Journal of Phytology 2010, 2(6): 42–46
Pharmacognosy
ISSN: 2075-6240
Available Online: www.journal-phytology.com

REGULAR ARTICLE

MEMBRANE STABILIZING POTENCY OF TWO *TEPHROSIA* SPECIES

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SUMMARY

Tephrosia maxima and *Tephrosia purpurea* Pers. is a pan tropical coastal shrub which contain flavonoids and isoflavanones. Since flavonoids exhibit good anti inflammatory activity an attempt has been made to evaluate its invitro anti inflammatory activity by means of HRBC membrane stabilizing method on three chloroform, ethyl acetate and methanolic extracts of the root of both the plants to identify the potent extract. The preliminary chemical tests revealed that all the extracts contain tannins and flavonoids. In the methanolic extract of both the plants presence of saponins was detected. In the invitro anti inflammatory activity screening it was observed that all the three extracts of both the plants showed significant HRBC membrane stabilization activity with regard to the standard hydrocortisone of 88.2% at 500 µg/ml. The methanolic extract of the plants was found be a better choice with a percentage protection of 79.49% and 79.01% at 500 µg/ml for *Tephrosia maxima* and *Tephrosia purpurea* respectively.

Key words: *Tephrosia maxima*, *Tephrosia purpurea*, Alsever solution, HRBC membrane stabilization

Sandhya S et al. Membrane Stabilizing Potency of Two *Tephrosia* Species. J Phytol 2/6 (2010) 42-46
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1. Introduction

Tephrosia is a genus in the Fabaceae family which is usually legume in nature. Many species in this genus are poisonous to fish, due to their high concentration of rotenone. In the last century, several *Tephrosia* species have been studied in connection with the use of rotenone as an insecticide and pesticide [1]. The different species of this genus are well known for its high content of flavonoids. *Tephrosia maxima* or *Galega maxima* L. is a prostate herb with 6-9 pairs of leaf lets. They are thin coriaceous, oblanceolate, entire, truncate-obtuse and mucronate. The flowers are pink in colour and are arranged in leaf opposed pseudoracemes. Sepals are appressed pubescent, standard sericeous and obovate. Pods are sparsely pubescent. The plant is distributed in wastelands and dry deciduous forests. The flowering and fruiting of the plant is usually throughout the year [2]. 7, 8-methylene dioxy isoflavone, maxima isoflavone H, were isolated from *Tephrosia maxima* along with the known isoflavone, maxima isoflavone B [3]. Another flavonone, maxima flavonone A, was also isolated from the chloroform extract of the roots of *Tephrosia maxima* along with maxima isoflavone J and T [4]. *Tephrosia purpurea* Pers. is a pan tropical coastal shrub that grows up to 1 m in height [5]. It is also known as ahuhu auhola, or hola [6]. The plant is an erect herb with leaf lets obovate, pubescent and ovate. It has pinkish blue or purple colored flowers. The fruits are pods and seeds are with strophiole in the middle. The roots of the plant are used in the treatment of various ailments like dyspepsia, diabetes, rheumatism, asthma, diarrhea, urinary complaints and cough. The whole plant is used to treat ulcers, fever and liver disorders. The pods are used in vomiting and inflammation [7]. Previous phytochemical investigations on this plant have shown the presence of coumarins, flavonoids and rotenoids, flavanones and isoflavanones and

quercetin [8]-[14]. Recently invented constituents are an isoflavone, 7,4 -dihydroxy-3 ,5 -dimethoxyisoflavone 1 , and a chalcone, -tephropurpurin 2,purpurin 3 , pongamol 4 , lanceolatin B 5 , maackiain 6 , 3-hydroxy -4- methoxy-8, 9-methylene-dioxypterocarpan 7, and medicarpin 8. Three novel flavonoids, (+)-tephrorins A and B and (+)-tephrosone, were isolated also isolated from *Tephrosia purpurea* [15], [16]. The toxic properties of the plant are due to the presence of flavonoids, rotenone and several of its isomers named deguelins. One of the deguelins, tephrosin, is poisonous to fish, but not to mammals. The leaves contain up to 2.5% rutin [17]. *T. purpurea* used for mediated chain reaction has been implicated in the centuries of Indian traditional medicine for the treatment various inflammatory disorders. It is considered beneficial for liver, spleen and kidney disorders and also it has property to cure all types of wounds. Experimental studies suggest that *T. purpurea* Linn exerts anti-ulcer, anti-oxidant, hepatoproductive and hypoglycemic activities [18]. It has been reported to possess hepatoprotective and mast cell stabilizing effect in various experimental models [19], [20].

2. Materials and Method

The plant materials were collected from Tirupathi at Talakona region and was authenticated by Dr. Madhava Chetty, S.V University, Tirupathi. UV-Spectrophotometer, [Elico]. Centrifugator, [Remi], Mumbai. Methanol, Finar Chem. Ltd, Mumbai; Chloroform and ethyl acetate, Qualigence Fine Chemicals Mumbai; Hydrocortisone Sodium Ranbaxy Laboratories Ltd.

