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PREPARATION, CHARACTERIZATION, PHYTOCHEMICAL ANALYSIS, ANTIBACTERIAL ACTIVITY AND WATER PURIFICATION OF HERBAL BEADS USING *LABLAB PURPUREUS* PEEL EXTRACT

Pooja K[1], Reshma A[1] and M. I. Niyas Ahamed[2]*

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Abstract

The objective of the current study is to conduct a phytochemical analysis, evaluate the antibacterial activity, and assess the effectiveness of Lablab purpureus L. peel extracts in water purification. Firstly, the extraction process is carried out to separate various substances by the use of a solvent. Following that, a phytochemical examination was conducted to determine the presence of different active ingredients in the extracts. The plant's phytochemical study reveals the existence of diverse constituents including sugars, alcohols, phenols, steroids, essential oils, alkaloids, tannins, flavonoids, saponins, coumarins, terpenoids, pigments, glycosides, and anthranoids. Lablab purpureus L. is a member of the fabaceae family and is commonly known as hyacinth bean, Dolichos bean, Seim bean, Egyptian bean, Kidney bean, and Indian bean. The antibacterial activity of hyacinth bean peel extracts against pathogenic bacteria, including gram positive (Staphylococcus aureus) and gram negative (Escherichia coli) bacteria, was evaluated under laboratory settings. Conducting a water purification analysis to effectively treat and reuse wastewater can help alleviate water constraint. The combination of population growth, heightened economic activity, and industrialization has led to a surge in the need for freshwater, as well as significant mismanagement of this valuable resource.

Keywords: *Lablab purpureus L*, Hyacinth bean peels, water treatment, Dolichos bean.

1 Introduction

For centuries, medicinal plants have played a crucial role in various cultures, serving as primary healthcare remedies. The knowledge surrounding these plant-based treatments likely evolved through trial and error, with essential cures passed down through generations. Different folkloric groups have attributed various common or local names to these plants, reflecting their widespread use across communities [1].

Water purification is essential for removing harmful chemicals, biological contaminants, suspended solids, and gases from contaminated water to make it safe for specific purposes. While most water purification focuses on disinfecting water for human consumption, it can also cater to medical, pharmacological, chemical, and industrial needs. Methods such as filtration, sedimentation, distillation, and chemical processes like flocculation and chlorination, as well as biological processes such as slow sand filters, are employed [2]. The process aims to reduce the concentration of particulate matter and dissolved materials, ensuring compliance with drinking water quality standards set by governments or international bodies.

Hyacinth Bean, a versatile crop grown for its beans, flowers, and leaves, finds extensive use in Africa, Asia, and the Caribbean. It serves various purposes such as food, forage, and soil enrichment through nitrogen fixation. Additionally, it's popular as an ornamental crop in the cut flower industry due to its vibrant flowers and purple peapods. Its twining vine nature makes it suitable for growth on sturdy trellises, preferably in well-drained, sunlit areas [3].

The plant's extracts have been utilized in skincare, with reported benefits for conditions like eczema. Similarly, Dolichos Bean, known as Lablab purpureus, has been cultivated for centuries due to its nutritional value, particularly its protein-rich immature green pods. In regions with protein deficiencies, this crop serves as a valuable food source and fodder [4-6]. It's also intercropped with other crops in tropical and subtropical regions, contributing to agricultural diversity and sustainability. Recent research focuses on utilizing Dolichos lablab peel powder as a natural coagulant for water treatment, aiming to determine optimal dosages and assess its effectiveness in treating water from Krishna Sagara Lake and wastewater, potentially offering eco-friendly solutions to water purification challenges [7].

***Corresponding author: e-mail** driniyasahamed@shctpt.edu,
[1,2]Department of Biochemistry, Sacred Heart College (Autonomous), Tirupattur - 635601, Tamilnadu, India.

2.3 Qualitative Phytochemical

Screening Phytochemical Analysis

The plant extract solutions were assessed for the existence of the phytochemical analysis by using the following standard methods [9-11].

2.3.1 Test for proteins

Millon's test: Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Ninhydrin test: Crude extract when boiled with 2 ml of 0.2% solution of Ninhydrin, violet color appeared suggesting the presence of amino acids and proteins.

2.3.2 Test for carbohydrates

Fehling's test: Fehling's test involves mixing equal volumes of Fehling A and Fehling B reagents, adding 2 ml to crude extract, and gently boiling it. The presence of reducing sugars is indicated by the appearance of a brick-red precipitate at the bottom of the test tube.

