BACTERICIDAL AND BACTRIOSTATIC ACTIVITY OF ISOLATED NAPHTHOQUINONE FRACTION OF *LAWSONIA INERMIS* AND SYNTHETIC LAWSONE ON *STAPHYLOCOCCUS EPIDERMIDIS*

Sujit T. Karpe*[1], Savita R. Kulkarni[2], Sohrab A.Shaikh[1] Anil M. Manikrao[1]

[1]Sahyadri College of Pharmacy, Methwade, Tal. Sangola, Dist. Solapur, India
[2]Bombay College of Pharmacy, Kalina, Santacruz (E), Mumbai, India.
*Correspondance:
E-mail: sujitkarpe80@gmail.com

Summary

Henna, in Hindi it is called as Mehndi, consists of leaves of small shrub known as *Lawsonia inermis* Linn. Syn. *Lawsonia alba*, (Lythraceae). The chief constituent reported in henna was Lawsone (2-hydroxy-1, 4-naphthoquinone). Lawsone reported to possess bactriostatic activity. The isolation of naphthoquinone fraction in view of isolation of lawsone from *Lawsonia inermis* was done by soxhlet extraction method and yield was found to be 1%w/w. Isolated naphthoquinone fraction from *Lawsonia inermis* leaves was studied for its antibacterial activity on *Staphylococcus epidermidis* by plate diffusion method at different concentration and compared with that of synthetic lawsone and tetracycline as a standard antibiotic. The present investigation reveals that isolated naphthoquinone fraction showed concentration dependent increase in both bactriostatic and bactericidal effect, synthetic lawsone showed concentration dependent increase in only bactriostatic effect where as tetracycline showed distinct bactericidal effect.

Key words: Bactericidal, Bactriostatic, Lawsone, *Lawsonia inermis.*

Introduction

Natural products once served mankind as the source of all drugs and higher plants provided most of these therapeutic agents. Today, natural products (and their derivatives and analogs) are still representing over 50 % of all drugs in clinical use, with higher plant-derived natural products representing 25 % of the total. [1] The World Health Organization estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care and about 85 % of traditional medicines involve the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs. [2]

The skin has a complex flora. Infections can results when there is a break down in the integrity of the skin or when the immune defense is compromised. Common skin infection include cellulitis, erysipelas, impetigo, folliculitis, furuncles and carbuncles.[3]

Staphalocccus epidermidis (coagulase negative *staphalococci*) is one of 33 known species belonging to genus staphalococcus.[4] *S. epidermidis* is usually nonpathogenic , patients with a compromised immune system are often at risk for developing an infection particularly nosocomial or community acquired but are more of threat to hospital patients. *S. epidermidis* is also a major concern for people with catheters or other surgical implants because it is known to cause biofilms that grow on these devices.[5] Infection can occur in dialysis patients or anyone with an implanted plastic device that may have been contaminated also responsible for causing endocarditis in patients with defective heart valves and in some cases sepsis can occur in hospital patients.[6]

Henna [7], in Hindi it is called as Mehndi, consist of leaves of small shrub known as *Lawsonia inermis* Linn. Syn. *Lawsonia alba*, (Lythraceae). The chief constituent found in henna is 2-hydroxy-1, 4-naphthoquinone called lawsone, which is soluble in water and acidic solution. Lawsone is reported to possess bactriostatic activity. [8] Henna reported to possess cytotoxic [9], anti-inflammatory [10], antibacterial [11] and wound healing activity. [12] In present study an attempt was made to isolate naphthoquinone fraction in view of isolating lawsone from *Lawsonia inermis,* and to investigate its antibacterial effect on *Staphylococcus epidermidis* by plate diffusion method at different concentration and compared with that of synthetic lawsone and standard antibiotic.

Materials and methods

The leaves of henna were purchased from Yucca enterprises, Mumbai and authenticated from the department of Pharmacognosy and Phytochemistry, Bombay College of Pharmacy, Mumbai. The identification, qualitative and quantitative tests were carried out according to standard texts [13-14] and the voucher specimens are preserved in department of Pharmacognosy. Synthetic lawsone (2-hydroxy-1,4-naphthoquinone) was purchased from Sigma Aldrich, USA. The strain *Staphylococcus epidermidis* was taken from Microbiology Laboratory, Bombay college of Pharmacy, Mumbai. All the chemicals used were of AR grade.

