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ABSTRACTS

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ANTIOXIDANT POTENTIAL OF INDIVIDUAL OR COMBINED DIETARY SUPPLEMENTS

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INTRODUCTION

An antioxidant may be defined as a substance that when present at low concentrations, compared with those of the oxidizable substrate, significantly delays or inhibits its oxidation [1]. Nowadays there are several commercial dietary supplements which claim for antioxidant activity.

In this work, the antioxidant activity (AA) of 17 dietary supplements (**Table 1**) available in different formulations (pills, capsules or bags), was evaluated by *in vitro* assays: scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, reducing power (RP) and inhibition of lipid peroxidation by preventing the formation of thiobarbituric acid reactive substances (TBARS). Furthermore, different combinations (8+10+17, 8+10, 8+17 and 10+17) of some samples were also tested in order to obtain potential synergistic effects.

Linear discriminant analysis (LDA) was used to categorize the condensed formulations (pills and capsules), the infusion bags and the combined samples, according with their antioxidant activity.

The Advanced Antioxidant Formula (AAF; sample 8) proved to have the highest antioxidant activity in all the assayed methods, either singly taken or included in mixtures. Furthermore, the mixtures containing this supplement revealed synergistic effects in 92% of the cases. The intake of antioxidant mixtures might provide some additional benefits.

MATERIAL AND METHODS

The samples were prepared using the available formulation (**Table 1**). Each sample was weighted and prepared in 200 mL of distilled water in order to obtain a stock-solution. Pills and the inner part of the capsules were dissolved, while bags were used after infusion. Several dilutions of each stock-solution were prepared to perform the antioxidant activity assays.

The antioxidant activity of the individual and combined samples was evaluated by DPPH radical-scavenging activity, reducing power and inhibition of lipid peroxidation in brain homogenates, using TBARS assay. The extract concentrations providing 50% of antioxidant activity or 0.5 of absorbance (EC_{50}) were calculated from the graphs of antioxidant activity percentages (DPPH and TBARS assays) or absorbance at 690 nm (RP assay) against extract concentrations. Trolox was used as standard [2].

All the assays were carried out in triplicate in three different samples of each supplement. The results are expressed as mean value±standard deviation (SD). One-way analysis of variance (ANOVA) was applied following Tukey's honestly significant difference *post hoc* test with $\alpha = 0.05$, coupled with Welch's statistic. The homoscedasticity of distribution was checked through Levene's test.

In addition, LDA was used as a supervised learning technique to classify the assayed antioxidant dietary supplements according to their antioxidant activity results. A stepwise technique, using the Wilks' λ method with the usual probabilities of F, was applied for variable selection, verifying which canonical discriminant functions were significant. To avoid overoptimistic data modulation, a leaving-one-out cross-validation procedure was carried out to assess the model performance.

RESULTS AND DISCUSSION

The AAF (sample 8) proved to have the highest antioxidant activity in all the assayed methods (**Figure 1**), providing the best results (lowest EC_{50} values), either singly taken or included in mixtures.

Table 1 – Identification of the dietary supplements and concentrations of the prepared stock-solutions.

Commercial designation	Formulation	Stock-solution (mg/mL)
(1) Selenium ACE	Pill	1.98
(2) Selenium Good' N Natural	Pill	3.07
(3) VitaBerry ACE	Pill	2.89
(4) Bioactive Antioxidant PharmaNord	Pill	5.29
(5) Anti-aging firming	Pill	3.68
(6) Kyolic aged garlic	Capsule	2.16
(7) Form +	Capsule	1.87

(8) Advanced Antioxidant Formula	Capsule	3.72
(9) Pycnogenol	Capsule	1.24
(10) Resveratrol	Capsule	3.48
(11) Green coffee Arkocapsules	Capsule	1.44
(12) Anti-aging supplement	Capsule	1.10
(13) Antioxidant Naturplan	Capsule	3.25
(14) Mangosteen extract	Capsule	4.24
(15) Vin rouge frut rouge	Bag	7.50
(16) Horsetail infusion	Bag	6.5
(17) Colesterol control	Bag	6.5

