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CONTRIBUTIONS AT THE PHARMACOGNOSTIC STUDY OF *ELAEAGNUS ANGUSTIFOLIA* L. SPECIES. NOTE I: POLIPHENOLIC COMPOUNDS STUDY OF FRUITS

***Laura BUCUR*[1], *Constanţa SAVA*[1], *Viorica ISTUDOR*[2]**
[1] Universitatea "Ovidius", Facultatea de Medicină Dentară şi Farmacie, Catedra de Farmacie,
str. Ilarie Voronca, nr. 7, **RO-900684 Constanta**
[2] Universitatea de Medicină şi Farmacie „Carol Davila", Facultatea de Farmacie,
str. Traian Vuia, nr. 7, **RO-020955 Bucureşti**

Abstract: Using like vitamins (A, E, C) and proteins sources, the *Elaeagnus angustifolia* L. fruits have a reach composition [1]. After a preliminary chemical study was identified the poliphenolcarboxilic acid (ODP) and flavonoides (flavonosides, proantocianosides, tannins), moreover saccharides and mucilage.

The quantitative analysis of ODP and certain flavonoides (flavonosides) was made using the spectrophotometric methods with a Turner apparatus beside caffeic acid and rutoside, like reference substances.

The quantitative analysis show a medium contents in flavonoides as 0,13% couched in rutoside and ODP as 0,06% couched in caffeic acid.

The antioxidant properties of ODP and flavonoides, known by reference materials [5], make to affirm that the drug could be interesting for capitalization in phytotherapie.

Introduction

Elaeagnus angustifolia L., Russian olive (*Elaeagnaceae*), is a specific plant in sandy soils area, spread in Dobrogea, on the sea cost area, but also in other region of the country [6].

The name of genus come by the Greek *élaia* who mean olive, because the fruit look like an olive and the name of specie suggest the slim aspect of the leaves.

The fruit covered by the modified cell compose a false drupe. The external tissue is floury and the internal tissue is woody. There have an oval train, roundabout 1 cm length yellowish colour covered by silver hairs, with a floury and sweet pulp. The seed is covered by a hard endocarp with 8 black stripes alternated with 8 brown stripes [6].

While participate of the same family with *Hippophäe rhamnoides* L., sea buckthorn, witch fruits are considered the Romanian ginseng, the Russian olive is not used in phytotherapie through the floury aspect of the pulp and the presence of scaly hairs. Anyway is considered a useful plant because it fixes the ecosystems in the sea cost area and the fruits are very appreciated by different species of birds, especial in the cold season.

The presence in a fruits of poliphenolic compounds (flavonozides and ODP), notified in reference materials [1] and the results of the preliminary research [3], establish us to deepen their study.

The research objectives consist of:

- verification the identity of studied drug;
- confirmation of the presence to the poliphenolic compounds (flavonosides and ODP) in the native drug;
- the establishment of the quality of the drug by determination the contents of solubles substances in ethanol, flavonozides and ODP.

Material and Methods

The drug is represented by the pulp of the fruits, reaping sunny period, in September 2004, from Constanta and environment. Separation the pulp to the hard kernel made manually, by scrap between fingers. The method using is pharmacognostic analysis.

Verification of the fruits identity was made by macroscopic examination and on powder microscopic preparation, clarified with cloralhidrat solution 80%. The hystochemical analysis was made on the powder microscopic preparation in Steimetz reagents.

The confirmation to the presence of flavonosides and ODP was made using specific identity reactions [4] with solution 5% obtaining by break-down the drug at the boiling with ethanol (P_1) and ethanol 50% (P_2).

The preliminary quantitative analysis (dried evaporation, soluble substances in ethanol) was made according FR X [7].

The quantitative analysis for flavonosides and ODP was realized by spectrophotometric method beside the etalon curves of rutoside and caffeic acid.

For flavonosides the method bases to obtaining the yellow internal complex with $AlCl_3$, who increases the yellowish colour of the solution.

For ODP, the method bases to the phenol properties to form with nitros acid the nitroso derived who izomerises to oxime. This last one has a low acid character and dissolve in alkaline solutions, with appearance a red colour [4].

The determinations was made with Turner apparatus using solution 5%. The reproducibility of this method was verified by statistic calculation (r = 0,995).

Results and Discussions

The fruits situated in raceme to the young branches (fig. 1 a), have a fluffy aspect, oval form, white-yellow colour, about 1 cm length, 0,7-0,8 cm diameter and floury and sweet pulp (Fig. 1b).

Fig. 1a: Young branches with fruits

Fig. 1b: Fruits (detail)

The microscopic examination show the big mezocarp cells (Fig. 2a) and hairs like a fan from epicarp. (Fig. 2b).

The hystochemical examination confirms the accumulation at the base of the hairs of the liposolubles compounds (probably resinic acids), coloured in red (Fig. 3).

The poliphenols quantitative analysis confirm the presence to flavonoides and ODP. The results obtaining are showed in Table 1.

