Evaluation of the photoprotective action of the active principles obtained from *TRYGON PASTINACA* liver

[1]FLORICA BUSURICU, [1]ANTOANELA POPESCU, [2]DOINA PAULA BALABAN, [1]TICUȚA PÂRJOL NEGREANU, [3]STELA ZAMFIRESCU
[1]University "Ovidius" Constanta, Faculty of Pharmacy
[2]University "Ovidius" Constanta, Faculty of Dentistry Medicine
[3]ICDOC Palas, Constanta
Present addresses: Faculty of Pharmacy, Univ. „Ovidius" Constanta, Str. Nufarului, nr. 2,

Abstract

The active principle was extracted from Trygon pastinaca (the fish from Black Sea) liver. By using the DPPH and DMPD methods the photoprotective action was demonstrated.

The results demonstrate a protective effect of anisole for the tasted oil when the dose level was 0.15mg for both methods. Comparing with the vitamin E, the tested principle presents a lower activity, but this activity is enough to have a photoprotective action (protective factor of 26.04% and 28.6 % - when DPPH and DMPD methods were used).

Keywords: *Trygon pastinaca* liver oil, photoprotective and antioxidant action, DPPH and DMPD methods.

Introduction

There are known some comparative studies of the mutagene action of the ionizing radiation, UV-radiation and of the nitrous acid. The oxygen, an indispensable element for human organisms, but can formatted of active radicals (free radicals- superoxid anion, hydroxyl radical and peroxyl radical; active species of oxygen-oxygen singlet $^1O_2$ and hydrogen peroxide-H_2O_2; organic free radical-alcoxil radical $RO^{\cdot}$ and peroxyl radical $ROO^{\cdot}$). The mutagene action of UV-radiation is accepted in irradiation cancer etymology and photodermatite. The mutagene potential of UV radiation is between 290-340 nm. The essential bio macromolecules degradation under reactive species action is: nucleic acids, proteins and lipids. When the natural photoprotective means (the cornos livel possesses reflex ion, diffusion and absorption of luminosity radiation) are beaten, it is necessary the external protection of the teguments by sun protective preparation. Efficient sunprotective products have to absorb UV radiation of 240-400 nm [1].

Some authors considered that same oils extracted from different fish species and algal have a good photoprotective/antioxidante effect [2, 3, 4]. The antioxidative ability of several natural compounds was cuantified by several methods. One of them uses the radical of 1,1-diphenyl-2-pycrylhydrazyl (DPPH) [5]. The same authors [6] used ABTS in order to assess the efficiency of antioxidant compounds from different vegetables developing a decolorization assay. However, a similar approach was followed by using *N,N d*imethyl- *p*-phenylenediamine (DMPD). In the presence of a suitable oxidant solution a colored DMPD radical cation is formed ($DMPD^{\cdot+}$). Antioxidant compounds, which are able to transfer a hydrogen atom to $DMPD^+$, cause a decoloration of the solution [7].

The aim of this paper was to obtain oil from the liver of *Trygon pastinaca* fish having some biological actions: healing, anti-inflammatory, photoprotective and antioxidant. In the same time, the confirmation of photoprotective action using the DPPH and DMPD methods were done.

Materials and Methods

Materials.
α-Tocopherol (Vitamin E), *N,N*-Dimethyl-*p*-phenylenediamine dihydrochloride (DMPD), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX), were purchased from Aldrich, Germany; α,α-diphenyl –β-pycryl-hydrazyl (DPPH) radical and dihydrochloride (Fluka). All solvents (hexane, methanol) and reagents (deionized water, acetate buffer pH = 7) were purchased from local suplliers.
Spectrophotometer measurements were recorded by using an UV-VIS Cintra 5 apparatus.
Oils extraction from Trygon pastinaca
The oils used in the analysis were extracted from liver of Trygon pastinaca (the fish from Black Sea), by using a Soxhlet apparatus. The extract was concentrated under reduced pressure by using rotatory evaporator (Turbo Vap ^{R} 500)
Vitamins identification. A, D, E vitamins from fish oil were identified by using a spectrophotometric method, with standard solution of vitamins (from local pharmacy) dissolved in hexane.
Scavenging Effect (%)

- Antioxidative ability - the DPPH method [5]

The antioxidant activity of oils was measured by using α,α-diphenyl-β-picrylhydrazil (DPPH) radical. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm. The color turn from purple to yellow as the reaction of DPPH with the antioxidant take place. The reaction mixture consisted of 3 mL hexane, 0.15 mL of fish oil was added in 0.3 mL methanolic solution of DPPH 1mM. For control sample the fish oil was excluded. The mixture for positive control (standard) consisted of 3 mL hexane, 0.3mL vitamin E methanol solution 1mM and 0.3 mL DPPH methanol solution. The mixture was shaken and stored at 20°C for 30 min and after the absorbance of the resulting solution was measured spectrophotometrically at 517 nm.
The ability to scavenge DPPH radical it was calculated by using the following equation:

$$\text{Scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \cdot 100$$

where A_0 was the absorbance of the control sample and A_1 was the absorbance in the presence of the sample of extract and standard.

