemical analysis of crude extract

Phytoconstituents	Ethanolic extract	Aqueous extract
Alkaloids	++	+
Glycosides	++	--
Saponins	--	--
Carbohydrates	--	+
Tannins & Phenolic compounds	--	+
Flavonoid	++	+
Phytosterols		
Proteins & amino acids	--	--
Triterpenoids		
Fixed oils & fats	--	--
Gums & mucilage	+	++

Whereas +: present, --: absent,

3.6 Thin Layer chromatography

The extracts of leaf of each solvent were subjected to TLC. All spots are colorless in day light but they are colored under UV light. The alcoholic extract shows the presence of alkaloids, glycosides and flavonoid. (See in table 4)

Table 4: Shows the TLC analysis of Ethanolic extract *R. discolor* Leaf

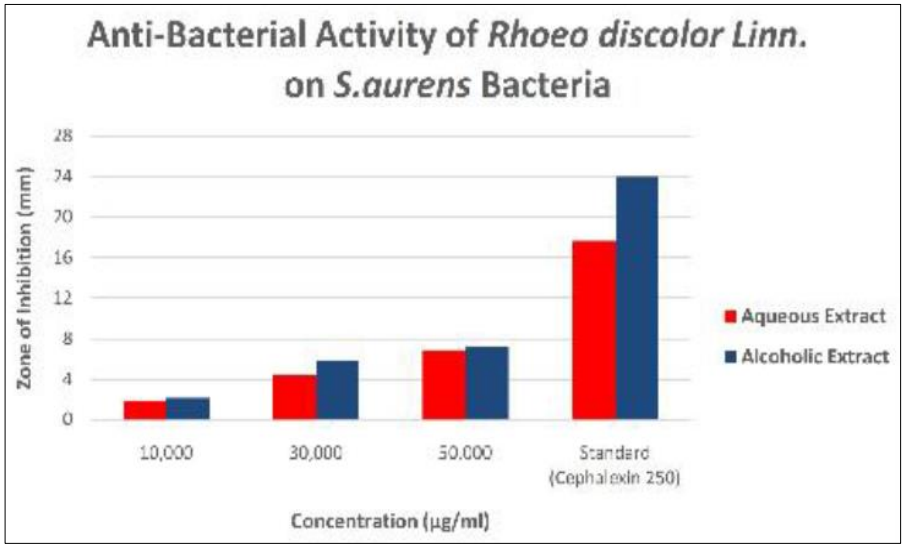
Phytoconstituents	Mobile phase	Spraying reagent	Rf value	Color of spot
Alkaloids	Tolune: Ethyl acetate: Dimethyl amine (70:20:10)	Dragandroff's reagent	0.91	Yellowish-orange
			0.85	Yellow
			0.74	brown
Glycosides	Ethyl acetate: Methanol: water (100:13.5:10)	Kedd reagent	0.78	brown
			0.54	brown
Flavonoid	Ethyl acetate: Formic acid: Glacial acetic acid (80:11:11:5)	Antimony trichloride	0.91	Yellow

3.7 Antibacterial assay

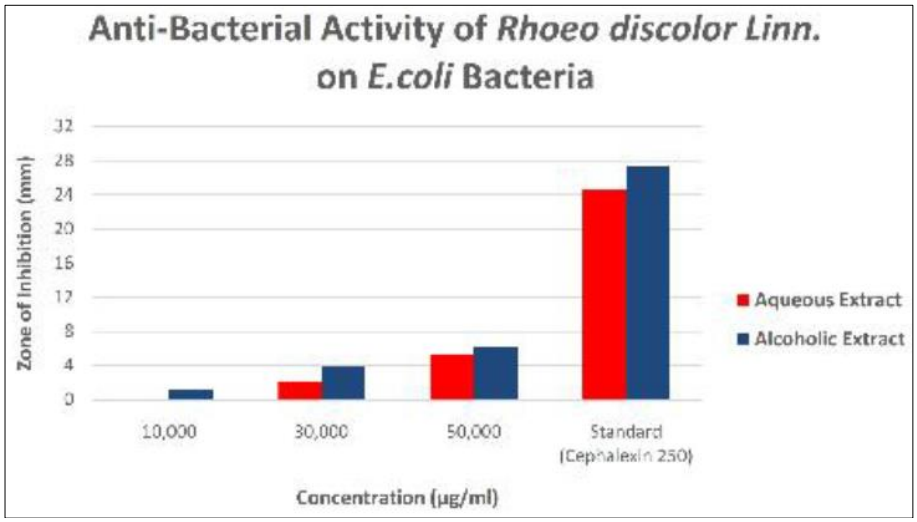
The results of agar well diffusion and minimum inhibitory concentration are shown in Table 5 indicates the antibacterial activity against pathogenic organisms exhibited by two extracts. The zones of inhibition were shown in the Figur-3 Comparatively ethanolic extract has shown the higher activity followed by aqueous extract. On B There was no zone of inhibition observed in both Aqueous and Alcoholic extract.

