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Antioxidant Activity of the Leaves of *Teucrium polium* sp. Aurasianum

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Abstract: The antioxidant activities of water, ethanol and ether extracts of the leaves of *teucrium polium* sp. Aurasianum (TPA) were determined by the thiocyanate method. The antioxidant activity of water extract was increased with the increasing amount of extract (200-1000 μg) added to the linoleic acid emulsion. Ether extract was the most effective antioxidant among the extracts. Like antioxidant activity, the reducing power of water extract was concentration dependent. However, ethanol extract was the highest in reducing power and ether extract was the lowest. The results obtained in the present study indicate that the leaves of (TPA) are a potential source of natural antioxidants. In addition, we could suggest that although the reducing power of a substance may be an indicator of its potential antioxidant activity, there is not necessarily a linear correlation between these two activities.

Key words: Antioxidant activity, reducing power, *Teucrium polium* sp. aurasianum

INTRODUCTION

Reactive Oxygen Species (ROS), sometimes called active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions (O_2^-) and Hydroxyl radicals (HO·), as well as non free radical species such as Hydrogen peroxide (H_2O_2) (Squadriato and Pelor, 1998; Halliwell, 1995). Exogenous sources of free radicals include tobacco smoke, ionising radiation, organic solvents and pesticides (Robinson *et al*., 1992).

In addition, reactive oxygen species have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes and cancer (Tanizawa *et al*., 1992; Suh, 1998). When produced in excess, ROSs can cause tissue injury. However, tissue injury can itself cause ROS generation (Auroma, 1998).

Nevertheless, all aerobic organisms, including human beings, have antioxidant defenses that protect against oxidative damages and numerous damage removal and repair enzymes to remove or repair damaged molecules (Davies, 1994; Sun *et al*., 1998). However, this natural antioxidant mechanism can be inefficient and hence dietary intake of antioxidant compounds is important (Duh, 1998; Halliewll, 1994).

There are some synthetic antioxidant compounds, however, it has been suggested that these compounds have some side effects.

In addition, it has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human disease (Rice-Evans *et al*., 1997).

Therefore, research for the determination of the natural antioxidants source is important.

Teucrium polium sp. Aurasianum is a plant of the family of the labiatae (Chadefaus *et al*., 1960).

This plant is very widespread in the Mediterranean basin and especially in Algeria. It is a plant whose plane sheets are distinctly crenelated, the stems and the green sheets in entirety, the height varies between 5 and 10 cm, it known under the name of Khayatat el Djrah (Ozenda, 1977).

Nevertheless, there is as yet no report concerning the antioxidant effect of this plant. Our main objective is the determination of potential natural antioxidant sources. However, the purpose of this particular study is the determination of antioxidant activities of various extracts of (TPA). As some effects of this plant have been reported, this plant was chosen.

MATERIALS AND METHODS

Preparations of Extracts Leaves of (TPA), collected in May at an Altitude of 900m in the place says El-Kouahi in the area of Ain Mlila in Algeria and were left on a bench to dry. A 15-gram dried sample was chopped into small parts in a blender and then extracted with 500 mL of boiled water by stirring for 30 min followed by filtration. Afterwards, the filtrate was freeze-dried. Ethanol extract was obtained as follows: 15g of dried and chopped leaves

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was extracted with 500 mL ethanol by stirring for 5 h. In the ether extraction, the same amount of sample was extracted with ether in a soxhlet apparatus until extraction solvents become colorless. Both of the extractions were followed by filtration and evaporation of the filtrate to dryness in vacuum.

Antioxidant activity: Antioxidant activity was determined by the thiocyanate method.

Each sample (containing 200-1000 μg extract) in 0.5 mL of distilled water was mixed with 2.5 mL of linoleic acid (Sigma) emulsion (0.02M, in 0.04M pH 7.0 phosphate buffer) and 2 mL of phosphate buffer (0.04M, pH 7.0) in a test tube and incubated in darkness at 37°C.

The amount of peroxide was determined by reading absorbance at 500 nm after coloring with $FeCl_2$ and thiocyanate at intervals during incubation (Yen and Chen, 1995).

α-Tocopherol (Sigma) was used as standard antioxidant.

Reducing power: Extracts (100-1000 μg) in 1 mL of distilled water were mixed with 2.5 mL of phosphate buffer (0.2M, pH 6.6) and 2.5 mL potassium ferricyanide $[K_3Fe(CN)_6]$ (1%) and then the mixture was incubated at 50°C for 30 min.

Afterwards, 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min.

Finally, 2.5 mL of upper layer solution was mixed with 2.5 mL of distilled water and 0.5 mL of FeCl3 (0.1%) and the absorbance was measured at 700 nm (Yen and Chen, 1995).

Increased absorbance of the reaction mixture indicated increased reducing power.

Statistical calculations were done by using Statistica for Windows 4.3 and SPSS 9.0.

Values of $p<0.05$ were considered to be significant and values of $p<0.01$ very significant.

