World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 7.409

FORMULATION AND EVALUATION OF ANTI-BACTERIAL CREAM OF *LABLAB PURPUREUS*

D. Prathyusha*, A. Madhu Bindu, Vallepu Samatha, Dugane Ashok, Kunchala Navya Sri, Parvathi Harini, Chenchu Ramya

India.

***Corresponding Author: D. Prathyusha**
India.

Article Received on 30/06/2025 **Article Revised on 21/07/2025** **Article Accepted on 11/08/2025**

ABSTRACT
The study focuses on the formulation and evaluation of an herbal antibacterial cream using methanolic extract of *Lablab purpureus* (Hyacinth bean), a plant known for its wide range of pharmacological properties. The extract was prepared using both maceration and soxhlation methods, with soxhlation yielding a higher quantity of bioactive compounds. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins and saponins, which are associated with anti-bacterial and therapeutic properties. A topical cream was formulated using the extract and evaluated for various parameters including pH, spread-ability, viscosity, homogeneity, washability and irritancy, all of which confirmed the formulations suitability for skin application. Anti-bacterial activity was tested using both cup plate method and disc diffusion method against Escherichia coli and Staphylococcus aureus. Results showed dose dependent anti-bacterial activity. The study concludes that *Lablab purpureus* extract can be effectively used in the development of the safe and natural topical anti-bacterial formulation.

KEYWORDS:
- Lablab Purpureus.
- Herbal Anti-bacterial cream.
- Phytochemical analysis.
- Methanolic extract.
- Maceration and Soxhlation extraction.
- Disc-diffusion method.

INTRODUCTION

Since ancient times, humans have turned to nature in their search for remedies to treat illnesses. The use of medicinal plants began as an instinctive practice, much like the behaviour observed in the animals. At that time, there was little understanding of the cause of diseases or knowledge about which plants could serve as effective treatments. As a result, early medicinal practices relied heavily on trial and error. Over time, however, the reason behind the effectiveness of the certain plants in treating specific aliments began to emerge. The shift led to a transition from purely empirical use to a more evidence- based and scientific approach in the use of medicinal plants.[1] Medicinal plants are among the oldest forms of the treatment, having been used for thousands of years in traditional healing properties was passed down through generations with communities.[2] By the eight century, it had made its way to Africa, carried from Southeast Asia. Since then, the plant had spread extensively, finding a place in the agricultural systems of numerous tropical and subtropical regions around the world.[3] Lablab purpureus ranks as the third most significant vegetable crop in the central and south-western regions of Bangladesh.[4] The plant demonstrates a broad spectrum of pharmacological effects, such as antimicrobial, antioxidant, anticancer, hypolipidemic, cardiovascular, central nervous system, respiratory, immune-modulating, anti-inflammatory, pain-relieving, fever-reducing and various other therapeutic activities.[5] Anti-bacterial resistance represents a critical and growing problem in todays medical and public health fields. It occurs when microorganisms such as bacteria, viruses, fungi and parasites develop the ability to resist the effects of drugs that were once effective in eliminating them or stopping their growth. As a result, standard treatments become less effective or even useless, leading to persistent infections, increased transmission of diseases, longer hospital stays and high medial costs. This adaption by microbes challenges the ability of health care systems to manage common infectious diseases and threatens the success of

the major medical procedures that relay on effective antibacterial therapy.[6] The primary aim was to protect the millions of lives threatened by serious infectious diseases caused by a range of pathogens and parasites. While antibacterial drugs have indeed been instrumental in saving countless lives, the widespread development of resistance mechanism causing organisms has significantly weakened the disease effectiveness of our existing treatment strategies.[7] Antimicrobial agents are structurally and functionally diverse group of low molecular weight compounds that disrupt bacterial growth, either by temporarily inhibiting their proliferation (bacteriostatic effect) or by killing the bacteria.[8]

