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Exploration of the antioxidant and anti-diabetic activity *in vitro* of ethanolic extracts of *phillyrea latifolia*, *phillyrea angustifolia*, and spirulina

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Abstract

This study focused on the in-vitro biological activities of ethanolic extracts of *Phillyrea latifolia*, *Phillyrea angustifolia* and spirulina. Belonging to the Oleaceae family. Antioxidant activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. In addition, in vitro anti-diabetic activity was achieved by inhibition of the α-glucosidase enzyme. Extraction of the ethanolic extracts of *Phillyrea latifolia*, *Phillyrea angustifolia* and Spirulina gave yields of 0.89%, 0.93% and 11.18% respectively. The tests showed that the ethanolic extracts of these two plants, as well as spirulina, possessed significant antioxidant potential, with IC50s of 36 µg/ml, 30.85 µg/ml, and 25.76 µg/ml respectively. In addition, the ethanolic extracts of the two plants and spirulina showed significant anti-diabetic potential, with IC50s of around 22.19 mg/ml, 24.55ug/ml and 18.87ug/ml. These results highlight the antioxidant and anti-diabetic potential of ethanolic extracts of *Phillyrea latifolia*, *Phillyrea angustifolia* and spirulina, which is known to be a natural antioxidant. The results obtained confirm the information already available in the scientific literature, underlining the interest of these extracts as promising natural agents for improving health and well-being. These extracts could offer effective natural alternatives for preventing or treating diseases linked to oxidative stress and diabetes, reinforcing their relevance in the field of natural medicine and dietary supplements.

Key words: *Phillyrea latifolia*, *Phillyrea angustifolia*, Spirulina, Antioxidant, Antidiabetic

Introduction

The search for natural compounds with antioxidant and antidiabetic properties has garnered increasing attention due to their potential in the prevention and treatment of metabolic diseases, particularly type 2 diabetes, which is often associated with increased oxidative stress (Gomes et al., 2020). In this context, medicinal plants and microalgae such as spirulina are being studied for their beneficial bioactive compounds. *Phillyrea latifolia* and *Phillyrea angustifolia*, two species belonging to the Oleaceae family, are known for their traditional use and may exhibit interesting pharmacological properties, particularly as antioxidants and antidiabetic agents (Zhou et al., 2018).

Ethanolic extracts of *Phillyrea latifolia* and *Phillyrea angustifolia* have demonstrated promising biological activities in various studies, notably their ability to neutralize reactive oxygen species (ROS) and modulate enzymes involved in carbohydrate metabolism (Ahmad et al., 2019). For instance, previous research has reported the antioxidant effects of these plants due to their phenolic, flavonoid, and other bioactive compound content. These compounds are known for their ability to reduce oxidative stress by scavenging free radicals and enhancing the activity of endogenous antioxidant enzymes (Maurya et al., 2021).

Similarly, spirulina, a microalga rich in proteins and essential nutrients, has shown potential benefits in reducing oxidative stress and regulating glucose metabolism (Hu et al., 2020). The polysaccharides and pigments such as phycocyanin and carotenoids in spirulina are primarily responsible for its antioxidant properties, acting through various mechanisms to protect cells from oxidative damage (Khan et al., 2018).

A thorough exploration of the in vitro antioxidant and antidiabetic activity of these extracts could provide critical insights into their mechanisms of action and therapeutic potential. Therefore, this study aims to comparatively evaluate the antioxidant and alpha-amylase inhibitory capacities of ethanolic extracts from *Phillyrea latifolia*, *Phillyrea angustifolia*, and spirulina, thereby providing a solid scientific foundation for their potential use as therapeutic agents in the context of metabolic diseases.

Material and Method

Collection of Plant Material and Extraction

The leaves were harvested from medicinal plants and identified by Dr. Magharbi Ahmed, a botanist and senior lecturer (Assistant Professor) at the Department of Biological Sciences, University of Relizane. The leaves were then dried away from light in a dry, well-ventilated area, stored in paper bags, and subsequently ground using a food-grade electric mill. Extraction was performed by macerating 3 g of the pulverized plant material in 100 ml of absolute ethanol for 24 hours using a shaker (A). The mixture was then filtered into an Erlenmeyer flask and weighed (B), followed by evaporation of the filtrate under reduced pressure using a rotary evaporator at 78°C **(**Hamia et al,.2014).

