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Exploring the Antibacterial Potential of *Tephrosia Purpurea* Extracts Against Tomato Spoilage Pathogens

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KEYWORDS

Tephrosia purpurea; Tomato spoilage bacteria; Antibacterial activity; Chloroform extract; MIC; Post-harvest preservation

ABSTRACT:

Tomato (Lycopersicum esculentum), a nutritionally rich vegetable widely consumed in raw and cooked forms, is highly susceptible to microbial spoilage due to its high moisture content. Spoilage caused by bacteria and fungi results in undesirable changes in taste, texture, and overall quality, leading to significant post-harvest losses. The present study aimed to evaluate the antimicrobial potential of different parts (stem, leaf, seed, and flower) of Tephrosia purpurea, a medicinal plant belonging to the family Fabaceae, against selected tomato spoilage bacteria. Plant extracts were prepared using aqueous, chloroform, and ethyl acetate solvents and assessed for antibacterial activity against Bacillus subtilis, Klebsiella pneumoniae, and Pseudomonas aeruginosa using the disc diffusion method. Minimum inhibitory concentration (MIC) assays were also performed to determine the potency of the extracts. Among the tested solvents, the chloroform extracts exhibited the highest antibacterial activity compared to aqueous and ethyl acetate extracts. Notably, chloroform seed and leaf extracts showed maximum zones of inhibition against B. subtilis and K. pneumoniae, while significant activity was also observed against P. aeruginosa. MIC analysis further confirmed the superior efficacy of chloroform extracts, particularly the seed extract, which demonstrated strong inhibition at lower concentrations. In contrast, aqueous and ethyl acetate extracts showed comparatively moderate activity. Overall, the findings suggest that chloroform extracts of Tephrosia purpurea, especially from seeds and leaves, possess potent antibacterial properties and may serve as a promising natural alternative for controlling tomato spoilage pathogens and enhancing post-harvest preservation strategies.

INTRODUCTION:

Agriculture is the backbone of our nation's economy. The role of agriculture in shaping the economy could be reflected in the large proportion of the population that depends on crops, which makes a significant contribution to raising the income of our nation. Tomatoes are consumed widely in both raw and processed forms (Moneruzzaman *et al*., 2008) and are enriched with carbohydrates, vitamins, and proteins. (Talvas *et al.*, 2010). It contains lower sugar content than other edible fruits. Tomatoes were not only used as food but also as a medicine, flavor ingredient, and

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antioxidant (Abhinaba, 2009). It has been estimated that about one-third of the tomatoes get spoiled before they reach the consumer (Mbajiuka and Emmanuel, 2014). *Tephrosia purpurea,* a medicinal plant, belongs to the family Fabaceae known as sharpunkha. It is distributed in India, Australia, China, and Sri Lanka up to 400 m to 1300 m altitude (Deshpandey *et al.,* 2003). It is one of the excellent gifts of nature, which contains all the essential phytochemicals that are required for both soil and good human health, and is being used as folk medicine because of its several properties, such as anticancer, antipyretic, antidiabetic, antiviral, and anti-inflammatory, etc. It is one of the most effective folk medicines for the treatment of inflammation as well as enlargement of the liver and spleen. This plant has also been used for the treatment of disorders related to the bowel, kidney, liver, and spleen (Zafar *et al.,* 2004; Rahman *et al.,* 1985). Several studies were carried out for the isolation of tomato spoilage organisms. Wogu and Ofuase (2014) isolated tomato spoilage bacteria from the tomatoes in Benin City, such as *Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella aerogenes, Salmonella typhi, Proteus mirabilis, and Staphylococcus aureus.* The current research was to analyse the antibacterial activity of T*ephrosia purpurea* Linn. against tomato spoilage pathogens.

1. Materials and Methods:

Plant Sample Collection:

Plant material of *Tephrosia purpurea* (Linn.), such as leaf, flower, stem, and seeds were collected. The plant was authenticated at the Rapinat Herbarium and Centre for Molecular Systematics, St.Joseph College, Tiruchirapalli-2, Tamil Nadu, India. The plant leaves, flowers, stem, and seeds were separated from the plant and dried in the shade. After drying, the plant materials were powdered and used for our studies.

