Research Article
CODEN (USA): IJPLCP

[Rayalu *et al.*, 4(2): Feb., 2013]
ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

# Phytochemical analysis, TLC profiling and antimicrobial activity of *Tephrosia purpurea*

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## Abstract

In the present study we carried out phytochemical analysis and anti microbial activity were performed on different solvent extractions like Hexane, ethyl acetate, methanol, ethanol and aqueous extract of *Tephrosia purpurea*. Among the all salvent extracts methanol, ethanolic and aqueous extracts have high phytochemicals, based on phytochemical and TLC profiles anti microbial activates was performed with some gram positive ,gram negative and few fungal strains in methanol, ethanolic and aqueous extracts. Based on increased concentration methanol extract shown potent activity against all strains.

Key-Words: *Tephrosia purpurea*, Phytochemical, TLC, Anti-microbia activity

## Introduction

From ancient times plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being (Aiyelaagbe *et al.,* 2000 and Nostro *et al,* 2000). With the rising prevalence of microorganisms developing resistance to antibiotics, there is an urgency to develop new antimicrobial compounds. Being nontoxic and easily affordable, there has been resurgence in the consumption and demand for medicinal plants (Jayashree and Maneemegalai, 2008).

*Tephrosia purpurea* belongs to the family Fabaceae, subfamily Faboideae, tribe Millettieae, and it is a highly branched suberect herbaceous perennial, up to 60 m in height with spreading branches; the leaves are imparipinnate, with narrow, oblanceolate leaflets; the flowers are red or purple in extra-axillary racemes, the pods are slightly curved, 3-4.5 cm long, grey, smooth and containing 5-10 seeds per pod (Warrier et al 1993, Orwa et al 2009) The plant grows abundantly in the upper Gangetic plains, and western Himalayas. The herb is commonly grown as a green manure in paddy fields in India and in tobacco and rubber plantation in other countries.

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It grows ubiquitously in all soils, sandy, rocky and loamy (Panda 2000).In India and South Africa, it is used as a fodder before flowering, but in Australia it is reported to cause livestock poisoning. In northern India, dry plants are collected for fuel. All parts of the plant have tonic and laxative properties. The dried plant is deobstruent, diuretic and useful in treating bronchitis, bilious febrile attacks and obstructions of the liver, spleen and kidneys. It is also recommended as a blood purifier, in the treatment of boils and pimples and is considered a cordial treatment. In southern India, a decoction of the fruit is given for intestinal worms and a fruit extract is used to relieve bodily pains and inflammatory problems. The roots are bitter and the decoction is used as a nematicide for treatment against *Toxocora canis* larvae which cause a lung disease in Sri Lanka; it is also used for treating dyspepsia, colic, and chronic diarrhoea and as an antihelminthic (Despande et al 2003, Lodhi et al 2006). Several reports of *T. purpurea* have demonstrated the presence of flavones, flavanones and prenylated flavonoids (Gupta et al 1980, Pelter et al 1981) chalcones (Pelter et al 1981, Sinha et al 1982, Ventakata et al 1984 and Chang et al 2000) and rotenoids (et al 1984 and Chang et al 2000). In continuation of our chemical investigation on some medicinal plants of the genus *Tephrosia* (Saxena et al 1997, Hegazy et al 2009) reinvestigation of the methylene chloride extract of aerial parts of *T.*

*purpurea* resulted in isolation and structural elucidation of three compounds: an aromatic ester 1, a sesquiterpene of the rare rotundane skeleton 2 and a prenylated flavonoid 3, isolated for the first time from this species.

## Material and Methods

**Plant material**

Leaves of Tephrosia purpurea were collected from fields near to nalamalla forest, A.P, India.

**Preparation of plant extracts**

All solvent extractions like Hexane, Ethyl acetate, methanol, ethanol and aqueous extract of *Tephrosia purpurea* leaves was prepared according to the method of Hossain et al 1992. 3Kgs of *Tephrosia purpurea* leaves were dried, powdered and then soaked in 10 lts of Hexane, Ethyl acetate, methanol, ethanol and aqueous. All Solvent extracts was evaporated by the process of Distillation procedure for recovery of solvent. and aqueous extract was evaporated in a rotavapour at 40-500C under reduced pressure. A 15-20% semisolid black and light greenish yellow material obtained was stored at 0-4$^0$C until used.

**Thin layer chromatographic study**

Silica gel 60 F254 – TLC aluminium sheets (Merck, Germany) were used for the thin layer chromatographic study and solvent system developed in solvent system (i)-Butnaol: acetic acid: ethanol: water (BAW) (50:10:10:30) Freshly prepared iodine spray reagents were used to detect the bands on the TLC plates.

**Preliminary phytochemical screening:**

The powdered plant material was subjected to preliminary phytochemical analysis to test the presence or absence of phytochemical constituents by the method of Harbone 1983.

**Preparation of the tested organisms**

**A) Preparation of standard bacterial suspensions:**

The average number of viable, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* organisms per ml of the stock suspensions was determined by means of the surface viable counting technique. About (108-109) colony-forming units per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

**B) Preparation of standard fungal suspensions**

The fungal cultures (*Aspergillus niger* and *Candida albicans* were maintained on Saboraud dextrose agar, incubated at 25ºC for 4days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in (100ml) of sterile normal saline and the suspension was stored in refrigerator till used.