Benedict's test: As 2 ml of Benedict's reagent was added to crude extract and heated, a reddish-brown precipitate developed, signifying the presence of carbohydrates.

Molisch's test: 2ml of Molisch's reagent were combined with crude extract, and the mixture was thoroughly shaken. Next, a cautious 2 ml of concentrated H_2SO_4 was gently poured along the test tube's side. The presence of carbohydrates was suggested by the appearance of a violet ring during the interphase.

Iodine test: There was a mixture of 2 ml of iodine solution and crude extract. The presence of the carbohydrate was identified by a dark blue or purple coloring.

2.3.3 Test for phenols and tannins

Crude extract was combined with 2ml of a 2% $FeCl_3$ solution. Phenols and tannins were characterized by a blue-green or black coloring.

2.3.4Test for flavonoids

Shinoda test: Concentrated HCl and a few pieces of magnesium ribbon were combined with crude extract was added drop wise. Pink, scarlet color appeared after a few Crude extract was mixed minutes which indicated the presence of flavonoids.

Alkaline reagent test: 2ml of a 2% NaOH solution was used to cure. When a few drops of diluted acid were added, the bright yellow color that had formed went colorless, indicating the presence of flavonoids.

2.3.5 Test for saponins

In a test tube, 5 ml of distilled water was combined with crude extract, and the mixture was agitated vigorously. It was believed that the production of stable foam indicated the presence of saponins.

2.3.6 Test for glycosidesLiebermann's test: 2ml of acetic acid and chloroform were combined with the crude extract. Ice was used to chill the concoction. A precise concentration of H_2SO_4 was introduced. When the hue changed from violet to blue to green, it meant that the glycone part of the glycoside, or the steroidal nucleus, was present.

Salkowski's test: Chloroform (2 ml) was combined with crude extract. After that, 2 ml of concentrated H_2SO_4 was added and given a gentle shake. The existence of a steroidal ring, or the glycone portion of the glycoside, was indicated by a reddish-brown colour.

Keller-Kilani test: Crude extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2 ml of concentrated H_2SO_4.The presence of cardiac glycosides was indicated by a brown ring at the interphase.

2.3.7 Test for steroid

Additions of concentrated H_2SO_4 were made after the crude extract and 2 ml of chloroform were combined. The presence of steroids was identified by a red hue formed in the bottom chloroform layer. Another experiment involved combining 2 millilitres of chloroform with crude extract. Next, the mixture was added to 2 millilitres of concentrated $H_2SO_4$and acetic acid, respectively. Steroid presence was revealed by the greenish color was developed.

2.3.8 Test for terpenoids

After dissolving the crude extract in 2ml of chloroform, it was dried out. After adding 2 ml of concentrated H_2SO_4, this was boiled for approximately 20 minutes. Terpenoids were indicated by a greyish tint.

2.3.9 Test for alkaloids

2ml of 1% HCl was combined with crude extract and heated gradually. The mixture was then supplemented with Wagner's and Mayer's reagents. The ensuing precipitate's turbidity was seen as proof that alkaloids were present.

2.4 Preparation of herbal beads

The sample will be collected in dried form. Take 50ml of 2-2.5% w/v sodium alginate. Add 50mg of sample. Mix it well together. Take glass syringe (size 22 needle) and fill the mixture. Now in 100ml of $CaCl_2$ leave the mixture in drop wise into it. The wet beads will form and allow it to dry in beads form.

2.5 Characterization of herbal beads

The prepared herbal beads will be characterized for its physico-chemical property includes

- UV-Visible Spectroscopy
- Fourier Transform Infrared (FT-IR) Spectroscopy

Finally novel herbal beads will be evaluated as Water Purifier in Column Experiment.

2.6 Antibacterial activity

Antibacterial screening of crude extracts was tested by the agar disc diffusion method. Two pathogenic bacterial strains including one gram- negative and one gram-positive bacteria were chosen for testing as they were maintained on nutrient agar media by streak plate method. Standard antibiotic was used as positive control and negative discs was used as negative control. Nutrient agar plate was prepared by pouring 15 ml nutrient agar media into Petric plates (100mm x 15 mm). After solidification of nutrient agar media, the media was inoculated with bacteria, cultured previously on liquid broth. The sample discs, antibiotic discs, negative control discs were gently placed on to bacteria-inoculated nutrient agar plate. The plates were inversely kept in an incubator at 37^0C for 24 hours. The antibacterial activity was determined by measuring the diameter of the zone of inhibition [12].

