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PHYTOCHEMICAL AND ANTIBACTERIAL EVALUATION OF *TEPHROSIA PURPUREA* AND ITS ACTIVE CONSTITUENT GALLIC ACID

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ABSTRACT

India is one of the hottest spot in the world in terms of biodiversity, but little is known about the chemical and pharmacological properties of most of the plants which needs to be explored. *Tephrosia purpurea* is one amongst the vital medicinal alternatives.In the present investigation, an attempt has been made to validate phytochemical composition of *Tephrosia* extract.Gallic acid is a good antioxidant and has been reported to possess various therapeutic activities. Thus, *Tephrosia* extract and gallic acid were screened against *Staphylococcus aureus*(gram +ve)and *E. coli* (gram -ve) for their antibacterial activities. Presence of different bioactive compounds were confirmed in *Tephrosia* extract by qualitative evaluation. Both *Tephrosia* extract and gallic acid possess strong antibacterial activity against gram +ve and gram –ve bacteria. Due to presence of several bioactive constituents and antibacterial activity, plant extract and gallic acid may be used as a therapeutic agent against a variety of diseases caused by bacterial infections.

Figure : 01 References : 11 Tables : 02
KEY WORDS : Antibacterial, *E. coli*, Gallic acid, Phytochemical, *S. aureus*, *Tephrosia purpurea*

Introduction

Antimicrobial resistance is one of the utmost serious public health threats resulting mainly by the excessive antibiotic use and abuse[1]. During the last decades, a rapid rise of bacterial resistance have been observed. Due to this alarming situation, several antimicrobial agents are losing their efficacy[2]. As a result, the therapeutic options for the treatment of infections have become restricted or even unavailable. According to the World Health Organization(WHO) infectious diseases are the second causeof death around the world[8]. Medicinal plants have been used from earlier times as the main and rich source of various defensive components especially antimicrobial agents.Plants are responsible for production of enormous array of functional relevant phytochemicals that exhibit a multiplicity of medicinal values[11].Majority of these compounds are plant secondary metabolites which are used as a defense mechanism against other microorganisms, herbivores and competitors[10]. The principal phytochemicals present in plants are phenolic compounds, alkaloids, lectins/ polypeptides and essential oils.

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TABLE-1 : Qualitative evaluation of phytochemicals in *Tephrosia purpurea* whole plant extract

Parameters	Biochemical tests	Reaction product	Result
Alkaloid	Meyer's test	Yellow colour precipitate	Present
	Wagner' test	Brown / Reddish colour precipitate	
	Hager's test	Yellow ppt	
Protein	Xanthoproteic test	Yellow colour	Present
	Ninhydrin test	Blue solution	
Carbohydrate	Fehling's test	Red colour precipitate	Present
	Benedict test	Orange red ppt	
Saponin	Forth test	Foam formation 1 cm layer	Present
	Foam test	Foam persistent for 10 minute	
Tannin	Gelatin test	White ppt	Present
	$FeCl_3$ test	Brownish Green / Blue Black	
Flavonoid	Alkaline reagent test	Intense yellow colour. Colourless adding acid	Present
	Lead acetate test	Yellow ppt	
	Schimado's test	Red colour	
	NH_3 test	Yellowcolour	
Phytosterols	Salkowski's test	Red colour	Present
	LibermanBurchard's test	Formation of brown ring at junction	
Glycosides	Boruetrager's test	Formation of red pink colour in ammonical layer	Present
Steroid	Acetic anhydride test	Violet changes into green/ blue	Present
Phenol	Ferric chloride test	Bluish black colour	Present
Terpenes	Chloroform test	Reddish brown interface (Junction)	Present
Diterpenes	Copper acetate test	Emerald-green colour	Present

Tephrosia purpurea is one amongst the vital medicinal alternatives. It is a common wasteland weed, which grows in poor soils and found throughout India. *Tephrosia purpurea* is one of the less explored species of flowering plant of family Fabaceae. It is commonly known as "Sharphunka" in Hindi, wild indigo in English and "Ghodakan" in Gujarati language. According to *Ayurveda* literature, this plant has also given the name of *"Wranvishapaka"* which means the property of healing all types of wounds and used in some preparations such as "Tephroli" and "Yakrifit" against liver disorders[7].The broad scope and power of the plant has been expanded recently with the discovery that it possesses spasmolytic, bronchodilator, vasorelaxant[6], antiulcer[4] and anticancer[5] potentials. Considering the potentiality of plants as a source of drugs with reference to antimicrobial agents, a systematic study was made to screen the phytochemical and antibacterial activity of *Tephrosia purpurea* extract and one of its active constituent gallic acid.

