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Evaluation of antifungal potential of *Tephrosia purpurea* (L) Pers an important medicinal plant of arid region

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Abstract
Tephrosia purpurea is an important medicinal plant of Indian arid region. This plant is used to treat dyspepsia, cough, asthma, fever, ulcers, skin diseases, anthelmintic, colic, and as a blood purifier. In ayurveda system it is also called sarva warnavishapak due to its excellent wound healing properties. The whole plants with roots are used as a medicine. Nowadays there is a trend from synthetic medicine to herbal medicine in all over world. Although From many decades, plants have been used for the ailment of diseases and to control microbes. In this respect, *T. purpurea*, screened in vitro for antifungal potential against four plant pathogenic fungi. Dried and powdered leaves, fruit and root of *Tephrosia purpurea* extracted with ethanol and water using soxhlet extraction apparatus. This extract was tested against four fungal pathogens namely, *Rhizoctonia solani, Fusarium solani, Fusarium moniliforme and Alternaria alternata* using poison food technique while, bavistin (a broad spectrum systemic fungicide) was used as a standard fungicide. The extract exhibited antifungal activity against selected fungi Thus, the current investigation leads to source of new antifungal compound in future.

Keywords: *Tephrosia purpurea*, fungal pathogens, extract, medicinal plant.

Introduction
Tephrosia purpurea is commonly known as Sarphonk or Sharpunkha in Hindi, Fish poison or Wild indigo in English [1]. It belongs to family, Fabaceae that has a pantropical distribution [2]. It is native to Australia, China, India, and Sri Lanka. It is found throughout India and Sri Lanka in poor soils [2]. It grows as common wasteland weed. [2]. It is also treated as green manure crop. *T. purpurea* is an erect or spreading annual or short-lived perennial herb, sometimes bushy, 40-80 cm tall, rarely up to 1.5 m. It is much branched, erect, perennial herb. Flowers are Reddish purple and fruit is flat pod with 5-6 seed. This genus included 400 species [4]. In northern India, dry *T. purpurea* plants are collected for fuel. Medicinally, all parts of the plant have tonic, laxative and antioxidant properties [5, 6]. In ayurveda system it is used in the treatment of spleen and liver enlargement and inflammation therefore, it is also called plihaśathru [7]. Due to its wound healing properties it is also called sarva warnavishapak means it has the property to healing all types of wounds [8]. The whole plants with roots is used as a medicine [9, 10]. This plant is used to treat dyspepsia, cough, asthma, fever, ulcers, skin diseases, anthelmintic, colic, and as a blood purifier [11]. Its root also shows good cytotoxic activity [12]. This genus is famous for prenylated flavonoids and also possess insect repellant, larvicidal, piscicidal, antimicrobial and anticancer properties [13, 14, 15]. These secondary metabolites are present in different species and responsible for their diverse pharmacological activities such as hepatoprotective, anti-diabetic, anti-oxidant, anti-hyperlipidemic, anti-ulcer, antibacterial and anti-fungal [16, 17, 18]. Nowadays there is a trend from synthetic medicine to herbal medicine in all over world [19]. Although From many decades, plants have been used for the ailment of diseases and to control microbes. In this respect, *T. purpurea*, screened in vitro for antifungal potential against four plant pathogenic fungi.

Materials and methods
The study was carried out at the Arid Forest Research Institute Jodhpur. Following steps were included to find out antifungal potential of selected plant species:

Collection of Plant Material
Plant material was collected from some places of Jodhpur District, Rajasthan, India. Plant samples were identified with the help of taxonomic literature, standard flora and herbarium. Collected material was washed thoroughly with running tap water followed by distilled water to remove dirt. After washing and cleaning, material was shade dried at room temperature and finely ground with help of grinder. Powdered material was stored in airtight bottles for further use in preparation of extract.

Preparation of Extracts
Two types of extract aqueous and alcoholic (Ethyl alcohol) were prepared from every collected plant part with the help of Soxhlet apparatus and dried with help of water bath and rotary evaporator respectively. Extract were dissolved in DMSO and solution of different concentrations (10, 20, 30, 40 & 50) were prepared. The effect of extract on selected fungi was tested in vitro by poison food technique [20].

