



Antibacterial Property of Crude extract of Phaseolus Vulgaris Plant Against Pathogenic E. Coli Causing Urinary Tract Infections (UTI)

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Abstract:- Urinary Tract Infections (UTI) are common infections that happen when bacteria, often from the skin or rectum, enter the urethra, and infect the urinary tract. The infections can affect several parts of the urinary tract. There are many medicinal plants which have shown antibacterial property against the pathogenic *E. Coli* causing UTI. **Objectives:** The antibacterial activity of the crude extract of plant *phaseolus vulgaris* (Green Bean) was observed against the UTI causing pathogenic *E.Coli* using Ethanolic extract and Distilled water extract at different concentrations to investigate its antibacterial activity. **Results** The antibacterial activity of *phaseolus vulgaris* crude extract was more pronounced against pathogenic *E-coli* in ethanolic extract. The inhibition activity was 17.00 mm at 20 μ l 22.00mm at 40 μ l and 25.00mm at 60 μ l gradient while distilled water extract didn't showed any Antibacterial activity. Presence of Phenols, Quinone, Alkaloids, Coumarin Tanins and Gums the phytoconstituents present in the crude extract were studied by chemical analysis. All were present except Tanin. **Conclusion:** *P. vulgaris* provides lead towards the exploration of antibacterial properties against UTI and other infections. The further enhancement in this study can be beneficial for treatment strategies for UTI.

Key Words: *Phaseolus vulgaris*, Antimicrobial activity, Urinary Tract Infection, Ethanolic Extract, Antibiotics

1.0 Introduction

Urinary Tract Infections (UTI) is a non-contagious infection. In UTI, any part of the urinary system which includes- kidneys, bladder, ureters, and urethra are infected. On the basis of infection area UTI is divided into two types: Bladder infection (cystitis) where the infection is in lower urinary tract, pelvic pressure, frequent painful urination, blood in urine and the other is Kidney infection (acute pyelonephritis) if the infection affects the upper section of urinary tract, causing the Back pain or side pain, High fever, Chills and Shaking, Nausea, Vomiting, Urethra (urethritis) - Burning with urination, Urine discharge, Vaginitis-infection of vagina (*Chandra et al.*).

As per the studies done till now, the average urine the human body excretes per day is 1.4L. and the normal pH value of urine is 5.8; colour- pale yellow to deep amber; bilirubin, RBC, protein and pus cells remain absent in Urine in normal conditions (*Schaffer et al.*). In case of any slight infection the characteristics of urine gets

altered and colour changes to deep yellow, cloudy, turbidity becomes high, The patient has strong urge of urination, burning sensation while urinating, passing frequent and small amount of urine, , strong-smelling urine accompanying pelvic pain, in women-especially in centre of pelvis and around the area of pubic bone. These symptoms indicates Urinary Tract Infection (***Sheffield et al.***).

If we look at the ratio of infection then almost 10% of the total world population experiences UTI at least once in their lifetime. In case of females the ratio is 1:2 whereas in males the ratio is 1:10. Females suffer more from UTI because of: **Female anatomy** having shorter Urethra i.e. approx(4.8-5.1)cm in length than in men i.e (18 to 20) which shortens the distance to be covered by bacteria to travel upto bladder, **Sexual activity-** Sexually active women tend to have more UTI than do women who aren't sexually active. **Birth control-** Women who have diaphragms for birth control may be at higher risks(***Sheffield et al.***). After menopause, a decline in circulating estrogen causes changes in urinary tract that makes you more vulnerable to infection.

Treatment of the UTI requires a good understanding of pathogenesis and the relation of host and bacteria. Low water intake is one of the reason for UTI, as it leads to the less flushing out of toxic material from the urinary tract. Consuming adequate amount of water during day time becomes less susceptible to the UTI as frequent urination cleans the urinary tract and reproductive organs making it less prone to the area for bacterial growth. (***Sheffield et al, Mambatta et al.***). The urogenital tract opening is near to anus is prone area of bacteria and helps the bacteria to transfer to urinary tract easily. (***Chandra et al, Schaffer et al.***). Till now it has been confirmed that the bacteria responsible for UTI includes:, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp.*, *Proteus spp.*, *Pseudomonas spp.*, *Enterococcus faecalis*, *Morganella spp.*, *Providencia spp.*, *Mycoplasma spp.*, *Staphylococcus aureus*, *Citrobacter spp.*, Out of these mentioned bacterias, *Escherichia coli*, *Proteus mirabilis* are the common bacterias responsible for UTI(***Ashraf A. Mostafa et al***)

2.0 Materials and Methodology

2.1 Site of Implementation of Study: The whole study was conducted in DNA Labs – A Centre for Applied Sciences, Dehradun, Uttarakhand , A molecular and Pathology Diagnostic and Allied sciences Research Laboratory.