Extraction Procedure: Henna was extracted by soxhlet extraction method.100 g of henna powder was extracted by using methanol in a soxhlet apparatus in menstrum to drug ratio of 5:1 till extract becomes colorless. The semisolid masses (about 20-25%w/w) were obtained after evaporation of solvent. Then the semisolid masses were separated into phenolic (sodium hydroxide soluble) and neutral fraction by using 3 % sodium hydroxide solution. The solution was filtered. The pH of filtrate was adjusted to 4.5 by using 1N hydrochloric acid and extracted with chloroform. Then the organic layer was washed with water, dried and then passed through anhydrous sodium sulfate and evaporated to obtain crude naphthoquinone extract (about 1%w/w). [15-16]

The above extract and synthetic lawsone were subjected to HPTLC analysis using HPTLC LINOMET 5 applicator and CAMAG 3 scanner with the help of Wincat software for characterization. The chromatograms were developed using toluene: acetone: acetic acid (9: 1: 0.1) as solvent system.

Preparation of Samples: 10mg of isolated naphthoquinone fraction of leaves of *Lawsonia inermis*, synthetic lawsone were dissolved in 10ml of methanol. The solutions were diluted to a concentration of 100mcg/ml, 200mcg/ml, 300mcg/ml, and 500mcg/ml with water.

Preparation of Test Culture: The test culture was prepared by inoculating 10ml of sterile nutrient broth with a loopful of culture of microorganism from nutrient agar slant. The culture was incubated at 37^0C for 18 hrs and this 18 hrs old culture was used for further experiment.

***Preparation of Petri plates*:** Petri plates, pipettes and borer were sterilized by dry heat in a hot air oven at 160^0C for 1 hour. The nutrient agar was sterilized by autoclaving at 121^0C and 15 lbs pressure for 15 minutes.

Plate Diffusion Method: The sterile nutrient agar was maintained at 40-45^0C to keep it in molten condition. The inoculation of the culture of microorganism was done by seeding method. About 0.5ml of culture was added in 300ml of nutrient agar. About 25ml of the molten nutrient agar was poured in each of the sterile petri plates aseptically.

The plates were allowed to set at room temperature for 1 hour. Holes were made in the medium with a sterile cork borer. The holes were then filled with 0.1ml of samples. The petri plates were then incubated at 37^0C for 24 hours. All the aseptic operations were carried out under the laminar air flow unit. The zones of inhibition were measured. [17]

Each sample was tested in duplicates. A negative control was carried out for aqueous methanol. A positive control was carried out without inoculating microorganism. And a standard antibiotic (Tetracycline) solution was also taken for comparison of the activity. The average zone of inhibition in mm inclusive of diameter of cup (6mm) were as shown in **Table 1** and photograph of zone of inhibition of the tested compounds were as shown in **Figure 1.**

Result and discussion

The isolated naphthoquinone fraction of *Lawsonia inermis* is characterized by HPTLC analysis using LINOMET 5 applicator and CAMAG 3 scanner with the help of Wincat software. The chromatograms were developed using toluene: acetone: acetic acid (9: 1: 0.1) as solvent system at 254nm and 366 nm and are compared with the chromatograms developed by synthetic lawsone. The chromatograms of isolated naphthoquinone fraction and synthetic lawsone are found to be similar. The chromatograms of isolated naphthoquinone fraction and synthetic lawsone are given in **Figure 1, 2, 3 and 4** and the peaks value are given in **Table 1**. Rf of spot on isolated naphthoquinone extract of *Lawsonia inermis* track matches with the Rf value of spot of std. Lawsone. The UV spectrum of spot present on isolated naphthoquinone fraction track and lawsone track are similar to each other.

Figure: 1. Chromatogram of Isolated naphthoquinone fraction of *Lawsonia inermis* at 254 nm

