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Research Article

EVALUATION OF ANTIMICROBIAL AND ANTI-HISTAMINE ACTIVITY OF THE AERIAL PARTS OF *TEPHROSIA PURPUREA* L.

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ABSTRACT
Tephrosia purpurea (Fabaceae) has been used as a medicinal plant in all over India. This plant is a much branched perennial herb. Roots are given orally against any type of poisoning such as snakebite and the aerial parts were used for hydrophobia, asthma, cough, heart disease and kidney problems. Anti-microbial activity of 50% alcoholic extract with different concentrations were tested against the fungal strains like *Aspergillus fumigates*, *Aspergillus niger*, *Ganoderma lucida* and *Candida albicans* and bacterial organisms like *Escherichia coli, Serratia mercescens, Staphylococcus aureus* and *Staphylococcus epidermis*. The 50% alcoholic extract of *Tephrosia purpurea* at 5mg, 10mg, and 20mg concentration showed antibacterial activity against *Escherichia coli*, *Serratia marcescens* and *Staphylococcus*. Extracts of *Tephrosia purpurea* at 5mg, 10mg, and 20mg concentration did not show positive antifungal activities against *Aspergillus fumigates, Aspergillus niger*, *Ganoderma lucida* and *Candida albicans*. Antihistamine activity of 50% alcohol extract of *Tephrosia purpurea* (TP) was evaluated in isolated guinea pig ileum. It was observed that different concentrations (2mg, 4mg and 8mg/ml) of TP extract antagonized the contraction of ileum induced by histamine. The extracts at 8mg/ml concentration expected maximum antagonistic. The results obtained with histamine in guinea-pig isolated ileum preparations are sensitive to histamine against like histamine at the lower concentration.

Key words: Antimicrobial activity, anti-histamine activity, induced guinea pig ileum, alcoholic extract of *Tephrosia purpurea*.

INTRODUCTION

Though many Indian medicinal plants are used in various Indian systems of medicine like Ayurveda, Siddha, Unani and Homeopathy, still almost over half of Indian medicinal plants are not exploited fully for the therapeutic activity or pharmaceutical aid. *Tephrosia purpurea*, belongs to the family Fabaceae. English name is Purple Tephrosia, vernacular name is Kolangi. It is a perennial herb found throughout the Indian subcontinent. The plant is 30-50cm height. Leaves are bipinnate, the flowers are pink and the effective parts used as medicine are leaves, stems & roots. The aerial parts of TP were used to treat pathological conditions like hydrophobia, asthma, cough, heart, lung diseases, kidney problems, mouth ulcer and piles. *Tephrosia purpurea* is a medicinal plant, considered highly useful in bilious febrile attacks and obstruction of liver and spleen[1]. According to Ayurveda literature, this plant has the property of healing all types of wounds. It is an important component of some preparation such as Tephroli and Yakrifit used for liver disorders[2,3,4]. The leaves are reported to be useful in jaundice[5]. *T.purpurea* has been shown to possess antimicrobial activity[6], insecticidal and repellent activity[7], antilithiatic activity[8], anti hyperglycemic and anti lipid peroxidative effect[9]. The plant was found to contain rutin, quercetin, lupeol, retinoid mainly degulin, elliptone, rotenone and tephrosin[10, 11]. *Tephrosia purpurea* is a common weed found in all parts of India and has been used as green manure in paddy cultivation. A preliminary ethnobotanical survey revealed that some communities in Thanjavur Taluk are using the aerial parts of *Tephrosia purpurea* in place of *Indigofera tinctoria* as the treatment for asthma and cough[12]. In present study antimicrobial activity and antihistamine activity of the aerial parts of *Tephrosia purpurea* were studied.

MATERIALS AND METHODS

Collection Of Plant Material

The aerial parts of *Tephrosia purpurea* were collected at Tamil University, Thanjavur. The collected aerial parts were dried under shade. These dried materials were mechanically powdered using 80 meshes and stored in a container.

Preparation Of Plant Extracts

5g of powdered material with 50% alcohol (50ml alcohol with 50 ml water) was shaken well occasionally for 6 hours and kept undisturbed for 18 hours. The liquefied extract thus obtained was concentrated in a vacuum pump and the percentage was calculated with the weight of the extract obtained. The extract was stored in a refrigerator and used for the present study.

Antimicrobial Activity

Well Diffusion Assay method[13]. The 50% alcohol extracts of *Tephrosia purpurea* were tested for their antibacterial and antifungal studies. The bacterial strains tested against various extracts were *Escherichia coli*, *Klebsiella pneumonia, Streptococcus pyogens* and *Citrobacter divergens* and fungal strains *Aspergillus niger*, *Aspergillus flavans, Candida albicans and Rhizopus* were tested.

Test Against Standard Controls

The commercially available antibiotic discs were used as standard controls for the entire test microorganism. The sensitivity patterns were recorded and the readings were interpreted according to the critical diameter given by National Committee for Clinical Standards.