2.2 Plant Used for the study

Phaseolus vulgaris belongs to the Family Fabaceae. Its generic name is ‘Phaseolus, introduced by Linnaeus in 1753, from the Latin *phaseolus*, a diminutive of *Phaseolus* turn borrowed from Greek φάσηλος 'cowpea', (**Loko et al**). It is also glossed as ‘kidney bean’, a new world crop. It is a herbaceous to woody annual and perennial vines in family Fabaceae containing about 70 plant species, all native to Americas, primarily Mesoamerica. (*Phaseolus vulgaris L.*) is an important grain legume crop grown in the central region of the Republic of Benin. (**Loko et al**). Its variants are nutritionally and economically important. (***Anderson et al, Loko et al***). Besides providing nutrition such as multifaceted carbohydrates, elevated proteins, dietary fiber, minerals, and vitamins, these also contain very rich variety of polyphenolic compounds with prospective health benefits.

2.3 Collection of Plant Sample

Plant sample *Phaseolus vulgaris* – green bean, were collected during the daytime from wild ,race course area, dharampur Dehradun, on 16 November 2021. Dehradun valley lies between latitude 30.345°N and longitude 78.029°E coordinates. When the plants were collected the temperature was ~ 19°C, and humidity was ~ 62%, 30gms of beans were collected for the crude phytoconstituents identification and antibacterial activity against

UTI causing *E.Coli*. Master plate of Escherichia coli (*E.Coli*) was obtained from DNA Labs- A Centre for Applied Sciences, Dehradun, Uttarakhand.

2.4 Plants Extract Preparation

The green bean were weighed in weighing machine. The collected plants were watery washed, disinfected with insecticides and bacteriosides, rinsed with distilled water and finally dried in shade with traditional method.. Each day the weight of bean was measured and once completely dried were grounded into a fine powder to pass 100 mm sieve(**Table No 1and Figure No 1**).

No. of days	Weight of the beans(grams(g)
Weight on the day of Sample collection	30.00 g
1 st day	27.22 g
2 nd day	22.36 g
3 rd day	17.00 g
4 th day	13.22 g
5 th day	11.25 g
Weight of Powdered leaves	11.00 g

Table No 1: Weight of beans recorded for 5 continuous days along with weight of powder

Depiction of decrease in weight of beans in duration of Drying

3.3 Solvent Extraction of *P.Vulgaris*

3.1.1 Ethanolic Extract and Distilled water Extract

10gm of dried powder was added into 35ml of EtOH Ethanol (1:7 w/v) and Distilled mineral water extract by 10gm of powder of sample was submerged into 50ml of Distilled water (10:50). with stirring for 48 h in Shaker,with intermediate shaking, filtered through double layers of muslin, centrifuged at 9000 rpm for 10 min and finally filtered again through Whatman filter paper No. (41) to attain a clear filtrate.

The filtrates were evaporated and dried at 40 °C under reduced pressure using rotatory vacuum evaporator. The extract yields were weighted, stored in a small bottles in fridge at 5 °C.

4.0 Test for Phytoconstituent Present in *P.Vulgaris*

For the phytoconstituent study the filtered extract obtained was used to study the presence of Phytoconstituents.

Phenols(ferric chloride test) or (Brayer's test)- Add in extract 3-4 drops of ferric chloride and the presence of Blue-black colour indicates presence of phenols. **Tannins**, Ferric chloride Add in 1 ml of extract, 0.1% ferric chloride and the result will be Brownish green or Blue-black colour. **Lead Acetate**, add in 2ml of extract, 1% lead acetate and the result will be yellow brown. **Terpenoids**, (lieberman test) –In 1ml extract add ethanol/acetic anhydride and few drops of H₂So₄ and the result will be Pink/violet colour. **Coumarin**, In 10%

of NaOH add chloroform and the result will be yellow colour. **Quinone, (HCL test)** In few ml of Extract add conc HCL and the result will be yellow colour precipitate. **Gums**, add few ml of H₂O and 2 ml of extract and it will swell up or adhesiveness.

Table 4: Different Gradient on Which Testing was Done

SPECIFICATION	<i>E.coli</i>
Media used	NAM
Incubation time	20min
Temperature	35°C

5.0 Bacterial Strains

E.Coli pure culture was obtained from DNA Labs- A Centre for Applied Sciences, Dehradun

5.1 Inoculum preparation

E.Coli bacterial strain was subcultured overnight at 35 °C in MacConkey and NAM Media,. The bacterial growth was harvested using 5 ml of sterile saline water, its absorbance was adjusted at 580 nm and diluted to attain viable cell count of 10⁷ CFU/ml using spectrophotometer.