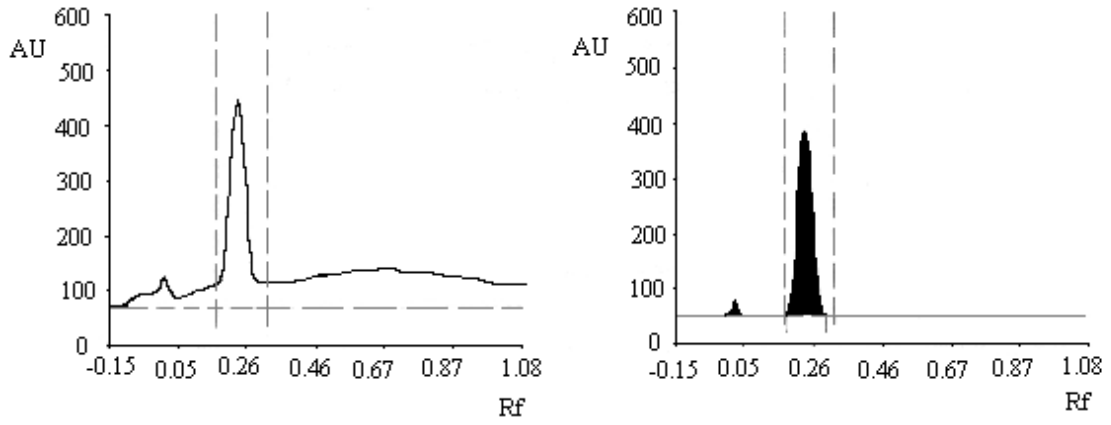


Figure: 2. Chromatogram of Isolated naphthoquinone fraction of *Lawsonia inermis* at 366 nm

