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In vitro antioxidant activity of chloroform extract of aerial parts of *Securinega leucopyrus* (willd.) Muell

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ABSTRACT

Antioxidant activity of the aerial parts of Securinega leucopyrus was studied for its free radical scavenging property on different in vitro models e.g. DPPH, nitric oxide and hydroxyl radical scavenging activity by using chloroform extract. The phytochemical screening has shown the presences of Steroids, Alkaloids, Triterpenoids, Flavonoids and Tannins.Invitro antioxidant activity revealed that the chloroform extract showed more activity .The present study on Securinega leucopyrus showed that the plant has moderate anti oxidant activity and it was compared with standard vitamin E.

Keywords: *Securinega leucopyrus*; Antioxidant activity; Nitric oxide scavenging activity; Hydroxyl radical scavenging activity; DPPH.

INTRODUCTION

Oxygen is essential for the survival of all on, this earth. During the process of oxygen utilization in normal physiological and metabolic processes approximately 5% of oxygen gets univalent reduced to oxygen derived free radicals like hydrogen peroxide, hydroxyl, nitric oxide and superoxide radicals. Free radicals are produced in large amount during metabolic disease conditions like diabetes, hypertension, atherosclerosis, urolithiasis, ulcers etc. This free radicals attack DNA, protein molecules, enzymes and cells leading to change in genetic material and cell proliferation (Cancer).Several anti oxidants of plants origin are experimentally proved and used as effective protective against oxidative stress. They play an important role in major health problems like cancer [1].Plants which contains carotenoids, flavonoids and Tannins can be utilized to scavenge the excess free radicals from human body [2]. *Securinega leucopyrus (willd.) Muell,* belonging to the family Euphorbiaceae commonly known as Bushweed, Indian snow berry, is a thorny woody shrub or a small tree distributed in different parts of India [3]. Its leaves and fruits are edible. The berry is sweet. The slender branches are reported to be utilized

for preparing wicker-baskets and for thatching [4]. Its leaves are having germicidal properties. In chattisgarh, the decoction of leaves is used to dress the cancerous wounds. It is used in combination with tobacco. The juice or paste of the leaves along with tobacco used to destroy worms in sores. Its leaf decoction useful externally in the treatment of piles and it is used to wash the wounds of cattle. It is used as popular veterinary medicine [5]. Even though *Securinega leucopyrus* has lot of potential medicinal uses; the study on this plant is very less. Considering the importance of this plant, the present study was undertaken.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade. 1, 1-diphenyl-2-picryl hydrazine DPPH was obtained from sigma chemicals USA, 1% Sulfanilamide, Dimethyl sulphoxide (DMSO), 2% Phosphoric acid 0.1%, Naphthyl ethylene diamine dihydrochloride (Sigma chemicals, U.S.A.) .Vitamin E, Ammonium molybdate (4mM) (E.merck (India) Ltd., Mumbai).Gallic acid (Rolex chemical industries, Mumbai.). sulfanilic acid, Follin-Ciocalteu Reagent (NICE chemicals Pvt.Ltd.).Sodium carbonate (Monsanto chemicals Ltd. India). n-Naphlylethylene diamine dihydrochloride ,Potassium dihydrogen Phosphate (Accord labs, Secunderabad), Disodiumhydrogen Phosphate, Potassium ferry cyanide, Trichloroacetic acid, Ferric chloride, sodium nitropruside (S.D.fine-chem.ltd.).

Plant material

The fresh parts of the plant *Securinega leucopyrus*, were collected from Tribal Women's Welfare Trust's, Herbal Garden, Thandarai, Chengalpattu (Dist), Tamil Nadu in the month of December and authenticated by National Institute of Herbal Science, West Tambaram, Chennai (PARC/2009/289).

Preparation of plant Extract

The aerial parts of *Securinega leucopyrus* were cut into small pieces dried in shade and made into a coarse powder. The powdered aerial parts of *Securinega leucopyrus* (3.0kg) were extracted exhaustively with chloroform for 72 hours followed by 48 hours and 24 hours. The solvents were pooled, distilled under vaccum and dried under vaccum dessicator and designated as SLCE.

