A study on the antioxidant capacity and prooxidant-antioxidant balance of different solvent extracts of *Thuja orientalis L*.

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Prooxidant-antioxidant balance (PAB) in variation concentration extracts of *Thuja orientalis L*. by a modification PAB assay and antioxidant capacity of this plant was evaluated. The assay is based on 3,3′,5,5′-tetramethylbenzidine and its cation, used as a redox indicator participating in two simultaneous reactions. PAB was determined in different solvent extract in comparison to vitamin C. The PAB value of extract decreased with increase in extract concentration. Highest amount of total phenolic compound was found in methanol extract whereas the Ethylacetate extract contained highest amount flavonoids compared to other extracts. This study demonstrate that T.orientalis exhibit antioxidant activity.

Key words: Antioxidant, PAB, extract, Thuja orientalis

در این مطالعه بالانس پراکسیدانت – آنتی اکسیدانت در غلظت های مختلف عصاره تویا با استفاده از روش PAB و ظرفیت آنتی اکسیدانی این گیاه مورد ارزیابی قرار می گیرد. اساس تست PAB بر TMB به دو صورت معمولی و کاتیون دار می باشد که همزمان وارد واکنش می شود. تست PAB برای حلال های مختلف عصاره در مقایسه با ویتامین C انجام شد. مقدار PAB عصاره با افزایش غلظت آن، کاهش می یابد. همچنین بیشترین مقدار فنل تام و فلاونوئید به ترتیب در عصاره اتانولی و اتیل استاتی مشاهده شد. این تحقیق فعالیت آنتی اکسیدانی تویا را نشان می دهد.

کلمات کلیدی: آنتی اکسیدانت، عصاره، گیاه تویا، PAB

Introduction:

Reactive oxygen species (ROS) include free radicals such as hydrogen peroxide (H_2O_2), super oxide anion ($^{\cdot}O_2^-$), and hydroxyl radical($^{\cdot}OH$) have been associated with carcinogenesis, coronary heart disease, and many other health problems related to advancing age [5]. Free radicals cause the oxidation of biomolecules (e.g., protein, amino acids, lipid and DNA) which leads to cell injury and death [6] Moreover, Oxidative stress is defined as an imbalance between prooxidants and antioxidants in favor of prooxidants [4].

Fruit and vegetables are the predominant sources of antioxidant vitamins (vitamin E, vitamin C, precursor of vitamin A i.e., b-carotene), which act as free radical scavengers, making these foods essential to human health [3].

Recently, scientific information reported on chemical and biological properties of T.orientalis remains limited. They isolated several biflavonoids and flavonoid glycosides from the fruits of *Thuja orientalis L.* and estimated their antioxidant and elastase inhibitory activities.[9]
The method was developed [1] which can measure the balance of oxidants and antioxidants simultaneously, by using 3,3′,5,5′-Tetramethylbenzidine (TMB) and two different kinds of reactions; one enzymatic reaction where the chromogen TMB is oxidized to a color cation by peroxides, and a chemical reaction where the TMB cation is reduced to a colorless compound by antioxidants; and given a redox index.
In this study, we determined the prooxidant–antioxidant balance in variation solvent of T.orientalis by a modified PAB assay; and its correlation with antioxidant such as vitamin C.
Because now the measurement of this prooxidant–antioxidant balance could be done rapidly, easily and cost-effectively in a clinical laboratory, the aim of this study was to investigate and present the prooxidant–antioxidant balance as a factor that could be estimated amount of antioxidant capacity of T.orientalis and differentiation between variation solvent extracts.

Matherial and method:

Chemicals

TMB powder (3,3′,5,5′-Tetramethylbenzidine, Fluka), peroxidise enzyme (Applichem: 230 U/mg, A3791,0005, Darmstadt, Germany), chloramine T trihydrate (Applichem: A4331, Darmstadt, Germany), hydrogen peroxide (30%) (Merck), Gallic acid, quercetin, ascorbic acid, Methanol, Ethanol, Water and Ethyl acetate as the solvent. All the other reagents used were reagent grade and were prepared in double distilled water.

Plant materials

The leaves of *T.orientalis* were collected from Gorgan, province of Golestan, northern Iran, in Juli. The cut into pieces, dried and then ground to powdered-form, which was then kept in an air-tight plastic bag until use.