Fig. 2a: Mezocarp cells

Fig. 2b: Hair like a fan

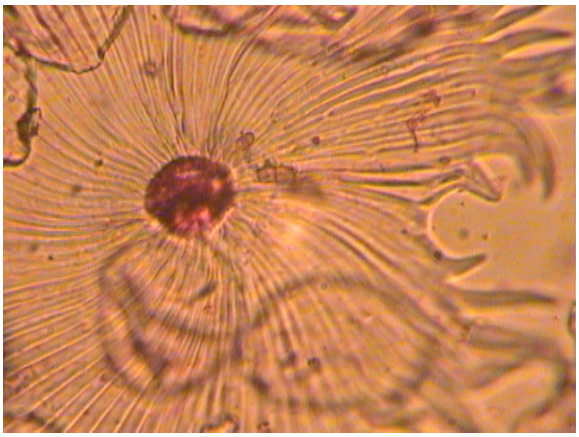

Fig. 3: Accumulation area of liposolubles compounds

Table 1: The identification of flavonosides and ODP

CRT. NR.	REAGENTS	PRODUCTS	INTENSITY	COMPOUNDS
1.	Arnow	Red colour	+	ODP
2.	HCl + Mg	Orage-red colour	+	Flavonosides
3.	$AlCl_3$	Increase the yellowish colour	+	
4.	NaOH	Increase the yellowish colour	++	
5.	Oxalic acid/ boric acid	UV fluorescence	+	

The preliminary determinations show a dried evaporation between 9.3314 – 9.9097% who corresponding to the stipulations of FR X (max. 12%) and a considerable contents in soluble substances in ethanol (21.8769-22.3847).

The quantitative analysis for flavonoides show a contents about 0.1328% couched in rutoside, calculated according to formula:

$$\% \text{ flavonosides (g/g)} = \frac{m_{rutoside} \times 10^{-3}}{m_{pv}} \times 100$$

were:

$m_{rutoside}$ = weight of rutoside

m_{pv} = weight of drug in samples solutions (5.0234g).

The results are presented in figure 4 and table 2.

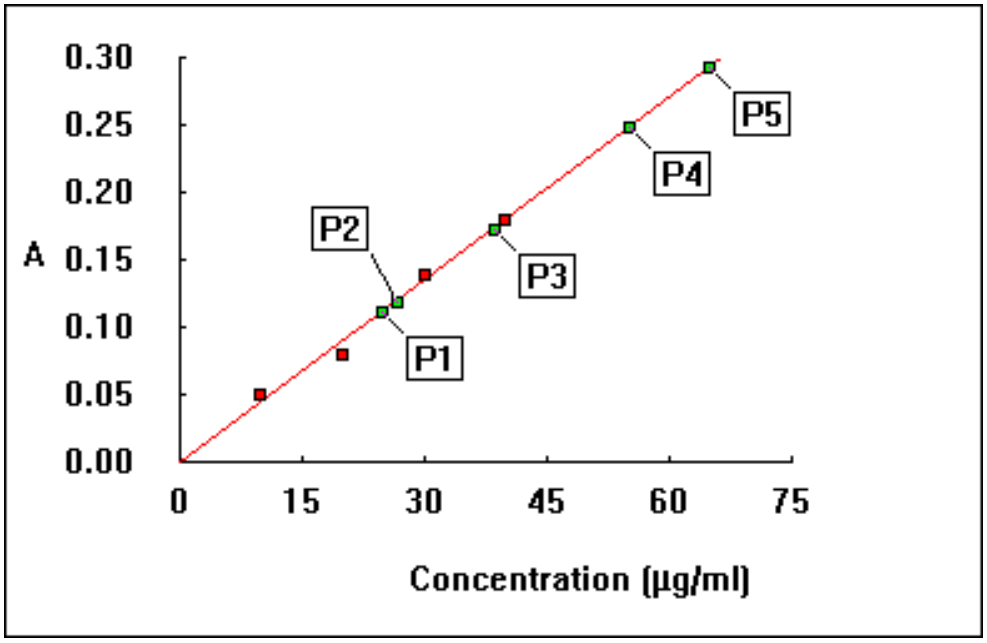

Fig. 4: Standard curve for flavonosides quantitative analysis (reference substance = rutoside) and graphic representation of a samples

Table 2: Results of flavonosides quantitative analysis

Sample	V_i sample mL	V_f sample mL	A 430 nm	C_f µg/mL ppm	C_i $C_i=C_f*V_f/V_i$ µg/mL (ppm)	$m_{rutoside}$ $C_i*100*10^{-3}$ $= C_i*0,1$ mg/100 ml	% flavone g/g
P1	1	5	0.112	24.88889	124.44	12.444	0.1328%
P2	2	5	0.120	26.66667	66.66	***6.666***	
P3	3	5	0.174	38.66667	64.44	***6.444***	
P4	4	5	0.249	55.33333	69.16	***6.916***	
P5	4.5	5	0.293	65.11111	72.34	7.234	
Samples mean P_2, P_3, P_4						***6.675***	

The quantitative analysis for ODP show a contents about 0.0598% couched in caffeic acid, calculated according to formula:

$$\% \text{ ODP (g/g)} = \frac{m_{\text{caffeic acid}} \times 10^{-3}}{m_{pv}} \times 100$$

were:

$m_{\text{caffeic acid}}$ = weight of caffeic acid

m_{pv} = weight of drug in samples solutions (5g).