DPPH radical scavenging activity

DPPH radical scavenging was measured according to the method of [6]. In brief 3ml reaction mixture containing 200µl of DPPH (100µ*M* in ethanol) and 2.8 ml of SLCE in ethanol was incubated at 37^0C for 30 min .Then the absorbance of test and standard mixtures was read at 517nm. The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical. The formula used was,

Percentage Inhibition = OD of control- OD of Test/ OD of control

Nitric oxide scavenging activity

Nitric oxide scavenging was measured according to the method of [7]. Nitric oxide (NO) radicals were generated from sodium nitroprusside solution at physiological pH. Sodium nitroprusside (1ml of 10mm) was mixed with 1ml of SLCE of different concentrations in phosphate buffer. The mixture was incubated at 37^0C for 150 min. To 1ml of the incubated solution, 1ml of griess reagent (1% Sulfanilamide, 2% Phosphoric acid and 0.1% Naphthyl ethylene diamine dihydrochloride) was added. Absorbance was read at 546nm and percentage inhibition was calculated by using the formula

Inhibitory ratio = $\frac{(C - T) \times 100}{C}$ …… (1)

Where, C is the absorbance of control
T is the absorbance of plant extract

Inhibition of Hydroxyl Radical

Hydroxyl radical scavenging activity was performed [8], by studying the competition between deoxyribose and test extract for the hydroxyl radical generated by Fenton's reaction. 1ml of reaction mixture containing 0.2 ml SLCE solution of different concentrations (10-1000 µg/ml) and 100µl of each of deoxyribose (28mM), ferric chloride (0.1mM,), EDTA (0.1mM), ascorbic acid (0.1mM), and hydrogen peroxide (2mM) in phosphate buffer (P^H 7.4, 20mM), Vitamin E (50, 100, 200, 400µg/ml) were incubated at 37^0C for 1hr. After incubation, Trichloroacetic acid (15%), and Thiobarbituric acid (1% w/v) in 0.25 N HCl were added. The reaction mixture was kept in a boiling water bath for 30 min, cooled, and the absorbance was measured at 532 nm.

RESULTS AND DISCUSSION

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The propagation of free radicals can bring about thousands of reactions and thus may cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals [9, 10]. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals.

DPPH is a relatively stable free radical the assay determines the ability of SLCE to reduce DPPH radical to the corresponding hydrazine by converting the unpaired electrons to paired ones. Antioxidants can act by converting the unpaired electrons to paired ones. The dose dependent inhibition of DPPH radical (Figure.1) indicates that SLCE causes reduction of DPPH radical in a stoichometric manner [11-13].

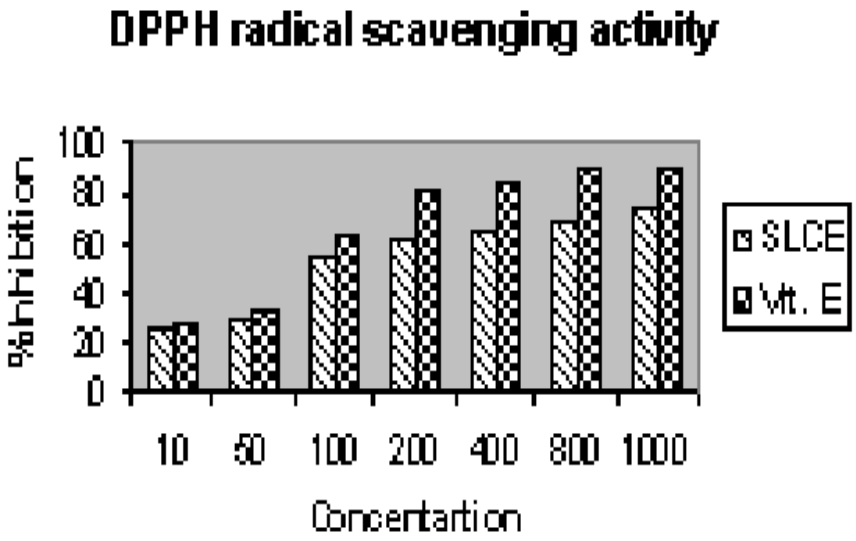

Figure 1

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and involved in the regulation of various physiological processes [14]. Excess concentration of NO is associated with several diseases [15, 16].