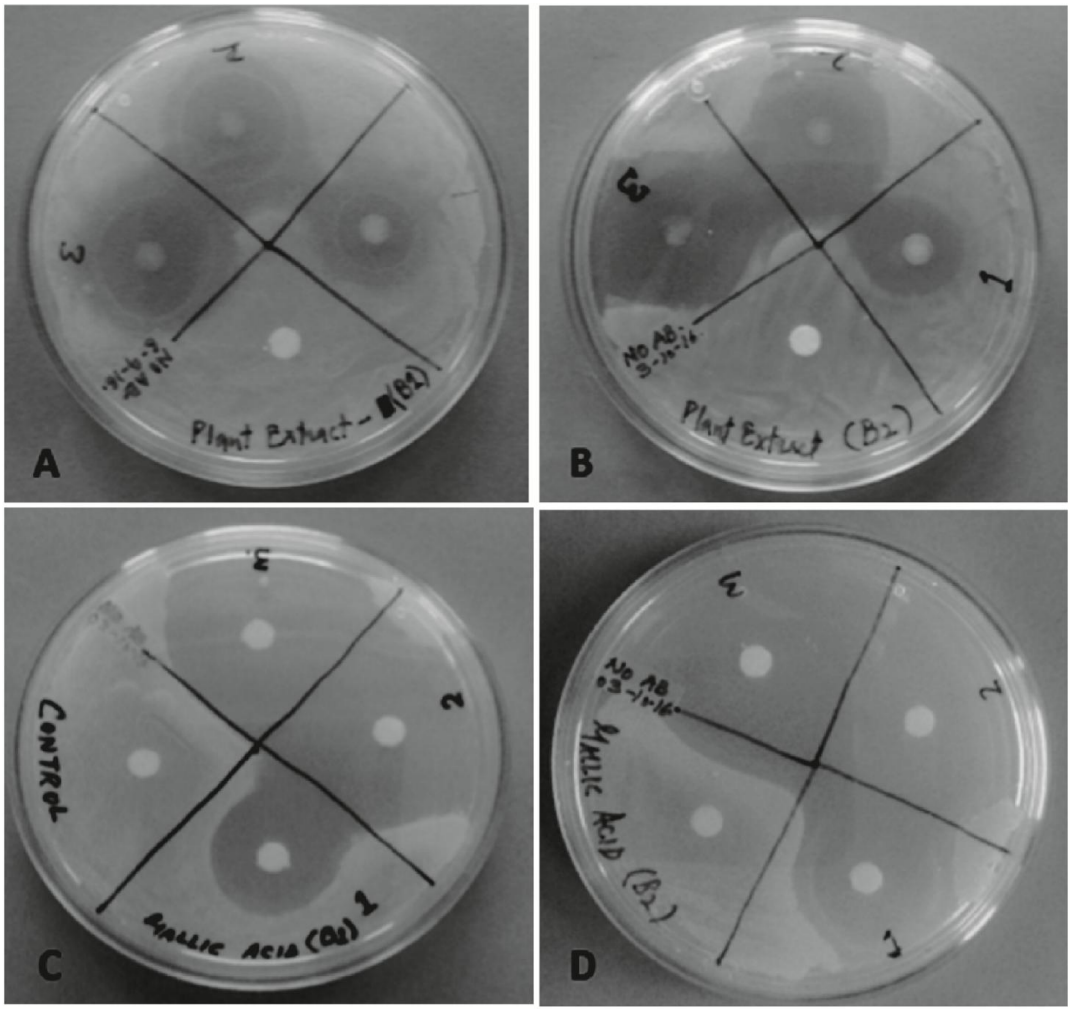

Fig.1: *Tephrosia* extract and gallic acid showing zone of inhibition against bacterial strains

A: *Tephrosia* extract against *S. aureus* strain , B: *Tephrosia* extract against *E. coli,* C: Gallic acid against *S. aureus* strain, D: Gallic acid against against *E. coli*

Materials and Methods

Whole plants of *Tephrosia purpurea* was collected from Guru Ghashidas University campus, Bilaspur (C.G.) India and was identified by botanist of the Department of Rural Technology and Social Development, Guru Ghasidas University, Bilaspur. Collected whole plant material was washed with distilled water and air dried at room temperature under shade, chopped in to very small pieces and processed further for extraction. The 70% ethanolic extracts was obtained using Accelerated Solvent Extractor (DIONEX ASE-150) at 20ºC and 15 atm. pressure. Obtained filtrates were dried by vacuum evaporator and extract was stored at 4°C for further study.

