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Research article

STUDIES ON *TEPHROSIA PURPUREA:* A PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL STUDIES

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ABSTRACT: Tephrosia purpurea is a species of flowering plant in the pea family, Fabaceae, which has a pantropical distribution. According to Ayurveda, the plant is anthelmintic, alexiteric, restorative, and antipyretic; it is used in the treatment of leprosy, ulcers, asthma, and tumors, as well as diseases of the liver, spleen, heart, and blood. A decoction of the roots is given in dyspepsia, diarrhea, rheumatism, asthma and urinary disorders. The root powder is salutary for brushing the teeth, where it is said to quickly relieve dental pains and stop bleeding. An extract, termed 'betaphroline' is claimed to promote release of endorphins, and finds use in certain cosmetic preparations. The ethanolic extract of Tephrosia purpurea leaves was used for its anti oxidant and antimicrobial activity. Tephrosia purpurea leaves extract has very well anti oxidant and anti microbial activity. The ethanolic extract of Tephrosia purpurea was checked for anti microbial activity against pathogenic bacteria such as *staphylococcus aures, pseudomonas aeruginosa* and fungi *Aspergillus niger.*
Key words: Tephrosia purpurea, Antioxidant activity, anti microbial activity, phytochemical screening.

INTRODUCTION

Tephrosia purpurea is an erect or spreading annual or short-lived perennial herb, sometimes bushy, 40-80 cm tall, rarely up to 1.5 m; indumentum sericeous, strigose or velutinous; stem slender, erect or decumbent at base [1, 2]. Leaves imparipinnate; stipules narrowly triangular, 1.5-9 mm x 0.1-1.5 mm; rachis up to 14.5 cm long, including the petiole of up to 1 cm; petiolule 1-3 mm long; leaflets 5-25, obovate to narrowly elliptical, terminal leaflet 7-28 mm x 2-11 mm, lateral leaflets 5-30 mm x 2-11 mm, acute at base, apex rounded to emarginate, venation usually distinct on both surfaces. Inflorescence an axillary or leaf-opposed pseudo-raceme, (1.5-)10-15(-25) cm long, sometimes with basal leaf-like bracts; flowers in fascicles of 4-6; bracts to fascicles and to flowers small, bracteoles usually absent; pedicel 2-6 mm long; flower 4-8.5 mm long, purplish to white; calyx campanulate, persistent, cup 1.4-2.3 mm x 1.5-3.2 mm, unequally 4-toothed, teeth pubescent inside; standard broadly ovate, 3.5-7.3 mm x 5-10 mm, clawed; wings 2.5-6 mm x 1.5-3.8 mm, auricled on vexillary side, clawed; keel 2.2- 4.5 mm x 2-3 mm, auricled on vexillary side, clawed; stamens 10, staminal tube 4-6 mm long, filaments alternately longer and shorter, free part up to 3.5 mm long, vexillary filament free at base, connate halfway, 5-8 mm long; style up to 4.5 mm long, upper half glabrous, stigma penicillate at base [3, 4].

Pod flat, linear, 2-4.5 cm x 3-5 mm, somewhat up-curved towards the end, convex around the seeds, flattened between, margins thickened, dehiscent with twisted valves, 2-8(-10)-seeded [5]. Seed rectangular to transversely ellipsoid, 2.5-5 mm x 1.8-3 mm, light to dark brown to black, sometimes mottled. T. purpurea is a very variable species and many subclassifications exist [6]. Most characteristic is the shape of its pod: convex around the seeds with a distinctive flat area in between. The name T. purpurea is often erroneously applied to the cultivated T. noctiflora Bojer ex Baker which has longer inflorescences, a very long carinal calyx tooth and reticulately ridged seeds [8-10].

For South-East Asia T. purpurea is subclassified as follows; (a) subsp. barbigera Bosman & de Haas: vexillary filament and staminal tube velutinous; occurring in the Philippines, New Guinea and Australia [11]. Based on flower and inflorescence lengths, further subdivided into 2 varieties: var. barbigera (flower 7-8 mm long, longest inflorescence 11-19.5 cm long) and var. rufescens Benth. (Flower 5-6 mm long, longest inflorescence 4.5-11 cm long). (b) subsp. purpurea: characteristics and distribution as described for the species; vexillary filament and staminal tube glabrous [12].

MATERIAL AND METHODS

Collection of Plant Materials

The fresh and healthy leaves of *Tephrosia purpurea* were collected. The plant specimens were identified in Department of Biotechnology, Sri krishnadevaraya University, Anantapuram.

Preparation of Leaf Extract of *Tephrosia purpurea*

The extraction of leaves of *Tephrosia purpurea* was carried out using known standard procedures. The leaves were dried in shade and powdered in a mechanical grinder. The powder (10.0 g) was initially defatted with ethyl alcohol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatman filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccator. The ethyl alcoholic leaf extract yields a dark reddish residue weighing 4.50 g (45.0% w/w). This crude extracts of ethylalcohol was used for further investigation for potential of antimicrobial properties.

Preliminary Phytochemical Screening

The leaf extracts were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. Air-dried and leaf powder was screened for the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, stroids, glycosides, anthraquinones, coumarin, saponins, gum, mucilage, carbohydrates, reducing sugars, starch, protein, and amino acids.