Oxygen reacts with excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals [9, 17]. In the present study, the extract competes with oxygen to react with nitric

oxide and thus inhibits the generation of the anions. SLCE had shown a dose dependant increase in Nitric oxide scavenging property (Figure.no.2.). The presence of flavonoids, tannins and steroids in these extracts may be responsible for Nitric oxide scavenging activity.

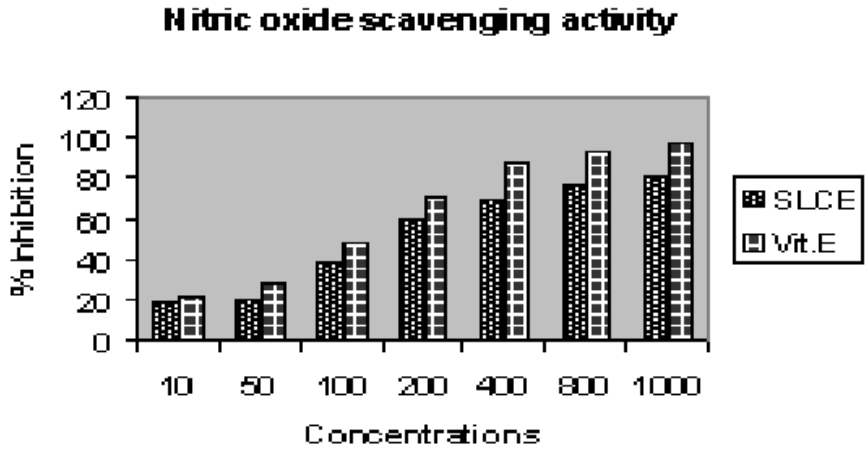

Figure 2

Ferrous salts can react with H_2O_2 and form hydroxyl radical via Fention's reaction. The iron required for this reaction is obtained either from the pool of iron or the heme containing proteins [9]. The hydroxyl radical $(OH)^-$ thus produced may attack the sugar of DNA deoxy causing ribose fragmentation, base loss, and DNA strand breakage [18]. The generation of $(OH)^-$ in fenton reaction is due to the presence of iron ions. When the Fe^{2+}/Fe^{3+} redox couple is bound by certain chelators, the $OH^{\bullet}$ fragmentation is prevented, where as the increased colour formation in the absence of crude extracts were observed in deoxy ribose assay. In this, the extract act as a chelator of iron ions , binding to them, & preventing the formation of free radicals, though the extracts not directly involved in the $OH^{\bullet}$ scavenging . The result indicates that the SLCE plays a major role in the inhibition of ribose fragmentation and hence the decreased colour formation in the deoxy rebose assay.

SLCE showed a dose dependant increase in hydroxyl radical scavenging property. The presence of flavanoids Tannins and steroids in these extracts may be responsible for Hydroxyl radical scavenging activity. The results were shown in figure.3.