Poison food technique
Starter culture of selected fungi was prepared in PDA medium. Plant Extract of different concentration was mixed with cooled molten media in conical flask and poured into petriplates and allowed to solidify at room temperature. A mycelium disk of 5 mm diameter was cut out from

periphery of actively growing fungus (4-7 days old culture) with the help of cork borer and aseptically plated at centre of each petriplate. Three replication of each treatment were maintained, Plate without extract act as negative control and plate with chemical fungicide (.2%) served as positive control. All petriplates were incubated at 25±1˚C for seven days. After incubation the effect of extract was determined by measuring the radial growth of fungi in test plate and compared with control plate. Colony diameter of fungus in each plate was measured in mm. The antifungal activity was assessed in terms of percentage inhibition.

The percentage inhibition was calculated with the help of following formula suggested by Vincet [21].

Inhibition Percent = I% = C-T/C X 100

C= Growth of mycelium in control plate (mm)

T=Growth of mycelium in treatment plate (mm) mean of three plates considered as final reading

Mean value and standard error mean were calculated for result of poison food technique and inhibition percentage calculated.

Result and Discussion

The result of this study clearly showed that *Tephrosia purpurea* has ability to inhibit selected fungi in vitro. Extract of leaves and fruit showed good antifungal activity while root extract could not show significant antifungal activity against target fungi. Both aqueous and ethanolic extract of *Tephrosia purpurea* leaves showed varied result against target fungi. All the ethanolic extracts showed wide range of activity against the targeted fungi as compared to aqueous extract which showed limited antifungal activity. The maximum percentage inhibition with aqueous extract (at 50%) for *Rhizoctonia solani*, was 42%, for *Fusarium solani* was 52% and *Fusarium moniliforme* was 25%. The maximum inhibition percentage with ethanolic extract (50%) for *Rhizoctonia solani*, was 73%, *Fusarium solani* was 60.2 % and for *Fusarium moniliforme* was 42% and for *A. alternata* it was 82%. It is clearly indicates that the ethanolic extract of *Tephrosia purpurea* leaves exhibited more antifungal properties against all fungi then aqueous extract. Effect of different concentration (10%, 20%, 30%, 40% and 50%) of ethanol extract on growth of fungi showed that inhibition of fungus growth increase with concentration of extract. The ethanolic extract *Tephrosia purpurea* leaves exhibit maximum inhibition against *A. alternata* (82%) followed *Rhizoctonia solani* (73%). All the concentration of ethanolic extract of *Tephrosia purpurea* leaves was found effective in inhibition of mycelia growth over the untreated control plate. However highest concentration of extract (50%) recorded maximum inhibition. The result of antifungal screening of aqueous and ethanolic extract of *Tephrosia purpurea* leaves, fruit and root are given in tables 1-6 and comparative effectiveness is shown with the help of graph1, 2 &3.

Table 1: Showing Inhibition Percentage of Ethanolic Extract of *T. purpurea* Leaves against Selected Fungi

Fungus species	Concentration of extract/Inhibition Percentage					
	Control	10	20	30	40	50
Rhizoctonia solani,	0	25	40.6	47	61	73
Fusarium solani	0	31	38	44.5	53	60.2
Fusarium moniliforme	0	14	18	22.8	29	42
Alternaria alternata	0	18	32	47	69	82

Table 2: Showing Inhibition Percentage of Aqueous Extract of *T. purpurea* Leaves against Selected Fungi

Fungus species	Concentration of extract/Inhibition Percentage					
	Control	10	20	30	40	50
Rhizoctonia solani,	0	6	10.2	18	33	42
Fusarium solani	0	13.5	18	39	43	52
Fusarium moniliforme	0	0	4	12	19	25
Alternaria alternata	0	0	0	0	0	0