The traditional Indian Ayurvedic system of medicine emphasizes the use of natural substances to support overall well being and prevent the onset of avoidable health problems. Rooted in ancient practices, ayurveda is widely recognized for its reliance on diverse herbal remedies aimed at restoring balance within the body and addressing a wide range of health issues. This holistic approach not only focuses on curing aliments but also on maintaining harmony between the mind, body and spirit through natural therapies.[9] Today herbal medicines hold a prominent place in the pharmaceutical industry due to their well-established therapeutic effects and minimal side effects.[10] Therefore, combining the reliable and effective herbal medicine system with modern conventional pharmaceutical practices has the potential to offer significant advantages. This integration could enhance the overall quality and accessibility of healthcare by supporting and reinforcing the foundation of primary healthcare services. By uniting traditional knowledge with modern science, a more comprehensive, safe and effective approach to patient care can be developed, ultimately improving health outcomes and expanding treatment options.[11] With this increasing demand for herbal pharmaceutical products, ensuring their quality has become essential. Currently, nearly 80% of the global population relies on herbal remedies for the treatment, prevention and management of various health conditions.[12]

MATERIALS AND METHODS

Plant material collection and authentication

Fresh plant material of lablab purpureus (commonly known as Hyacinth bean) was collected from the local agricultural field (Kasala village/ Medak district/ Telangana/ India) during the summer season of the year 2025. The plant was identified and authenticated by a botanist from koti was deposited in the herbarium for the future reference.

The collected plant parts Leaves and stem, were thoroughly washed with running tap water to remove soil and dust, followed by rinsing with distilled water. The cleaned material was then shade -dried at room temperature for 2 weeks until a constant weight was achieved. The dried plant material was then pulverized using a mechanical grinder and stored in a air tight container at room temperature until further use in extraction and formulation processes.[13,14]

MACERATION

This method is simple and less equipment was used but, it takes longer

- We weighed the 20 grams of dried plant powder
- Mixed with 200 ml of the methanol in a clean glass container
- Soak the mixture, stand for 24 hours with occasional shaking
- After 24 hours, filter the mixture through Whatman filter paper
- Re- macerate the residue with fresh solvent for better yield
- Concentrate the filtrate using a water bath at 40-50 c to remove the solvent

SOXHLATION

- Weigh the 50 grams of the dried Lablab purpureus powder
- Place the powder in a filter paper thimble and insert into the Soxhlet chamber
- Pour 250ml of the methanol in to the round-bottom flask
- Setup the Soxhlet extractor with a condenser and heat source
- Heat the solvent gently. It will evaporate, condense and siphon through the plant material repeatedly
- Continue the cycle for 6-8 hours or until the solvent in the siphon tube appears colorless.
- Let the extract cool. Filter the mixture through Whatman filter paper
- Concentrate the extract using a rotary evaporator or a water bath 40-50c to remove the solvent

PHYTOCHEMICAL SCREENING

One gram of the ethanolic extract was dissolved in 100 ml of methanol and then used for preliminary phytochemical screening to identify the types of phyto-constituents present.[15]

Test for Alkaloids

- **Mayer's test**: Take 2ml of extract, 2ml of Mayer's reagent was added
- **Wagner's test:** For 2ml of the extract, 2ml of Wagner's reagent was added.
- **Dragendroff's test:** To the extract, add few drops of Dragendroff's reagent

Test for Saponins

- **Foam test:** To the plant extract add few ml of water and shake vigorously
- **Dry foam test:** To the water add few ml of extract shake vigorously and add a mixture of oil to it and boil it for 5 mins
- **Lead acetate test:** The extract was tested with a

lead acetate solution

- **Keller killiani test:** To the extract add acetic acid and sulfuric acid

Test for tannins

- **Ferric chloride test:** 2ml of the extract, add few drops of ferric chloride solution (5%)
- **Lead acetate test:** To the 2ml of the extract, add few drops of lead acetate solution

Test for Flavonoids

- **Shinoda test (Magnesium Hydrochloride reduction test):** To the test solution(2ml), few reagent of magnesium ribbon were added and concentration hydrochloric acid was added drop wise.
- **Sodium Hydroxide test:** To the plant extract, add NaOH and add drop wise dilute HCl.[16]

PREPARATION OF ANTI-BACTERIAL CREAM

We prepare 10g cream base using the oil-in-water emulsion type, which is suitable for herbal cream.