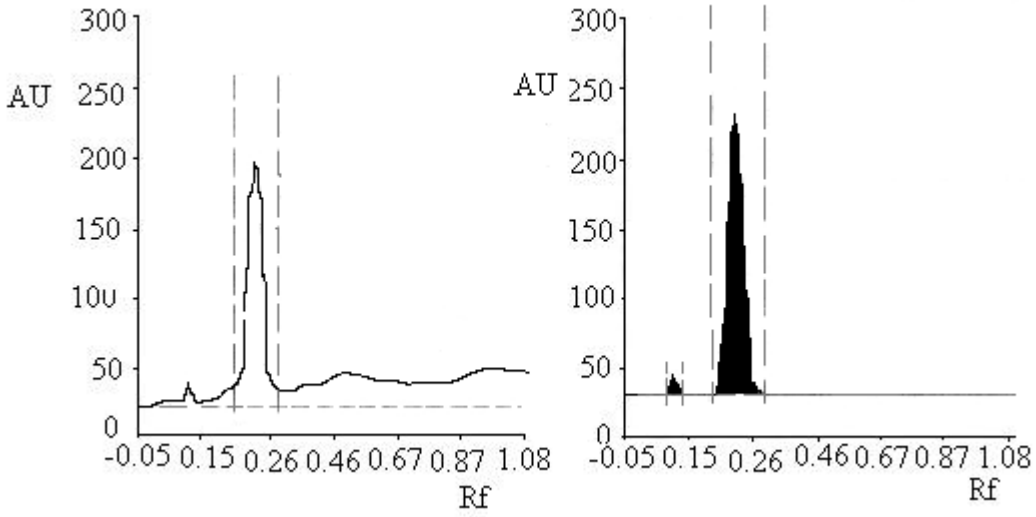


Figure: 3. Chromatogram of Standard Lawsone at 254nm

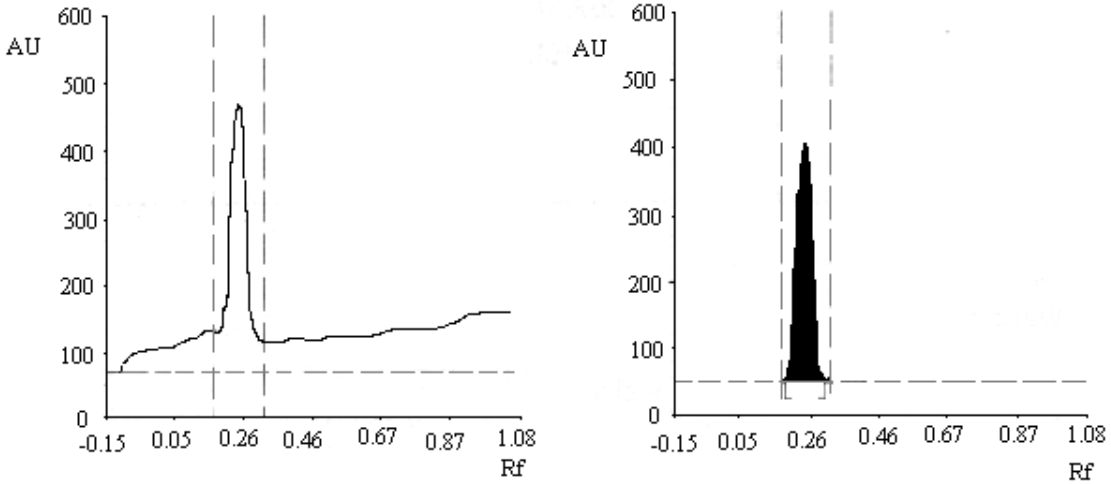


Figure: 4. Chromatogram of Standard Lawsone at 366nm

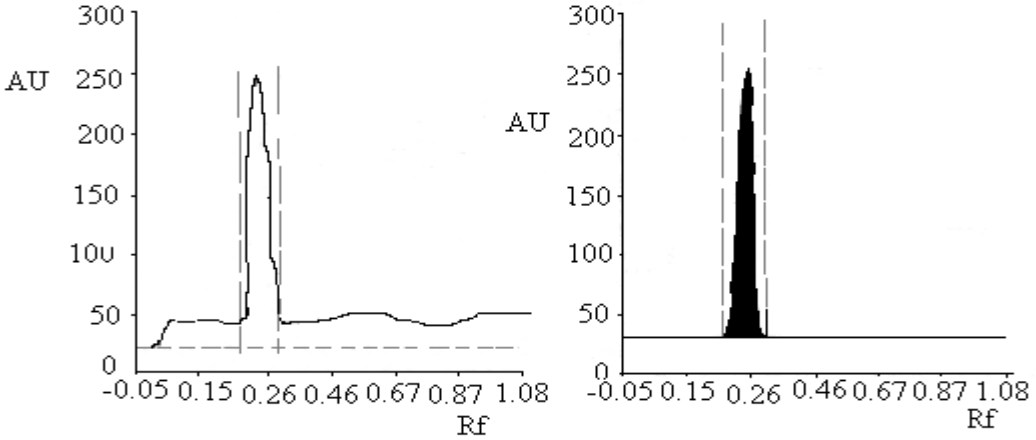


Table: 1. Peak table of isolated naphthoquinone fraction of *Lawsonia inermis* and synthetic lawsone at 254nm and 366nm

Sr. no.	Drug	Rf of the Peak	
		At 254 nm	At 366 nm
1.	Isolated naphthoquinone fraction	0.270	0.266
2.	Synthetic Lawsone	0.270	0.268

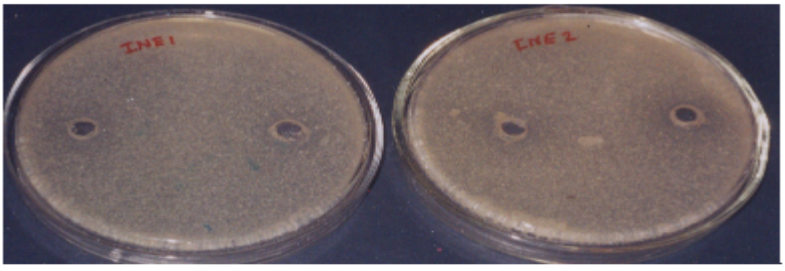
Antimicrobial activity of isolated naphthoquinone fraction of henna and synthetic lawsone was done at different concentration range (100mcg/ml to 500 mcg/ml) on *Staphylococcus epidermidis* by using plate diffusion method. All the activities of the isolated naphthoquinone fraction of henna and synthetic lawsone were compared with that of standard antibiotic (Tetracycline) and the results are given in **Table 2**.

Table -2: Bactericidal and bactriostatic activity of isolated naphthoquinone fraction of *Lawsonia inermis* and synthetic lawsone

Test solution	Conc. (mcg/ml)	Zones of inhibition in mm.	
		Bactericidal activity	Bactriostatic activity
Control	--	--	--
Standard Tetracycline	50	26	--
Isolated naphthoquinone extract of henna	100	12	17
Isolated naphthoquinone extract of henna	200	12	20
Isolated naphthoquinone extract of henna	300	15	25
Isolated naphthoquinone extract of henna	500	16	27
Synthetic Lawsone	100	--	19
Synthetic Lawsone	200	--	20
Synthetic Lawsone	300	--	25
Synthetic Lawsone	500	--	29

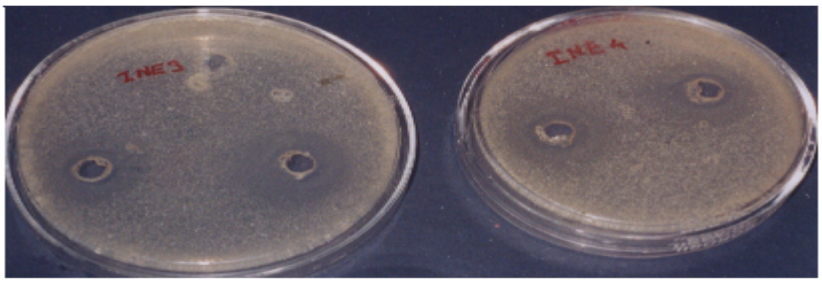
The photographs of Petri plate showing bacteriostatic zones as well as bactericidal zones are given in **figure number 5, 6, 7**.

