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Antioxidant and anti-diabetic effects of hydromethanolics extracts from *Olea europea* and *Erythreae centaurium*

W. Kouadri Boudjelthia[1], K. Hammadi[1], M. Kouidri[2], N. Djebli[1], A. Noui[2], M. Cheurfa[2]

[1]*Laboratoire de Pharmacognosie Api-phytothérapie, Département de Biologie; Université de Mostaganem, Algeria*

[2]*Laboratoire de Bio Ressources naturelles, Université Hassiba Benbouali de Chlef, Algeria*

Key words: *Oleaeuropeae L, Erythreae centaurium* L., polyphenols, antioxidant activity, antidiabetic activity.

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Abstract

This work represent a part of the valorization of the hydro-methanolic extract of the leaves of *Olea europeae* L. and flowering tops of *Erythreae centaurium* L. by the evaluation of antioxidant and antidiabetic activities of polyphenols.The antioxidant power of these extracts was evaluated in vitro by the DPPH test, after having quantified the total polyphenols. It appears that they have a capacity to trap the DPPH radical with an IC50 of 0.475 mg / ml and 0.495 mg / ml. for the extract of *Olea europeae* L. and *Erythreae centaurium* L. respectively.The α-amylase test has been recommended for the evaluation of the in vitro antidiabetic effect of olives and small cornflower, the results obtained show a high capacity for inhibition of the enzyme α-amylase with a percentage of more than 80% for the Olea europeae L extract and about 60% for the *Erythreae centaurium L* extract.The present study has shown that the antioxidant activity and inhibitory effect of α-amylase hydro-methanolic extracts of both plants are strongly related to its richness in polyphenols that can be exploited in the pharmaceutical field.

* **Corresponding Author:** W. Kouadri Boudjeltia ✉ wafa.twinty@yahoo.f

Introduction

Research in recent decades has shown that oxidative stress promotes many human diseases by potentiating the appearance of certain multifactorial diseases such as rheumatism, cardiovascular diseases and diabetes [1, 2].

Diabetes is a metabolic disorder characterized by chronic hyperglycemia And a clinical triad associating polyphagia, polydipsia and polyuria, resulting either in a lack of insulin secretion or a defect in glucose uptake [3].

Hyperglycemia is the consequence of the production of free radicals that reduce the activity of glyceraldehyde-3-phosphate dehydrogenase (glycolysis enzyme).

The glucose flux will be deviated to another metabolic pathway, the pathway of glucosamine which leads to some of the transactional effects (activation of gene expression) contributing to the development of diabetic complications. In other words, the excess of glucose in the extracellular medium causes an acceleration of the production of free radicals at the level of the respiratory chain [2].

An upsurge of interest has been noted in the biological effects of natural substances included in the fight against oxidative stress and diabetes such as Berberis vulgaris, Zygophyllumgeslini and many others [4,5].

Olea europea L, known under the vernacular name "Zeitoun" is a perennial plant belongs to the family of oleaceae Landing all the Mediterranean region including Spain, Italy, France, Tunisia and Algeria [6,7].

The therapeutic value of olive leaves has been correlated mainly with its content of polyphenols, mainly oleuropein [8,9].

The centaury is an annual herb belonging to the family Gentianales, it grows in Europe, North Africa and West Asia [10].

Erythreaecentaurium is known for its antipyretic, anti-inflammatory and even antidiabetic efficacy [11,12].

In this case, the present study focuses on the evaluation of the antioxidant and antidiabetic activity in vitro of Olea europea L and Erythreaecentaurium L for their valuations as medicinal antidiabetic plants.

Material and methods

Plant material

The leaves of the Olea Europeae L and the flowering tops of the Erythreaecentaurium L were harvested the month of March 2015 from the region of Chlef Algeria. The leaves are converted to powder using a sprayer. After drying in the dark and at room temperature.

Preparation of the hydromethanic extract

The preparation of the hydromethanolic extract of Olea Europeae Let from Erythreaecentaurium L was carried out by maceration of 10 g of plant material in a 50% aqueous-alcoholic solution. After 72 hours of contact, the extract obtained is filtered and then concentrated to dryness (T ° = 40 ° C.) by a rotavapor. The resulting residue is stored at 4 ° C [13].