Preparation of Plant extract:

The different parts of the *Tephrosia purpurea* plant leaf, flower, stem, and seeds were collected and dried at a temperature of 2-3 days and further dried at 60^0 C. The dried leaf, flower, stem, and seeds were powdered and extracted with solvents (Aqueous, Ethyl acetate, and Chloroform) separately and incubated at room temperature for 48 hours with stirring at regular intervals. The extract was filtered with the Whatman filter paper and then dried using a rotary evaporator. The filtrate was stored in a screw cap bottle at-200 °C for further use.

Isolation of Pathogens

Different microbial strains were evaluated to evaluate the antimicrobial effect of *Bacillus subtilis* (MTCC 121), *Klebsiella pneumoniae* (MTCC 96), and *Pseudomonas aeruginosa* (MTCC 2488). The strains were obtained from Jamal Mohamed College, Trichy, Tamil Nadu, India, and maintained in the agar slants.

Standardization of Inoculum:

In this study rapid inoculum preparation method was used to facilitate the estimation of MIC within 5 hours. Organisms from the semi-solid nutrient medium were inoculated into broth media. After 4 hours of incubation, a subculture was done on nutrient agar slant, which was then incubated overnight. The overnight cultures were used for the antibacterial activity.

Antibacterial activity test (Disc diffusion method):

Disc diffusion method was carried out for antimicrobial susceptibility testing according to the standard method to assess the presence of antimicrobial activities of the plant extract (Kavitha and Manoharan,2006; Kumar *et al.,* 2007; Anitha *et al.,* 2012). Muller-Hinton agar (MHA) plates were prepared. An overnight nutrient broth culture of test organisms was seeded over the MHA plates using a sterile cotton swab so as to make a lawn culture. The disc, which has been impregnated with aqueous extract of the leaf were placed on the MHA with the control disc and subjected to antimicrobial screening. The plates were incubated at 370 °C for 24 hours, depending on the species of bacteria used in this test. After the incubation, the plates were examined for an inhibition zone. Discs usually consist of absorbent paper impregnated with **an** antimicrobial agent. It was most convenient to use Whatsman No1 filter paper for preparing the disc. Dry discs of 6 mm diameter were prepared and sterilized in an autoclave. These paper discs were impregnated with the sample in bacterium. The plates were incubated at 37^0 C for 24 hours. The zone of inhibition around each disc was measured, and the diameter was recorded.

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Tryptone soya broth for the modified micro dilution method by incorporating phenol red indicator:

The tryptone soya broth for the modified micro dilution method by incorporating phenol red indicator was prepared with Pancreatic digest of casein (17 g), Papain's digest of soya meal (3g), Dibasic potassium phosphate (2.5g), and Glucose (2.5g). Instead of sodium chloride Phenol red solution of about 50 ml was added to this medium. The above ingredients were added to 950 ml of distilled water (for the dilutions of antimicrobial agents). Sterilization was done by autoclaving at 121^0 C for 20 minutes. The media was stored in the refrigerator at 4°C.

Preparation of 0.2% Phenol Red solution:

One gram of phenol red was dissolved in 10 ml of 0.1 M NaOH and 20 ml of distilled water. It was heated gently to dissolve all the phenol red, and to this, 10 ml of 0.1 M HCl was added. Then it was made up to 500 ml by adding distilled water. This gives a 0.2% Phenol red solution. For use, 5 ml of 0.2% solution was taken in 100 ml of media.

Preparation of Micro trays:

The microtitre trays were sterilized by immersing in 2% glutaraldehyde solution overnight sterilization and the plates were rinsed twice with sterile water and finally with sterile distilled water. The plates were dried under UV exposure. The sterilized plates were either used immediately or packed and kept at 4^0 C.

Preparation of antimicrobial agents:

Different concentrations of antibacterial agent (*Tephrosia purpurea*) ranging from 2µg/ml to 256µg/ml were prepared in the medium containing modified Tryptone soya broth.

Dispensing antimicrobial dilution into microtrays:

50 µl of different antimicrobial concentrations (*Tephrosia purpurea* stem, seed, leaf, and flower) from 2µg/ml to 256µg/ml were distributed with a calibrated dropping pipette or multichannel pipette. Modified Tryptone soya broth without the antimicrobial concentration and with bacterial suspension was added to the 12^{th} well in all rows, and it acts as growth control. 100 µl of tryptone soya broth without microorganisms, without antimicrobial concentration, was added to the 11^{th} well of the first row, which acts as a medium control.

