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The dosage of polyphenols in *Elaeagnus angustifolia L.* fruit extracts

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ABSTRACT

A large number of ornamental and medicinal trees and shrubs possess very interesting biological properties finding applications in diverse domains including in food, medecine and pharmacy. In the present work, we have chosen the medicinal *Elaeagnus angustifolia* because it is the less frequently used in our country because of the ignorence of its nutritional and medical values. We are interested in the dosage and the study of the composition in secondary metabolites in different fruits extracts of *Elaeagnus angustifolia* including flavonoids, tannins and alkaloids. Phytochemical essays done on *Elaeagnus angustifolia* fruit extracts revealed the presence of flavonoids, tannins and the complete absence of alkaloids. Quantitaive analysis on TLC of the different extracts revealed the presence of a unique type of of flavonoids which is the rutin. The quantitaive analysis showed that the methanolic extract is rich with total flavonoids with a tenor of 3,94 ± 0,07 mg E AG/g of extract followed by dichloromethane and aqueous extracts with the value of 2,9 ± 0,7 mg E AG/g of axtract and 2,32 ± 0,48 mg EAG/g of extract. Flavonoids and tannins spectrophotometry dosage showed the presence of these substances in the polar extracts and their absence in the apolar extracts.

KEYWORDS

Elaeagnus angustifolia;
Polar extracts;
Apolar extracts;
Polyphenols;
Flavonoids;
Tannins.

INTRODUCTION

Traditional remedies made of plants were used for a long time without knowing to what were due their beneficial actions, it's difficult to define the molecules responsible of the pharmacological action[3].

The medicinal plants represent inexhaustible sources for natural bioactive substances and compounds. In fact, secondary metabolites are and remain the object of numerous *in vivo* as well as *in vitro* researches, especially searching for new natural constituents as the phenolic compounds.

For the need of this study, we have chosen *Elaeagnus angustifolia* among the medicinal plants

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because it is the less frequently used in our country because of the ignorance of its nutritional and medical values.

Phytochemical studies *in vivo* done on the fruit aqueous phase of the species *Elaeagnus angustifolia*, showed its richness in flavonoids, terpenoids with anti-inflammatory and antinociceptives (analgesic) effects[1].

In traditional Iranian medicine, *Elaeagnus angustifolia* fruits and flowers are prescribed for the treatment of nausea, vomiting, icterus, asthma and flatulence (Hosseinzadeh et al., 2003).

Elaeagnus angustifolia fruit is used as an analgesic agent in order to relieve rheumatism and arthritis pains[19].

Its extracts are traditionally used against a multitude of illnesses, especially as antipyretics, antineuroceptive and as antidiarrhoeics[1].

Elaeagnus angustifolia bear the vernacular name of Russian olive as it reminds olive tree with its silver habit and its leafage. *Elaeagnus angustifolia L.* belongs to the family of *Elaeagnacees* native of several regions: south Europe, central Asia and Himalaya. It was introduced into North America during the colonial-age and widely planted western United States[15]. In Algeria, *Elaeagnus angustifolia* is subspontaneous and cultivated as roads sides ornaments[22]. It was also introduced and systematically planted in the following locations: Djelfa, Biskra, Relizane, Mascara and the south of Tennes and Cherchelle[11].

The fruits of the Russian olive are small egg-shaped drupes, alike small olives, of a reddish yellow colour widely consumed fresh or used in different seasoning preparations[4]. According to Dzhangaliev et al, they are very rich with potassium and phosphorus salts, and vitamin C, A and E (Boudraa et al). Several studies showed that *Elaeagnus angustifolia* species fruits are very rich with several bioactive constituents[2,4] having important biological activities ayant des activités biologiques importantes.

Polyphenols of the fruit pulp are in the majority made of catechin, gallocatechin. epigallopetroleum ether, proantocyanidins and flavonols[17].

Our work inscribes within the context of a contribution to a better knowledge of this medicinal plant of the Aures Algerian region and to perform qualitative and quantitative analysis of its content in polyphynols, flavonoids, tannins and alkaloids of different aqueous and organic extracts of the edible part of *Elaeagnus angustifolia* fruit.