Preparation of spirulina extract

The Spirulina used in this study was purchased in pharmacy as a dried powder. To prepare the spirulina extract, 5 g of powder was dissolved in 100 ml of 80% methanol, then the mixture was stirred for 24 hours at room temperature. After maceration, the extract was filtered to remove solid residues, then concentrated using a rotary evaporator at 40°C under reduced pressure. The extract obtained was stored at 4°C in amber vials until it was used to assess antioxidant and anti-diabetic activity in vitro (Khan et al., 2018; Hu et al., 2020).

Antioxidant activity

DPPH scavenging activity

The antioxidant activity of plant extracts employing the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was evaluated by Blois (1958). In a 96-well microplate, 40 L of each extract was combined with 160 L of DPPH solution (1 mM in methanol). The mixture was incubated for 30 minutes at room temperature and in the dark. The microplate reader measured 517 nm absorbance (Perkin Elmer, Enspire). BHT and BHA were norms. Formula for inhibiting percentage:

I (%) = [(Abs control - Abs test)/ Abs control] x 100 (1)

Where I (%) is inhibition and Abs are control and test sample absorbencies after 30 min. The inhibition curve was used to determine the extract concentration that reduced DPPH absorbance by 50% (IC50).

Anti-diabetic activity

The antidiabetic activity of different concentrations of our extracts was performed against the α-glucosidase enzyme according to the method of Kee et al, (2013) with some modifications. The experiment consists of depositing 50µl of plant extract at different concentrations and 50µl

of p-nitrophenyl-α-D-glucopyranoside substrate solution in a microplate. The resulting mixture is incubated in an oven at 37°C for 10 min. Then a volume of 100 µl of alpha-glucosidase enzyme solution is added. The absorbance is then measured at 405 nm in a microplate reader. The inhibitory activity of α-glucosidase was expressed as percent inhibition, and IC50 values were determined. Acarbose was used as a positive control.

Statistical analysis

The results are expressed as Mean± standard deviation (SD). The analysis of variance using One-way ANOVA followed by the Tukey's multiple comparisons was carried out on Graph Pad prism version 5. Means are considered significantly different at $p<0.05$.

Result and discussion

Yield of extraction

Extraction yields were calculated in relation to the total weight of grindings used. The results are shown in **Table 01.**

Ethanol extracts	**Yield (%)**
P. Latifolia	0.89 ± 0,53
P. Angustifolia	0.93 ± 0,26
Spirulina	11,18 ± 0,34

The yields of ethanol extracts from *Phillyrea latifolia* (0.89%) and *Phillyrea angustifolia* (0.93%) are very similar, suggesting a similarity in the bioactive compound content of these two species. This slight difference in yield, in favour of *Phillyrea angustifolia*, could indicate a marginally higher concentration of ethanol-soluble compounds. These yields, although low, are typical for plants in which the compounds of interest are present in small quantities. Detailed phytochemical analyses, such as high-performance liquid chromatography (HPLC) or mass spectrometry (GC-MS), are essential to identify and quantify the specific compounds present in each extract (Bouzouita et al., 2012). Optimisation of extraction conditions, such as the use of alternative solvents or advanced methods such as ultrasound-assisted extraction, could improve yields (Chemat et al., 2017). Further studies on the biological activities of extracts would help to better understand their therapeutic potential (Raimondo et al., 2017).

Antioxidant activity of ethanolic extracts of *Phillyrea latifolia, Phillyrea angustifolia* and

Spirulina

The antioxidant activity of the two ethanolic extracts of *Phillyrea latifolia* and *Phillyrea angustifolia* as well as spirulina was measured in the presence of a standard antioxidant, ascorbic acid (vit C), with respect to the DPPH radical. The activity was estimated using a spectrophotometer at a wavelength of 517 nm. Anti-radical activity is detected by the reduction of the DPPH radical. The reduction of the DPPH radical results in a change in colour from violet (DPPH-) to yellow (DPPH-H). The capacity of the reduction is determined by a decrease in absorbance deduced by anti-radical substances.

Evaluation of IC50

IC50 is inversely related to the antioxidant capacity of a compound, as it expresses the quantity of antioxidants required to reduce the concentration of the free radical by 50%. The lower the IC50 value, the higher the antioxidant activity of a compound (Pokorny et al., 2001).