The bacterial and fungal pathogens were obtained from the Microbiology Laboratory, Sea Horse Hospital Pvt., Tiruchirapalli. Test bacterial strains were seed over the Muller Hinton agar plates and Sabourach's dextrose agar plates were prepared for fungi aseptically. In wells, drugs (0.5ml) were injected using a micropipette for all concentration.

Separately the plates were incubated at 39°C for 48 hours. The plates were observed for the elevating zone around the well. The zone of inhibition was calculated by measuring the diameter of their inhibition zone around the well (in mm)

including the well diameter. Reading was taken in three different fixed directions in all three replicates and the average values were calculated.

Antihistamine activity

Materials of instruments

Kymograph and smoked drum, Frontal lever, L.Stand, T-Rod, X-Blok, Screw lip, Marriott bottle, Rubber tubes, Tuberculin's syringe 26 no needle, Droppers, Thermometer, Thread and needle(non-stretch nylon),Surgical gloves, Acrylic Board, Dissection kit: Scalpel, Forceps, Scissors, Dissection, Pins, Tape, Microscope& Petri dish.

Drug preparations

Extract preparation

To 60 mg portion of the extracts (same TP 50% alcohol) were scraped off from the bottom of the container and placed in a motor and pestle. To this added 2ml of distilled water and triturated well. This mixture was the maid up 6ml of distilled water. This process gave a stock solution of 10 mg/ml. This solution was tested against the guinea pig ileum preparation.

Histamine

A stock solution of 5mg/ml was made with triode solution. This concentration was added to the bath and used as a standard drug.

Animals

Selection Of Animal's Species

The Health Adult male guinea pigs (460g; Hartley strain) were kept separately in individual polypropylene cage with stainless steel hopper. The females were nulliparous and non-pregnant.

Housing and feeding conditions: The temperature in the experimental animal room 22±3°C. Although the relative humidity was 30% and preferably not exceeding 70% other than during room cleaning and the aim was 50-60%. Lighting used artificially, the sequence being 12 hours light and 12 hours dark. The animal was chosen individually. For feeding, conventional laboratory diets was used with an unlimited supply of drinking water. The study was performed under CPCSEA guidelines and IAEC.

Preparation Of Animals

The animals were uniquely identified and kept in their cages for five days prior to dosing for acclimatized to the laboratory conditions. During acclimatization the animals were observed for ill health.

Perfusions apparatus (Morgan et al., 1961)[14]

In this system the tissue was suspended in a 20cm (internal dimensions) water jacketed chamber with a coarse glass filter disk sealed into the lower portion. A mixture of moistened O_2: CO_2 (95:5) was delivered by small diameter tubing to the lower portion of the chamber by aerator.

Methods

For the preparation of tissues, adult male guinea pigs (460g: Hartley strain) were killed by a blow to the head and exanguinated. The abdomen region was opened an identification ileo-cecal junction. The lumen of ileum were removed, the intact tissue and rubbed preparation in which the blood had been removed by vigorously rubbing the luminal surface with filter paper. A piece of ileum was excised (approximately 3-4) by using surgical suturing needle tied a thread at each end. One of the threads was tied to the hook of the aeration tube and the other to frontal writing level. The ileum was mounted in 30 ml organ bath under a load of 500g. The tissues were allowed to equilibrate for 90 min in Tyrode solution (composition in Mm): NaCl 139.2, KCL 2.5, $CaCl_2$ 1.8, and $MgCl_2$ 0.49, NaH_2PO_4 0.4, Glues 5.5, pH 7.4 and gassed with 5% CO_2 and 95% O_2 at 37°C. During the equilibration period the bath fluid was exchanged every 10 min with fresh Tyrode solution. All protocols were applied to both intact and rubbed preparations.

Drug Injected Order For 50% Alcoholic Extract Of TP

Stabilization -----> Histamine (500ng) -----> Washing ------> Histamine (1µg) -----> Histamine (2µg) -----> Washing -----> Histamine (4µg) -----> Washing Sample (2mg) + Histamine (500ng) -----> Washing ------> Sample (4mg) + Histamine (500ng) -----> Washing ------> Sample (8mg) + Histamine (500ng) -----> Washing.

RESULT

Anti-microbial activity of 50% alcoholic extracts at different concentration against the fungal strains *Aspergillus fumigates*, *Aspergillus niger*, *Ganoderma lucida* and C*andida albicans* and bacterial organism *Escherichia coli, Serratia marcescens*, *Staphylococcus aureus* and *Staphylococcus epidermis* was evaluated. 50% alcoholic extracts of *Tephrosia purpurea* at 5mg, 10mg and 20mg concentration showed antibacterial activity against *Escherichia coli, Serratia marcescens* and *Staphylococcus epidermis*. Extracts of *Tephrosia purpurea* at 5 mg, 10 mg and 20 mg antifungal activity against all the extracts did not show positive activity against A*spergillus fumigatus*, *Aspergillus niger*, *Ganoderma lucida* and *Candida albicans*. (Table-1:Fig.1)

Anti Histamine activity of 50% alcohol extraction of TP was evaluated guinea-pig ileum Table (Fig 2). It was observed that different concentration with (2mg, 4mg & 8mg/ml) of TP extraction antagonized the contraction of ileum induced by Histamine in dose dependent women. The extract at 2 mg/ml concentration has the maximum antagonistic (Table-2).