- Antioxidative ability - DMPD method [7]

DMPD, 100 mM, was prepared by dissolving 209 mg of DMPD in 10 mL of deionized water; 1 mL of this solution was added to 100 mL of 0.1 M acetate buffer, pH 5.25, and the colored radical cation ($DMPD^{\cdot+}$) was obtained by adding 0.2 mL of a solution of 0.05 M ferric chloride (final concentration 0.1 mM). One milliliter of this solution was directly placed in a 1-mL plastic cuvette and its absorbance at 505 nm was measured. Standard solutions of the TROLOX were prepared as follows: 1 mg/mL of TROLOX was prepared by dissolving 0.1 g of TROLOX in 100 mL of methanol. Fifty microliters of standard antioxidants or of fish oil (mixture of 3 mL hexane and 0,15 mL of fish oil) were added in the spectrometric cuvette and after 10 min at 25°C under continuous stirring the absorbance at 505 nm was measured. The buffered solution was placed in there ference cuvette. A dose-response curve was derived for

TROLOX, by plotting the absorbance at 505 nm as percentage of the absorbance of the uninhibited radical cation solution (blank) according to the equation:

$$\text{inhibition of } A_{505}\ (\%) = (1 - \frac{A_f}{A_0}) \cdot 100$$

where: A_0 is the absorbance of uninhibited radical cation; A_f is the absorbance measured 10 min after the addition of antioxidant samples.

Antioxidant ability of fish oil was expressed as TEAC (TROLOX equivalent antioxidant capacity) according to DMPD method, using the calibration curve plotted with different amounts of TROLOX (Figure 5).

Statistical Analysis. All data were expressed as mean ± SD (n=3) by using *Origin 8* test. Mean values do not differ significantly.

Results and Discussions

Proton radical-scavenging action is one mechanism of oxidation. DPPH has a proton free radical and shows characteristic absorption at 517 nm. When it encounters proton radical scavenger, its purple color fades rapidly [7], suggesting that antioxidant activity of fish oil is due to its proton donating ability. The fish oil was less able to scavenge radicals than α-Tocopherol. The value of scavenging effect (%) are depicted in Table 1.

Table 1. Scavering effect (%) by DPPH method

Fish oil / vitaminE added (mg)	Fish oil scavenging effect (%)				Vitamin E scavenging effect (%)			
	Scavenging effect (%)	Standard deviation	Confidence intervals for mean 95%		Scavenging effect (%)	Standard deviation	Confidence intervals for mean 95%	
			min.	max.			min.	max.
0.02	2.45	0,05568	2,4	2,51	3.7	0,10583	3,62	3,82
0.05	6.7	0,05292	6,64	6,74	6.9	0,23259	6,69	7,15
0.1	8.4	0,0500	8,35	8,45	15.7	0,45574	15,23	16,14
0.2	15.62	0,26665	15,41	15,92	26.8	0,40447	26,34	27,1
0.5	20.88	0,23516	20,87	21,12	**35.2**	0,56	34,8	35,84
1	24.14	0,29597	23,94	24,48	33.4	0,54781	32,79	33,85
1.2	25.36	0,50587	25,01	25,94	31.2	0,22539	31,06	31,46
1.5	**26.04**	0,48662	25,48	26,36	31.6	0,4613	31,08	31,96
1.75	24.66	0,56666	24,01	25,05	31.8	0,39038	31,42	32,2
2	22.4	0,8864	21,41	23,12	32.2	0,31575	31,97	32,56

Each value is a mean of triplicate analyses ±SD. Mean values do not differ significantly.

In order to establish the optimal concentration value for best antioxidant effect were used (fish oil and E vitamin) in the concentration of 0.02; 0.05; 0.1; 0.2; 0.5; 1.0; 1.5; 2.0 (mg). The scavenging percentage on the DPPH radical was 26.04% for oil from *Trygon pastinaca* extract at the dose level of 1.5mg and 35.2 % for α-tocopherol at the dose 0.5 mg (Fig.1).

