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ANTICANCER ACTIVITY OF METHANOLIC EXTRACT OF *TEPHROSIA PURPUREA* (LINN.) PERS LEAVES

P. Nagaveni*, G. Krishna Mohan[1] and Switi B. Gaikwad[1]

*Department of Pharmacognosy Pulla Reddy Institute of Pharmacy Domadugu(v), Gummadidala(M), S-angareddy(D), Hyderabad.

[1]Department of Pharmacognosy, Centre for Pharmaceutical Sciences, Institute of Science and Technology, JNTUH, Hyderabad 500 085, India.

***Corresponding Author: P. Nagaveni**

Department of Pharmacognosy Pulla Reddy Institute of Pharmacy Domadugu(v), Gummadidala(M), S-angareddy(D), Hyderabad.

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ABSTRACT

Tephrosia purpurea (Linn.) Pers belonging tofamily Fabaceae is a much branched, erect, perennialherb. It is commonly known as **Sharapunkha**in Sanskrit, Vempalli in Telugu, andWild Indigo in English. Traditionally, plant has been reported for the treatment of different ailments like anthelmintic, antipyretic, diseases of liver, spleen, heart, blood, tumours, ulcers, leprosy, asthma etc. According to Unani system of medicine, root is diuretic, enriches blood, and cures diarrhoea, useful in bronchitis, asthma, liver, spleen diseases, inflammations, pimples, piles, syphilis and gonorrhoea. Leaves are used as a tonic and a promising appetizer. Present study was an attempt to find out the anticancer potential of methanol extract of *T. purpurea* leaves for antimitotic activity by ***Allium cepa*** method and **brine shrimp lethality** test using ***Artemia salina***eggs.Results suggest that methanol extract (10 mg/ml) was found to possesscomparable antimitoticactivity withstandard drug Methotrexate (0.1 mg/ml) and for brine shrimp lethality test, 200 µg/ml of extract has shown significant activity.

KEYWORDS: *Tephrosia purpurea*, Fabaceae, Sharapunkha, antimitotic, brine shrimp lethality test, Methotrexate.

INTRODUCTION

Medicinal plants are natural resources yielding valuable herbal products which are often used in the treatment of various diseases and ailments. Herbs are staging a comeback andherbal 'renaissance' was happening all over the globe. Herbalism and folk medicine, both ancient and modern have been the source of much useful therapy.[1-3]

Tephrosia purpurea (Linn.) Pers belonging tofamily Fabaceae is a much branched, erect, perennialherb. It is commonly known as **Sharapunkha**in Sanskrit, Vempalli in Telugu, andWild Indigo in English. Traditionally, plant has been reported for the treatment of different ailments like anthelmintic, antipyretic, diseases of liver, spleen, heart, blood, tumours, ulcers, leprosy, asthma etc. According to Unani system of medicine, root is diuretic, enriches blood, and cures diarrhoea, useful in bronchitis, asthma, liver, spleen diseases, inflammations, pimples, piles, syphilis and gonorrhoea. Leaves are used as a tonic and a promising appetizer.[4-7]

Chemical constituents roots contain tephrosin, dengulin, quercetin, isotephrocinand rotenone. In the roots and leaves 2.5% rutin is found. A new β-hydroxychalcopurnone, Isolonchocarpin, pongamol, Lanceolatin A, lanceolate B, karanjin, kanjone and β-sitosterolis isolated from roots.[8]

MATERIALS AND METHODS

Procurement of plant material

Fresh leaves of*Tephrosia purpurea* were collected from the Neradikonda, Adilabad district (Telangana), India and authenticated at Department of Botany, Osmania university, Hyderabad.

Extraction

Dried and powdered leaves were subjected to the cold maceration using methanol as solvent. The extracts were concentrated in open water bath. Extracts obtained were stored in air tight container and used further for Preliminary Phytochemical Screening and anticancer screening.

ANTIMITOTIC ACTIVITY

Antimitotic activity was evaluated by using the meristematic cells of *A. cepa* roots.*Allium cepa* bulbs were divided into 3 different groups and each group containing 5 root tips. *Allium cepa* bulbs were sprouted in wet sand at room temperature. When the roots were about 5 mm long the bulbs were placed on vials containing the extracts (10mg/ml, and 20mg/ml) followed by the immersion of roots in the extracts. The

duration of extract treatments for each bulb was 1 and 3 hrs respectively.

The sprouted roots were also treated with distilled water (Control) and Methotrexate (0.1 mg/ml, standard drug). One hour later the root tips were cut and transferred to fixing solution of 45% acetic acid and 95% ethanol in the ratio of 1:3 v/v (10- 12 hrs) followed by warming the root tips in 1 N HCl in oven at 50°C for 15 min and then stained with carmine stain. The slides were observed under microscope to record the number of non-dividing and dividing cells. Same procedure was after 3 hrs extract treatment. Mitotic index was calculated using the following formula as per standard procedures. Five replicates were prepared for each concentration of test drugs.[9-11]

Mitotic Index = (Number of dividing cells/ Total number of cells) x 100

BRINE SHRIMP BIOASSAY

Brine shrimp bio assay was determined by using *Artemia salina* eggs. Brine shrimps were divided into 7 different groups and each group containing 10 brine shrimps.