RESULTS AND DISCUSSION

In the present study, antioxidant activity was determined by the thiocyanate method in that the amount of peroxides formed in emulsion during incubation was determined by measuring absorbance at 500 nm. High absorbance is an indication of high concentrations of formed peroxides.

The antioxidant activity of water extract of (TPA) leaves increased with an increasing amount of extract. A similar property was determined with ether or ethanol extract.

As can be seen in Fig. 1, there is no clear difference between the control and the sample containing 200 μg extract.

However, peroxidation is suppressed about 6 h in the presence of 400 μg or 500 μg extract and after that it begins to increase. In the presence of 1000 μg extract or 500 μg α-tocopherol, the peroxidation process is delayed for about 12 h.

In order to determine the statistical significance of the above results, SPSS 9.0 software was used. After two way variance analysis, which showed that there was a statistically significant difference ($p = 0.000$), multiple comparison was carried out by LSD. There were statistically highly significant differences between the control and 1000 μg extract or control and 500 μg α-tocopherol ($p = 0.000$ for both). It was also interesting

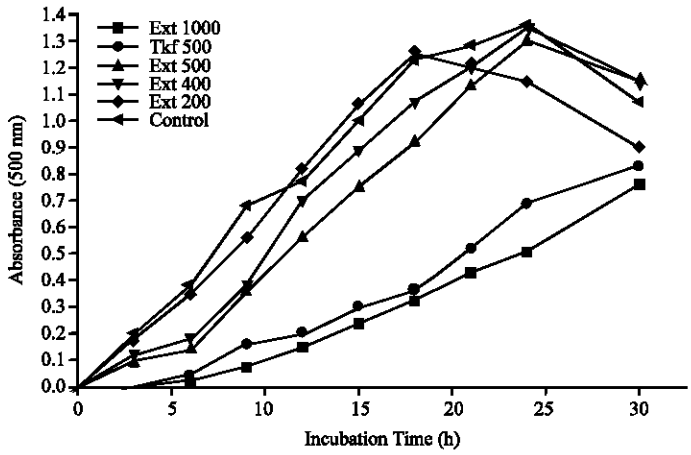

Fig. 1: Antioxidant activity of lyophilized water extracts of the leaves of (TPA) (Numbers following ext indicates the μg of extract added to the emulsion and tkf 500 = 500 μg α-tocopherol)

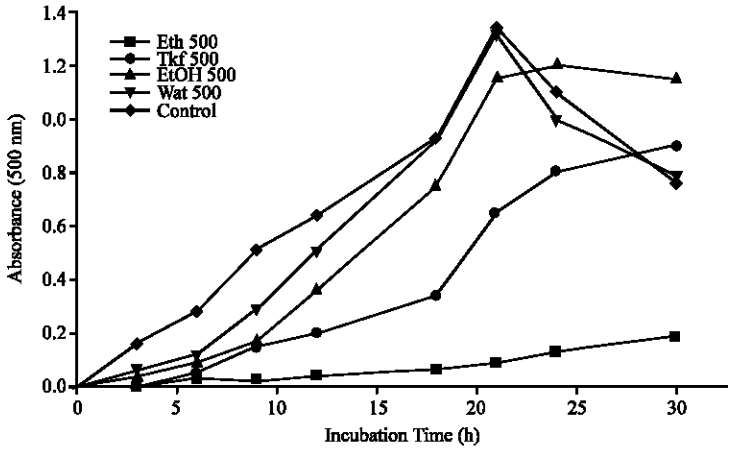

Fig. 2: Antioxidant activities of d ried ether, ethanol extracts and lyophilized water extract of the leaves of (TPA). In each there was 500 μg of indicated dried extract or α-tocopherol while in the control there was no extract. (Tkf = α-tocopherol; Waqt = water; Eth = ether; EtOH = ethanol)

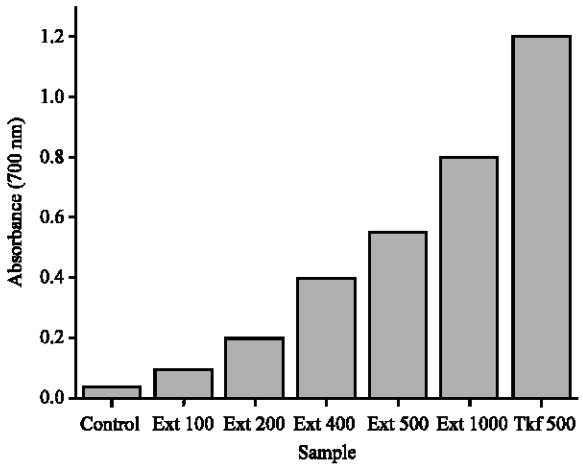

Fig. 3: Reducing power of lyophilized water extract of the leaves of (TPA). (Numbers following ext indicates the μg of extract added to the emulsion and Tkf500 = 500 μg of α-tocopherol)

to determine that 1000 μg extract or and 500 μg α-tocopherol were significantly different from 500 μg extract ($p = 0.000$ and $p = 0.002$, respectively).

However, the difference between 1000 μg extract and 500 μg α-tocopherol was not significant ($p = 0.503$).