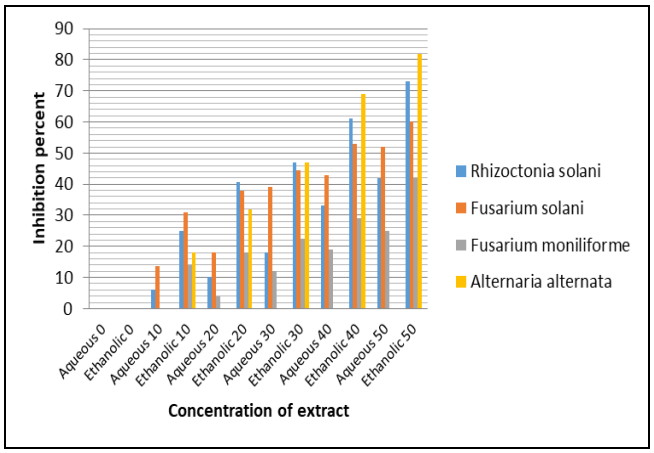

Fig 1: Showing Comparison of Aqueous and Ethanolic Extract of *Tephrosia purpurea* Leaves against Selected Fungal Species

Table 3: Showing inhibition percentage of ethanolic extract of *T.purpurea* fruit pod against selected fungi

Fungus species	Concentration of extract/Inhibition Percentage					
	Control	10	20	30	40	50
Rhizoctonia solani,	0	11.8	36.33	48	64	77
Fusarium solani	0	18	30.02	46.4	59.	64.6
Fusarium moniliforme	0	31.5	38	42.6	58.4	68.5
Alternaria alternata	0	10	17.5	22.8	35.6	48.4

Table 4: Showing inhibition percentage of aqueous extract of *T.purpurea* fruit against selected fungi

Fungus species	Concentration of extract/Inhibition Percentage					
	Control	10	20	30	40	50
Rhizoctonia solani,	0	0	10.8	15.6	29.6	32.4
Fusarium solani	0	0	4	12	19.8	25
Fusarium moniliforme	0	9.6	18.6	21.5	28.6	36.8
Alternaria alternata	0	0	7.8	15.6	21.4	29

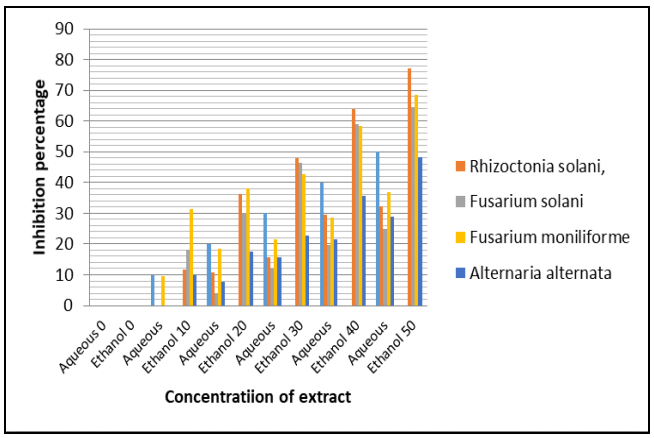

Fig 2: showing comparison of aqueous and ethanolic extract of *T.purpurea* fruit against selected fungal species

Table 5: Showing inhibition percentage of ethanolic extract of *T. purpurea* root against selected fungi

Fungus species0	Concentration of extract/Inhibition Percentage					
	Control	10	20	30	40	50
Rhizoctonia solani,	0	10.2	18.5	29	34	51.4
Fusarium solani	0	9.6	18.6	21.5	28.6	36.8
Fusarium moniliforme	0	18	26	32	48	62
Alternaria alternata	0	0	11.5	19	29.4	38

Table 6: Showing inhibition percentage of aqueous extract of *T. purpurea* root against selected fungi

Fungus species0	Concentration of extract/Inhibition Percentage					
	Control	10	20	30	40	50
Rhizoctonia solani,	0	4.6	9.2	17	26	33
Fusarium solani	0	0	6	14	19.8	25
Fusarium moniliforme	0	2.5	8	14	26	31
Alternaria alternata	0	0	7.8	15.6	21.4	29