Test Microorganisms and Growth Media

Staphylococcus aureus (MTCC 3160), Pseudomonas aeruginosa (MTCC 1688) and fungal strain Aspergillus niger (MTCC 1785) were chosen based on their clinical and pharmacological importance. The bacterial strains obtained from Department of Microbiology, Osmania University, were used for evaluating antimicrobial activity. The bacterial and fungal stock cultures were incubated for 24 hours at 37°C on nutrient agar and potato dextrose agar (PDA) medium, respectively, following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), whereas the fungi were grown in Sabouraud dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°C.

Antimicrobial Activity

Whatman No: 1 filter paper discs of 6mm diameter are prepared and autoclaved by keeping in a clean and dry Petri plate. The filter paper discs were soaked in plant extracts for 6 hours are taken as test material. After 6 hours the discs were shade dried. The concentrations of leaf extracts per disc are accounted for 0.1 grams/1ml. Subsequently they are carefully transferred to spread on cultured Petri plates. Filter paper discs immersed in ethanol, benzene, distilled water are prepared and used as control.

Testing of antimicrobial activity

To test the antimicrobial activity on agar plates, LB agar medium was prepared using the ingredients mentioned above. The medium was sterilized at 121°c for 30 min's. The agar test plates were prepared by pouring about 15ml of the medium into 10cm Petri dishes under aseptic condition and left undisturbed for 2hrs to solidify the medium. 1ml of inocculum (containing suspension) of P.aeruginosa and Sta.aureus was poured to the respective plates separately containing solidified agar media. Six replicates were maintained. The prepared sterile whatman no :1 filter paper discs of 6mm diameter were impregnated with the extracts and shaken thoroughly and this test plates incubated for a period of 48 hrs in BOD at 37ºC for the development of inhibitory zones and the average of 2 independent readings for each organism in different extracts were recorded. The control Petri plates and also maintained above respective cultures

Measuring the diameter of inhibition zone

The inhibition zones were lead after 1 day at 37ºc for bacteria. The diameter of the inhibition zone was measured and recorded with the aid of plastic ruler. 7 paper discs placed in 1 Petri plate.

RESULTS AND DISCUSSION

It was found that ethyl alcoholic extracts of *Tephrosia purpurea* leaves contained tannins, flavonoids, saponins, triterpenoids, steroids, glycosides, anthraquinones, reducing sugars, carbohydrates, proteins, and amino acids. (Fig-1 to 3, table-1 and 2).

Antimicrobial activity

Ethanolic extract of *Tephrosia purpurea* leaf

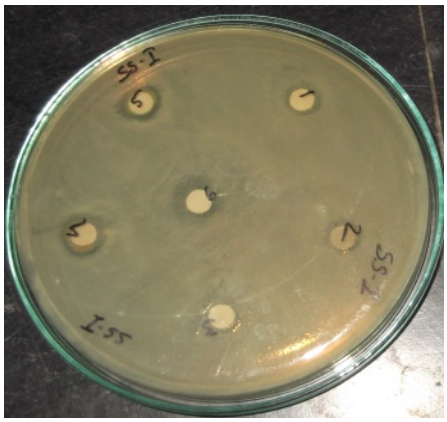

Fig 1: *Pseudomonas aeruginosa*

Fig 2: *Staphylococcus aureus*

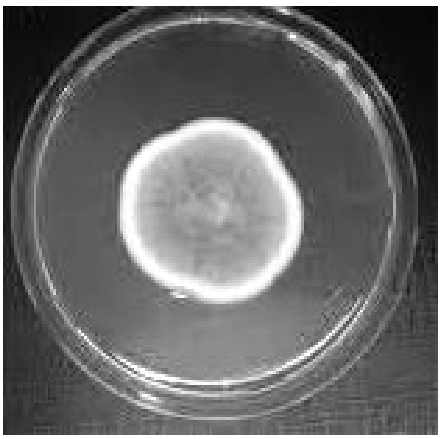

Fig 3: Aspergillus niger

Table 1: Inhibitory activities of leaf extract of *Tephrosia purpurea* on microorganisms

Plants	Zone of inhibition (MIC)		
	Pseudomonas aeruginosa (-ve) (mm)	*Staphylococcus aureus* (+ve) (mm)	*Aspergillus niger* (mm)
Tephrosia purpurea	1.8	2.6	1.2

Phytochemical Screening of Plant leaves in Different Extracts

Table 2: *Tephrosia purpurea* phyto chemical Screning

S.No	Secondary metabolites	Hexane	Ethyl acetate	Ethanolic	Aqueous
1	Steroids	+	++	++	+
2	Triterpenes	-	+	+	-
3	Saponins	-	-	-	-
4	Tri terpinoidal saponins	-	-	-	-
5	Alkaloids	+	++	+ +	+
6	Carbohydrates	-	-	+	+
7	Flavonoids	+	+	++	+
8	Tannins	+	++	+	+
9	Glycosides	+	++	++	+
10	Polyphenols	+	++	++	++

CONCLUSION

In the present study it was found that *Tephrosia purpurea* leaf extract has an excellent antimicrobial activity. The pathogenic bacteria like Pseudomonas aeruginosa, Staphylococcus aureus and fungus Aspergillus niger were inhibited in presence of the leaf extracts of *Tephrosia purpurea* ethanolic extract. Therefore the future studies should be aimed to exploit this plant to be used as one of the best medicinal plant is controlling pathogenic bacteria.

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