Ethanol extract had higher activity than water extract, but the difference was not statistically significant ($p = 0.883$). Although they were able to suppress oxidation for about 6 h, their antioxidant activities were not statistically different from that of the control ($p = 0.606$, for control and water extract; $p = 0.507$ for control and ethanol extract).

Nevertheless, the most effective antioxidant activity was shown by ether extract ($p = 0.000$ for control and ether extract) (Fig. 2).

It was also interesting to find that ether extract had even higher antioxidant activity than α-tocopherol ($p = 0.006$).

Hence it was able to delay peroxidation for 30 h. Like antioxidant activity, the reducing power of water extract was also concentration dependent. Hence the reducing power of extract is increased as amount of extract increased (Fig. 3).

Even in the presence of 100 μg extract, the reducing power was significantly higher than that of the control ($p = 0.02$), in which there was no extract.

Unlike antioxidant activity, the reducing power of ether extract was the lowest one.

However, even this extract had significant reducing power activity ($p = 0.04$ between the control and ether extract).

Among the extracts, ethanol extract had the highest reducing power activity (Fig. 4).

Although α-tocopherol was more effective than ethanol extract, this difference was not statistically significant ($p = 0.074$).

It was interesting to find that although ether extract had the highest antioxidant activity; it was less effective in reducing power. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir, 1995). However, the antioxidant activities of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging (Diplock, 1997).

Thus, although ether extract has low reducing power, it could have high total antioxidant activity.

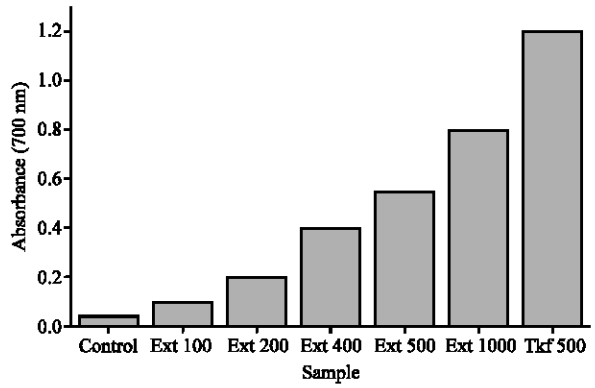

Fig. 4: Reducing power of dried ether extract, ethanol extract and lyophilized water extract of the leaves of (TPA).In the each of that there was 500 μg of indicated dried extract or α-tocopherol (Tkf) while in the control there was no extract

The present study suggests that the leaves of (TPA) might be a potential source of natural antioxidant.

REFERENCES

Auroma, O.I., 1998. Free radicals, oxidative stress and antioxidants in human health and disease JAOCS, 75: 199-212.

Chadefaud, M., L. Emberger, 1960. Traité de Botanique Systématique. Tome (2nd Edn.), Paris VI.

Davies, K.J.A., 1994. Oxidative Stress the Paradox of Aerobic Life. Biochem. Symp., 61: 1-34.

Diplock, A.T., 1997. Will the Ôgood fairiesÕ please prove to us that vitamin E lessens human degenerative diease? Free Rad. Res., 27: 511-532.

Duh, P.D., 1998. Antioxidant activity of Burdock: Its scavenging effect on free-radical and active oxygen. JAOCS, 75: 455-463.

Halliwel, B., 1994. Free radicals, antioxidants and human disease: Curiosity, cause or consequence. The Lancet, 344: 721-724.

Halliwell, B., 1995. How to Characterize an Antioxidant: An update. Biochem. Soc. Symp., 61: 73-101.

Meir, S., J. Kanner, B. Akiri and S.P Hadas, 1995. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J. Agric. Food Chem., 43: 1813-181.

Ozenda, P., 1977. La Flore du Sahara. Ed. Paris.

Rice-Evans, C.A., J. Sampson, P.M. Bramley and D.E. Hollowa, 1997. Why do we expect carotenoids to be antioxidants *in vivo*. Free Rad. Res., 26: 381-398.

Robinson, E.E., S.R.J. Maxwell and G.H.G. Thorpe, 1997. An investigation of the antioxidant activity of black tea using enhanced chemiluminescence. Free Rad. Res., 26: 291-302.

Squadriato, G.l. and W.A. Pelor, 1998. Free rad. Oxidative chemistry of nitric oxide: The roles of superoxide, peroxynitrite and carbon dioxide. Biol. Med., 25: 392-403.

Sun, J., Y. Chen, M. Li and Z. Ge, 1998. Role of antioxidant enzymes on ionizing radiation resistance. Free Rad. Biol. Med., 24, 589-593.

Tanizawa, H., Y. Ohkawa, Y. Takino, T. Miyase, A. Ueno, T. Kageyama and S. Hara, 1992. Studies on natural antioxidants in citrus sp. I. Determination of antioxidative activities of citrus fruits. Chem. Pharm. Bull., 40: 1940-1942.

Yen, G.H. and H.Y. Chen, 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agric. Food Chem., 43: 27-32.