EXPERIMENTAL

Plant material

It is made of the *Elaeagnus angustifolia L.* fruits collected from the region of Batna during September and October 2007. The fruits were at first stoned then the edible part (fruit) was dried in dark, under the lee of light at ambient temperature. After drying, the edible parts were ground into fine powder that was used in the preparation of extracts.

The different l'*Elaeagnus angustifolia L.* fruit parts are shown in figure 1.

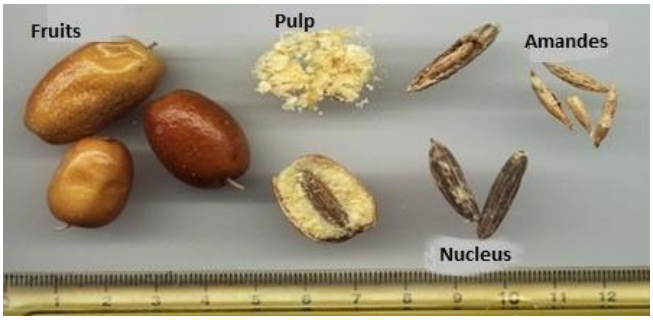

Figure 1 : Different parts of *Elaeagnus angustifolia L.* fruit.

Preparation of *Elaeagnus angustifolia L.* fruit extracts

According to Diallo et al[8] method, different extracts types were prepared from des ground fruits using solvents with increasing polarity (petroleum ether, dichloromethan, methanol). In the end of the extraction, the three organic extracts were concentrated in vacuum rotavapor at 37C°, 40C° and 50C° respectively. After the concentration, these extracts were dried in the open air. While an aqueous maceration at 10% was also made for the aqueous extract. After filtration, the extract is lyophilized.

Analysis of *Elaeagnus angustifolia* fruit extracts

Qualitative analysis of *Elaeagnus angustifolia* fruit extracts

Preliminary tests

The detection of the presence of flavonoids, of tannins and of alkaloids in the extracts *Elaeagnus*

angustifolia fruit was carried out with coloured reactions: cyanidin reaction, ferric chloride essay and potassium mercuriteraiodure for the flavonoids, tannins and alkaloids respectively[7,8,10].

Thin layer choromatography

Elaeagnus angustifolia fruit extracts analysis was carried out on silice gel plates with fluorescent indicator according to Diallo et al[8] modified method. The four extracts were dissolved in their original solvent. The analysis of polar extracts (AQ, MET) were made a separation system BAW (butanol/ ethyl acetate /water) with the proportions (60/15/35). So for the apolar extracts (DCM, ET), the system (petroleum ether / ethyl acetate) with the proportions (8/2) were used. After migration, the plates were dried, then visualized with four revealing systems: physical revelation under UV at 254 nm and 366 nm ; and chemical revelation with sulphuric vanillin and ethanolic DPPH at 2,4% solution for extracts detection having antioxidant activity[14].

Quantitative analysis *Elaeagnus angustifolia* fruit extracts

Total polyphenols assay

The dosage of total polyphenols in *Elaeagnus angustifolia* fruit extracts was done according to folin ciocalteu method[23]. The absorbance was read at 765 nm with an UV spectrophotometer after 2 hours of incubation. The concentration of total polyphenols is calculated from the regression equation of the set of calibration solutions assessed with gallic acid (0-200 µg / ml). The results are given in milligram of acid gallic equivalent per one gram of extract (mg EAG/g of extract).

Flavonoids assay

The aluminium chloride method[3] is used for flavonoids quantification in different extracts. The absorbance is read at 430 nm. After 10 minutes incubation, the flavonoids concentrations are deduced from the set of calibration solutions assessed with quercetine (0-35 µg/ml) and are expressed in milligram of quercetine equivalent per gram of extract (mg EQ/g of extract).

Condensed tannins assay

The dosage of condensed tannins in *Elaeagnus angustifolia* fruit extracts was relized according to vanillin method of Broadhurst et Jones (1978), modified by Heimler et al.[13]. The principal of this assay is based on the fixation the vanillin aldehydic group on the carbon 6 of the A cycle of petroleum ether to form a red chromophore complex that absorbs at 500 nm[21]. After 15 minutes incubation, the absorbance is measured at 500 nm. The concentration of condensed tannins (proanthocyanidin s) is deduced from the set of calibration solutions assessed with petroleum ether (0-30 µg /ml) and are expressed in milligram of petroleum ether equivalent per gram extract (mg CatE/g).