Preparation of extracts

For the partitioning by solvent, The power of *T.orientais* (1g) was subjected to extraction with 150 ml of methanol, ethanol, water and ethyl-acetate, three times, 24h each.

subject

The aim of this study, therefore, was to determine the antioxidant capacity and activities of the variation solvent extract of *T.orientalis* leaves.
Different concentration (0-12 mg/ml) of each extracts were prepared and the anti-oxidant activities of the isolated compounds from *Thuja orientalis L.* were evaluated by PAB assays, and compared with the well-known reference antioxidants, vitamin C.

Determination of total phenolic compounds and flavonoid

Total phenol content was determined by Folin-Ciocalteau method [7]. The methanol, ethanol, water and ethyl acetet solution of the extract (100 µg µl-1) was mixed with 2.5 ml of 0.2N Folin- Ciocalteau reagent for 5 min and 2.0 ml of 75 g l-1 sodium carbonate was then added. The absorbance of the reaction mixture was measured spectrophotometrically at 760 nm after 2 h of incubation at room temperature. The result was expressed as gallic acid equivalent.
Total flavonoid was estimated as previously described [2]. Briefly, 0.5 ml solution of the extract in methanol, ethanol, water and ethyl acetat (100 µg µl-1) was separately mixed with 1.5 ml of solvent, 0.1 ml of 10 % aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water, and then left at room temperature for 30 min. The absorbance of the reaction mixture was measured at

415 nm in a double beam spectrophotometer. Total flavonoid content was calculated as quercetin from a calibration curve.

Prooxidant–antioxidant balance (PAB) assay

A modified PAB was applied based on a previously described method [1]. The standard solutions were prepared by mixing varying proportions (0–100%) of 250 μM hydrogen peroxide with 3 mM uric acid (in 10 mM NaOH).

Sixty mg TMB powder was dissolved in 10 mL DMSO; for preparation of TMB cation, 400 μL of TMB/DMSO was added in 20 mL of acetate buffer [0.05 M buffer, pH 4.5], and then 70 μL of fresh chloramine T (100 mM) solution was added into this 20 mL, mixed well, incubated for 2 h at room temperature in a dark place; 25 U of peroxidase enzyme solution was added into 20 mL TMB cation, dispensed in 1 mL and put at −20 °C; in order to prepare the TMB solution 200 μL of TMB/DMSO was added into 10 mL of acetate buffer [0.05 M buffer, pH 5.8];

the working solution was prepared by mixing 1mLTMBcation with 10 mL of TMB solution, incubated for 2 min at room temperature in a dark place and immediately used. Ten microliters of each sample, standard or blank (distilled water) were mixed with 200 μL of working solution, in each well of a 96 well plate, which was then incubated in a dark place at 37 °C for 12 min; at the end of the incubation time, 100 μL of 2 N HCl was added to each well; and measured in an ELISA reader at 450 nm with a reference wavelength of 620 or 570 nm. A standard curve was provided from the values relative to the standard samples.

The values of the PAB are expressed in arbitrary HK units, which are the percentage of hydrogen peroxide in the standard solution. The values of the unknown samples were then calculated based on the values obtained from the above standard curve.

Result:

Total phenol and flavonoid contents

Total phenol compounds are reported as gallic acid equivalent by reference to standard curve (y = 0.0379x + 0.0004, r2 = 0.9995).

As assumed, amount of the total phenolics was very low in aqueous water extract (5.503± 0.18) On the other hand, methanol extract has been found to be rich in flavonoids with a value of 32.47± 0.44 mg quercetin/g. The amounts of, quercetin and gallic acid are shown in Table 1.

Table 1: *Total phenol and flavonoid content of extracts.*

Extract(100μg/μl)	Gallic acid	Quercetin
T-H_2O	5.503±0.13	6±0.58
T-Et	7.078±0.34	40.11±3.53
T-Me	8.063±0.05	32.47±10.03
T-EtAc	5.635±0.25	41.29±8.24

PAB assay

The PAB value of ascorbic acid and each extracts were evaluated separately. In all samples, following increase of extract concentration, the related PAB value was decreased (figure1) significantly (Table2). Decreasing PAB assay means increasing antioxidant which is a favourite condition.

Based on PAB value curve the activity of ascorbic acid as an antioxidant is stronger than other extracts especially in very low concentration.