**In vitro testing of extracts for antimicrobial activity:**

**Testing for antibacterial activity**

The cup-plate agar diffusion method was adopted according to to assess the antibacterial activity of the prepared extracts. 0.6 ml of standardized bacterial stock suspensions (108-109) colony- forming units per ml was thoroughly mixed with 60 ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into sterile Petri dishes. The agar was left to set and in each of these plates 4 cups, 10 mm in diameter, was cut using a sterile cork borer No. 4 and the agar discs were removed. Alternate cups were filled with 0.1ml of each extracts using microtiter-pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 hours. Two replicates were carried out for each extract against each of the test organism. Simultaneously addition of the respective solvents instead of extracts was carried out as controls. After incubation the diameters of the results and growth inhibition zones were measured, averaged and the mean values were tabulated.

**Testing for anti-fungal activity**

The same method as for bacteria was adopted. Instead of nutrient agar, yeast and mould extract agar was used. The inoculated medium was incubated at 25ºC for two days for the *Candida albicans* and three days for *Aspergillus niger*.

## Results and Discussion

**Phytochemical activity**

Extracts were prepaired based on their polarity like Hexane, ethyl acetate and methanol, ethanol and aqueous, Phytochemical screening of the solvent extracts of *Tephrosia purpurea* shown in (table-1). Secondary metabolites like Steroids, Saponins, Triterpenes, Alkaloids, Carbohydrates, Flavanoids, Tannins, Glycosides and Polyphenols were tested in all extracts. Steroids, triterpenes, Alkaloids, Tannins, Glycoside and falvanoids were present in all solvent extracts where as polyphenols were present in methanol, ethanol and aqueous extracts, Carbohydrates are present in ethanol extract and are completely absent in all the extracts. Saponins are absent in all salvent extracts (Table 1).

**Antimicrobial activity**

The antimicrobial activity was performed based on their phytochemical results. Among the all extracts, methanolic, ethanolic and aqueous extracts were selected for antimicrobial activity and tested against few Gram- positive, Gram- negative and fungal species (*Bacillus subtilis, Bacillus cereus and Staphylococcus aureus and Micrococcus roseus, Salmonella typhi,*

*Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa, Asperillus niger and Candida tropicali.* The results revealed that the extracts showed moderate to high antimicrobial activity against all the tested microbial strains. The antimicrobial activity was evaluated from the zone of inhibition (Table-2). With increase in concentration of the extracts from 25 to 100 mg/ml, an apparent increase in antimicrobial activity was observed in all the extracts. Among various solvent extracts studied, Methanol extract at a concentration of 100 mg/ml showed the highest degree of inhibition followed by ethanol and water extracts (Table 2).

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**Table 1: Phyto-chemical screening of the extracts**

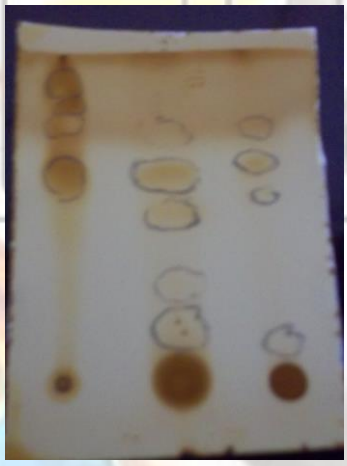
| S./No | Compounds | *Tephrosia purpurea leaves extracts* | | | | |
|---|---|---|---|---|---|---|
| | | **Hexane** | **Ethyl acetate** | **Methanol** | **Ethanol** | **Aqueous** |
| 1 | Steroids | + | ++ | + | + | ++ |
| 2 | Triterpenes | + | + | + | + | + |
| 3 | Saponins | – | – | – | – | – |
| 4 | Alkaloids | + | + | ++ | ++ | + |
| 5 | Carbohydrates | – | – | – | ++ | – |
| 6 | Flavonoids | + | + | ++ | +++ | +++ |

| | | | | | | |
|---|---|---|---|---|---|---|
| 7 | Tannins | + | + | + | ++ | ++ |
| 8 | Glycosides | + | + | ++ | ++ | ++ |
| 9 | polyphenols | – | – | + | + | + |

**TLC profiles**

Hexane extract of TF *

Ethyl acetate extract of TF*



Methanol extract of TF *

Ethanol extract of TF*

***Fig 1: All samples are spotted in triplicate form**

**Table 2: *Invitro* antimicrobial activity of bark extracts of *Tephrosia purpurea***

| S/No | Micro organisms | Zone of Inhibition (mm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Methanol extract (mg/ml) | | | Ethanol extract (mg/ml) | | | Aqueous extract (mg/ml) | | |
| | | 100 | 50 | 25 | 100 | 50 | 25 | 100 | 50 | 25 |
| 1. | *Bacillus subtilis* | 16 | 15 | 10 | 13 | 12 | 7 | 12 | 6 | – |
| 2. | *Bacillus cereus* | 16 | 12 | 10 | 12 | 8 | – | 10 | – | – |
| 3. | *Staphylococcus auras* | 17 | 12 | 9 | 8 | – | – | 10 | – | – |
| 4. | *Micrococcus roseus* | 15 | 9 | – | 14 | 9 | – | 12 | – | – |
| 5. | *Salmonella typhi* | 14 | 10 | 7 | 13 | 6 | – | 12 | 10 | – |
| 6. | *Klebsiella pneumoniae* | 12 | – | – | 15 | 11 | 9 | 12 | 6 | – |
| 7. | *Escherichia coli,* | 19 | 15 | 10 | 14 | 10 | 6 | 18 | 13 | 8 |
| 8. | *Pseudomonas aeruginosa* | 15 | 9 | – | 12 | 9 | – | 12 | – | – |
| 9. | *Aspergillus niger* | 16 | 13 | 6 | 16 | 12 | 7 | 10 | 8 | 6 |
| 10. | *candida tropicali* | 10 | – | – | 13 | 7 | 9 | – | – | – |