In this test, brine shrimp (*Artemia salina*) eggs were hatched in artificial sea water (38g/l of sea salt). The brine shrimp test (BST) bioassay experiment is performed according to the procedure described by Meyer. After 48 hr. of incubation, 10 brine shrimps were transferred to each sample vial using pipette and artificial seawater was added to make 5-ml. sample vials were previously prepared by dissolving specific concentration of test drugs (50,100,200,400µg/ml) with different dilutions. Methotrexate was used as standard drug, caffeine as a positive control. Artificial sea water as control.The Survivors were counted after the 24 hrs drug treatment. Three replicates were prepared for each concentration of test drugs.[1,9,12-18]

Statistical Analysis

The results were expressed as mean ± S.E.M. Data was analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. Value of P less than 5% (i.e. $p<0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Methanol extract of *Tephrosia purpuria* leaves shown the presence of Alkaloids, Carbohydrates, Flavonoids, Phenols, Steroids, Saponins, and Tannins.

Effect of Leaves extracts on Antimitotic activity

An agent that prevents or disrupts mitosis is called as Antimitotic agent. Antimitotic constituents can stop the mitosis in anywhere of the cell cycle. Methanol extract of *Tephrosia purpuria*leaves reduced mitotic index significantly after 1 and 3 hour extract treatment (10 mg/ml at 3hr) which was comparable with mitotic index of methotrexate (0.1 mg/ ml) (Figure.1).Mitotic index of leaves extract 62.6% and P value ($P<0.0001$) was observed.

Mitosis was normal in the control roots. The mitotic index of leaves extracts (20mg/ml) treated roots were significantly lowered than the mitotic index of the control. Leaves extract (20mg/ml at 3hr) exhibited significant Antimitotic activity as reduction in mitotic index in leaves extract 64.8% and P value ($P<0.001$) was observed. Mitotic index of methotrexate was found to be 64.6% and 62.4%, after 1 and 3 hours treatment respectively.

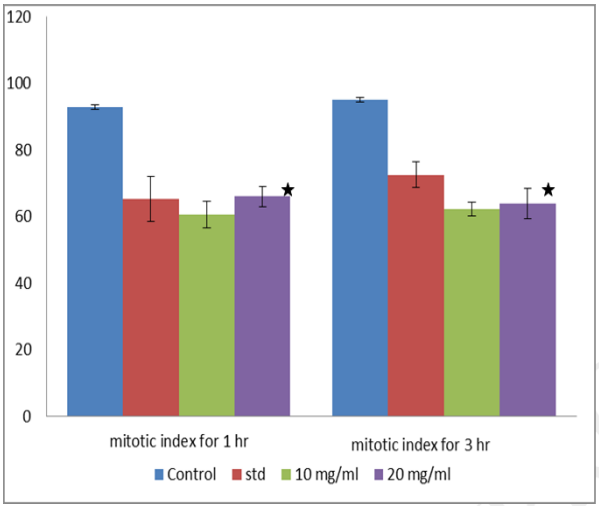

Figure.1: Antimitotic Effect of Leaves extracts.

Effect of leaves and stems extract on Brine Shrimp Lethality Test

The brine shrimp test represents a rapid, inexpensive and simple bioassay for testing plant extract lethality, which in most cases correlated reasonably well with cytotoxic and anti-tumour properties. In this test numbers of survivals were counted. Larvae were considered dead if they did not exhibit any movement during several seconds of observation. Brine shrimp can survive up to 48 hrs without food as they still feed on their yolk sac.

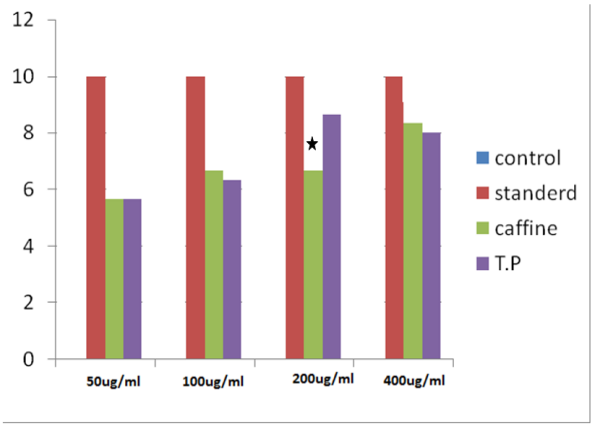

Figure.2: Number of brine shrimps dead after 12 hrs with different concentration of extracts.

The results shown in fig 2 indicates that the lethality shown by methanol extracts (P<0.001) of leaves extracts in the concentration of 200µg/ml was significant lethality showed. The standard drug i.e methotrexate at the concentration of 50,100µg/ml (P<0.0001) and caffeine at the concentration of 50µg/ml after 24 hours.

This test was performed by using *Artemia salina* eggs in different concentration of extracts and standard drug. Number of shrimps dead after24 hours were counted. The lethal concentration of tests resulting in 50% mortality of brine shrimps.

CONCLUSION

Methanol extract of leaves *Tephrosia purpuria*have shown the presence of the Alkaloids, Steroids, Phenols, Flavonoids, Tannins and Carbohydrates and also showed significant anti-cancer activity inthe two models in lower doses. *Tephrosia purpuria* leaves extracts were showed significant activity which was compared with standard. The obtained results i.e., presence of phytochemical constituents may be responsible for the anticancer activity.

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