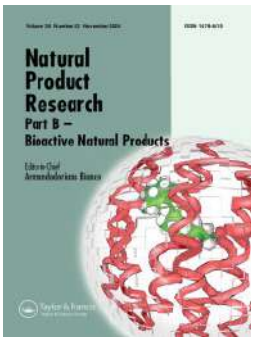

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Assessing Algerian *Ilex aquifolium* L.'s phenolic compounds, antioxidant and enzyme inhibitory properties to find a new natural remedy for Alzheimer's, diabetes and skin diseases

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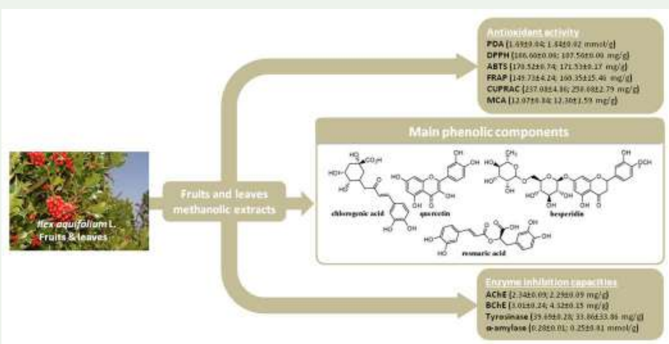
ABSTRACT

This research explored the impact of methanolic extracts from the fruits and leaves of *Ilex aquifolium* L., a rare Algerian species, on several enzymes, focusing on tyrosinase, α-amylase, α-glucosidase, and cholinesterases, as well as their antioxidant potential *in vitro* assays, including phosphomolybdenum, DPPH, ABTS, CUPRAC, FRAP, and MCA. HPLC-DAD analysis identified chlorogenic acid, rosmarinic acid, hesperidin, and quercetin as predominant phenolic components. Both extracts exhibited notable antioxidant properties, up to 250.08 mg TE/g for fruits and 237.08 mg TE/g for leaf extracts. Enzyme inhibitory assays demonstrated significant inhibition against AChE and BChE with maximum activities of 2.34 mg GALAE/g in leaves and 4.32 mg GALAE/g in fruits. Additionally, considerable tyrosinase inhibition was observed in leaves at 39.69 mg KAE/g. These findings highlight *I. aquifolium* L. as a valuable source for investigating natural antioxidants and enzyme inhibitors, particularly for possible cosmetics and food additives applications.



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1. Introduction

Natural antioxidants, such as polyphenols, flavonoids, and related compounds, are crucial in mitigating oxidative damage caused by reactive oxygen and nitrogen species in dietary and biological systems (Krimat et al. 2014). Alzheimer's disease is a neurological condition that impairs cognition and causes memory loss (Erdogan et al. 2010). Research has suggested that phenolic compounds possess neuroprotective effects and may halt the progression of Alzheimer's disease. Their antioxidant properties are believed to reduce oxidative stress, which is implicated in the development and progression of this condition (Syed 2022; Alejandro et al. 2023). In diabetes management, phenolic compounds have garnered interest due to their ability to modulate key enzymes involved in glucose metabolism, thereby helping to manage blood sugar levels and related complications (Himangshu et al. 2022). Furthermore, these compounds show promise in addressing skin disorders, like dermatitis and aging-related changes, by potentially reducing oxidative stress and inflammation, paving the way for the development of natural skincare products (Nasser 2023).

The genus *Ilex* (Aquifoliaceae), commonly known as 'English holly' or 'holly', is an ornamental tree for Christmas celebrations. It is widespread in temperate and tropical regions and comprises about 600 species (Dacheng et al. 2013). In Algeria, the genus encompasses one species known as *Ilex aquifolium* L., locally called 'Tguersalt' or 'Kerrouch attani', synonymous with *I. aquifolium* var. *barcinonae* Pau. This species, a 2–10 metres tall shrub or tree, has green bark, leathery leaves, clustered white or pinkish flowers, and red fleshy fruits with 4–5 triangular stones (Quezel and Santa 1963). Traditionally, infusion from various plant organs (leaves, flowers, and roots) has been employed to treat illnesses such as liver, gastrointestinal tumours, malaria, and inflammation. *I. aquifolium* is mainly used in the United Kingdom, Europe, and temperate regions of Asia and Africa (Nahar et al. 2005; Nurgun et al. 2009; Sudhir et al. 2012). The presence of terpenoids, saponins, and polyphenols (especially flavonoids) is a distinctive feature of *Ilex* species (Sun et al. 2000). Nevertheless, many plants within this genus have yet to be thoroughly investigated chemically or scientifically for their respective activities. Thus, comprehensive research is needed to explore such constituents' biological activity and mechanisms of action.