RESULTS AND DISCUSSION

Preparation of extracts from *Elaeagnus angustifolia* fruits

The use of solvents with different polarity allows the separation of the compounds according to their solubility degree in extraction solvent. This extraction has allowed to obtain four crude extracts: etheric extract (EpE), dichloromethanic extract (DCME), methanolic extract (MetE) and aqueous extract (AqE). Each extract was characterized with its aspect, its colour and its yield in relation to the dried powder. These elements are represented TABLE 1.

TABLE 1 : Aspects, colours and yields of *Elaeagnus angustifolia* pulp extracts by different solvents.

Extract	Aspect	Colour	Yield (%)
Ep	Oily	Yellowish green	0,35
DCM	Oily	Dark green	0,40
Met	Pasty	Honey colour	8
Aq	Lyophilised	Yellowish white	30,46

In the sight of this table, we have noticed that the highest yield is obtained with the aqueous polar and methanolic extracts with 30,46% and 8% respectively and the lowest yield with the dichloramethanic apolar and etheric extracts with 0,40% and 0,35% respectively.

Analysis of *Elaeagnus angustifolia* fruit extracts

Qualitative analysis of *Elaeagnus angustifolia* fruit extracts

Preliminary tests

The results of these phytochemical tests are reported in the TABLE 2.

The phytochemical essays performed on the extracts of *Elaeagnus angustifolia* fruit revealed the presence

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of flavonoids, tannins and the absence of alkaloids.

These results are in accordance with the ones obtained by other authors that the phytochemical analysis gave spositive results for flavonoids and negative results for alkaloids[1].

The predominance of tannins in *Elaeagnus angustifolia* fruit was showed by Kusova et al[16].

TABLE 2 : Characterisation of the chemical groups in the extracts of *Elaeagnus angustifolia* fruits.

Chemical groups	Extracts	Results of tube reactions
Flavonoids	Met	+ + +
(Cyanidin reaction)	Aq	+ +
	DCM	-
	Ep	-
Tannins (reaction with	Met	+ +
aluminium chloride)	Aq	+
	DCM	-
	Ep	-
Alkaloids	Met	-
(Mayer reagent)	Aq	-
	DCM	-
	Ep	-

(+++): Positive reaction, (++): Fairly positive reaction, (+) : Suspicious reaction (-) : Negative reaction

We noticed also the presence of flavonoids and tannins in the polar extracts (Aq and Met) and the absence of these substances in the apolar extracts (DCM et Ep).

Thin layer choromatography analysis

For a partial characterisation of the different extracts of the pulp fruit of *Elaeagnus angustifolia*, a thin layer choromatography (TLC) was realized following Diallo et al method[8]. For the polar extracts, the solvent system used (n-butanol/ acetic acid) water (12 :3 :7 V/V/V) allowed to abtain a very good chromatographic separation using three standards which are petroleum ethere, quercetine and rutin. The revelation is realized with a mixute of sulfuric vanillin after the observation under ultraviolet light. Only methanolic extract was put on the thin layer choromatography, aqueous extract was eliminated as it doesn't migrate with different systems.

The use of different solvents at different polarity allowed to separate these compounds according to their solubility degree in extraction solvent. The results of thin layer choromatography of the methanolic extract are mentioned in the TABLE 3.

TABLE 3 : Results of the thin layer choromatography of the methanolic extract of *Elaeagnus angustifolia* fruit.

	N°spot	FR	Colour	254 nm	366 nm
Quercetine		0,91	Orange yellow	Visible	Blue
Rutin		0,66	Light yellow		-
Petroleum ethere		0,54	Red		-
Met	1	0,64	Orange yellow	3 spots	A blue spot
	2	0,54	Light yellow		
	3	0,77	Blue	Blue	
	4	0,96	Mauve	Fluorescents	

The orange yellow colouring of the spot with RF 0,64 orientate us to the presence of a flavonoid which is rutin, by comparison of their frontal ratio (rutin FR= 0,66).