Determination of total polyphenols

The estimation of the total extractable phenolic compounds content was based on the Folin-ciocalteu spectrophotometric assay using a set of reagents [14]. The Folin-ciocalteu reagent is a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) [15].

0.2 ml of each extract and 0.8 ml of sodium carbonate (7.5 g / l) were added after stirring for 2 minutes to 1 ml of Folin-ciocolteau solution. After 30 minutes of incubation, protected from light, at room temperature, the reagent undergoes a color change from yellow to blue following the oxidation of the polyphenols. This coloration, the intensity of which is proportional to the levels of the phenolic compounds

present in the medium, gives a maximum absorption at 760 nm [16, 17].A standard curve is realised in parallel under the same operating conditions using gallic acid as a positive control at different concentrations [15].

The total phenol concentration of the extracts was expressed in milligrams of gallic acid (AG) equivalents per gram of plant.

Determination of flavonoids

The determination of the flavonoid content in the hydromethanolic extracts of Olea Europea L and Erythreaecentaurium L was carried out by spectrophotometry using the aluminum chloride method [18].

1ml of the methanol solution of AlCl3 was added to 1ml of the two samples. After a 40 minute incubation at room temperature and in darkness, the absorbance was measured at 420 nm [19].

The results obtained are expressed in equivalent milligrams of catechin per gram of plant.

Dosage of tannins

The condensed tannins are determined by the vanillin method in an acid medium [20].

1.5ml of methanolic solution of vanillin was added to 1ml of each extract and 0.75ml of concentrated hydrochloric acid. After 20 minutes of incubation at room temperature, the absorbance was read at 550 nm.

The tannin concentration is expressed in milligrams of catechin equivalents per gram of the plant.

Evaluation of antioxidant activity in vitro

The antioxidant activity of the two extracts was measured using the DPPH radical.

After a 30 minute incubation of a mixture of 1ml of DPPH solution and 0.1ml of the extract of each plant at different concentrations, the absorbance was measured at 517 nm [21]. Ascorbic acid was used as a positive control.The percent inhibition of DPPH was calculated using the following equation:% inhibition of DPPH = [(A1-A2) / A1] × 100, of which A1 and A2 represent the absorbance of the DPPH solution with and without the extract respectively [22].

The results are expressed as percentage inhibition of DPPH and IC50 (50% inhibitory concentration of the DPPH radicals).

Evaluation of in vitro antidiabetic activity

The antidiabetic activity of the hydroalcoholic extracts of *Olea Europea L* and *Erythreaecentaurium L* was evaluated using the α-amylase enzyme. 0.2ml of α-amylase solution and starch-based substrate solution was added to 0.2ml of two extracts at different concentrations. After a 5 minute incubation, 0.6ml of DNSA (3-5 Dinitro-Salicylic) was recommended to stop the reaction.

The evaluation of the effect of the extracts on the activity of α-amylase is based on the spectrophotometric reading at 540 nm [23].

The results are expressed as percentage inhibition according to the following formula:% inhibition = [(Absorbance control-Absorbance extract) / Absorbance control] × 100.

Results and discussion

Content of total polyphenols, flavonoids and tannins

The total phenol, flavonoid and tannin contents of *Olea Europeae L* leaf extracts and *Erythreaecentaurium L* flower arrangements are shown in Table 1:

The results of determination of the total polyphenols obtained are expressed in mg EAG / g of dry matter.

The highest content of polyphenols was measured in thehydromethanic extract of *Olea Europea*L leaves with a value of 36.040 +/- 0.901 mg EAG / g MS, this value is close to that of [24].

Table 1. Rates of polyphenols, flavonoids and tannins in the extract of *Olea Europeae L* and *Erythreaecentaurium L.*

Studied plant	Total Polyphenols mg EAG/g MS	Flavonoïds mg EC/g MS	Tanins mg EC/g MS
Olea Europea L	36.040+/-0.901	27.730+/-0.360	6.299+/-0.167
Erythreaecentaurium L	12.692+/-0.696	24.870+/-0.718	3.183+/-0.104

While this content does not exceed 12.692 +/- 0.696 mg EAG / g MS in the extract of flowering heads of *Erythreaecentaurium L*, however the work done by [25]. found a value of 22.28 + / -1.07 mg EAG / g MS.

Polyphenols are phytochemicals with high antioxidant activity [26], implying their use in antidiabetic herbal medicine.