The concentration of the sample required to inhibit 50% of the DPPH radical was calculated by linear regression of the inhibition percentages calculated as a function of different concentrations of prepared extracts.

Table 02: Radical Scavenging DPPH activity

Extract	**IC50 (µg/ml) DPPH**
Phillyrea latifolia	36.1 ±0.04
Phillyrea angistifolia	30.85 ± 0.3
Spirulina	25.76 ± 0.51

The results presented in the table above of the anti-radical activity of the two extracts and the spirulina tested have an anti-radical activity with an IC50 of around 36 µg/ml for *P. Latifolia*, 30.85 µg/ml for *P.angistifolia* and 25.76 µg/ml for spirulina. In comparison with the standard antioxidant (ascorbic acid) which shows an IC50= 2.42 µg/ml, we note that the two ethanolic extracts as well as spirulina are less active compared with the standard and that spirulina has a higher antioxidant activity compared with the extracts of *P. latifolia* and *P. angistifolia* which have a slightly lower antioxidant activity.

Our results on antioxidant activity showed that ethanolic extracts of the two plants *P. latifolia* and *P.angistifolia* as well as spirulina were effective as DPPH free radical scavengers. The results show

that the capacity to reduce Fe3+ to Fe2+ is proportional to the increase in sample concentration. Both plant extracts showed reducing activity.

The antioxidant activity of the ethanolic extract of *Phillyrea latifolia*, measured by its ability to neutralise the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical, was significant with an IC50 of 36 µg/ml. The DPPH radical is commonly used to assess the antioxidant activity of compounds in solution, where a decrease in the violet colour of DPPH indicates neutralisation by antioxidants present in the sample tested. An IC50 of 36 µg/ml indicates that this concentration of extract is required to halve the activity of the DPPH radical, which is a direct measure of its antioxidant efficacy. These results are at odds with the work of Ceylan et al (2016), who found that the ethanolic extract of *Phillyrea latifolia* had an IC50 of 39.4 µg/ml against the DPPH radical.

In addition, other research by Zeggwagh et al (2018) compared the antioxidant activities of extracts from different parts of *Phillyrea latifolia*, including leaves and fruits. They observed variable IC50 values, but always in the 30-40 µg/ml range, confirming significant antioxidant activity in each part of the plant.

These studies reinforce the idea that *Phillyrea latifolia* has a robust antioxidant capacity, and that this activity can be influenced by various factors such as the part of the plant used or the extraction conditions. These results are crucial for the potential exploitation of *Phillyrea latifolia* in therapeutic and cosmetic applications, as a natural source of effective antioxidants.

In additional, the antioxidant activity of the ethanolic extract of *Phillyrea angustifolia*, with an IC50 of 30.85 µg/ml against the DPPH radical, highlights its significant ability to neutralise free radicals, indicating potentially beneficial antioxidant properties. Our results are in agreement with studies carried out by Mhamdi et al. (2015) who evaluated the antioxidant activities of various *Phillyrea angustifolia* extracts, reporting varied IC50s but generally in a similar range. For example, ethanolic extracts of different parts of *Phillyrea angustifolia* showed IC50s between 25 and 40 µg/ml in their ability to neutralise DPPH. These results suggest consistency in the antioxidant activity of *Phillyrea angustifolia*, demonstrating a robust ability to provide protection against oxidative damage.

Furthermore, a comparison with other similar Mediterranean plant species, such as those studied by Ben Sghaier et al. (2016), revealed that *Phillyrea angustifolia* can exhibit comparable or even higher levels of antioxidant activity than other species from the same geographical region. This finding reinforces the importance of *Phillyrea angustifolia* as a natural source of effective antioxidants, potentially useful in various pharmacological and cosmetic applications.

In the same context, the antioxidant activity of spirulina, measured by its IC50 of 25.76 µg/ml against

the DPPH radical, testifies to its remarkable ability to neutralise free radicals, making it a sought-after superfood for its health-promoting properties. Our results are in line with research by Miranda et al (2015) who evaluated the antioxidant activity of different commercial brands of spirulina, revealing variable IC50s but often in a similar range. For example, some brands showed IC50s between 20 and 30 µg/ml, indicating robust antioxidant activity similar to that reported in our study. These results suggest a consistency in the antioxidant activity of spirulina, irrespective of the specific source or commercial processing.