Inoculation of the Trays

Before inoculation of the trays, the bacterial suspension was further diluted in a 1:50 dilution (0.1 ml of suspension in 4.9 ml of TSB media containing Phenol Red). From this, in 50 dilutions, 50 µl of inoculum was added to each well containing 50 µl of diluted antimicrobial agent. The final concentration should be approximately 5×10^5 CFU/ml (5×10^4 CFU well) because the antimicrobial agent was diluted 1:2 with the inoculum; the final concentration will be one-half that which was originally dispensed. For example, if the original concentration is 64 µg/ml, then after the addition of the inoculum, the concentration will be 32 µg/ml.

Incubation of the Trays

After the inoculation, the trays were stacked one above the other to minimize evaporation. These stacks were then placed in an aluminum box with tightly sealed tops. The base of each bin was covered with wet cotton, which gives the humidity in the box, and these boxes were placed in an incubator at 350 °C for 3 hours. The results were observed from the third hour onwards.

RESULT:

Tephrosia purpurea (Linn.) plants were collected (Figure-1) and assayed for antimicrobial activity by the disc diffusion method. The antibacterial activity was tested against leaf, flower, stem, and seed samples using MTCC bacterial cultures of Bacillus subtilis (MTCC 121), Klebsiella pneumoniae (MTCC 96), and Pseudomonas aeruginosa (MTCC 2488) in both polar (Aqueous and Ethyl acetate extracts) and nonpolar (Chloroform) solvents. The antibacterial activity of the aqueous, chloroform, and Ethyl acetate extracts of *T. purpurea* stem, leaf, flower, and seeds was evaluated against the above-mentioned MTCC bacterial cultures. (Table-1-3). The antibacterial activity of the aqueous extract of *Tephrosia purpurea* was more effective against *B. subtilis* (St-10 mm, L-12 mm, S- no zone of inhibition, F-10 mm), *P. aeruginosa* (St-11 mm, L-11 mm, S- no zone of inhibition, F- no zone of inhibition) and *K. pneumoniae* (St-10 mm, L-13 mm, S-11 mm, F-6 mm) (Table 1, Figure 2). The antibacterial activity of the chloroform extract of *T. purpurea* stem was more effective against *B. subtilis* (St-8 mm, L-10 mm, S- 22

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mm, F-5 mm), *P. aeruginosa* (St-22 mm, L-14 mm, S-20 mm, F- 30 mm), and *K. pneumoniae* (St-21 mm, L-20 mm, S-32 mm, F- 25 mm) (Table 2, Figure 3).

The antibacterial activity of the Ethyl acetate extract of *T. purpurea* stem was more effective against *B. subtilis* (St-10 mm, L-10 mm, S-10 mm F-10 mm), *P. aeruginosa* (St-6 mm, L-11 mm, S- 10 mm, F- 14 mm) and *K. pneumoniae* (St-6 mm, L-5 mm, S-9 mm, F- 12 mm) (Table 3, Figure 4). Compared with aqueous and ethyl acetate extracts, the chloroform extract gave the maximum zone of inhibition in the *B. subtilis* (St-39 mm, L-37 mm), *K. pneumonia* (S-32 mm), and *P. aeruginosa* (F-30 mm). The overall results are compared with aqueous, ethyl acetate, and chloroform extracts of leaf, flower, stem, and seeds. The results concluded that the chloroform extracts gave the maximum zone of inhibition in the *B. subtilis* (St-39 mm, L-37 mm), *K. pneumoniae* (S-32 mm), and *P. aeruginosa* (F-30 mm). The overall results are compared with aqueous, ethyl acetate, and chloroform extracts of leaf, flower, stem, and seeds. The results concluded that the chloroform extracts gave the maximum zone of inhibition in the *B. subtilis* (St-39 mm, L-37 mm), *K. pneumoniae* (S-32 mm), and *P. aeruginosa* (F-30 mm).