Table 3. Values of IC50 of extracts.

Plant extract	IC_{50}expressed mg/ml
*Olea europaea*L. extract	0.475
*Erythraeacentaurium*L. extract	0.495

Numerous biological activities attributed to antioxidant properties have been recognized by the flavonoids. These latter have the capacity to react with several reactive oxygen species [27].

A value of 27,730 +/- 0.360 mg EC / g MS was recorded for the hydroalcoholic extract of Oleaeeuropea L. This value is remarkable compared to that of the extract of Erythreaecentaurium L (24,870 +/- 0.718 mg EC / g MS).

Table 3. Percent inhibition of α-amylase by extracts of *Olea europaeaL.* and *Erythraeacentaurium L.*

	1.6mg/ml	2.4mg/ml	3.1mg/ml	4.8mg/ml	6.4mg/ml
Olea europaea L.	82.78%	83.33%	83.52%	88.82%	89.38%
Erythraeacentaurium L.	15.57%	50.55%	56.78%	58.61%	59.71%

Phenolic polymers are represented by hydrolyzable and condensed tannins. Olive leaves have a condensed tannin content of about 6.299 +/- 0.167 mg EC / g DM, according to the study by [28]. these are present with a percentage of 1%.

Evaluation of antioxidant activity in vitro

For rapid and direct evaluation of the antioxidant activity, the DPPH radical is used [29]. The antiradical activity of the hydroalcoholic extract of Olea europeae L and Erythreaecentaurium L was evaluated spectrophotometrically at 517 nm.

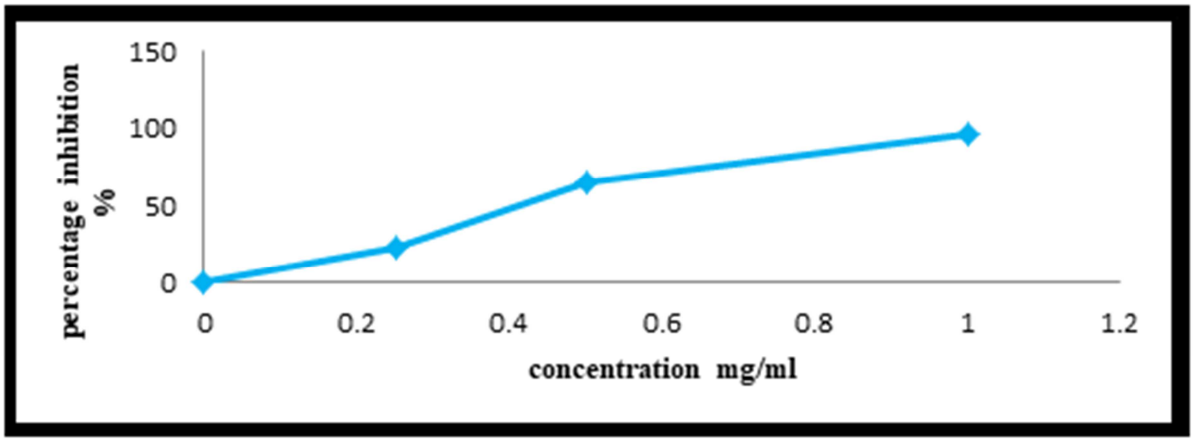

Fig. 1. Percentage inhibition of the free radical DPPH as a function of the different concentrations of the hydroalcoholic extract of *Olea europaea L.*

The results obtained are expressed as a percentage of inhibition of the free radical DPPH as a function of the concentrations of the extracts or by IC 50 which is inversely proportional to the antioxidant power of a compound [30].

According to this study, it was observed that the extract of both plants has antiradical activity proportional to the concentration of polyphenols.

The extract of *Olea europeae L* showed the highest activity at low concentrations compared to the *Erythreaecentaurium L* extract with a 96.22% inhibition percentage at a concentration of 1 mg / ml.