The results are presented in figure 5 and table 3.

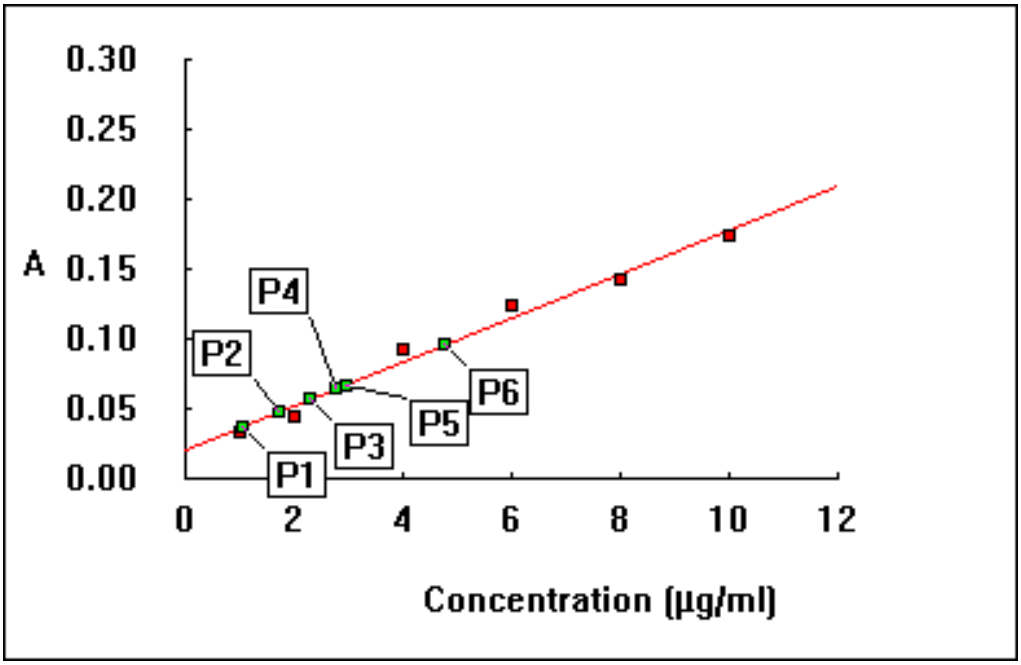

Fig. 5: Standard curve for ODP quantitative analysis (reference substance = caffeic acid) and graphic representation of a samples

Table 3: Results of ODP determination

Sample	V_i sample mL	V_f sample mL	A 510 nm	C_f µg/mL ppm	C_i $C_i=C_f*V_f/V_i$ µg/mL (ppm)	$m_{caffeic\ acid}$ $C_i*100*10^{-3} = C_i*0,1$ mg/100 ml	% ODP g/g
P1	0.5	10	0.038	1.06329	21.26	2.126	0.0598%
P2	0.6	10	0.049	1.75949	29.32	***2.932***	
P3	0.7	10	0.058	2.32911	33.27	3.327	
P4	0.9	10	0.065	2.77215	30.80	***3.080***	
P5	1	10	0.068	2.96203	29.62	***2.962***	
P6	2	10	0.097	4.79747	23.98	2.398	
Samples mean P_2, P_4, P_5						***2.991***	

Conclusions

The quantities of flavonosides (0.13%) and ODP (0.06%) are low but we could say that *Elaeagni fructus* would be capitalized in phytotherapie for antioxidants properties.

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CONTRIBUȚII LA STUDIUL FARMACOGNOSTIC AL SPECIEI *ELAEAGNUS ANGUSTIFOLIA* L. NOTA 1 STUDIUL DERIVAȚILOR POLIFENOLICI DIN FRUCTE

(Rezumat)

Utilizate ca sursă de vitamine (A, E şi C) şi de proteine, fructele speciei *Elaeagnus angustifolia* L., ce reprezintă hrana păsărilor până iarna târziu, s-a presupus că ar prezenta şi un bogat conținut în alte principii active. În urma unui studiu chimic preliminar s-au evidențiat acizi polifenol-carboxilici (ODP-uri) şi flavonoide (flavonozide, proantocianozide, cateholi).

Determinarea cantitativă a ODP-urilor şi a unora dintre flavonoide (flavonozide), s-a efectuat prin metode spectrofotometrice, la un aparat TURNER, față de acidul cafeic, respectiv rutozidă, ca substanțe de referință.

În urma acestora s-a stabilit un conținut mediu de 0,13% flavone exprimate în rutozidă şi de 0,06% ODP-uri exprimate în acid cafeic.

Proprietățile antioxidante ale acizilor polifenol-carboxilici şi flavonelor, cunoscute din literatura de specialitate, ne determină să afirmăm că produsul ar putea prezenta interes pentru a fi valorificat în fitoterapie.