The Minimal inhibitory concentrations were analyzed for the different pathogens using phenol red indicator through micro titre plate. The modified microdilution techniques were used to analyze the results in a very short period of time. In our research, *T. purpurea* plant parts (leaf, flower, stem, and seeds) were used as an antimicrobial agent. We used the solvents of aqueous, ethyl acetate, and chloroform for analyzing the MIC. The zone of inhibition, which showed above 13 mm in the disc diffusion method, was further analyzed by the modified microdilution method to conclude the MIC values for the pathogens. Each row in a microtitre represents one organism. The MIC results were observed for *P.aeruginosa, K. pneumoniae,* and *B.subtilis.* The colour change from red to yellow indicates the growth of the organisms, whereas no colour change indicates that the antimicrobial agents of *T. purpurea* are resistant. The chloroform extracts of the seed showed the best results for *B. subtilis* and *K. pneumoniae, with* < 1 µg; the leaf showed < 8 µg in *P. aeruginosa*. The polar extract of aqueous and ethyl acetate (seed, leaf, flower) showed the < 128 µg for *B. subtilis, K. pneumoniae,* and *P. aeruginosa.*(Table-4,Figure-5) The overall results concluded that the seed and leaf (*T. purpurea)* extracts of chloroform observed the highest inhibitory activity.

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Fig 1: *Tephrosia purpurea* plant with stem, leaves, flowers, and seeds

TABLE 1 Antibacterial activity of aqueous extract of *Tephrosia purpurea* (zone of inhibition)

S.No	Bacterial strains used	Aqueous Extract of *Tephrosia purpurea*					
		Standard Value	Standard Mean	Observed Value of Stem (St)	Observed Value of Leaf (L)	Observed Value of Seed (S)	Observed Value of Flower(F)
1.	*Bacillus subtilis*	19-26	23	10	12	10	11
2.	*Pseudomonas aeruginosa*	19-23	23	11	11	12	5
3.	*Klebsiella pneumoniae*	16-20	20	10	12	12	10

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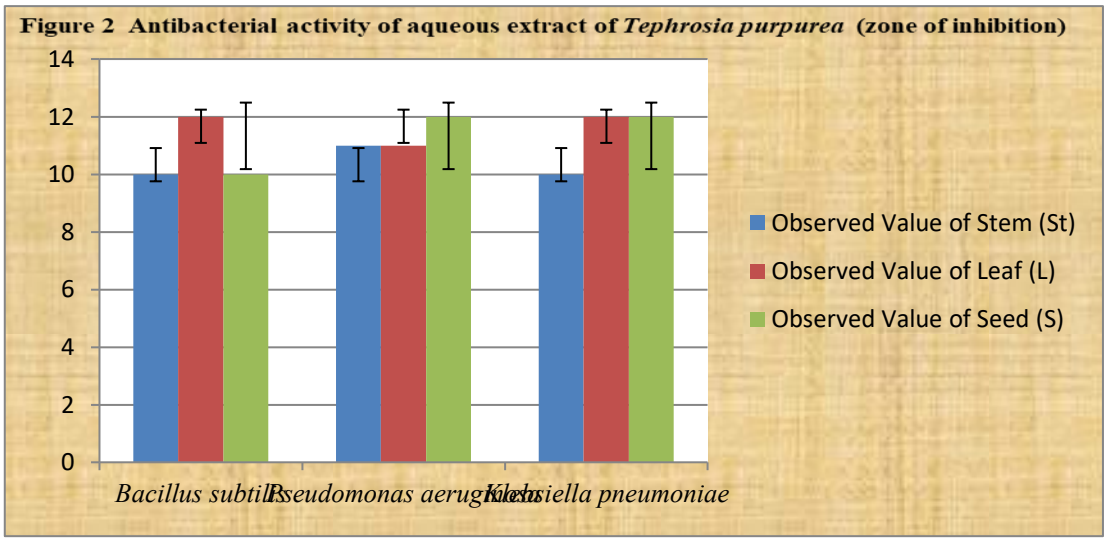

Fig 2: Antibacterial activity of aqueous extract of *Tephrosia purpurea* (zone of inhibition)

TABLE 2: Antibacterial activity of Chloroform extract of *Tephrosia purpurea* (zone of inhibition)

S.No	Tomato Spoilage Pathogens	Chloroform Extract of Tephrosia *purpurea*					
		Standard Value	Standard Mean	Observed Value of Stem (St)	Observed Value of Leaf (L)	Observed Value of Seed (S)	Observed Value of Flower (F)
1.	*Klebsiella pneumoniae*	19-26	23	10	11	10	11
2.	*Pseudomonas aeruginosa*	19-23	23	22	14	20	30
3.	*Bacillus subtilis*	16-20	20	8	10	22	5