However, as far as we know, neither the phytochemical investigations nor the biological properties of the Algerian species *I. aquifolium* L. have been conducted. Pursuing our studies on the Algerian flora, the current investigation reports the phenolic compositions, antioxidant potential, and enzyme inhibition effects of *I. aquifolium* fruits and leaves connected with Alzheimer's disease, diabetes, and skin disorders.

2. Results and discussion

The methanolic extraction of 15 g of *I. aquifolium* L. leaves and fruits yielded 3.25 and 1.04 g, respectively, and was subjected to antioxidant activity and enzyme inhibition investigations. Antioxidant activity was examined using electron transfer assays like ABTS/TEAC, CUPRAC, DPPH, Folin-Ciocalteu, and FRAP. These methods involve chromogenic redox reagents to measure the reduction ability of antioxidants (Apak et al. 2007).

2.1. Total bioactive components

Plant polyphenols are highly regarded for their potent antioxidant properties and ability to prevent various disorders associated with oxidative damage, including cancer (Dai and Mumper 2010).

The total phenolic content (TPC) and the total flavonoid content (TFC) in both extracts were determined, and the results are shown in Table S1. The fruits and the leaves contained comparable amounts of total phenols (44.95 mg GAE/g of extract and 41.16 mg GAE/g of extract, respectively). The fruit extract showed a minimum amount of flavonoids (7.03 mg RE/g of extract), whereas the leaf extract contained 26.51 mg RE/g of extract.

In a previous research conducted by Zwyrzykowska et al. (2015), the aqueous methanol extract derived from the leaves of the same species was determined to include a total of 27,169 mg GAE/g of phenolic compounds. The investigation carried out by Berté et al. (2011) on *I. paraguariensis* species revealed that the spray-dried extract contained 178.32 mg GAE/g of extract, while the dehydrated leaves contained 96.16 mg GAE/g of extract. Another study (Bassani et al. 2014) performed on *I. paraguariensis* A. announced that the total polyphenols of aqueous extract changed from 349.92 to 428.31 mg GAE/L, and the total flavonoid content shifted from 268.06 to 421.75 mg CTE/L.

The total content of bioactive components can be changed within samples taken from the same plant, influenced by factors such as the season of the year when the plants are collected and the organs of the plant being used. Moreover, variation in the extraction techniques makes evaluations more complicated, as well as the solvents used and how results are reported (Zengin et al. 2020). Therefore, it is impossible to reasonably compare the mentioned studies due to the variation in solvents, employed plant organs, and the methods used for plant extraction. Also, the variations in value might be attributed to botanical and geographical provenance.

2.2. Phenolic compounds

It is worth noting that HPLC is presently regarded as the dominant and most efficient method for analysing phenolic compounds (Dai and Mumper 2010). The phenolic components of the two methanolic extracts under investigation were determined using the HPLC-DAD method. This involved comparing the different components' retention times and UV spectra with established standards under identical chromatographic conditions. Sixteen compounds were used as standards, and ten were detected in at least one studied sample. The structures of the principal components are shown in Figure S1. As seen in Table S2, the dominant compounds for the leaves' MeOH extract were chlorogenic acid (726.47 mg/kg extract) followed by rosmarinic acid (278.17 mg/kg extract) (cafeic acid esters) and hesperidin (74.76 mg/kg extract) (flavonoid), while fruits extract was rich in chlorogenic acid (887.89 mg/kg extract), rosmarinic acid (78.44 mg/kg extract) and hesperidin (18.96 mg/kg extract). Meanwhile, neither rosmarinic acid nor hesperidin was detected in the same species analysed by Zwyrzykowska et al. (2015), who found rutin to be a major component besides chlorogenic acid. It has been stated that chlorogenic acid, which was stated with the greatest rate, has beneficial effects on oxidative stress, cancer, cardiovascular disorders, diabetes, and hepatoprotective features (Maalik et al. 2016).