150g of the root of both the plants were taken, cleaned and dried. It was powdered coarsely and packed to make a thimble in the soxhlet apparatus. The extraction was carried out using the solvents chloroform, ethyl acetate and methanol at a temperature 15-20^{o}C for 48hrs. Each time while changing the solvent the thimble was taken out and the root powder was dried to make it solvent free. The four different extracts were collected and concentrated by distillation at reduced temperature. A preliminary chemical test was performed to identify the chemical nature of the constituents present in the three extracts of both the plants [21], [22]. Evaluation of the anti- inflammatory activity on the root extracts of *Tephrosia* species was performed by HRBC membrane stabilization method. Human blood was taken and mixed with equal volume of sterilized Alsever solution. Alsever solution contains dextrose, sodium citrate, sodium chloride in water. The blood was centrifuged and the packed cells were washed with isosaline and 10% v/v suspension was made with isosaline. The drug samples were prepared by suspending the residues in hot water. The assay mixture contained the drug, 1 ml phosphate buffer , 2 ml hyposaline, 0.5 ml HRBC suspension, hydrocortisone sodium was used as the reference drug. Instead of hyposaline 2 ml of distilled water used in the control. All the assay mixture were incubated at 37˚C for 30 minutes and centrifuged at 300 rpm for 20min. The hemoglobin content in the supernatant solution was estimated using spectro photometer at 560nm. The percentage hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization was calculated using the formula,

$$\text{Percentage protection} = \frac{100 - \text{Optical density of drug treated sample}}{\text{Optical density of control}} \times 100$$

3. Result

The preliminary chemical tests revealed that all the extracts contain tannins and flavonoids. In the methanolic extract of both the plants presence of saponins was found [Table 1, 2].In the invitro anti inflammatory activity screening it was observed that all the three extracts of both the plants showed significant activity when compared to the standard hydrocortisone [Table 3, 4]. Among the three extracts of both the plants the methanolic extract was found to possess better activity with the percentage protection of 79.49% and 79.01% at 500 µg/ml for *Tephrosia maxima* and *Tephrosia purpurea* respectively.

Table 1: Preliminary chemical analysis of *Tephrosia maxima* root extract

Phytochemicals	Chloroform extract	Ethyl acetate extract	Methanolic extract
Carbohydrates	+	-	+
Amino acids	-	-	-
Proteins	+	+	+
Alkaloids	-	-	-
Tannins	+	+	+
Flavonoids	+	+	+
Saponin	-	-	+
Steroids	-	-	-
Glycosides	-	-	-

Table 2: Preliminary chemical analysis of *Tephrosia purpurea* root extract

Phytochemicals	Chloroform extract	Ethyl acetate extract	Methanolic extract
Carbohydrates	-	+	+
Amino acids	-	-	-
Proteins	+	+	+
Alkaloids	-	-	-
Tannins	+	+	+
Flavonoids	+	+	+
Saponin	-	-	+
Steroids	-	-	-
Glycosides	-	-	-

Table 3: HRBC Membrane stabilizing activity of *Tephrosia maxima*

Concentration of Drug(µg/ml)	SH		CE		EAE		ME	
	O.D	%Protection	O.D	%Protection	O.D	%Protection	O.D	%Protection
Control	1.236	-	1.236	-	1.236	-	1.236	-
100	0 .063	83.8	0 .0740	74.92	0.089	73.71	0.0994	72.86
200	0.059	84.9	0.0612	75.95	0.077	74.40	0.0834	74.16
300	0.057	85.4	0.0579	76.22	0.068	75.4	0.0717	75.11
400	0.051	86.9	0.041	77.59	0.052	76.7	0.0441	77.39
500	0.046	88.2	0.038	77.83	0.045	77.26	0.0175	79.49

Table 4: HRBC Membrane stabilizing activity of *Tephrosia purpurea*

Concentration of Drug(µg/ml)	SH		CE		EAE		ME	
	O.D	%Protection	O.D	%Protection	O.D	%Protection	O.D	%Protection
Control	1.236	-	1.236	-	1.236	-	1.236	-
100	0 .063	83.8	0 .0975	73.02	0.088	73.79	0.0945	73.26
200	0.059	84.9	0.755	74.80	0.071	75.16	0.0700	75.24
300	0.057	85.4	0.068	75.40	0.063	76.01	0.0668	75.50
400	0.051	86.9	0.057	76.29	0.048	77.43	0.0322	78.30
500	0.046	88.2	0.037	77.91	0.039	78.05	0.0247	79.01

SH-standard hydrocortisone, CE-Chloroform extract, EAE-ethyl acetate extract, ME-methanolic extract

4. Discussion

The lysozomal enzymes released during the inflammation produce a variety of disorders. The extra cellular activity of these is said to be related to acute or chronic inflammation. It was found that the NSAIDS act either by inhibiting these lysozomal enzymes or by stabilizing the lysosomal membranes. The same mechanism might be followed by the chemical constituents present in these extracts like tannins and flavonoids which are well known for their anti inflammatory activity. In this research work it was found that all the extracts of both the plants showed potent HRBC membrane stabilization activity with good percentage protection. The methanolic extract of the plants was found be a better choice. This may be due to synergistic effect produced by saponins in these extracts.