In a clean beaker add beeswax and liquid paraffin. Gently heat to 70-75 ºC until beeswax is fully melted (Oily Phase) Take another beaker mix borax with distilled water, Heat to 70-75ºC until borax dissolves completely (Aqueous Phase). Slowly add hot aqueous phase into the oil phase in motor and pestle, triturate vigorously to form an emulsion, stir continue stirring as it cools down to 40º (Emulsification). Add Lablab purpureus extract dissolved in a few drops of rose water and preservative. Triturate gently until the cream becomes smooth and uniform, once cooled transfer the cream into sterile glass or plastic container. Label and store in a cool and dry place.[17]

INGREDIENTS	QUANTITY (10gm)	FUNCTION
Lablab purpureus extract	0.02g	Anti-Bacterial agent
Beeswax	3.2g	Emulsifying agent
Liquid paraffin	10ml	Emollient
Borax	0.16g	Emulsifier
Preservative	0.02g	Prevents microbial contamination
Rose water	1ml	Fragrance
Distilled water	Q.S.	Solvent

EVALUATION TESTS[18]

The evaluation tests for an antibacterial activity cream formulated with Lablab purpureus are essential to ensure its safety, efficacy, stability and quality. These tests are performed as part of the formulation and evaluation study. Some of the tests we performed are:

1. **Physical evaluation:** The formulated anti-bacterial cream was evaluated for its physical characteristics including colour, odour, texture, and consistency.
2. **pH determination:** To ensure the skin compatibility, the Ph of the cream was determined by dispersing 1 gram of the formulation in 10 ml of distilled water and measuring it with a digital pH thermometer
3. **Spread-ability:** The spread-ability of the cream was evaluated to assess its ease of application on the skin. A fixed quantity of cream was placed between two glass slides and a standard weight was applied for a few minutes.
4. **Washability:** Washability was tested by applying a small amount of cream on the skin and rinsing with tap water after a few minutes.
5. **Homogeneity:** The homogeneity of the cream was assessed by visual and microscopic observation to check for the uniform distribution of ingredients.
6. **Viscosity:** Viscosity was measured using Brookfield viscometer to evaluate the cream thickness and flow properties.
7. **Irritancy test:** A specific area of 1 cm^2 was delineated on the left-handed dorsal surface. To provide even coverage, the cream was administered gently, and application time was carefully noted for precise evaluation. Any indications or erythema, edoema or irritating effects were closely examined and reported after intervals of up to 24 hours Make the region on the dorsal surface of the left hand. This test aids in determining whether the cream formulation may cause skin sensitivity or irritation.

ANTI-BACTERIAL ACTIVITY

To prepare Nutrient agar media, weigh the nutrient agar grams, dissolve this media in the distilled water about 100 ml, adjusting the pH to 7.0 and sterilizing the mixture in an auto clave at 121ºC for 15 minutes. After autoclaving the nutrient agar is poured into the sterile petri dishes and allow to solidify. For both cup plate method and disc diffusion method, fresh bacterial cultures such Escherichia coli and Staphylococcus aures are prepared by inoculating them into the nutrient broth and incubating for 18-24 hours at 37ºC. Once the agar plates are ready, the surface is uniformly swabbed with the bacterial suspension using a sterile cotton swab to ensure even growth wells of about 6mm in diameter are then made in the agar using a sterile cork borer and also disk papers are in 3mm in diameter. The lablab purpureus extract which is got from the soxhlation using methanol is introduced into the wells at different concentrations and impregnated discs are carefully placed on the inoculated agar media using sterile forceps. The Plates are incubated at 37ºC for 24 hours. After the incubation, the anti-bacterial activity is assessed by measuring the diameter of the clear zones around the wells and disc,

which indicate the effectiveness of the extract is inhibiting bacterial growth.