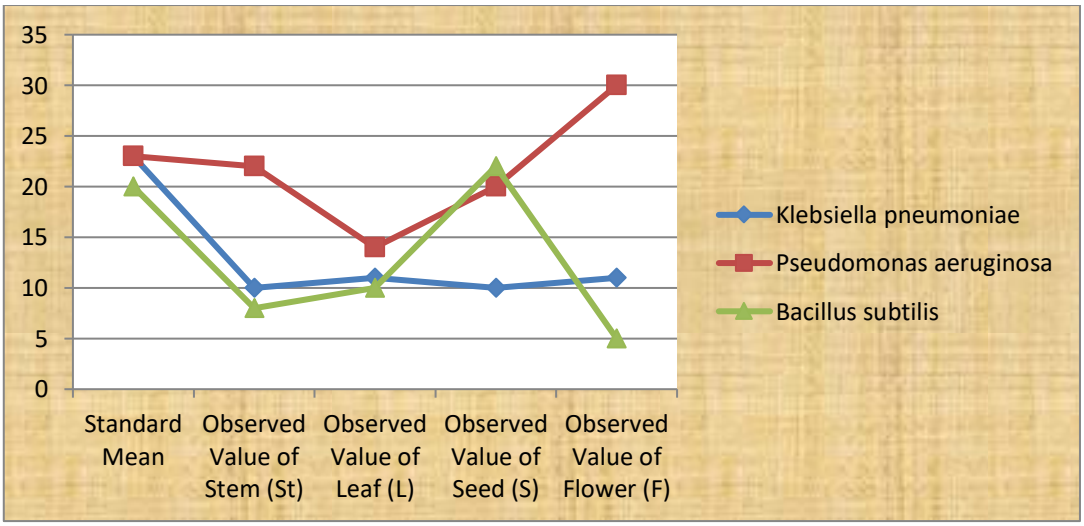

FIG 3: Antibacterial activity of Chloroform extract of *Tephrosia purpurea* (zone of inhibition)

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TABLE 3: Antibacterial activity of Ethyl acetate extract of *Tephrosia purpurea* (zone of inhibition)

S.No	Tomato Spoilage Pathogens	Ethyl Acetate Extract of *Tephrosia purpurea*					
		Standard Value	Standard Mean	Observed Value of Stem (St)	Observed Value of Leaf	Observed Value of Seed	Observed Value of Flower
1.	*Klebsiella pneumoniae*	19-26	23	5	10	10	12
2.	*Pseudomonas aeruginosa*	19-23	23	6	11	10	14
3.	*Bacillus subtilis*	16-20	20	10	10	10	10