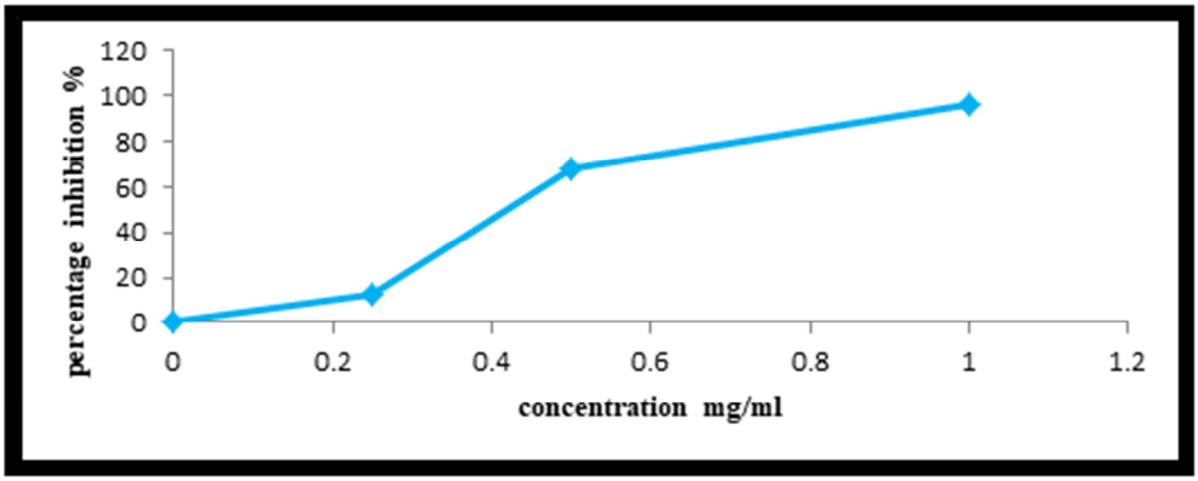

Fig. 2. Percentage inhibition of the free radical DPPH as a function of the different concentrations of the hydroalcoholic extract of *Erythraeacentaurium L.*

The hydroalcoholic extract of *Olea europeae L* and from *Erythreaecentaurium L* has a moderate antioxidant capacity with an IC50 of 0.475 and 0.495 respectively (Table 2).

These results are consistent with those of Gracia and al in 2000 ,Polzonetti and al in 2004, which conclude that continuous phenols in olive leaf extract have an enormous capacity for free radical scavenging.

According to[25],the polyphenols of the small knapweed have an effective antiradical capacity of 79.29 +/- 1.22 TE / g DM. As well as results[31], demonstrate that the antioxidant properties of the small knapweed are interesting.

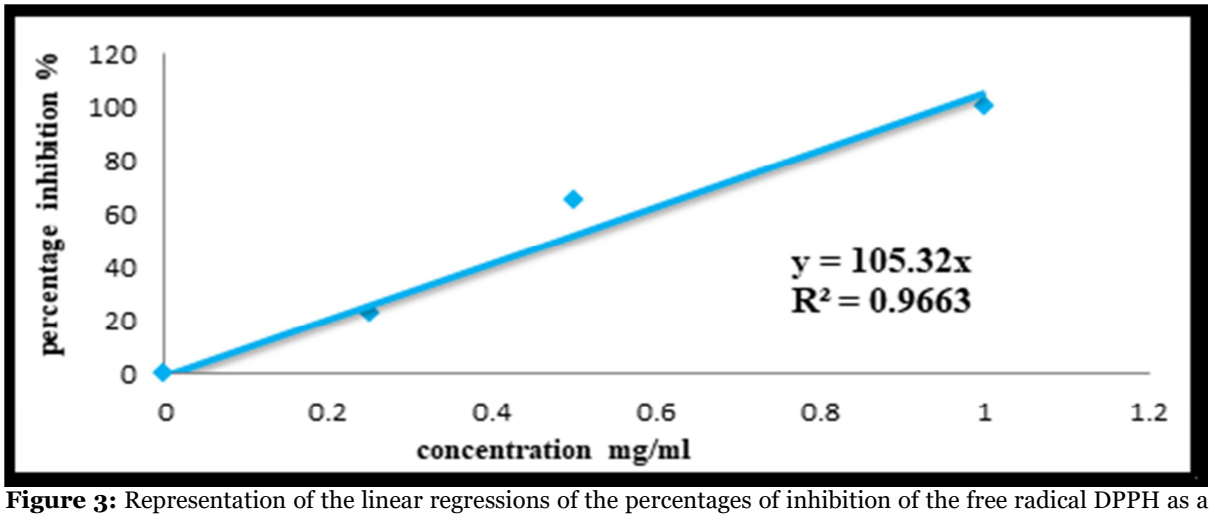

Figure 3: Representation of the linear regressions of the percentages of inhibition of the free radical DPPH as a function of the different concentrations of the hydroalcoholic extract of olive tree.