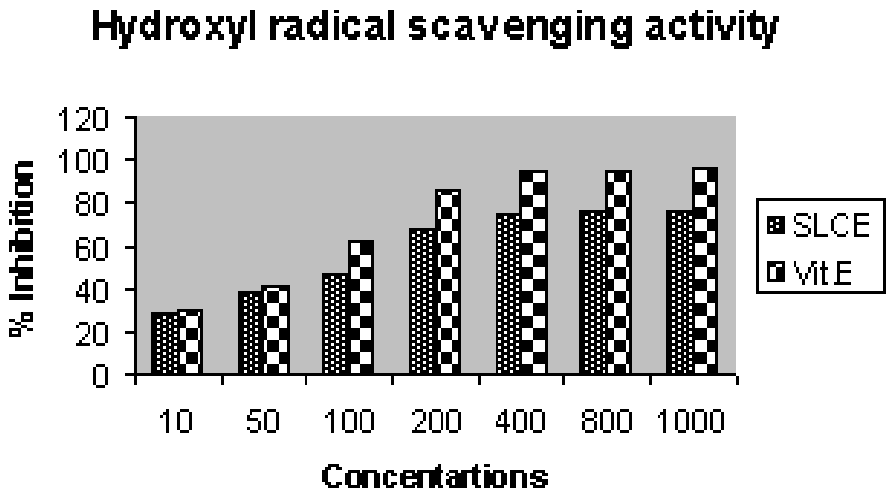

Figure 3

The free radical scavenging property of the crude extracts of plant against DPPH, Nitric oxide and Hydroxyl radical scavenging activity is clearly understood from the results of this chapter. The antioxidant activity of SLCE seems to be due to presence of flavanoids, tannins.

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CONCLUSION

The antioxidant activity of *Securinega leucopyrus* seems due to presence of flavanoids, tannins, which may act in similar fashion as reductones by donating the electron and reacting with free radicals to convert them to a more stable product and terminate free radical chain reaction. It is apparent from the present study that the *Securinega leucopyrus* not only scavenge off the free radicals but also inhibits the generation of free radicals.

It may conclude that SLCE have a significant antioxidant activity. These results are encouraging enough to isolate the active constituents present in the extract.

REFERENCES

[I] Nishigaki, H Oku, K Yogi, *Journal of Clinical and Nutrient Biochemistry*, **1992**, 13, 23.
[2] S Kumar, SA Dwivedi, S Kakreja, AK Sharma, R. Tewari, *Journal of medicinal and Aromatic plant sciences*, **2000**, 22 / 4A, 23/ 1A.
[3] ES Ayensu, World medicinal plant resourses. In conservation for productive agriculture VL Chopra, TN Khoshoo, edds. ICAR, New Delhi, **1996**, 11, 2.
[4] KR. Kirtikar, BD Basu, Indian medicinal plants, 5, 97.
[5] LM Bakshu, AJ Ram, RBV Raju, *Fitoterapia*, **2001**, 72, 8.
[6] T Yohozawa, CP Chen, E Dong, T Tanaka, GT Nonaka, I Nishioka, Biochemical pharmacology,**1998**, 56, 212.
[7] WK. Alderton, CE. Cooper, RGK. Nowels, *Journal of Biochemistry*, **2001**, 357, 593.
[8] J Robak, RJ Gryglewski, *Biochemistry Pharmacology*, **1998**, 378.
[9] R S Cotran, V Kumar & T Collins, In Robbins pathological basis of diseases, 6th ed.,Thomson Press(I)Ltd, Noida,1999;pp.1-43
[10] BP Yu, E A Suescun & S Y Yang, *Mech Ageing dev*, **1992,** 17.
[11] C Sanchez Moreno, *Food Sci Tech Int*, **2002** 8,122.
[12] C Sanchez Moreno, Larrauri J & Saura-Clixto F, *J Sci Food Agric*, **1999,**79,1301.
[13] T Vani**,** M Rajani, S Sarkar, CJ Shishoo, ***Int J Pharmacog***. **1997**, 35,313.
[14] H Lata & G K Ahuja, *Ind J physio&Alied Sci*, **2003,** 57,124.
[15] A.Ialenti, Moncada S& Di Rosa, *Br J Pharmacol*, **1993**,110,701.
[16] R Ross, *Nature*, **1993,** 362,801.
[17] G .S. Sainani , J .S. Manika, R.G. Sainani., *Med Update*, **1997**, 1, 1.
[18] T Kaneko, S Tahara & M Matsu, *J Free Radic Bio Med*, **1996**, 23, 76.