Physico-Chemical Characterization of Amaranth Extracts from Romanian Vegetal Sources with Antioxidant and Antiinflammatory Activities

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For prevention and treatment of degenerative diseases caused by oxidative stress, as atherosclerosis, cancers, diabetes, Alzheimer disease, rheumathoid arthritis and osteoarthritis, the concerns of scientists were oriented in the last years towards the study of natural compounds with highly antioxidant pharmaceutical potential and with minimal secondary effects. These compounds may play important role in inhibiting both free radicals and oxidative chain-reactions within tissues and membranes. Amaranthus spp was rediscovered as a most promising plant that may provide a complex range of active substances with high antioxidant and antiinflammatory activities. This paper described the studies performed for physico - chemical characterization of bioactive extracts obtained from indigenous Amaranthus seeds, using HPLC / DAD, HPLC / MS / MS, GC and ICP - MS advanced analysis techniques.

Keywords: bioactive extracts, squalene, flavonoids, phenolic acids, tocopherols, fatty acids, macro and microelements

In the last years, Amaranthus spp was rediscovered as a benefic plant to health, because of its complex composition of biologically active substances.

Among the cited bioactive compounds in scientific publications, lipids, proteins, fatty acids, tocopherols, squalene, phenolic compounds, vitamins, minerals with diuretic, hypotensive, antipyretic, antioxidant, antidiabetic, antiinflammatory activities can be mentioned [1 - 11].

The aim of our studies was to develop analytical metods for assay of compounds of antioxidant and anti-inflammatory activities of Amaranth extracts, complex bioactive ingredients from pharmaceutical products used for prevention and treatment of degenerative diseases caused by oxidative stress as atherosclerosis, reumathoid arthrits and osteoarthrits.

Experimental part

Materials and reagents

Bioactive extracts obtained by extraction from Amaranthus species

Reference substances: High-purity standards (min.98 %) squalene, tocopherols, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, chlorogenic acid, rutin, quercetin, apigenine, kemferol

HPLC solvents (Merck): ethanol, methanol, i-propanol

Physico-chemical measurements

The new methods were developed using HPLC / DAD, HPLC / MS / MS, GC and ICP - MS advanced analysis techniques.

HPLC / MS / MS Method for identification of squalene

HPLC/MS/MS proposed method used an Agilent 6410 Triple Quad chromatograph with triple quadrupole mass spectrometer detector and chromatographic conditions as: ZORBAX SB–C18(150 x 0.5 mm) column, mobile phase methanol – i-propanol – acetic acid (920 : 80 : 0.5), flow rate 20 µL/min, injected volume 8 µL, carrier gas temperature 350°C, carrier gas flow 10 l / min, the mass range: 100 – 1000.

HPLC Method for assay of tocopherols

HPLC proposed method used a HPLC - La Chrom Merck with DAD detector and chromatographic conditions as: ZORBAX Eclipse XDB - C18 (150 x 4.6 x 5)column , mobile phase methanol and water (95:5 V/V), flow rate 1.2 mL/min, detection 280 nm, temperature 25°C, injected volume 20 µL.

HPLC / DAD Method for assay of flavonoids and phenolic acids

HPLC proposed method used a HPLC Elite La Chrom Merck with DAD detector and chromatographic conditions as: Inersil ODS -3 (250 X 4.6 X 5) column, mobile phase methanol and water (50 : 50, V/V), flow rate 1.0 mL/min, detection 289 nm and 330 nm, injected volume 20 µL.

GC Method for assay of squalene and fatty acids

GC proposed method used a GC – 6890N Agilent Technologies with split-splitless injector and FID detector and chromatographic conditions as: capillary column - HP 88 (88% cyanopropyl – methyl polysiloxane) (60m x 0.25mm, 0.20µm), 2 gradient steps (the first step: initial temperature: 150°C, 10 min; final temperature: 175°C, 1 min; rate: 1 °/min.; the second step: final temperature: 220°C, 5 min; rate: 10 °/min.), split ratio 20:1, carrier gas nitrogen – flow = 1.5 mL/min., evaluation method external standard

ICP - MS Method for determination of macro and microelements

ICP-MS conditions: apparatus Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) type ELAN DRC-e Perkin Elmer Inc., furnace type L3/12/B170 Nabertherm GmbH, references multielement standard STD 3.