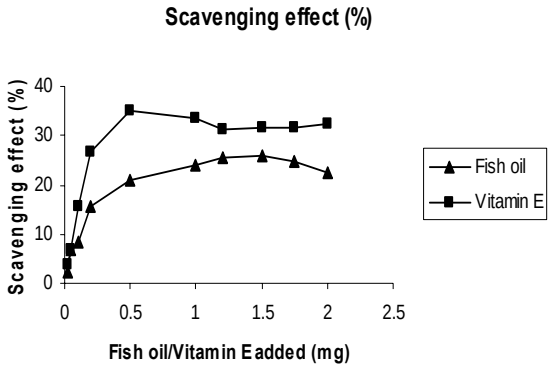

Figure 1 Scavenging effects of fish oil and α-Tocopherol on DPPH radical

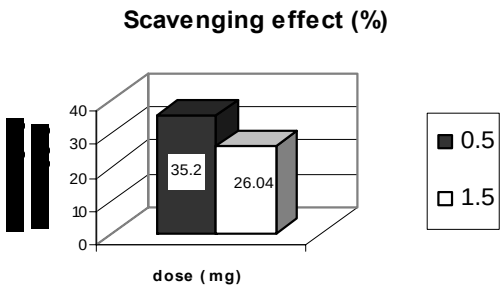

Figure 2 Scavenging effects of fish oil

The radical scavenging activity of the extract and positive controls decreased in the following order: α-tocopherol > fish oil (Fig.2).

The absorbtion spectra of the oil extracted from *Trygon pastinaca* at 200-400 nm correspond to a „screen substance" characteristic for photoprotective pharmaceutical products (Fig 3, 4 and 5). The presence of A and E vitamins in the fish oil composition, explains the fish liver oil antioxidant and photoprotective properties.

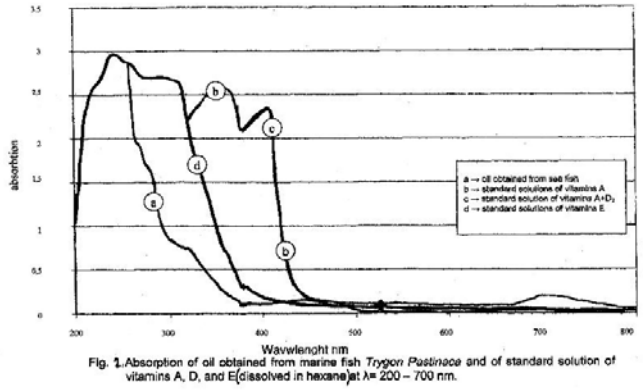

Fig. 1.Absorption of oil obtained from marine fish *Trygon Pastinace* and of standard solution of vitamins A, D, and E(dissolved in hexane)at λ= 200 – 700 nm.

Figure 3. Absorbtion of fish oil and of standard solution of vitamins A, D and E at 200-700nm.

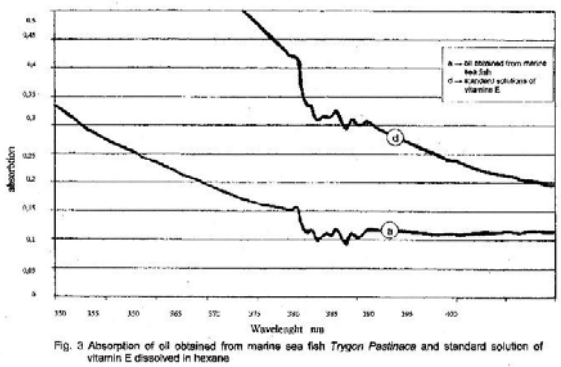

Fig. 3 Absorption of oil obtained from marine sea fish *Trygon Pastinace* and standard solution of vitamin E dissolved in hexane

Figure 4. Absorbtion of fish and standard solution of vitamin E at 350-400nm

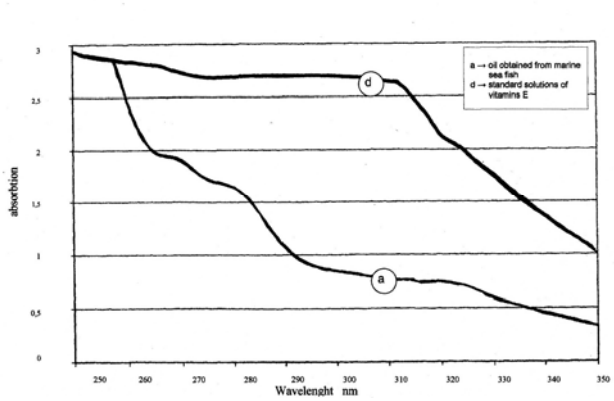

Figure 5. Absorbtion of fish and standard solution of vitamin E 250-350nm

Antioxidants from the fish oil are mainly lyphophilic compounds and their antioxidant activity could be well evaluated by using the DMPD method. Results obtained are reported in Table 2. The antioxidative efficiency was expressed in TEAC (TROLOX equivalent antioxidant activity) according to the method by using the calibration curve plotted with different amounts of TROLOX (Figure 6). The standard deviation was very low and the dose-response curve is highly reproductible. The absorbance inhibition at 505 nm is linear between 0.2 and 11 μg of TROLOX.