5. Conclusion

The HRBC membrane stabilizing property of *T. maxima* and *T. purpurea* were found to be promising. This may due to the tannins and flavonoid fractions in the extracts. Further studies on isolated compounds should be performed to establish the chemical responsible for the activity.

Acknowledgement

The authors are thankful to the Principal and management of Nalanda College of Pharmacy for their constant help and support in conducting this work to full satisfaction.

References

1. U.S. Food & Drug Administration (2006-03). *FDA Poisonous Plant Database*. U.S. Food & Drug Administration. http://vm.cfsan.fda.gov/~djw/pltx.cgi?QUERY=tephrosia. Retrieved 2008-01-21.
2. Venkata Rao E, Sree Rama Murthy.1985. Further studies on the isoflavones of *Tephrosia maxima*. Phytochemistry, 24(4): 875-876.
3. Venkata Rao E, Y Rajendra Prasad, M Sree Rama Murthy. 1994. A Prenylated flavonone from *Tephrosia maxima*. Phytochemistry, 37(1): 111-112 .
4. *WHO Traditional medicine strategy* 2002-2005, World Health Organization, Geneva.
5. Perry, L.M. 1980. Medicinal plants of East and South East Asia, MIT Press, Cambridge.
6. Arnold, M.D, L Harry. 1968. Poisonous Plants of Hawaii, Charles E. Tuttle Co, Tokyo, Japan.
7. Madhava Chetty, K Sivaji, K Tulasi Rao. 2008. Flowering Plants of Chitoor Dist, A.P, India. Student Offset Printers, Thirupathi.
8. Rajani, P, P.N Sharma. 1988. A coumestone from the roots of *Tephrosia hameltonii.* Phytochemistry, 27: 648.
9. Vankata, R. E, R Ranga. 1984. Two flavonoids from *Tephrosia purpurea.* Phytochemistry, 23:2339.
10. Sree Rama Murthy, M.E Venkata Rao. 1985. Maxima Isoflavone J: a New O-Prenylated Isoflavone from *Tephrosia maxima.* J. Nat. Prod., *48(*6): 967–968**.**
11. Leng Chee Chang, Clarissa Gerhäuser, Lynda Song, Norman R. Farnsworth, John M. Pezzuto Douglas Kinghorn A. 1997. Activity-Guided Isolation of Constituents of *Tephrosia purpurea* with the Potential to Induce the Phase II Enzyme, Quinone Reductase., J. Nat. Prod., *60(*9): 869–873**.**
12. Gupta R.A, M Krishnamurti, Parthasarathi. 1980. Purpurin, A flavanone from *Tephrosia purpurea* seeds. J. Phytochemistry, 19:1264.
13. Rao E.V, M.S.R Murthy, R.S Ward. 1984. Nine isoflavones from *Tephrosia maxima* isolated from the aerial parts and roots. Phytochemistry, 23:143.
14. Saxena V.K, A. Choubey. 1997. A neoflavonoid glycoside from *Tephrosia purpurea* stem. Fitoterapia, 68: 359–360.
15. Chang L.C, A Gerh,C ¤user, L Song, N.R Farnsworth, J. M Pezzuto, A.D Kinghorn. 1997. Activity-guided isolation of constituents of *Tephrosia purpurea* with the potential to induce the phase II enzyme, quinone reductase. J. Nat. Prod., 60(9): 869-73.
16. Leng Chee Chang, Daniel Chávez, L.S Lynda, R.F Norman, M John, Pezzuto, A

Douglas Kinghorn. Absolute Configuration of Novel Bioactive Flavonoids from *Tephrosia purpurea.* Org. Lett., 2 4: 515–518.

17. Orwa, C.A, R Mutua Kindt, R Jamnadass, R, S Anthony. 2009. Agroforestree Database: a tree reference and selection guide version 4.0 (http://www.world agroforestry.org/sites/treedbs/treedatab ases.asp).1- 5.
18. Gopalakrishnan S, E Vadivel, K Dhanalakshmi. 2010. Anti-inflammatory and analgesic activities of *Tephrosia purpurea* Linn. aerial and root extracts. Journal of Pharmacy Research, *3(5):1103-1106.*
19. Murthy M.S.R, M Srinivasan. 1993. Hepatoprotective effect of *Tephrosia purpurea* in experimental animals. Indian J Pharmacol., 25:34-6.
20. Gokhale A.B, V.J Dikshit , A.S Damle, K.R Kulkarni, M.N Saraf. 2000. Influence of ethanolic extract of *Tephrosia purpurea* Linn. on mast cells and erythrocytes membrane integrity. Indian J Exp Biol. 38:837-40.
21. Khandelwal, K.R, Practical Pharmacognosy 2005. Nirali Prakashan, Pune.
22. Kokate, C.K. Practical Pharmacognosy. 2003. 4th ed. Vallabh Prakashan, New Delhi.
23. V.R. Ravikumar, M. Dhanamani, Sudhamani. 2009. In-vitro anti-inflammatory activity of aqueous extract of leaves of *Plectranthus amboinicus* (Lour.) Spreng, Ancient Science of life, 28(4): 10-12.