Composition of media

S.no	Composition of media	Amount (grams)
1	Peptone	0.5
2	Beef extract	0.3g
3	Yeast extract	0.4g
4	Agar	1.5G
5	Sodium chloride	0.5g
6	Distilled water	100
7	pH at 25ºC	7.4 ±0.2

RESULTS AND DISCUSSION

Maceration

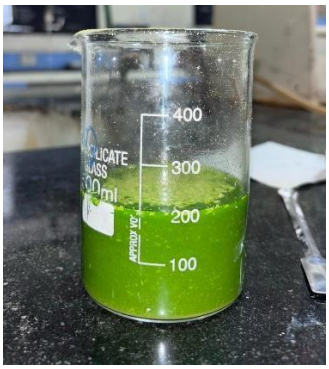


Fig. 1: Soaking the powder in methanol

Fig:2 Evaporating the solvent after soaking

Fig:3 Extract formed after evaporation for 24 hours

Soxhlation

Fig. 4: Soxhlation.

Fig. 5: Evaporating the solvent after soxhlation.

Fig. 6: Extract formed after evaporation.

Phytochemical tests

TEST	RESULT
1. Detection of Alkaloids	
Mayers Test	-
Wagner's Test	+
Dragendroff's Test	+
2. Detection of Saponins	
Foam Test	+
Dry foam Test	+
Lead Acetate Test	+

Keller-Killiani Test	+
3. Detection of Tannins	
Ferric Chloride Test	+
Lead Acetate Test	+
4. Detection of Flavonoids	
Sodium Hydroxide Test	+
Shinoda Test	+

PREPARATION OF CREAMS

Fig:7 Cream

Fig: 8 Cream with plant extract

Fig. 9: Anti-bacterial cream.

EVALUATION TESTS

1. **Physical appearance**: The physical appearance of the cream was observed to have a smooth, uniform texture with a green colour and pleasant herbal odour. There was no phase separation or grittiness, indicating that the formulation was physically stable and well-prepared.
2. **pH determination:** The pH value was found to be within the ideal range of the topical application that is 6.2 suggesting that the formulation is non irritating and safe for human skin.
3. **Spread-ability:** The cream showed good spread-ability which is essential for uniform application on the skin.
4. **Washability:** The cream was easily washable, leaving no sticky or greasy residue. This property makes the formulation convenient.
5. **Homogeneity:** The cream appeared to be free from the lumps, air bubbles or phase separation, indicating a homogeneous and stable mixture.
6. **Viscosity:** The results showed for the viscosity of the cream was within the acceptable limits that is 40,000 centi-poise, ensuring safe of application without being too thicky.
7. **Irritancy test:** After the application of the cream the irritancy test was observed to be no irritancy, such as redness or swelling on the skin.

Anti-bacterial activity

Fig:10 Anti-bacterial activity against E. coli by using cup plate method.

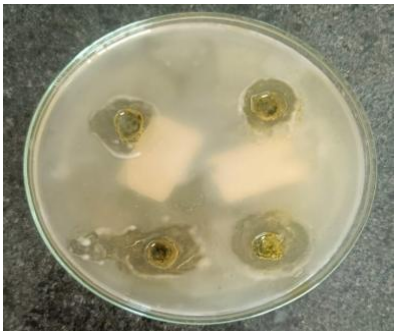


Fig:11 Anti-bacterial activity against Staphylococcus. A by cup plate method.