Table 2: *P value.*

PAB vale	HK
Vitamin C	Pearson co. = -0.985 P = 0.00011
T-Me	Pearson co. = -0.993 P = 0.0008
T-Et	Pearson co. = -0.986 P = 0.0008
T-H_2O	Pearson co. = -0.982 P = 0.00016
T-EtAc	Pearson co. = -0.975 P = 0.00010

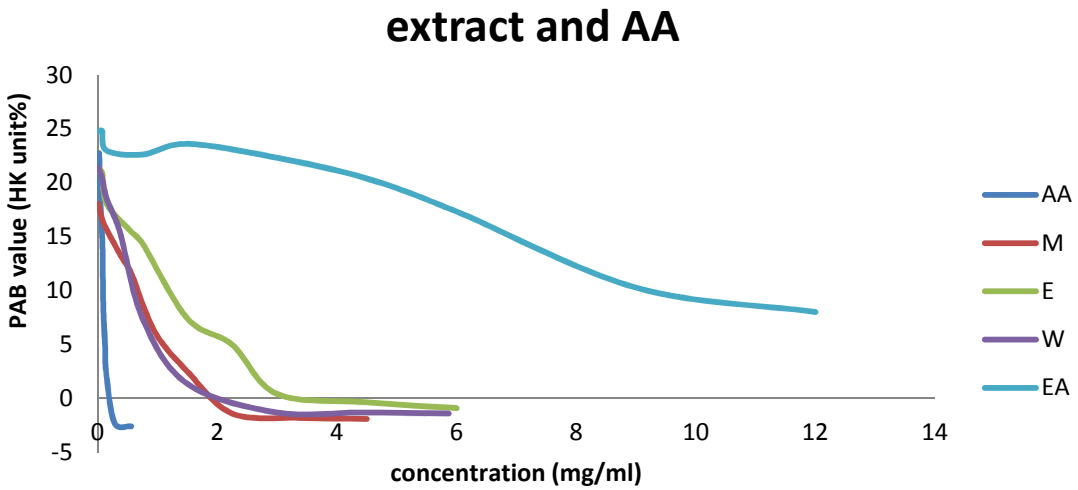

Figure 1: *PAB Value of extracts and Vitamin C.*

In this study, the results are depicted in different figures. the Figure 2, shows the PAB value of extracts (methanol, ethanol, water and ethyl acetate) comparing with each other. According to the Figure T-water extract has the best antioxidant activity followed by T-methanol, T-ethanol, T-ethyl acetate, respectively.

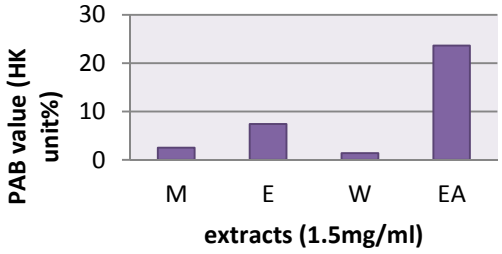

Figure 2: the PAB value of extracts (methanol, ethanol, water and ethyl acetate)

The figure 3 shows compared concentrations of extracts while their PAB values are equalized with vitamin C. The lowest amount of PAB value was found in the water extract compared to vitamin C.

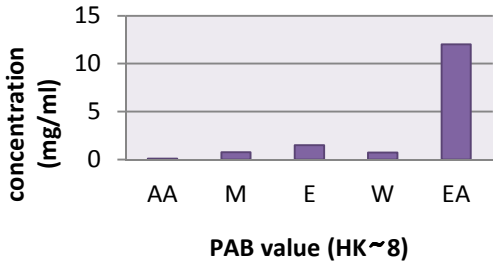

Figure 3: the PAB value was about 8 unit and concentration compared toghether

Conclusion:

The content of total phenolic was carried out based on the absorbance values of the various extract solutions, compared with the standard solutions of gallic equivalents as described above [8]. The result show that methanol extract phenol from Thuja orientalis better than other solvent followed by, ethanol, ethyl acetate and water, respectively.

As shown in table 1, total flavonoid of extracts of this plant was found to decrease in the order, ethylacetat> ethanol> methanol> water extract.

In conclusion the best PAB value of extracts was found in T-H_2O extract, according to Table2 and 3,

means this extract change TMB cation to TMB solution (colourless). etethylacetate extract was shown different behaviour. In this solvent, the best decreasing PAB value was found in very high concentration.

figure 1 shows antioxidant activity of extracts to compared with vitamin C that demonstrate all of the extracts have antioxidant activity, but in different concentration.

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