According to Selon Yanez[24], rutin is a flavone (quercetine-3-rhamnosyl glucoside) that has antioxydant proved properties.

Quantitative analysis of *Elaeagnus angustifolia* fruit extracts

Total polyphenols assay

The results of the total polyphenols assay (TABLE 4) in the different extracts of *E. angustifolia* fruit pulp show that all the extracts contain phenolic compounds with the methanolic extract being the richest with these compounds. The values present the average of three assays ± SD.

TABLE 4 : Content of the total polyphenols in the pulp of de *Elaeagnus angustifolia* fruit.

Extract	Polyphenols
Met	3,94 ± 0,07
DCM	2,9 ± 0,7
Ep	1,96 ± 0,7
Aq	2,32 ± 0,48

In a study made on *Elaeagnus angustifolia* species, Mavlyanov et al,[17] has found that the levels of total polyphenols is of 4,5%. This is a close value to ours, obtained in the methanolic extract.

According to a study performed by Patthamakaporn et al., in[18], the variation of the polyphenols content could

be due to conservation conditions of the fruit in cold.

On the other side, the level of fruit polyphenols of the gender *Elaeagnus* decreases with the degree of maturation. This parameter was explained by Sakamura et Suga[20] and Bekker et Gluchencova[4].

Flavonoids and tannins assay

The results of the dosage of the flavonoids and tannins tenors are reported in the TABLE 5. The values obtained represent the average of two assays ± SD.

The determination of the level of flavonoids shows that the AqE contains 0,15 ±0,06 mg QE/g of extract followed by the metE with 0,13±0,06 mg EQ/g of extract.

TABLE 5 : Flavonoids and tannins tenors in the extracts of *Elaeagnus angustifolia* fruit.

Extract	Flavonoids content [a]	Tannins [b] content
Met	0,13 ± 0,06	1,51 ± 1,15
Aq	0,15 ± 0,06	2,57 ± 0,35
DCM	-	-
Ep	-	-

[a]mg of quercetin equivalent per gram of extract
[b]mg of petroleum ether equivalent per gram of extract

The Aq extract is also the richest in tannins with 2,57±0,35 mg cat E /g of extract followed by par the Met extract with 1,51±0,08 mg catE / g of extract. This could be a confirmation of the astringent taste of our fruit.

From the tenors obtained in this study, we can tell that *Elaeagnus angustifolia* fruit constitutes a non negligible source of tannins. The importance of tannins drogues is linked to their astringent properties[6], having the power to stop haemorrhages[12] and fight infections[9].

These results are in accordance with those found in the literature on the richeness of the *Elaeagnus angustifolia* fruit with bioactives substances in particular tannins[16].

The low flavonoids content in all extracts may be explained by the decrease of the polyphenolic compounds during the maturation as demonstrated by a study made by Sakamura et Suga[20] on fruits of the species *E. multiflora* and *E. umbellata*.

CONCLUSIONS

In the present work, we have been interested to different dosages of secondary metabolites of different *Elaeagnus angustifolia* fruit extracts.

From a phytochemical point of view, we have noticed that highest yield is obtained with aqueous extract (30,46 ?), whereas the lowest one had been obtained with petroleum ether and dichloromethane extracts (0,35 ; 0,40) respectively.

The characterization reactions have underlined the presence of flavonoids and condensed tannins in the polar extracts and their absence in the apolar extracts of *Elaeagnus angustifolia* fruit. However, alkaloids were absent in all extracts.

Thin layer choromatography has allowed us to reveal a flavonoid which is the rutin but this result remains relative.

The dosage of flavonoids and tannins has allowed us to confirm the results obtained futher to characterisation reactions, concerning the presence of the substances in the polar extracts and their absence in the apolar ones.

Regarding the quantitative analysis of total polyphenols, our results show that all polar and apolar extracts contain the phenolic compounds but the methanolic extract is the richest with polyphenols with a tenor of 3, 94 ± mg. However, we have noticed a low tenor of polyphenols in the petroleum ether extract.

Of course, this results obtained *in vitro* during this study constitute only the first step in the search of new bioactive substances. Complementary studies are necessary to be able to confirm the underlined performances.

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