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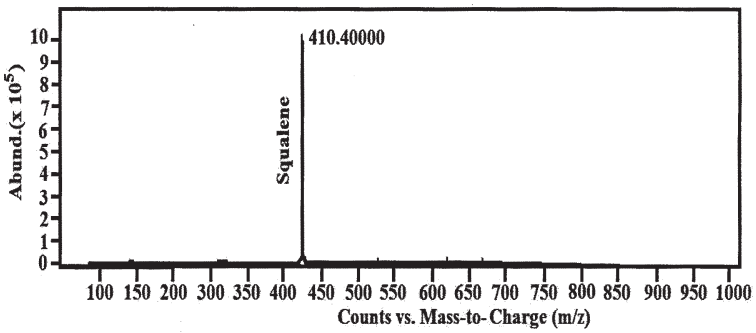

Fig.1. HPLC / MS / MS chromatogram for identification of squalene from bioactive oils

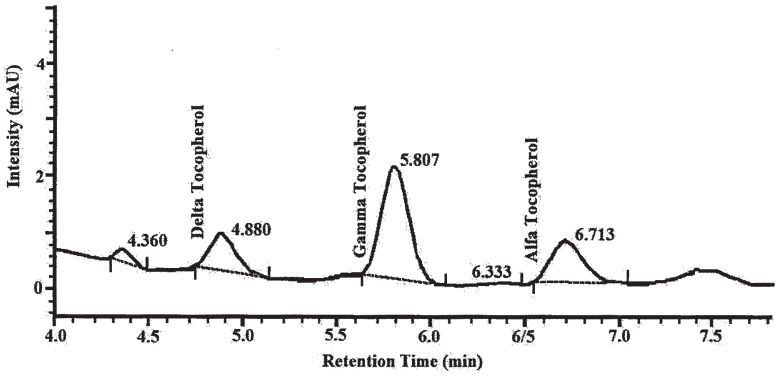

Fig.2. HPLC chromatogram for assay of tocopherols from bioactive oils

Tocopherols	mg %
δ –Tocopherol	2.14
α -Tocopherol	2.02
γ -Tocopherol	2.59

Table 1
TOCOPHEROLS COMPOSITION OF AMARANTH EXTRACTS

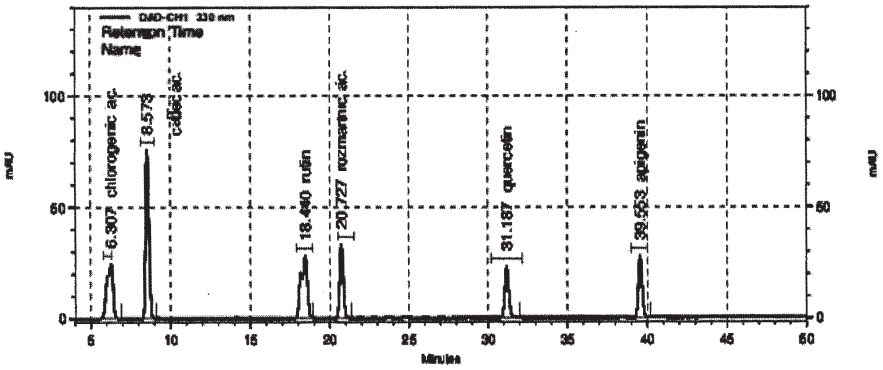

Fig.3. HPLC chromatogram for assay of flavonoids and phenolic acids from Amaranth extracts

Compounds	g %
Chlorogenic acid	0.02
Rutin	0.346
Quercetin	0.100
Apigenin	0.055
Kempherol	0.109