Phytochemical analysis

Various chemical tests were performed on ethanolic extract of *T. perpurea* for semiquantitative identification of phytochemical constituents present in it. Detection of protein and amino acids was done by xanthoproteic test and ninhydrin test. Fehling's test and Benedict test indicated the presence of reducing sugars. Presence of saponins was identified by froth test and foam test. Extract was treated with Wagner's reagent, Meyer's reagent and Hager's test to identify the presence of alkaloids. Extract undergone ferric chloride test to know the presence of phenols. Acetic anhydride test was used to indicate the presence of steroids in *T. purpurea*. Presence of terpenoids was identified by chloroform test. Sodium hydroxide test indicated the presence of flavonoids. Intense yellow color precipitate due to lead acetate test and NH_3 test indicated the presence of flavonoids in extract. Schimodo's test indicated the presence of flavonoids. $FeCl_3$ induced brownish green/blue coloration indicated the presence of tannins, whereas gelatin test gave white ppt for the same. Salkowski's test and LibermanBurchnad's test were applied to know the presence of phytosterols in extract. Bouretrager's test was applied for indication of glycosides. Copper acetate test was used to know the presence of diterpenes.

Antimicrobial activity screening

Bacterial cultures of *Escherichia coli* and *Staphylococcus aureus* were maintained on nutrient agar (NA, Hi-Media) at 37°C.About 25 ml of sterile Muller-Hinton Agar was poured into Petri-plates and allowed to set. Different dosages of *Tephrosia* extract and gallic acid (10, 20 and 30µg) dissolved were added on a 10 mm filter paper, dried and sterilized by ultraviolet lamp for 60 min. Pathogenic bacterial cultures were spread on LB agar plates. Drug samples were loaded on filter paper discs and empty disc was used as control. The plates were placed in a 37 °C incubator for 24 h. Then inhibitory action of tested samples on the growth of the bacteria was determined by measuring diameter of inhibition zone.

Results and Discussion

In the present study, qualitative analysis indicates the presence of various bioactive components in *T. purpurea* extract of Chhattisgarh origin. Different qualitative tests were carried out to confirm presence of various functional groups of phytochemicals like alkaloids, flavonoids, terpenoids, saponins, glycosides, polyphenols etc.

TABLE- 2: Zone of inhibition and MIC values of *Tephrosia* extract and gallic acidagainst *S. aureus* and *E. coli.*

	***Tephrosia purpurea* extract (1mg/ml)**					**Gallic acid (1mg/ml)**				
	Cont	**10 µg**	**20 µg**	**30 µg**	**MIC**	**Cont**	**10 µg**	**20 µg**	**30 µg**	**MIC**
S.aureus	0	09	13	19	2.5 µg	0	18	25	30	1.5 µg
E. coli	0	10	12	17	2.0 µg	0	19	23	29	1.5 µg

End color product of chemical reaction was used as an index for the presence or absence of phytochemicals (Table-1). Most of them were found in whole plant extract of *T. purpurea*. Bioactive components possess well defined physiological role to protect organism from different infections and environmental stress. Alkaloids, triterpenoids, steroids and saponins possess antibiotic and analgesic activity[9]. Flavonoids possess well known antiinflammatory, antiallergic, antiviral, antispasmodic and diuretic properties, whereas tannins are strong antimicrobial and antiviral agents[3]. Phenolic compounds are well known powerful antioxidants, capable of breaking chain reactions due to free radical scavenging activity.

For anti-bacterial assays, *Tephrosia* extract and gallic acid were dissolved in methanol and found effective against both *Staphylococcus aureus* and *Escherichia coli*. The minimum inhibition concentration value of *Tephrosia* extract and gallic acid for *S. aureus & E. coli* strain ranged to 2.5, 2 and 1.5, 1.5µg respectively. The antibacterial activity observed was in a concentration dependent manner. The zone of inhibition and MIC values of the two selected agents against bacterial pathogen is shownin (Table-2, Fig. 1- A,B,C,D).

Due to the emergence of multi-drug resistance pathogenic bacteria as well as undesirable side effects of certain antibiotics have triggered immense interest in the search for new antimicrobial drugs of plant origin. Antibacterial activity of *Tephrosia* extract may be due to the presence of various phytochemical constituents in them. The broad spectrum of antibacterial activity by *Tephrosia purpurea* may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease such as tuberculosis etc.

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