Evaluation of in vitro antidiabetic activity

The results shown in Table 3 determine the effect of the extracts of Olea europeae L and Erythreaecentaurium L on the activity of α-amylase in vitro, expressed as percent inhibition of the enzyme.

The results obtained in Table 3 show that the hydro-methanolic extract of *Olea europeae L* has a higher percentage inhibition in high concentration (89.38%) compared with *Erythraeacentaurium L* which inhibits the α- amylase less than 60%.

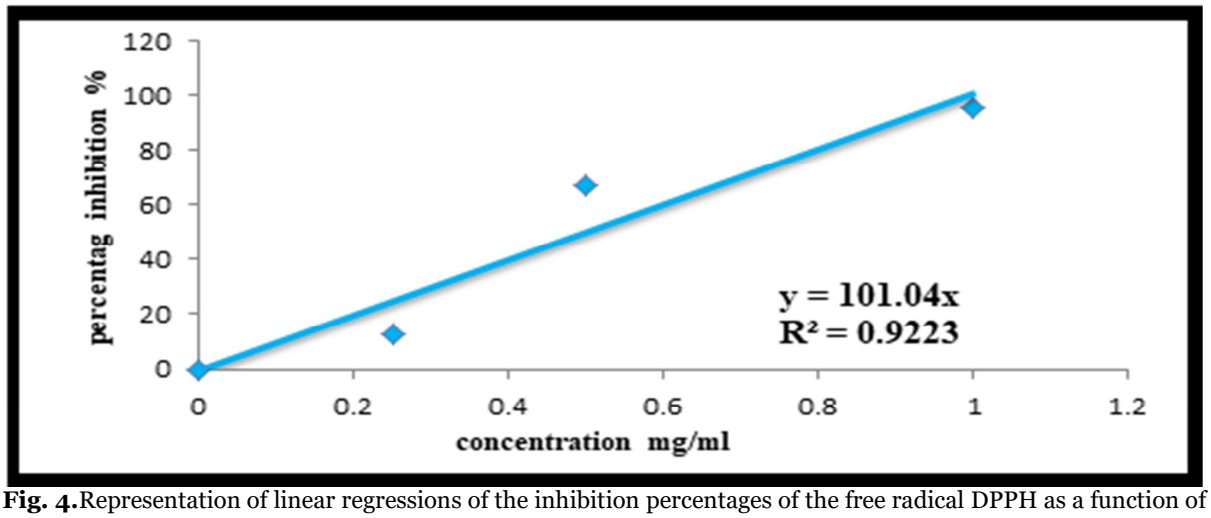

Fig. 4. Representation of linear regressions of the inhibition percentages of the free radical DPPH as a function of the different concentrations of the hydroalcoholic extract of the small centaury.

The inhibitory effect of these two extracts reveals that the percentage of inhibition is proportional to the concentration, the higher the concentration, the higher the percentage inhibition.

It is wise to note that tannins have an action on α-amylase, are able to bind to digestive enzymes and inhibit them [32].

[33] showed that the administration of the aqueous extract of Olea europaea L to rats rendered diabetic by alloxane showed a high tissue tolerance to glucose. Ethobotanical surveys carried out by [34] and by [35] reported the use of *Erythraeacentaurium L* in the treatment of diabetes, as well as the administration of the hydro-alcoholic extract of this plant. normoglycemic rats reduce the level of glucose in the blood [36].

Conclusion

An enhancement of medicinal plants as a source of natural bioactive substances has been carried out recently. As a result, many studies are increasingly interested in antioxidant and antidiabetic effects of natural origin.

The present study has highlighted the richness of the hydromethanic extract of the leaves of *Olea europeae* L and flowering tops of *Erythreaecentaurium L* polyphenols including flavonoids and tannins and an important antioxidant and an effect interesting inhibitor on the activity of α-amylase.

On the basis of these data, the realization of other in vitro studies (fractionation and identification of some major components) and in vivo, is recommended for a better appreciation of the pharmacological effects of these two plants.

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