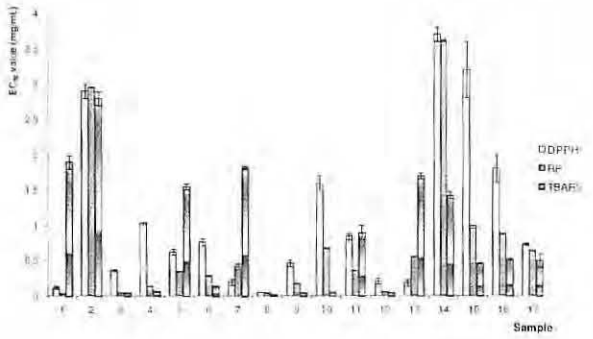

Figure 1 – Antioxidant activity, expressed as EC_{50} values (mg/mL), of the studied dietary supplements.

Furthermore, for DPPH radical scavenging activity, as well as for reducing power assays, the selected mixtures were always synergistic (increase of antioxidant capacity). The synergist effect predominated also in the TBARS inhibition assay, being observed in 75% of the mixtures; 8+10 demonstrated an additive effect (**Table 2**).

Regarding LDA, the assayed dietary antioxidant supplements proved to have distinctive features, derived from being condensed or infusion formulas.

Table 2 – Theoretical[a] (Theor.) *versus* experimental (Exp.) values of antioxidant activity EC_{50} (mg/mL) of the combined samples of dietary supplements.

Mixtures	DPPH		RP		TBARS	
	Theor.	Exp.	Theor.	Exp.	Theor.	Exp.
8+10+17	0.78±0.04	0.14±0.02	0.18±0.03	0.07±0.01	0.45±0.01	0.07±0.01
8+10	0.81±0.05	0.07±0.01	0.04±0.01	0.04±0.01	0.36±0.01	0.06±0.01
8+17	0.39±0.01	0.09±0.01	0.25±0.04	0.06±0.01	0.34±0.01	0.07±0.01
10+17	1.15±0.05	0.84±0.01	0.26±0.04	0.09±0.01	0.66±0.01	0.31±0.01

The effect was considered synergistic when the EC_{50} experimental values were more than 5% lower than theoretical values.

Regarding LDA, the assayed dietary antioxidant supplements proved to have distinctive features, derived from being condensed or infusion formulas. Furthermore, it is relatively clear that the tested combinations retain an antioxidant profile highly similar to the presented by the condensed formulas (pills or capsules) included in those mixtures (**Figure 2**).

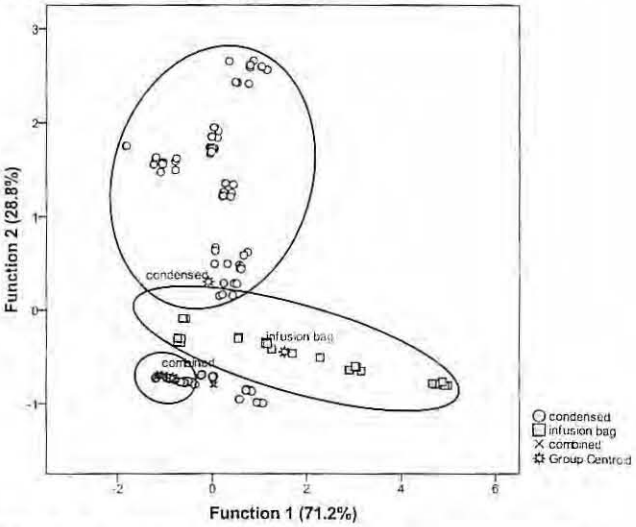

Figure 2 – Discriminant functions coefficients defined according with the antioxidant activity EC_{50} values of the dietary supplements.

CONCLUSIONS

AAF revealed to be the most active supplement. In addition, the mixtures containing this supplement revealed synergistic effects in 92% of the cases.

The antioxidant activity of these mixtures was closely related with that presented by the condensed formulations when assayed individually. Thereby, the same beneficial effects might be achieved ingesting lower quantities of the condensed supplements.

REFERENCES

[1] Gutteridge JMC. Rev. Clin. Gerontol., **4**: 279-288, 1994.
[2] Guimarães R et al., Food Chem. Toxicol., **48**: 99-106, 2010.

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