2.7 Water purification property

The preparation of the stock solution of the standard involved dissolving 10 mg of potassium permanganate (KMnO4) at a concentration of 1M in 100 ml of double-distilled water. In order to reduce the amount of turbidity in water, the stock solution is diluted, and the herbal beads that have been manufactured are employed [13].

3 Results and Discussion

3.1 Phytochemical analysis of peel extract:

Qualitative Phytochemical analysis was performed to determine the presence of phytocomponents on the aqueous extract of *Lablab purpureus* peel

Table 1: PHYTOCHEMICAL ANALYSIS OF AQUEOUS EXTRACT AND ETHANOL EXTRACT OF *Lablab purpureus* PEEL EXTRACT

SI.NO.	PHYTOCHEMICALS	AQUEOUS EXTRACT
1.	Carbohydrates	+
2.	Tannins	+
3.	Saponins	-
4.	Alkaloids	+
5.	Flavonoids	-
6.	Glycosides	+
7.	Quinones	+
8.	Phenols	-
9.	Terpenoids	+
10.	Steroids	+

[Symbol (+) indicates positive and (-) indicates Negative]

To detect the presence of several Phytochemicals, namely carbohydrates, phenols, flavonoids, quinones, steroids, tannins, saponins, terpenoids and alkaloids. The results are given in Table 1. Carbohydrates, tannins, alkaloids, glycosides, quinones, terpenoids and steroids were present in the aqueous peel extract. The aqueous extract doesn't contain the saponins, flavanoids and phenols [14].

AQUEOUS EXTRACT

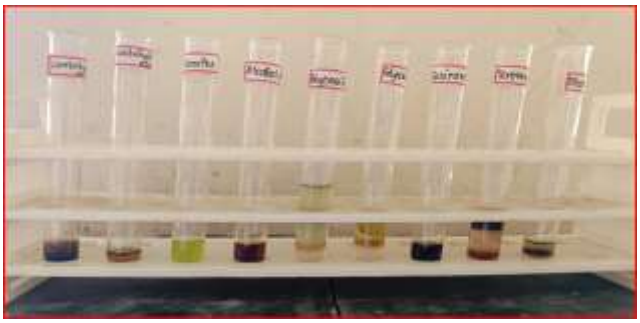


Figure 1: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF AQUEOUS PEEL EXTRACT OF *Lablab purpureus*

3.2 PREPARATION OF HERBAL BEADS USING *Lablab purpureus* peel

The herbal beads were prepared for the purification of water turbidity using *Lablab purpureus* peel which has extremely high properties in water purification [15-18]. The phytocomponents present in the plant helps in purifying the water turbidity. The prepared herbal beads were measured an average size of 4mm.

Figure 2: Dried beads

Figure 3: Size of a bead- 4mm

3.3 UV- Visible Spectroscopy

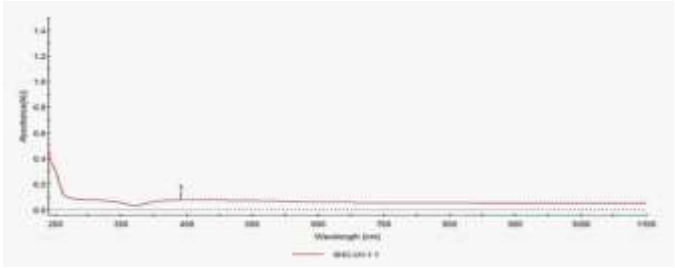


Figure 3: UV-Visible Spectroscopy

UV-visible absorption spectroscopy is an analytical technique that measures the number of discrete wavelengths of UV or visible light that absorbed by or transmitted through a sample [19-20]. The Lablab purpureus beads absorption spectra shown in graph. It has an observing absorption band at about 391.3 nm.

Table 2- UV – Visible absorption spectrum of herbal beads.

Name	Peak(nm)	Peak (AU)
Herbal Beads	391.3	0.08

3.4 FTIR Spectrum of herbal beads

The Fourier-transform infrared spectroscopy analysis shows the functional group present in the Herbal beads.