Fig:12 Anti-bacterial activity against E. coli by Disc diffusion method

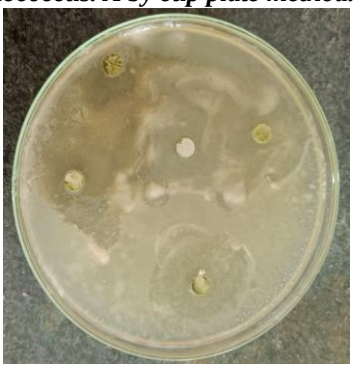
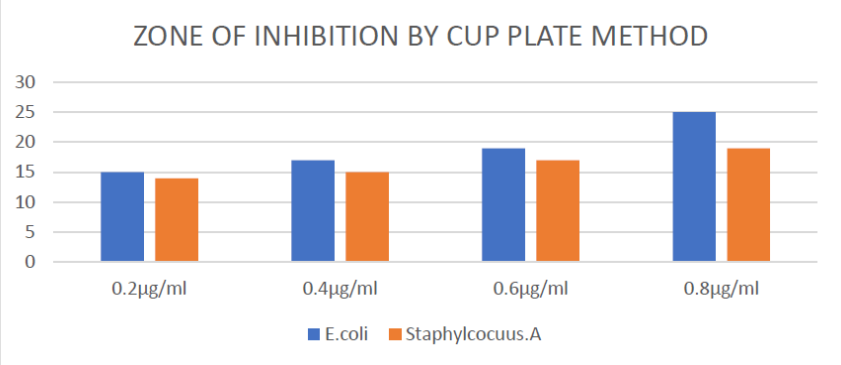
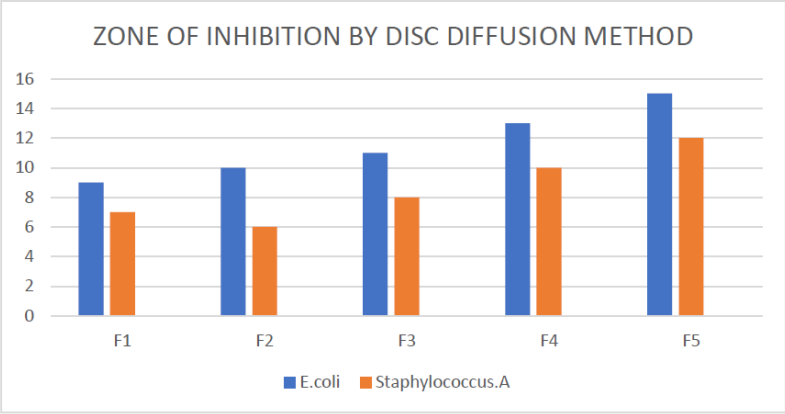


Fig: 13 Anti-bacterial activity againststaphylococcus. Disc diffusion method



Graph 1: Different concentrations of extract was taken, antibacterial activity was performed against E. coli and Staphylococcus by cup plate method.



Graph 2: 10µg/ml of concentration of extract was taken, Anti-bacterial was performed against E. coli and staphylococcus by disc diffusion method.

DISCUSSION

Maceration

The maceration of Lablab purpureus leaves and stem was carried out using methanol 95% in a 1:10 ratio at room temperature for 72 hours. After extraction, the solution was filtered using whatman no.1 filter paper. The filtrate was dark green liquid and was concentrated using a rotary evaporator to obtain a semi-solid extract. The extract was stored in an amber colour bottle at 4c until further use.

Soxhlation

The dried powder of Lablab purpureus (Leaves and stem) was subjected to Soxhlet extraction using methanol as the solvent. The process was carried out for approximately 5-6 hours until the solvent in the siphon tube appeared clear, indicating complete extraction. After completion, the extract was concentrated using a rotary evaporator and further dried to obtain a thick, semi-solid mass. The extract was stored in an air tight container at 4c for further cream formulation and anti-bacterial studies.

Phytochemical analysis

The Phytochemical analysis of the plant extract was carried out to detect the presence of various bioactive constituents using standard chemical tests. The result confirmed the presence of alkaloids, saponins, tannins and flavonoids in the extract. Among all the tests only the Mayer's test shows the negative results, remaining all the tests show positive results. Which the keller- killiani test shows it present the cardiac glycosides, in tannins Ferric chloride test and lead acetate. These compounds are known for their anti-oxidant and ani-microbial properties. Where the flavonoid indicates the anti-inflammatory and anti-oxidant properties.