Table 5: Zone of inhibition (mm) of micro organisms by well diffusion method of *R. discolor*

Bacterial strains	*S. aureus*	*B. subtilis*	*E.coli*
Ethanolic extract (µg/ml)			
10,000	2.19	--	1.21
30,000	5.89	--	3.89
50,000	7.25	--	6.23
Aqueous extract (µg/ml)			
10,000	1.86	--	00
30,000	4.39	--	2.21
50,000	6.84	--	5.33
Standard (Cephalexin) (1000µg/ml)			
	12.54	13.56	14.43



Graph 1: Antibacterial assay on *S. aureus*



Graph 2: Antibacterial assay on *E. coli*

4. Conclusion

The improvement in the quality control and standardization of herbal drugs has led to the development of effective quality medicines from plants. The present investigation hits the pharmacognostical evaluation of crude drug for judging acceptability or rejection of crude drugs in the medicines. *Roheo discolor* is one of the most ancient folk medicine. The pharmacognostical investigation on physicochemical analysis indicated its potential for medicinal value, among the two different extract of the leaf. Ethanol extract is showing the good source of bioactive compounds like phenols, flavonoids, tannins, alkaloids, glycosides etc. The plant may be a good source of minerals to treat number of diseases that are mainly caused due to the deficiency of those minerals and can be utilized in Ayurvedic medicine system to cure diseases. The antibacterial property of *R. discolor* can is attributed. Based on the results from the experiment, the researchers concluded that the concentration of the extract from the leaves of Rhoeo discolor Hance does not possess antibacterial activity against gram-positive *Bacillus subtilis* due to the absence of zone of inhibition. It was also concluded that the extracts does not possess an efficacious antibacterial activity against gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* due to the presence of ≤16 mm diameter of clearing zone which was interpreted by the criteria [19] as resistant. The presence of clearing zone, however, may be due to the presence of various active principles as seen in the study

By above all these parameters we can build up a suitable plant profile which paves way for further studies on the plant for the presence of bioactive compounds and their biological activity.

5. Acknowledgment

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6. Conflict of interest

The authors have not declared any conflict of interests.

7. References

1. Kadam PV, Deoda RS, Shivatare RS, Yadav KN, Patil MJ. Pharmacognostic, phytochemical and physicochemical studies of Mimusops elengi stem, bark (Sapotaceae). Scholars Research Library 2012;4(2):607-613.
2. Khanvilkar V, Patil L, Kadar V. Standardization of chitrakadi vati: An Ayurvedic polyherbal formulation. International Journal of Pharmaceutical Sciences and Drug Research 2014;6:303-309.

3. Fazal H, Ahmad N, Khan MA. Physico-chemical, phytochemical evaluation and DPPH- scavenging antioxidant potential in medicinal plants used for herbal formulation in Pakistan. Pakistan Journal of Botany 2011;43:63-67.
4. Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction Technologies for medicinal and aromatic plants. International Centre for Science and High Technology Trieste 2008, 21-25.
5. Argal A, Pathak AK. CNS activity of *Calotropis gigantea* roots. J Ethnopharmacol 2006;106:142‑5.
6. Fawcett CH, Spencer DM. Plant chemotherapy with natural products. Annu Rev Phytopathol 1976;8:403‑18.
7. Kato K. Cytological Studies of Pollen Mother Cells of Rhoeo discolor, Hance with Special Reference to the Question of the Mode of Syndesis. Botanical Institute, Science Department, Kyoto Imperial University. Memoirs of the College of Science, Kyoto Imperial University. Series B 1930;5:139-161.
8. Freytag A. Contribution on the development of a new cancer test. (i). On the influence of carcinomatous mouseascites on resting plant cells (Kohlrabi, Rhoeo discolor). Z Gesamte Inn Med 1963;18:1018-24.
9. González-Ávila M, Arriaga-Alba M, De la Garza M, Hernández-Pretelín MC, Domínguez-Ortíz MA, Fattel-Fazenda S *et al*. Antigenotoxic, antimutagenic and ROS scavenging activities of a Rhoeo discolorethanolic crude extract. Toxicology *in vitro* 2003;17:77-83.
10. García-Varela R, García-García RM, Barba-Dávila BA, Fajardo-Ramírez OR, Serna-Saldívar SO, Cardineau GA. Antimicrobial activity of Rhoeo discolor phenolic rich extracts determined by flow cytometry. Molecules 2015;20:18685-18703.
11. Idaka E, Ogawa T, Kondo T, Goto T. Isolation of highly acylated anthocyanins from commelinaceae plants, zebrina pendula, rhoeo spathacea and setcreasea purpurea Agric. Biol. Chem 1987;51:2215-2220.
12. Mosa AI, Emara AA, Yousef JM, Saddiq AA. Novel transition metal complexes of 4-hydroxy-coumarin-3-thiocarbohydrazone: Pharmacodynamic of Co(Iii) on rats and antimicrobial activity. Spectrochim. Acta A Mol. Biomol. Spectrosc 2011;81:35-43.
13. World Health Organisation, Quality control methods for herbal materials, Geneva: World Health Organisation 2011.
14. Practical Pharmacognocy by Dr. K.R. Khandelwal and Dr. Vrundhasethi, Nirali Publication, 26th edition.
15. Tyler V, Brady L, Robber J. Pharmacognosy, Varghese Company, India 1977, 103-141.
16. Khadbadi SS, Deore SL, Baviskar BA. Experimental Phytopharmacognosy. 1st edn. Nirali Prakashan, Pune 2011, 4.7-4.8.
17. Bladt S. Plant Drug Analysis (A thin layer chromatography atlas). 2nd edition, published by springer, New Delhi 2004, 100, 196. Sethi PD. HPTLC, 1st edn, CBS Publishers, New Delhi 1996, 1-66.
18. Tannu G, Gupta A, Kumar S, Singh K. Antimicrobial activity of Nerium oleander stem extract. International Journal of Pharmaceutical Professional Research 2011;2(1):210-211.
19. Amarvani Kanjikar P, Ramesh Londonka L. Pharmacognostic Evaluation, Phytochemical Screening and Antimicrobial Activity of Stem Bark of *Ficus Krishnae.* International Journal of Pharmacognosy and Phytochemical Research 2017;9(5):733-738.
20. Coyle MB. Manual of antimicrobial susceptibility testing. Washington, DC: American Society for Microbiology 2005.