The relationship calculated within this range for the standard compound is:

A_{505} (inhibition) = 5.3 (μg of TROLOX) + 7.0; r^2 = 0.987.

To evaluate the sensibility of the DMPD method, the system was tested by using the standard solution 1mg/mL of TROLOX and DMPD: $FeCl_3$ molar ratio of 10:1 [6]. The results demonstrate a protective effect of anisole by 28.6% for the tasted oil when the dose level was 0.15mg. Comparing with the results obtained by DPPH method, the tested active principle presents a similar activity without any important differences. According to these results the tested oil can be used as a possible active principle for the preparation of some pharmaceutical products used in dermatological and cosmetically domains.

Table 2. Antioxidant activity of fish oil by DMPD method

Antioxidant activity of fish oil	
Inhibition of the A $_{505\ nm}$ **(%)**	TROLOX equivalent antioxidant activity (TEAC μg)
28.60	3.75

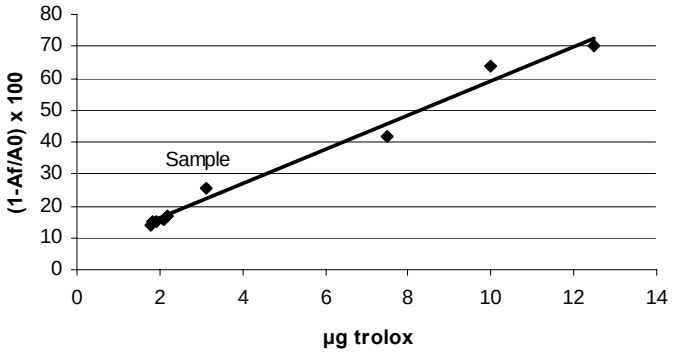

Figure 6. Degree of inhibition of the absorbance at 505 nm as a function of the TROLOX concentration**.**

Conclusions

The bioactive principle isolated from *Trygon pastinaca* fish, demonstrates a photoprotective action and allow us to recommend the marine source investigation as a potential source to obtain the external use pharmaceutical products (photoprotective ointments) and also for the internal use pharmaceutical products (as antioxidant).

Acknowledgment

We are gratefully to Prof. Alberto Ritieni for the helpful discussion and for his technical support.

References

1. STOICESCU I., SARAH NICA A., Efficacité de la sunprotective oitmants dans le traitement des affections dermatologiques, *Archives of the Balkan medical Union*, **36**(1), 43-47, 2001.
2. BUSURICU F., ZAMFIRESCU S., The "in vivo" study of the pharmacological potential of the ointments which contain fish oil from *Trygon pastinaca*, *Archives of the Balkan medical Union*, **43**(2), 113-117, 2008.
3. BUSURICU F., BALABAN D. The obtaining of marine active principle from the brown algae *Cystoseira barbata* with antioxidants properties, *Bulletin of the Transilvania University of Brasov*, Transilvania University Press, 200-203, 2005.
4. ROBERT W.G., GORDON M.H., WALKER A.F. Effect of enhanced consumption of fruit and vegetables on plasma antioxidant status and oxidative resistance of LDL in smokers supplemented with fish oil, *Europ.J.Clin. Nutr*, **57**, 1303-1310, 2003.
5. FOGLIANO V., VERDE V., RANDAZZO G., RITIENI A., Methods for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines, *J. Agric. Food Chem.* **47**, 1035-1040, 2002.
6. PRYOR W.A., CORNICELLI J. A., DEVALL L.J., TAIT B., KVEDI B., WITIAK D.T., Rapid screening test to determine the antioxidant potencies of natural and synthetic antioxidants, *J. Org Chem*, **58**, 3521-3522, 1999.
7. HUANG S.W., HOPIA K., SCHWARZ E.N., FRANKEL E.N., GERMAN J.B., Antioxidant activity of a-tocopherol and TROLOX in different lipide substrates: bulk oil vs. oil-in-water emulsions, *J Agric Food Chem.* **44**,444 – 452, 2004.