Table 3 Conditions and Media provided for Bacterial Culture

Extracts	Gradient I	Gradient II	Gradient III
Ethanolic Extract	20µl	40µl	60µl
Distilled Water Extract	20µl	40µl	60µl

5.2 Antibacterial activity of plants extract

The disk diffusion method is used to evaluate antimicrobial activity of each plant extract. DMSO (dimethyl sulphoxide). For ethanolic extract 10 µL of crude was added in 10 µL of DMSO. DMSO is added in the crude because it is polar aprotic solvent that dissolves both polar and non-polar compounds due to which the results are effectively shown for distilled water extract 10 µL of crude phytochemical was added in 10µL of DMSO in the MCT for proper storage. The antibacterial activity of *Phaseolus vulgaris* distilled water and ethanolic extracts were evaluated by agar well diffusion method. The bacteria were streaked over the entire surface evenly. Well, were punched on the bacterial plate using sterile borer of 8mm. the plates were allowed to dry for 5 minutes. Extracts each of 20µL, 40µL, and 60 µL were dispensed into each well using sterile micro pipette. DMSO was used as positive control and overnight incubation was given at 37°C. After 24 hours, the antibacterial activity was determined by measuring the diameter of zone of inhibition. These compounds were used in different gradients for the testing of the effectiveness of the phytochemicals/Phytoconstituents and observe the difference in the inhibition zone.

6.0 Result and Discussion

6.1 Phytoconstituent Present in *P. Vulgaris*

Phenols, Tanins were absent while The formation of yellow coloured precipitate showed the presence of Quinone. The adhesiveness appearance indicated the presence of GUMS, The appearance of yellow colour at the end of test detected the presence of Coumarin. The creamy precipitate formation at the end of test indicated the presence of Alkaloids .

Table:6 Result of Phytoconstituents present in *P. Vulgaris*

S.no	PHYTOCONSTITUENTS	INFERENCE
1	Phenols	Positive(-ve)
2	Tannins	Negative(-ve)
3	Quinone	Positive(+ve)
4	Alkaloids	Positive (+ve)
5	Coumarin	Positive (+ve)
6	Gums	Positive (+ve)

In the present study we demonstrated anti-bacterial activity of *Phaseolus vulgaris*. However, detailed study is required to find out specific bioactive compounds responsible for anti-bacterial and anti-microbial property through various advanced technique. Data gives insight about the anti-microbial activity of medicinal plants, an alternative to the antibiotics. In the era of modern medicine, drugs of plant origin are widely being produced and used access and also cheaper than the one synthesized in the laboratory in artificial circumstances.

Figure: 1



Figure: 2





Figure: 3



Figure:4

Figure 1-4 Phytoconstituent test Results

6.2Anti-bacterial activity of *Phaseolus vulgaris*

Anti-bacterial activity of *Phaseolus vulgaris* against different UTI causing bacterias was concluded. The antibacterial activity and inhibition of bacterial growth of plant extract were examined against bacterial strain *E.coli*. The inhibition zone of the activity of the plant solvent was calculated with the help of diameter measuring laboratory scale.

Antibacterial properties for solvents extract of *Phaseolus vulgaris* was against UTI causing gram bacteria. *Phaseolus vulgaris* is a valuable plant source of antimicrobial as well as anti-bacterial properties which will be used in integrated with some antibiotics to treat many bacterial and fungal diseases. The extract shows its antibacterial activity against UTI causing *E.coli*, bacteria. In agar well diffusion assay, the average zone of inhibition of ethanolic extract against *E.coli*, is 17.00 mm, 22.00mm, 25.00mm respectively. Whereas distilled water extract solvent didn't show antimicrobial activity.



Fig 5: Zone of Inhibition of ethanolic extract and distilled water extract

The result obtained from the sample extract of solvents are represented as follows:

Table 7: Inhibition zone in different gradients against *E.coli*

EXTRACTS	20µL	40µL	60µL
	CRUDE + DMSO	CRUDE + DMSO	CRUDE + DMSO
ETHANOLIC EXTRACT	17mm	22mm	25mm
DISTILLED WATER EXTRACT	-VE	-VE	-VE

7.0 Discussion and Conclusion

Phytochemical compounds are present in various plants which are used in treatment of infectious or chronic disease for a long time, plant extracts have antimicrobial feature. Plants have a good therapeutic potency (*Sasidharan et al.*).

In this study, *Phaseolus vulgaris* plant extract and its activity proved to have anti-bacterial potency against the *E.coli* bacteria. In today's time, antibiotics are broadly used to treat different infectious diseases. Antibiotics have more side effects and their excessive use has made the bacterias more resistant against the antibiotic. In this study one idea can reduce the bacterial growth. We concluded that many types of antibiotics can be integrated with *Phaseolus vulgaris*, plant extract. This plant extract with different solvent gradient will have huge potential to enhance the antimicrobial and antibacterial properties against many bacteria's and fungal species which are responsible for many dangerous diseases like UTI. The study showed that some solvent gradients extracted from the *Phaseolus vulgaris* showed some low antibacterial activity whereas some showed good antibacterial activity. Ethanolic extract showed the best results against *E.coli*. In today's scenario, it can be concluded that in coming future people will trust herbal and plant-based medicines and products and will believe in Ayurveda as done in ancient times.

Conflict of Interest: None

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