The current study's findings align with those of Paluch et al. (2021), who detected elevated amounts in water extracts of leaves from (*I. aquifolium* L., *I. aquifolium Argentea Marginata,* and *I. paraguariensis*). This further illustrates the impact of solvent choice, the extraction method utilised, and the variation in the geographical environments and collecting period on the phenolic content levels obtained.

2.3. Antioxidant capacity

Epidemiological investigations have established a relationship between high polyphenol consumption and a lower risk of many disorders, including cardiovascular disease and certain types of cancer (Hu 2011). Here, we conducted diverse assays to determine the antioxidant properties of *I. aquifolium* L.'s methanolic extracts.

2.3.1. Total antioxidant capacity (TAC)

The phosphomolybdenum assay (PDA) is a widely employed technique for estimating antioxidant capacity using inexpensive and efficient reagents. This capacity indicates the extract's potential health benefits and effectiveness in preventing oxidative damage (Prieto et al. 1999). The results of the Phosphomolybdenum assays are presented in Table S3. Consequently, the antioxidant capacities of the methanolic samples exhibited a high degree of similarity, with 1.84 mmol TE/g for *I. aquifolium* fruits and 1.69 mmol TE/g for *I. aquifolium* leaves. The absorbance value of Trolox in the assay was 0.63 at a concentration of 1 mg/mL.

2.3.2. Radical scavenging potentials (DPPH and ABTS assay)

The radical scavenging potential is another important indicator of antioxidant activity. It indicates the ability of the extracts to neutralise free radicals, which are unstable molecules that can cause cellular damage (Pisoschi and Negulescu 2012). The findings of the radical scavenging activity are given in Table S3. As shown in Table S3, both leaves and fruit extracts have a remarkable DPPH radical scavenging activity (106.66 mg TE/g) and (107.56 mg TE/g), respectively. Considering the ABTS radical scavenging assay, the capacity of leaf extract (170.52 mg TE/g) is similar to that of fruit extract (171.53 mg TE/g). At a concentration of 0.1 mg/mL, Trolox's inhibition values in DPPH and ABTS tests were 42.57% and 28.10%, respectively. In another study, Bassani et al. (2014) established that the H_2O extract of *I. paraguariensis* presented a high DPPH radical scavenging activity (80.02%). Besides, Berté et al. (2011) found that the IC_{50} is 2.52 mg/mL using DPPH radical scavenging to study the antioxidant capacity of *I. paraguariensis* species. In addition, researchers have studied the antioxidant effect of *I. paraguariensis* species and reported that the IC_{50} value was 27.3 µg/mL using the DPPH assay (Milioli et al. 2007).

2.3.3. Reducing antioxidant capacities (FRAP and CUPRAC)

FRAP and CUPRAC are valuable procedures employed to check the reduction power activity of *I. aquifolium* species, and the results are given in Table S3. The decreasing power activity of the methanol extract of *I. aquifolium* fruits (FRAP: 160.35 and CUPRAC: 250.08 mg TE/g) was higher than the results acquired for *I. aquifolium* leaves (FRAP: 149.73 and CUPRAC: 237.08 mg TE/g). The absorbance values of Trolox in CUPRAC and FRAP assays were 0.36 and 0.66, respectively, at a concentration of 0.1 mg/mL.

2.3.4. Metal chelating activity (MCA)

The extracts' chelating properties were assessed using EDTA as a reference standard (mg EDTAE/g extract). Based on the reported results in Table S3, the metal chelating activity of *I. aquifolium* leaves (12.07 mg EDTAE/g extract) is very close to that of *I. aquifolium* fruit extract (12.30 mg EDTAE/g extract). The inhibition value of EDTA in the metal chelating assay was 59.87% at a concentration of 40 µg/mL.