Figure -5: Bactericidal and bactriostatic activity of isolated naphthoquinone fraction of *Lawsonia inermis*



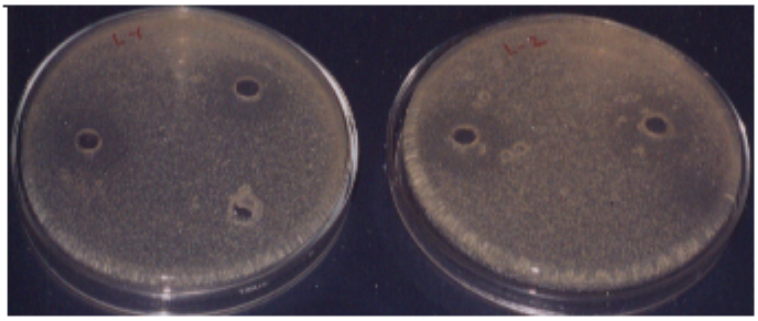
Isolated naphthoquinone fraction (100mcg)

Isolated naphthoquinone fraction (200mcg)

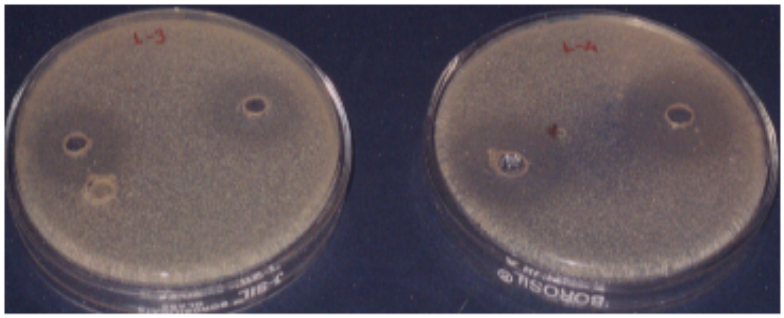


Isolated naphthoquinone fraction (300mcg) Isolated naphthoquinone fraction (500mcg)

Figure 6: Bactericidal and bactriostatic activity of synthetic lawsone

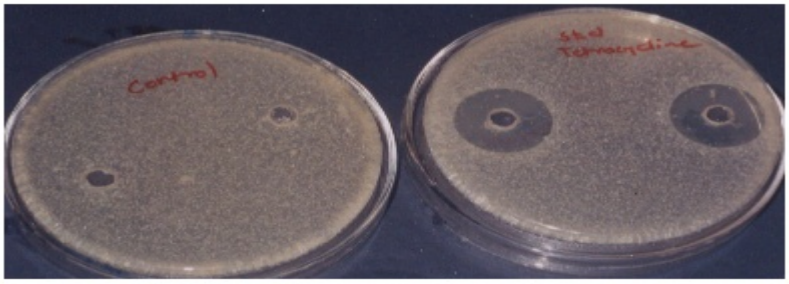


Lawsone (100mcg) Lawsone (200mcg)



Lawsone (300mcg) Lawsone (500mcg)

Figure 7: Bactericidal activity of tetracycline and positive control



Control Tetracycline (50mcg) (Standard antibiotic)

Isolated naphthoquinone fraction shows concentration dependent increase in both bacteriostatic as well as bactericidal activity **(Fig. no. 01)**. Synthetic lawsone shows concentration dependent increase in only bacteriostatic activity **(Fig. no. 02)**. The bactericidal effect of isolated naphthoquinone fraction at all concentration is less than that of standard antibiotic (50mcg/ml).

Standard antibiotic shows distinct bactericidal zone of inhibition of about 26 mm **(Fig. no. 03)** and not shows the bacteriostatic zone of inhibition however isolated naphthoquinone fraction show very narrow zones of bactericidal area which was followed by relatively broad distinct bacteriostatic zone of inhibition that is the number of colonies of bacteria in these area were distinctly less than that of other part of the plate. The synthetic lawsone shows very prominent bacteriostatic zone of inhibition which is almost similar to that of bacteriostatic zones of inhibition of isolated naphthoquinone fraction and not shows bactericidal zone of inhibition. So, this will suggest that the isolated naphthoquinone fraction of *Lawsonia inermis* consist chiefly lawsone and other constituents of henna which may probably show the bactericidal action.