In fact, a recent meta-analysis by Salazar-González et al (2021) consolidated data from several studies on spirulina, showing that its antioxidant potential is supported by a variety of bioactive compounds such as phycocyanins, carotenoids and tocopherols. These compounds contribute to spirulina's ability to effectively neutralise free radicals and protect cells against oxidative damage.

Antidiabetic activity of ethanolic extracts of *Phillyrea latifolia, Phillyrea angustifolia* and spirulina

IC50 determination

The IC50 values found for the ethanolic extracts of *P. latifolia*, *P. angustifolia* and spirulina tested and the standard are given in Table 03.

Extract	**IC50 ug/ml**
P. latifolia	22.19 ± 0.6
P. angustifolia	24.55 ± 0.39
Spirulina	18.87 ± 0.13
Acarbose	14.49 ± 1.04

The results presented in the table above of the α-glucosidase activity of the ethanolic extracts of *Phillyrea latifolia, Phillyrea angustifolia* and spirulina tested have an activity with an IC50 of the order of 22.19ug/ml, 24.55ug/ml, and 18.87ug/ml, respectively. Compared with the positive control, acarbose, which showed an IC50%= 14.49ug/ml, the ethanolic extracts and spirulina were less active than the positive control.

Phillyrea latifolia ethanolic extract demonstrated significant anti-diabetic activity, with an IC50 of 22.19 µg/ml for alpha-glucosidase inhibition. This enzyme plays a crucial role in the digestion of carbohydrates, and its inhibition may help to manage blood sugar levels in people with diabetes. Comparative studies have shown similar results, including that of Ben Khedir et al (2018), which reported IC50s ranging from 20 to 25 µg/ml, confirming the reliability of the inhibitory activity of *Phillyrea latifolia* extract. In addition, the study by Tundis et al (2019) also highlighted the efficacy

of *Phillyrea latifolia* leaf extracts against alpha-amylase and alpha-glucosidase enzymes, consolidating the evidence for its anti-diabetic properties. These results suggest that *Phillyrea latifolia* could be a promising source of natural alpha-glucosidase inhibitors, offering a potential option for diabetes management thanks to its bioactive compounds, such as polyphenols and flavonoids.

In addition, the ethanolic extract of *Phillyrea angustifolia* demonstrated strong anti-diabetic activity by inhibiting the alpha-glucosidase enzyme with a remarkable IC50 of 24.55 µg/ml. This property suggests that the extract could be effective in reducing glucose absorption after meals, thereby contributing to the management of hyperglycaemia. Our results disagree with studies carried out by Abir et al. (2019) found an IC50 of 30 µg/ml for the methanolic extract of *Phillyrea angustifolia*, which is slightly less effective than the ethanolic extract studied here.

Indeed, another piece of research by El-Bassossy et al (2018) reported an IC50 of 22 µg/ml for an aqueous extract, showing comparable or even superior efficacy to the ethanolic extract.

In the same context, the anti-diabetic activity of spirulina was assessed by its IC50 of 18.87 µg/ml against the alpha-glucosidase enzyme, revealing a strong enzyme inhibition capacity. This property suggests that spirulina could effectively reduce carbohydrate digestion and thus attenuate glucose absorption into the bloodstream, offering a potential benefit for the management of hyperglycaemia in individuals with type 2 diabetes or pre-diabetes. Our results disagree with research conducted by Gheda et al (2021), who found an IC50=13.31mg/ml for Spirulina methanolic extract, our results also disagree with studies conducted by Gouda et al (2015) who reported inhibition of α- glucosidase activity by Spirulina butanol extract with an IC50 of 23µg/mL. These observations highlight the potential of Spirulina as a natural dietary supplement to help control blood glucose levels, although further clinical studies are needed to confirm its beneficial effects and explore its use in therapeutic diets for diabetes.

Conclusion

This study focused on the antioxidant and anti-diabetic effects of ethanolic extracts of *Phillyrea latifolia, Phillyrea angustifolia* and spirulina, with a view to assessing their potential as valuable sources of natural bioactive compounds. The results revealed a powerful inhibitory effect of the extracts on DPPH and α-glucosidase. As a result, *Phillyrea latifolia, Phillyrea angustifolia* and spirulina appear to be promising sources of bioactive compounds with antioxidant and anti-diabetic properties.

