Research Article

In Vitro Antioxidant Study of Extracts of *Tephrosia spinosa (L.F) Pers*.

R. Xavier Arulappa[1*] and K. Ilango[2]

[1]Research Scholar, Karpagam University, Coimbatore, Tamil Nadu, India.
[2] Department of Pharmaceutical Chemistry, SRM college of Pharmacy, SRM University, Kattankulathur, Kancheepuram , Tamil Nadu, India.

ABSTRACT

In the present investigation ,an attempt has been made to investigate the invitro antioxidant potential of chloroform and methanol extract of Tephrosia Spinosa (L.f) pers .The Nitric oxide assay method has been performed at different doses (20-100µg). The results of the present study shows that the methanol extract of Tephrosia Spinosa possess antioxidant activity through ABTS radical scavenging activity. The prelimnary phytochemical investigation indicates the presence of flavanoids and flavono glycosides. The results are found to be significant when compare with the standard ascorbic acid. Further studies are required to determine the mechanism and isolation of active constituents involved in the antioxidant activity .

Keywords: Tephrosia Spinosa, ABTS and antioxidant activity.

INTRODUCTION

Free radicals had been implicated in several human diseases e.g. atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, ageing, inflammatory response syndrome, respiratory diseases and cancer [1-4]. Many herbal plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species such as singlet oxygen, superoxide, hydroxyl radicals and peroxy nitrite [5-6]

Tephrosia Spinosa (L.f) pers belongs to the family fabaceae [7] and it is a stiffy throny shrub, known as mullukolinji commonly found in south India on dry barren lands on the coast and inland to the hills of Coimbatore, Madurai and Tirunelveli districts. The phytochemical studies revealed the presence of flavanoids [9]. It is used in traditional system of medicine for anti-rheumatic, antipyretic, indigestion, antidiarrheal, anti-inflammatory, anthelminitic and to control excessive thrist[10]. No systematic studies on anti-oxidant activity have been reported on *Tephrosia Spinosia*. Hence an effort has been made to establish the antioxidant activity.

Experimental

Material and methods

The aerial parts *Tephrosia Spinosa* was collected The aerial parts *Tephrosia spinosa* was collected from Madurai district in 2009 and authenticated by Dr. D. Stephen who is the taxonomist in American college, Madurai. A voucher Specimen(KMCP/RXA/CC-0280) was deposited in the department of Pharmacognosy, K.M. College of pharmacy, Uthangudi, Madurai for future reference. The air dried aerial parts of plant material was ground into coarse powder using cutter mill and then stored in an air tight container for further use.

Preparation of Extract

The coarse powdered plant material was defatted with hexane using cold maceration process and further subjected to extraction with chloroform followed by methanol successively by cold maceration for five days until complete extraction was effected. It was then concentrated under reduced pressure at 50^{o}c and finally dried in desiccators. The chloroform and methanol extracts were used for antioxidant activity.

ABTS Assay

The scavenging activity of the test sample was tested using $ABTS^+$ assay. The method was described by Re *et al*., 1999 with a slight modification. The $ABTS^+$ radical solution was prepared by mixing 14mM ABTS stock solution with 4.9 mM ammonium per sulphate and incubating 16h in the dark at room temperature until the reaction was stable. The absorbance of the $ABTS^+$ solution was equilibrated to 0.70±0.02 by diluting with ethanol at room temperature. To 1ml of the $ABTS^+$ solution various concentration of the test sample (20-100µg/ml) was added. The absorbance was measured at 734nm after 6minutes. The percentage inhibition of absorbance was calculated and plotted as a function of the concentration of standard and sample to determine the antioxidant concentration. Ascorbic acid was used as a standard.

$$\text{ABTS Scavenged (\%)} = \frac{(A_{cont} - A_{test})}{(A_{cont})} \times 100$$

Where,

A_{cont} is the absorbance of the control reaction.

A_{test} is the absorbance in presence of the extracts.

The antioxidant activity of extracts are expressed as IC_{50}. The IC_{50} value is defined as the concentration(µg/mL) of extracts that inhibits the formation of ABTS radicals by 50% .The results antioxidant activity of extracts of using ABTS free radical scavenging method are shown in Fig.1 and Table No :1.