The probable mechanism of antibacterial activity of lawsone is proposed. [8] It is supposed that lawsone acts on the –SH groups in the cellular development. This is supported the fact that cysteine acts as an antagonist to its action. Synthetic lawsone and isolated naphthoquinone fraction are reported to show immunostimulant activity. [15, 18] Thus the static action on growth of microorganism and its immunostimulant activity by increasing the WBC count can be helpful in elevating the host immune response towards infection. Since it is reported that antibiotics are largely ineffective in clearing biofilms but the drug of choice is vancomycin to which rifampin or aminoglycosides can be added. The mentioned antibiotics are known to have serious side effects. The study result reveals that Isolated Naphthoquinone Fraction of *Lawsonia inermis* and Synthetic Lawsone have promising bactericidal and bactriostatic activity against *Staphylococcus epidermidis which is known to* induce biofilms in patients with implants.

Thereby we conclude that the plant extract of henna in the Isolated Naphthoquinone Fraction and synthetic lawsone can be considered as an alternative to the antibiotics in treating the *Staphylococcus epidermidis* induced infections with immuno-compromised patients.

References

1. Balandrin NF, Kinghorn AD, Farnsworth NR. Human medicinal agents from plants. Eds. ACS symposium series; 1993, 534.
2. Farnsworth NR, Wislson EO. Biodiversity ED. National academy press. Washington, DC.
3. Stulberg DL, Penrod MA, Blatny RA. Common Bacterial Skin Infections. Am Fam Physician. 2002; 66:119-124.
4. Queck SY and Otto M (2008). Staphylococcus epidermidis and other Coagulase-Negative Staphylococci. *Staphylococcus: Molecular Genetics*. Caister Academic Press.
5. Salyers, Abigail A, Whitt, Dixie D. Bacterial Pathogenesis: A Molecular Approach. 2nd ed. ASM Press, Washington, D.C. 2002.
6. http://www.mayoclinic.com/health/endocarditis/DS00409.
7. Indian council of medical research. Medicinal plant of India. 1987; 2: 138-143, 471-478.
8. Marco A, Biffi G, Zaavag L. Bacteriostatic action of quinones on normal and resistant strains of *S. aureus.* Sperimentale. 1951; 100: 481-91, CF:CA:45:4776.
9. El-Merzavani MM, El-Aaser AA, Attia MA, El-Duweini AK, Ghazal AM. Screening system for Egyptian plants with potential anti-tumor activity. Planta Medica. 1979; 36: 150-5.
10. Singh S, Shrivastava NM, Modi NT, Saifi AQ. Antiinflammatory activity of *Lawsonia inermis,* Current Science. 1982; 51(9): 470-1

11. Radhika U, Prathap Kumar KVL, Geetha Sadhana P, Rao KNV, Shanmuganathan S. Antibacterial studies of *Lawsonia inermis,* Indian journal of Natural Product. 18(1): 30.
12. Patil KS, Mandawgade SD. Wound healing activity of leaves of *Lawsonia alba,* Linn. Journal of Natural remedies. 2003; 129(3/2): 129-133.
13. Datta SC, Mukerji B. Pharmacognosy of Indian leaf drugs, Pharmacognosy Laboratory Bulletin. 1952; 24-6.
14. Indian Herbal Pharmacopoeia, Revised new edition, Published by IDMA, Mumbai, 2002.
15. Jiny VK, Karpe ST, Kulkarni SR. Immunomodulatory effect of *Adathoda vasica, Alkanna tintoria, Lawsonia inermis.* Indian Drugs. 2005 June; 42(6): 345-52.
16. Jiny VK, Karpe ST, Kulkarni SR. The effects of methanolic extract, isolated naphthoquinone fraction of *Lawsonia inermis* leaves and Synthetic Lawsone on mitosis in *Allium cepa* root tips. Indian Drugs. 2009 July; 46(7): 546-9.
17. Pharmacopoeia of India, Vol. II, Govt. Of India, Ministry of Health and Family Welfare, New Dehli, A-105.
18. KulKarni SR, Karande VS, Study of Immunostimulant activity of naphthoquinone extract of leaves of *Lawsonia alba* Linn, Indian Drugs, 1998 March; 25(3): 397-403.