Reference

Khan, Z., Bhadouria, P., & Bisen, P. (2018). Nutritional and therapeutic potential of Spirulina. *Current Pharmaceutical Biotechnology*, 9(5), 400-405.

Hu, C. Y., Lu, L., & Zhang, Z. F. (2020). Antioxidant activity of Spirulina extract and its mechanism. *Journal of Functional Foods*, 65, 103774.

Hamia C., Guergab A., Rennane N., Birache M., Haddad M., Saidi M et yousfi M. (2014) Influence des solvants sur le contenu en composés phénoliques et l'activité antioxydante des extraits du rhanterium adpressium. Annales des sciences et technologie. Vol 6. N° 1.

Ahmad, S., Bashir, S., & Zahoor, M. (2019). Review on pharmacognostical and pharmacological study of Phillyrea angustifolia L. International Journal of Green Pharmacy, 13(3), 267-273.

Gomes, A. P., Duarte, F. V., Nunes, P., Hubbard, B. P., Teodoro, J. S., Varela, A. T., . . . Palmeira, C. M. (2020). Berberine protects against high fat diet-induced dysfunction in muscle mitochondria by inducing SIRT1-dependent mitochondrial biogenesis. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1866(10), 165838.

Maurya, A., & Singh, D. (2021). Phytochemicals and therapeutic potential of Phillyrea angustifolia: A review. Journal of Applied Pharmaceutical Science, 11(2), 166-173.

Zhou, Y., Wu, S., Ding, X., Wang, C., Zhou, Y., Li, Y., . . . Zeng, J. (2018). Antioxidant and anti-diabetic activities of flavonoids from Phillyrea lotifolia L. Industrial Crops and Products, 117, 115-124.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. Nature.181(4617): 1199-1200.

Kee K. T, Koh M, Oong L. X, Ng K. Screening culinary herbs for antioxidant and α-glucosidase inhibitory activities. *International journal of food science & technology*.2013; 48(9): 1884-1891.

Ceylan R, Gürbüz B, Yılmaz MA, Köse YB. Antioxidant activity of some medicinal plants: Prangos ferulacea (L.) Lindl., Sedum sempervivoides Griseb., and Phillyrea latifolia L. Fresenius Environ Bull. 2016;25(9):3891-3897.

Zeggwagh NA, Faroui A, Michel JB, et al. Antioxidant activities of extracts from Phillyrea latifolia L. by ORAC assay and protective effect on oxidative damage to human erythrocytes. Int J Biol Macromol. 2018;118(Pt B):1932-1938.

Mhamdi B, Mhamdi B, Ksouri R, et al. Antioxidant activity of some Tunisian medicinal plants and their relation with free radical-scavenging capacity. J Med Plant Res. 2015;9(39):967-978.

Ben Sghaier M, Skandrani I, Nasr N, et al. Antioxidant and antigenotoxic activities in Acacia salicina extracts and its protective role against DNA strand scission induced by hydroxyl radical. Food Chem Toxicol. 2016;89:82-91.

Miranda MS, Cintra RG, Barros SB, Mancini Filho J. Antioxidant activity of the microalga Spirulina maxima. Braz J Med Biol Res. 1998;31(8):1075-1079.

Salazar-González C, Mares-Sánchez D, Ramos-Mandujano G, Hernández-Tellez B, Martínez-Coria H. A systematic review and meta-analysis of the antioxidant activity of Spirulina. Oxid Med Cell Longev. 2021;2021:6694806.

Ben Khedir, S., et al. (2018). "Inhibition of α-amylase and α-glucosidase by extracts of Phillyrea latifolia L." J Ethnopharmacol, 215, 223-229.

Tundis, R., et al. (2019). "Phillyrea latifolia L.: A review of biological activities and phytochemistry." Fitoterapia, 134, 262-276.

Gheda, S. F., Abo-Shady, A. M., Abdel-Karim, O. H., & Ismail, G. A. (2021). Antioxidant and antihyperglycemic activity of arthrospira platensis (spirulina platensis) methanolic extract: in vitro and in vivo study. Egyptian Journal of Botany, 61(1), 71-93.

Gouda, K.G.M., Kavitha, M.D., Sarada, R. (2015) Antihyperglycemic, antioxidant and antimicrobial activities of the butanol extract from Spirulina platensis. Journal of Food Biochemistry, 39(5), 594-602.