Overall, the phytochemical screening confirms that the plant extract contains several important secondary metabolite, which may contribute to its potential anti-bacterial activity and therapeutic properties.

Anti-Bacterial cream

The herbal cream formulated using *Lablab purpureus* extract was smooth, homogeneous and non-greasy in nature. The cream exhibited good spread-ability and showed no signs of phase separation or grittiness. The colour of the cream was greenish due to the plant extract and it had a pleasant smell odour.

Anti-bacterial activity

Cup plate method

The cup plate method showed that Lablab purpureus extract dose-dependent anti-bacterial activity. As the concentration increased from 02 to 0.8 µg/ml, the zone of inhibition also increased for both E.coli and Staphylococcus aureus. E. coli showed a higher sensitivity compared to staphylococcus aureus. This proves extract is very effective, especially against Gram - negative bacteria.

Disc diffusion method

The disc diffusion method that all the discs of Lablab purpureus cream exhibited anti-bacterial activity. The zone of inhibition increased from f1 to f5, indicating improved effectiveness with higher concentrations pr better formulation. E. coli showed slightly greater sensitivity than staphylococcus aureus in all. F5 shows the highest activity.

CONCLUSION

The present study demonstrates that Lablab purpureus possesses significant antibacterial activity and can be effectively used in the formulation of a herbal cream. The extract obtained through soxhlation showed the presence of important phytochemicals such as alkaloids, flavonoids, tannins and saponins., which contribute to its anti-microbial potential. The formulated cream exhibit ideal physical properties, including good spread-ability, stability and a pH suitable for topical application. Both the cup plate method and disc diffusion methods confirmed that the extract and its cream formulations were effective against Escherichia coli and Staphylococcus aureus, with E. coli showing slightly greater potential activity, Among the tested methods.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the support provided by the department of pharmaceutics and pharmacognosy, Pulla Reddy Institute of Pharmacy/JNTUH University for offering the necessary facilities and infra structure to carry out this research work. We extend our sincere thanks to our supervisor D. Prathyusha and A. Madhu bindu, for their valuable guidance, encouragement and constructive suggestions throughout the study.

REFERENCES

1. Biljana Bauer Petrovska; HISTORICAL REVIEW OF MEDICINAL PLANTS USAGE; Pharmacognosy reviews, 6 : 11.
2. Mariangela Marrelli; MEDICINAL PLANTS; 2021, Volume.10; https://doi.org/10.3390/plants10071355.
3. Sheikh Ashikur Rahman; ANTIBACTERIAL AND CYTOTOXIC ACTIVITY OF SEEDS OF WHITE HYACINTH BEAN (Lablab purpureus L. sweet white); JABET, 2018; 1(2): 49-54.
4. Brigitte L. Maass, Maggie R. Knox, S.C. Venkatesha, Tefera Tolera Angessa, Stefan Ramme, Bruce C. Pengelly; LABLAB PURPURUES- A CROP LOST FOR AFRICA; Tropical Plant Biol., 2010; 3: 123-135. DOI 10.1007/s12042-010-9046-1.
5. Ali Esmail Al- Snafi; THE PHARMACOLOGY AND MEDIAL IMPORTANCE OF DOLICHOS LABLAB (Lablab purpureus)- A REVIEW, Feb 2017; 7, Issue 2 Version.1: 22-30.
6. Mostafa E. Elshobary, Nadia K. Badawy, Yara Ashraf, Asmaa A. Zatioun, Hagar M. Masriya Mohamed M. Ammar, Nourhan A. Mohamed, Sohaila Mourad and Abdelrahman M. Assy; COMBATING ANTIBIOTIC RESISTANCE: MECHANISMS, MULTIDRUG- RESISTANCE

PATHOGENS AND NOVEL THERAPEUTIC APPROACHES: AN UPDATED REVIEW; Pharmaceuticals, 2025; 18(3): 402. https://doi.org/10.3390/ph18030402.