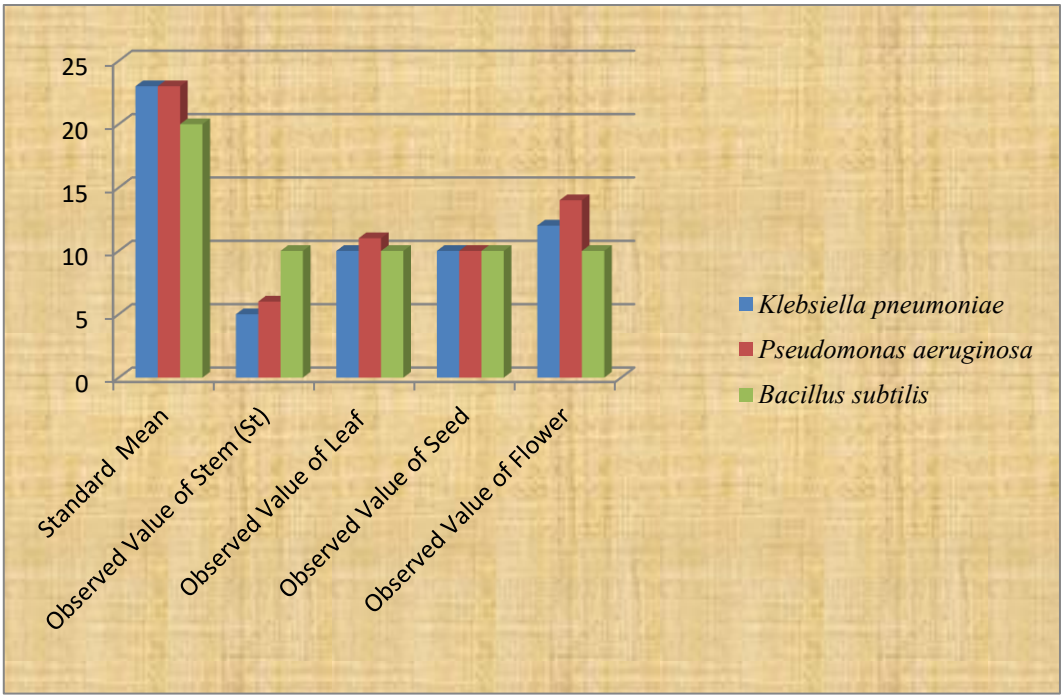

FIG 4: Antibacterial activity of Ethyl acetate extract of *Tephrosia purpurea* (zone of inhibition)

TABLE 4: Volumetric dilutions of plant leaf, flower, stem, and seeds are as follows:

Extracts of *Tephrosia purpurea* (Antibacterial solution)		Media	Intermediate concentration (µg/ml)	Final concentration (µg/ml)
Volume (ml)	Concentration (µg)	Volume (ml)		
3.2	2000	1.8	1280	256
1	1280	4	256	128
1	256	1	128	64

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1	**256**	**3**	**64**	**32**
1	**256**	**7**	**32**	**16**
1	**32**	**1**	**16**	**8**
1	**32**	**3**	**8**	**4**
1	**32**	**7**	**4**	**2**
1	**4**	**1**	**2**	**1**

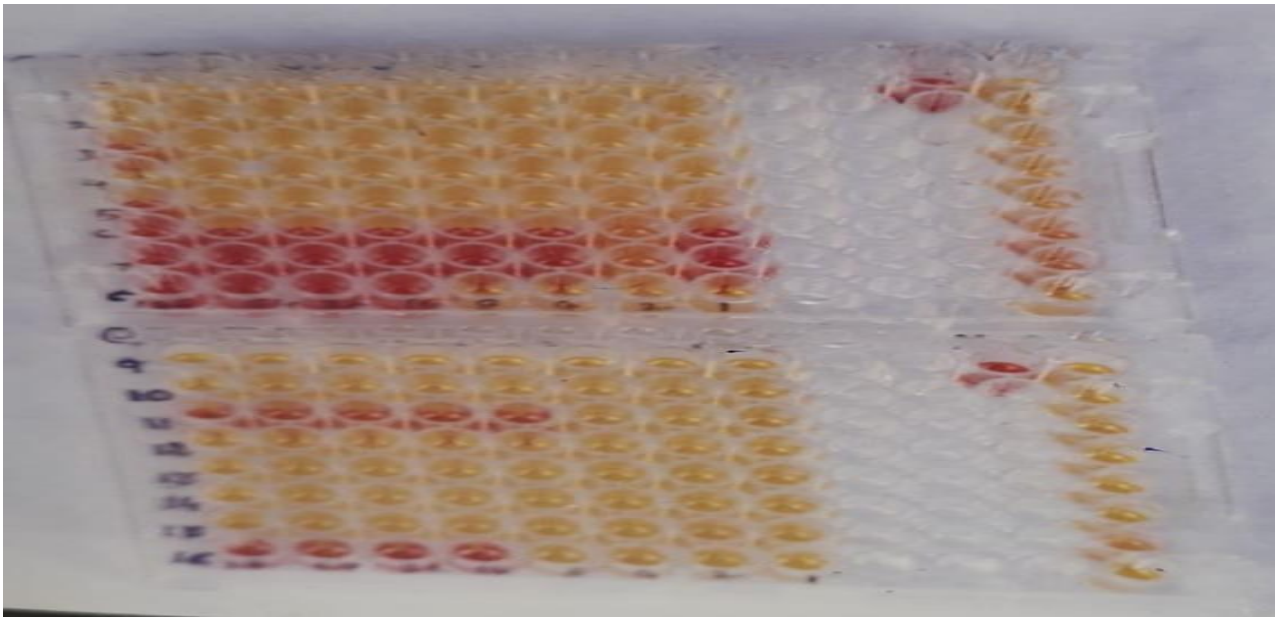

Figure 5 Minimal Inhibitory Concentration of *Tephrosia purpurea* Extracts for the bacterial strains by Microtitre Plate method