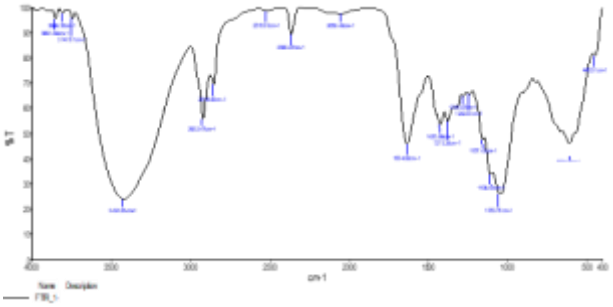


Figure 4: FT-IR Spectroscopy graph

Table.3 FT-IR Spectrum of *Lablab purpureus* herbal beads

S. No	Absorption Band (cm^{-1})	Indication
1.	3741.21 (cm^{-1})	O-H stretching
2.	3424.35 (cm^{-1})	N-H stretching
3.	2922.47 (cm^{-1})	S-H stretching
4.	2365.67 (cm^{-1})	O=C=O bending
5.	2856.40(cm^{-1})	S-H stretching
6.	2050.48 (cm^{-1})	N=C=S stretching
7.	1634.82 (cm^{-1})	C=C stretching
8.	1425.84 (cm^{-1})	C-H bending
9.	1379.36 (cm^{-1})	O-H bending
10.	1281.57 (cm^{-1})	C-O stretching

3.5 Antibacterial activity

Antibacterial screening of crude extracts was tested by the agar disc diffusion method. Two pathogenic bacterial strains including one gram- negative (*Escherichia coli*) and one gram-positive *Staphylococcus aureus*) bacteria were chosen for testing as they were maintained on nutrient agar media by streak plate method [21-23]. Standard gentamycin was used as positive control and DMSO was used as negative control. Nutrient agar plate was prepared by pouring 15 ml nutrient agar media into Petric plates (100mm x 15 mm). After solidification of nutrient agar media, the media was inoculated with bacteria, cultured previously on liquid broth. The sample discs, antibiotic discs, negative control discs were gently placed on to bacteria-inoculated nutrient agar plate. The plates were inversely kept in an incubator at 37^0C for 24 hours. The antibacterial activity was determined by measuring the diameter of the zone of inhibition.

ANTIBACTERIAL ACTIVITY OF *Lablab purpureus* PEEL EXTRACT AND HERBAL BEADS

(Figure 5: *Staphylococcus aureus* and *Escherichia coli*)

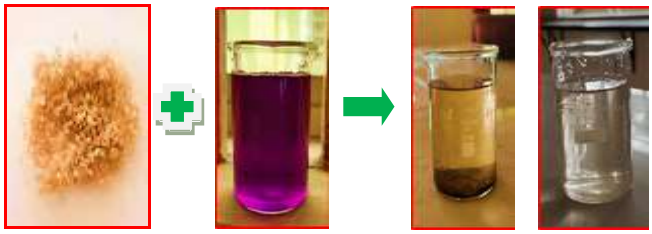
The above result shows the inhibition of microorganisms in positive control is greater compared to the aqueous extract and herbal bead of *Lablab purpureus* peel [24-27].

Table 4- Antibacterial Activity of *Lablab purpureus*

Particulars	*E.Coli*	*S.aureus*
***Lablab purpureus* Beads**	-	-
***Lablab purpureus* Aqueous Extract**	25 mm	-
Positive control (Gentamicin)	13 mm	25 mm

Water Purification Property

The water purification property of *Lablab purpureus* herbal beads was proven by the standard KM4nO solution. Compared to the absorption noticed on the day 1, day 2 interaction studies highlighted the maximum absorption of $KMnO_4$.Thus I conclude that herbal beads prepared using Lablab purpureus peel has significant property of water purification [28-30].



Lablab purpureus beads (Day 1) Stand $KMnO_4$ Purified water $KMnO_4$ + Beads

Figure-6 purification process

Table-5 Colorimetric Analysis

	Standard KMnO4	$KMnO_4$+ Beads (Day 1)	$KMnO_4$+ Beads (Day 2)
OD at 540 nm	0.91	0.21	0.02

4 Conclusions

An aqueous extract of *Lablab purpureus* was made in accordance with the existing literature. Both the phytochemical analysis and the preparation of the herbal beads for the research investigation were finished. By using UV-visible spectroscopy, the signature absorption peak at 391.3 nm was verified. The chemical components contained in the produced herbal beads were thoroughly elucidated by FTIR spectroscopy. When compared to the positive control (gentamicin), the microorganisms, specifically *E. coli* and *Staphylococcus aureus*, showed no antibacterial action. *Lablab purpureus* herbal beads were found to have the ability to purify water when tested with the standard KM4nO solution. Analyses of phytochemicals, herbal bead formation, ultraviolet-visible spectroscopy, Fourier transform infrared spectroscopy, antimicrobial, and water purification activities of *Lablab purpureus* peel were conducted on Day 2 of the interaction investigations, which revealed the highest absorption of $KMnO_4$.

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Conflict of Interest:

None Declared.

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