RESULTS AND DISCUSSION

The obtained results of absorbance and percent inhibition showed decrease in the concentartion of ABTS radical due to the scavenging ability of extracts and ascorbic acid as a reference compound A .100µg/ml of CETS ,METS and ascorbic acid exhibits 51.89% and 91.26 % inhibition.

CONCLUSION

The chloroform and methanol extracts of tephrosia spinosa have antioxdiant activity. further studies are required to isolate the possible phytochemical constituents which may be responsible for anti oxidant activity.

Table 1: ABTS Radical Scavenging activity of extract of *Tephrosia Spinosa*

Concentration	STANDARD			T. S. CHOLOROFORM			T.S .METHANOL		
20.000	65.931000	65.308000	64.686000	99.151000	98.981000	98.812000	98.698000	98.755000	98.812000
40.000	65.931000	65.535000	65.139000	99.321000	99.208000	99.095000	99.264000	98.981000	98.698000
60.000	65.931000	65.988000	66.044000	98.981000	98.925000	98.868000	96.321000	96.095000	95.869000
80.000	100.000000	66.667000	33.333000	98.812000	98.642000	98.472000	93.435000	93.265000	93.096000
100.000	100.000000	94.907000	89.813000	98.698000	98.585000	98.472000	82.569000	82.400000	82.230000

Concentration	STANDARD			T. S. CHOLOROFORM			T.S .METHANOL		
20.000	65.30833	0.3594014	3	98.98133	0.09786231	3	98.755	0.03290851	3
40.000	65.535	0.2286306	3	99.208	0.06523998	3	98.981	0.1633907	3
60.000	65.98766	0.03262042	3	98.92467	0.03262262	3	96.095	0.13048	3
80.000	66.66666	19.24511	3	98.642	0.09814849	3	93.26534	0.09786011	3
100.000	94.90667	2.940732	3	98.585	0.06523998	3	82.39967	0.09786011	3

EC50	0.9491	8.061	2.172

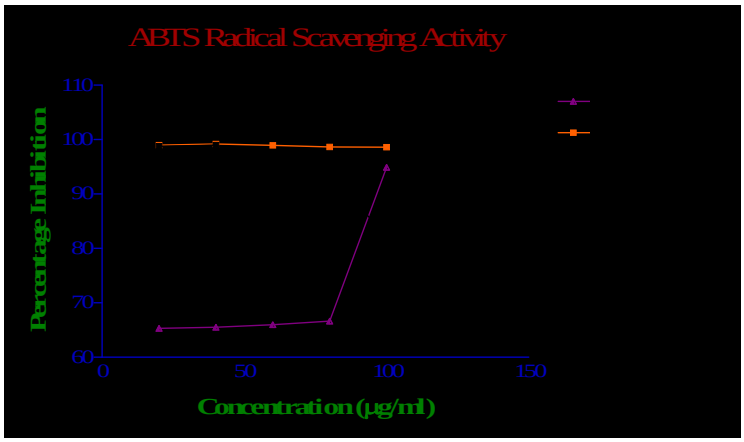

Fig. 1:

REFERENCES

1. Halliwell B. Lancet. 1994;344:721-724.
2. Gutteridge J and Halliwell B. Biochem J. 1984;219:1-4.
3. Pourmorad F, Hoseeinimehr SJ and Shahabimajd N. Afr J Biotech. 2006;11:1142-1145.
4. Halliwell B and Gutteridge J. Free Radicals in Biology and Medicine, Japan Scientific Press, Tokyo,1989;23.30
5. Dasgupta N and De B. Food Chem. 2004; 88:219-224.
6. David JM,. Barreisors AIBS and David JP. Pharm Biol. 2004;42:36-38.
7. Akthtar Husain O, Virmani P and Singh K. Dictionary of Indian medicinal Plants, Director, Central Institute of medicinal and Aromatic plants. 1992:455.
8. The flora of presidency of madras by Gamble J.S. Vol. I Aplard and son Ltd. London, 320-321.
9. Venkato Rao E and Rajendra Prasad. Phytochemistry. 1992;31(6):2121-2122.
10. The wealth of India – A dictionary of Indian raw materials and industrial Products Vol. X, Publication and information directorate, CSIR, New Delhi. 2001:155.
11. Ref: Re R, Pellegrini N, Proteggente A, Pannala A, Yang and Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad Biol Med. 1999;26:1231-1237.