2.4. Enzyme inhibitory activity

The study of enzyme inhibition is of significant importance in pharmacological research, specifically in addressing the prevalence of world health issues such as Alzheimer's disease and diabetes mellitus. The key enzyme inhibitory theory is a well established method that uses natural products to prevent the harmful effects of synthetic inhibitors and decrease the occurrence of these significant health problems (Waltenberger et al. 2016).

Within this context, the impact of extracts derived from the Algerian species *I. aquifolium* on inhibiting cholinesterases (AChE and BChE), tyrosinase, α-amylase, and α-glucosidase was examined. Table S4 presents the results. These activities were presented in the equivalent of standard inhibitors: galanthamine for AChE and BChE, kojic acid for tyrosinase, and acarbose for α-amylase and α-glucosidase. The methanol extract of the leaves revealed significant inhibitory effects on cholinesterases (AChE: 2.34 and BChE: 3.01 mg GALAE/g), with slight variations in comparison with the fruit extract (AChE: 2.29 and BChE: 4.32 mg GALAE/g). Several investigations have mentioned that terpenoids and phenolics possess considerable cholinesterase inhibitory effects (Orhan et al. 2007; Stasiuk et al. 2008). Accordingly, *Ilex* species could be regarded as potential inhibitors of AChE and BChE. The inhibition values of galanthamine in AChE and BChE tests were 93.86% and 43.28%, respectively, at a concentration of 5 µg/mL.

Moreover, exploring the studied species' anti-diabetic activity involved assessing their ability to inhibit α-amylase and α-glucosidase (Table S4). In α-amylase inhibitory effect, leaves and fruits demonstrated notable inhibitory effects with values of 0.28 and 0.25 mmol ACAE/g, respectively. However, both of them were not active against α-glucosidase. The inhibition values of acarbose in amylase and glucosidase tests were 62.86% and 29.60%, respectively, at 1 mg/mL concentrations. According to Custódio et al. (2015) and Unuofin et al. (2019), a significant correlation has been observed between elevated phenolic levels and increased inhibitory activity of α-glucosidase in plant extracts. The study by Unuofin and Lebelo (2021) provides more evidence supporting the modulatory impact of phenolic compounds on glucose metabolism and their significance in treating type 2 diabetes mellitus (T2DM). Nevertheless, it's noteworthy to highlight that information on inhibiting these enzymes by species within the genus *Ilex* is very scarce.

Also, it is reported that triterpenoids and phenolics exhibited anti-diabetic activities (Etxeberria et al. 2012). Hence, the relationship between the existence of these constituents in *Ilex* species and their detected anti-diabetic effect may be established.

The effectiveness of an extract depends on the solvent used, and this was confirmed by the findings of Zengin et al. (2014), who declare that petroleum ether and ethyl acetate extracts of *Sideritis galactic* showed considerable inhibition of α-glucosidase and α-amylase compared to the methanol and water extracts.

Additionally, The methanol extract of the fruits (33.86 mg KAE/g) displayed a lower level of tyrosinase inhibitory effects compared to the leaves extract (39.69 mg KAE/g). The inhibition value of kojic acid in the assay was 94.96% at a concentration of 1 mM. The samples' potent inhibitory actions were probably attributed to their high phenolic content, particularly ferulic acid. Several studies have shown the potential protective effects of various phenolic compounds, such as ferulic acid and catechin, against the mentioned public health issues (Higdon and Frei 2003; Mamiya et al. 2008). Meanwhile, various studies have found no association between the phenolic content and enzyme inhibitory activities. This indicates that phytochemicals have complicated interactions and may exhibit synergistic or antagonistic effects (Dalar and Konczak 2013).

3. Experimental

See Supplementary Material.

4. Conclusion

In conclusion, our research demonstrated that the species had remarkable chemical profiles and important biological effects. This work is the first to investigate the Algerian species *I. aquifolium* L. phenolic profile and related biological activities. Our findings suggest that *I. aquifolium* could be a valuable resource for developing novel phytopharmaceuticals or nutraceuticals, and this work could serve as a foundation for further research on this species.

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