7. Chowdhury M. Hasan, Debprasad Dutta and An N. T. Nguven; REVISITING ANTIBIOTIC RESISTANCE: MECHANISTIC FOUNDATIONS TO EVOLUTIONARY OUTLOOK; Antibiotics, 2021; 11: 40. https://doi.org/10.3390/antibiotics110110040.
8. Engeline Van Duijkeren, Anne – Kathrin Schink, Marilyn C. Roberts, Yang Wang, Stefan Schwarz; MECHANISMS OF BACTERIAL RESISTANCE TO ANTIMICROBIAL AGENTS; https://journals.asm.org/journal/spectrum on 3 July 2025 by 2409: 40f0:1d:9f7c:1596:7230:5318: a3bd.
9. Satish Dubey, Ashwini Kumar Dixit; PRECLINICAL EVIDANCE OF POLYHERBAL FORMULATIONS ON WOUND HEALING: A SYSTEMATIC REVIEW ON RESEARCH TRENDS AND PERSPECTIVES; Journal of Ayurveda and Integrated Medicine, 2023; 14: 100688; https://doi.org/10.1016/j.jaim.2023.100688.
10. Sandhiya V and Ubaidulla U; A REVIEW ON HERBAL DRUG LOADED INTO PHARMACEUTICAL CARRIER TECHNIQUES AND ITS EVALUATION PROCESS, 2020; 6: 51.
11. Neha Singh, Meenakshi Garg and Rajni Chopra; AN OVERVIEW OF HERBAL FORMULATIONS: FROM PROCESSING TO PHARMACOVIGILANCE; Singh et an., ijpsr, 2023; 14(2): 562-578.
12. Ghongade Govind, APTE Madhavi; REVIEW OF HERBAL DRUG FORMULATIONS AND ITS EVOLUTIONS, Jan-Mar. 2019; 8(1).
13. Md. R. Hossain, Azharul I. Biplob, Syeda r. Sharif, Abdul M. Bhuiya, Abu Sadat M. Sayem; ANTIBACTERIAL ACTIVITY OF GREEN SYNTHESISED SILVER NANO PARTICLES OF Lablab purpureus FLOWERS EXTRACT AGAINST HUMAN PATHOGENIC BACTERIA; Trop J Nat Prod Res, August 2023; 7(8): 3647-3651.
14. Priya S, Jenifer S; ANTIBACTERIAL ACTIVITY OF LEAF AND FLOWER EXTRACT OF Lablab purpureus AGAINST CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS, 1(2).
15. Dilara Parvin, Mir Muhammad Nasir Uddin, Md.Siddiqul Islam, Salma Parvin, Mohammad Shahriar; PHYTOCHEMICAL SCREENINGS, THROMBOLYTIC ACTIVITY AND ANTIMICROBIAL PROPERTIES OF LEAF EXTRACTS OF LABAB PURPUREUS, 2013; 1(2).
16. Usha Vishwakarma, Kumar Suryanshu, Harsh Badode, Gulshan Dhakad, Hariom Parihar, Jayash Kumar Underiya, Dr. Jagdish Rathi; PHYTOCHEMICAL ANALYSIS OF LABLAB PURPUREUS L.SEED EXTRACTS AND LEAVES; 2023IJCRT, November 2023; 11(11).
17. Tanesh Sahu, Tarun Patel, Sagar Sahu, Bina Gidwani; SKIN CREAM AS TOPICAL DRUG DELIVERY SYSTEM: A REVIEW; Sahu et al., J Pharma Bio1 Sci., 2016; 4(5): 149-154.
18. Jayashree Modi, Sunayaya Rathore, Sunil Dwivedi, Gauravkant Saraogi; FORMULATION AND EVALUATION OF MULTIPURPOSE HERBAL CREAM; International Journal of Newgen Research in Pharmacy & Healthcare, June 2024; 2.