Table 2
CONTENTS OF PHENOLIC CONSTITUENTS OF AMARANTH EXTRACTS

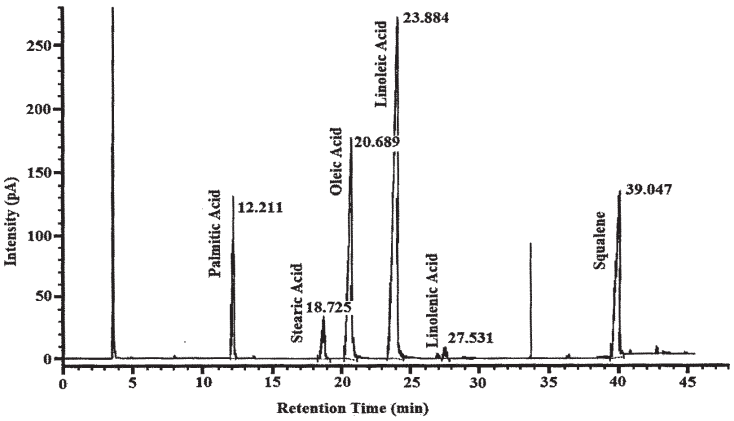

Fig.4. GC chromatogram for assay of squalene and fatty acids from bioactive oils

Results and discussions

The results obtained by the proposed methods show the presence of a complex range of compounds with antioxidant / antiinflammatory activities in investigated Amaranth extracts, as: tocopherols, phenolic compounds, squalen, saturated and unsaturated fatty oils and minerals too.

Compounds	T_R	Linearity range	Correlation coeficient (R)	g %
C16 : 0 (palmitic acid)	12.28	0.4176 - 8.3520	0.00039	3.11
C18 : 0 (stearic acid)	18.85	0.0781 - 1.5626	0.99954	0.59
C18 : 1c (oleic acid)	20.84	0.8149 - 16.2980	0.99944	3.67
C18 : 2c (linoleic acid)	24.10	0.7977 - 15.9534	0.99942	7.05
C18 : 3α (linolenic acid)	27.65	0.1108 - 2.2168	0.99943	0.17
Squalene	39.63	0.2344 - 4.6872	0.99888	97.99

Table 3
CONTENTS OF SQUALENE AND FATTY ACIDS FROM BIOACTIVE OILS

Table 4
CONTENTS OF MACRO AND MICROELEMENTS OF AMARANTH EXTRACTS

Minerals	mg / 100g
Macroelements	
Mg	2.38
Ca	0.52
Na	1.43
K	1.05
Microelements	
Mn	0.69
Zn	0.90
Cu	0.32
Cr	0.13
Co	0.01
Fe	17.54
Se	0.007

Identification of squalene

Using the HPLC / MS / MS proposed method in the investigated Amaranth extracts, squalene was identified. In figure 1 the HPLC / MS chromatogram is presented.

Squalene was detected as $[M + H^+]$ (m / z 411, 4) and $[M + NH_4]^+$ (m / z 428.4); t_r 21 min.

Assay of tocopherols

Using a HPLC / DAD proposed methods, in investigated Amaranth extracts were identified and evaluated α, β, γ tocopherols, as can be seen in figure 2 and table 1.

Assay of flavonoids and phenolic acids

Using a HPLC / DAD proposed method phenolic compounds were identified and evaluated as can be seen in figure 3 and table 2.

Assay of squalene and fatty acids

In table 3 and figure 4 the composition of squalene and fatty acids of investigated extracts obtained by GC methods are presented.

Determination of macro and microelements

Using a ICP-MS proposed method macro and micro-elements were determinated as can be seen in table 4.

Conclusions

New simple and selective HPLC - DAD / MS, GC and ICP - MS methods were developed to determine the content of some compounds with antioxidant / antiinflammatory proprieties as squalene, fatty acids, tocopherols, phenolic compounds and the content of minerals in extracts from indigenous Amaranthus spp.

These methods can be used in quality control of new pharmaceutical products with Amaranth extracts as bioactive ingredient.

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