Row number	Organism
1,8,9,11,14	*Staphylococcus aureus*
2, 4,6,7,10,15	*Proteus vulgaris*
3,5,13	*Pseudomonas aeruginosa*
12	*Bacillus subtilis*

The MIC values for 6, 7 rows were < 1 µg whereas1,2,3,4,5,9,10,12,13,14,15,17,18 rows were < 128 µg. The 8th, 11th, and 16th were 32 µg, 8 µg, and 16 µg, respectively, for phenol red. The overall results compared with aqueous, ethyl acetate, and chloroform extracts of leaf, flower, stem, and seeds, the chloroform extracts of leaf 37 mm and 39 mm in seed were observed in *Proteus vulgaris,* which exhibited the highest inhibitory activity.

DI SCUSSION:

Medicinal plants contain a large amount of antimicrobial agents. A wide range of medicinal plant parts are extracted and used as raw drugs and for other medicinal uses (Srivastav *et.al.,* 1996). The use of plant

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material control pathogenic microorganisms is an alternative method to chemotherapeutic agents, as most of the chemical drugs are not affordable. (Aqil *et.al.,* 2006). *Tephrosia purpurea* plants possess a wide range of medicinal properties. In the present work, the antibacterial activity was tested in the aqueous, chloroform, and Ethyl acetate extracts of *Tephrosia purpurea* stem, leaf, flower, and seeds against the MTCC bacterial cultures of *Bacillus subtilis* (MTCC 121), *Klebsiella pneumoniae* (MTCC 96), and *Pseudomonas aeruginosa* (MTCC 2488). According to Rangama *et al*. (2009, the water extract of roots, leaves, and pods of *T. purpurea did* not seem to possess considerable antimicrobial properties on any of the pathogens tested. In the present study, the chloroform extract of *Tephrosia purpurea* seed was observed to have a very high zone of inhibition in *Bacillus subtilis* (39 mm), 37 mm (leaf), and *Klebsiella pneumoniae* (32 mm).

Selvamaleeswaran P. *et al (*2009 studied that *among* the two crude extracts of *Tephrosia purpurea*, the acetone and ethanol root extract showed good antimicrobial activity against *Salmonella typhi* and *Proteus mirabilis*. Leaf extract of acetone showed moderate inhibitory activity against the *Staphylococcus aureus and Proteus mirabilis*. The root extract of *Tephrosia purpurea* has a good antimicrobial activity compared to the leaf extract. In the present investigation, chloroform extract of *Tephrosia purpurea* seed and leaf showed the highest inhibition activity against *B. subtilis* and *P. aeruginosa.*

Gayathri Devi *et al*. (2014 reveled that the methanol extract in the various parts of *Tephrosia purpurea* showed maximum inhibitory activity (66 percent). The order of DPPH radical scavenging ability of different extracts of *Tephrosia purpurea* was methanol, ethanol, chloroform, acetone, aqueous, and petroleum ether, respectively. In the present study, the chloroform extract of *Tephrosia purpurea* gave the maximum activity against the pathogens in Seed 92%, Leaf 90 %, and flower 85% can be obtained even after less than 5 hours of incubation. The modified micro dilution method may be an alternative to the conventional disc diffusion method to analyse more samples, especially for a rapid group of pathogens. The result of the antimicrobial activity of *Tephrosia purpurea* chloroform extracts from the seed showed the best results for *B. subtilis* and *K. pneumoniae, with* < 1 µg; leaf showed < 8 µg in *K. pneumoniae*, and *P. aeruginosa* observed < 64 µg in the flower. The polar extract of aqueous and ethyl acetate (seed, leaf, flower) showed the < 128 µg for *K. pneumoniae*, *P. aeruginosa,* and *B. subtilis.* The overall results concluded that the seed and leaf (*Tephrosia purpurea)* extracts of chloroform observed the highest inhibitory activity of *Tephrosia purpurea* plants.

CONCLUSION:

Tephrosia purpurea (Linn.) plant exhibits a lot of medicinal properties in its stem, leaves, flowers, and seeds. The present study showed the antibacterial activity of the seed, leaves, flower, and stems extracts from various solvents of *Tephrosia purpurea* against pathogenic organisms. When using this plant as a green manure and in pharmacological uses, it brings a wide range of antibacterial effects against the pathogens and can be used as a natural pathogen controller.

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