DISCUSSION

Anti Microbial Activity

50% alcoholic extract of aerial parts of TP were screened against human pathogenic method. The 50% alcoholic extract of aerial parts of TP showed antimicrobial activity against *Escherichia coli, Serratia marcescens* and *Streptococcus epidermis*. Maximum zone of inhibition was observed at 50% alcoholic concentration. TP extract did not show any activity against other bacteria and fungi species used.

Anti-Histamine Activity

The observation that there was an increase in sensitivity to several contractile agents in guinea-pig isolated ileum preparation has been made by a number of investigators[15]. The results obtained with histamine in guinea-pig isolated ileum preparations are sensitive to histamine against at the lower concentration. Our study failed to produce maximum response to this contractile agent. This investigation reveals that the samples which partially antagonist is an agent which serves to inhibit the release or action of histamine. The extracts of TP 50% alcohol can be used to describe any histamine antagonist.

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Table - 1. Antimicrobial activity of aqueous 50% alcohol extracts of *Tephrosia purpurea* aerial parts

Organism/ Samples	Zone of inhibition (in mm)		
	5mg	10mg	20mg
Escherichia coli	0.7	0.9	0.6
Serratia marcescens	0.6	0.6	0.7
Staphylococcus aureus	-	-	-
Staphylococcus eapidermis	0.5	0.6	0.5
Aspergillus fumigatus	-	-	-
Aspergillus niger	-	-	-
Ganoderma lucida	-	-	-
Candida albicans	-	-	-

Table - 2. Height for concentration response curve of Histamine and sample with histamine

Drug and Treatment	Height of the concentration response curve (cm) (TP 50% Alcohol)
Histamine (500ng)	1
Histamine(1 µg)	1.4
Histamine(2 µg)	2.3
Histamine(4µg)	3.3
Sample(2mg)+ Histamine(500ng)	1.5
Sample(4mg)+ Histamine(500ng)	1.2
Sample (8mg)+ Histamine(500ng)	1.0

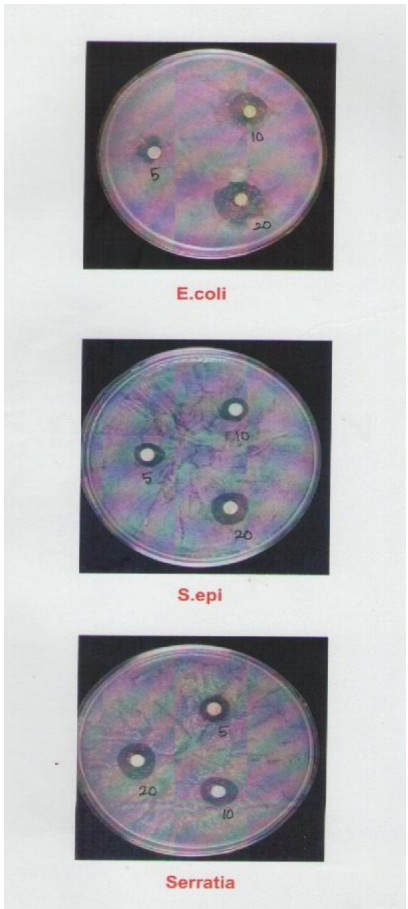

Figure 1: Antimicrobial activity of Zone inhibition of 50% alcohol extracts of *Tephrosia purpurea* L.

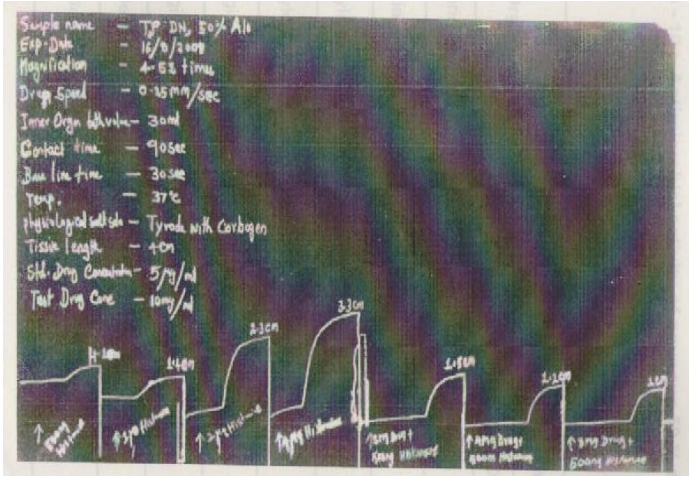

Figure 2: Effect of *Tephrosia purpurea* 50% alcohol extract against histamine on isolated ileum

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