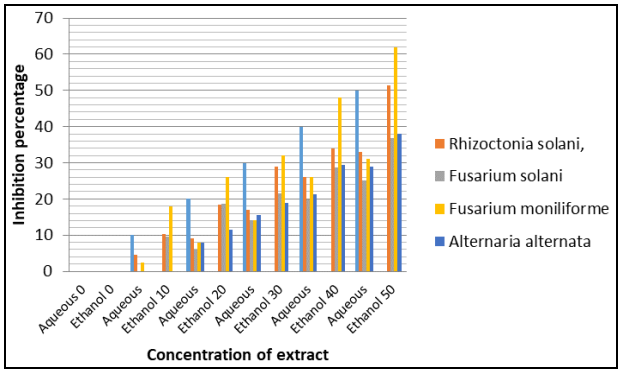

Fig 3: Showing comparison of aqueous and ethanolic extract of *T.purpurea* root against selected fungal species

In our literature survey less work was found on antifungal activity of species from genus Tephrosia on plant pathogenic fungus, however much work done on pathogenic bacteria and fungi of humans. Only three species of this genus are known to possess antifungal potential. The methanolic extract of *Tephrosia purpurea* showed significant activity against *A. Niger* and *C. ablicans* [22]. *Trphrosia Hiderbrandtii* showed antifungal activity against *C. cucumerium*. The activity was found to be related to a chemical compound isolated from its root [23]. *Tephrosia tinctoraia* also showed actIvity against *A. niger, C. ablicans.* The methanolic extract was found to be more active against the These fungi. However methanolic extract showed no activity against *S. cerevisiae* [24]. It was also studied that various solvent extracts of *Tephrosia purpurea* leaves (Methanol, Ethyl alcohol, Chloroform and Hexane) showed antifungal activity against fungal strain *A. niger and Alternaria* the ethyl alcohol extract showed maximum zone of inhibition against *Aspergillus and Alternaraia.* [25]. these results were in agreement with the findings of our studies. The antimicrobial activity was enhanced with increased doses. The antimicrobial properties exhibited by the plant could be due to the presence of mixtures of active constituents which show a broad spectrum antifungal activity [26]. Due to these active constitute *T. purpurea* is also used as tonic, antidiarrheal, leprosy, laxative, antivenom, and antiulcer [27]. *Tephrosia purpurea* leaves, pods and roots were also studied for their antimicrobial against 3 standard cultures (*Staphylococcus aureus, Pseudomonas aeruginosa, E. coli* and one clinical isolate of *Candida spp.* Ethanolic root extracts of *Tephrosia purpurea* were found effective against *Pseudomonas aeruginosa* and two other Pseudomonas strains and two coli form strains[28]. Researcher isolated flavonoids from the plant has been reported to have antimicrobial activity. Phytochemical investigations on *Tephrosia purpurea* proved the presence of various phytoactive constituents such as glycosides, rotenoids, isoflavones and flavanones [29]. Methanolic extracts of *Tephrosia purpurea* showed excellent antibacterial and antiviral activity [30]. Antifungal activity of Tephrosia purpurea also investigated against *Pythium debaryanum* and found encouraging result [31]. The ethanol extract of *Tephrosia purpurea* whole plant showed significant antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus subtitils* as we found that antifungal activity was shown by root, fruit and leaves of *Tephrosia purpurea* [1]. The leaves of *T. purpurea* also exhibited antibacterial activity against *Staphylococcus aureus, Escherichia coli* and *Candida albicans* [32]. The methanol, ethanolic and aqueous extracts of *Tephrosia purpurea* were reported with high phytochemicals [33]. The chloroform root extract of *T. calophylla* were tested for anti-bacterial and anti-fungal activity and showed moderate activity. *Tephrosia purpurea* exhibited anti-fungal activity against 61 endophytic fungus strains [34]. The activity of these extracts increased with increasing concentrations as our study also proved [35, 36].

Conclusions

The present study leads to conclusion in nutshell that ethanolic extract of *Tephrosia purpurea* leaves contain significant antifungal potential against selected plant pathogenic fungi. Further investigations are suggested for *in vivo* effect and chemical composition of this extract.

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