



Phytochemical analysis, antioxidant and anti-*Candida albicans* activities of *Annona cherimola* Mill. fruit pulp

Bachir BENARBA ^{1,*}, Okba Ibnou Nafaa MENDAS ², and Setti RIGHI ²

¹ Research Laboratory on Biological Systems and Geomatics, Faculty of Nature and Life Science, University of Mascara, Algeria ² Department of biology, Faculty of Nature and Life Science, University of Mascara, Algeria.

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ABSTRACT

Aims: The main objectives of the present study were to characterize the phytochemical profile of *Annona cherimola* Mill, to assess its antioxidant characteristics and its antifungal activity against *Candida albicans*. **Methods and Material:** Aqueous decoction, aqueous infusion, aqueous maceration, and methanolic maceration were screened for the presence of phytochemicals and to quantify content of phenolics, tannins, and flavonoids. Furthermore, antioxidant activity was assessed using DPPH and FRAP assays, as well the assessment of antifungal activity for the different extracts. **Results:** Results showed that phenols, tannins, flavonoids, and saponins were present in the four extracts. The aqueous maceration extract presented the highest total phenolic content (3.687 mg GAE/g of extract). Decoction extract showed the lowest phenolic content 2.504 mg GAE/g. Besides, infusion showed the most important total flavonoids content (2.567mg CE/g). The most relevant total antioxidant activity was found for decoction (lowest IC₅₀ 12.61 mg/ml AAE). The aqueous maceration exhibited the less antioxidant activity (IC₅₀= 21.98 mg/ml AAE). The best scavenging activity was observed for decoction (IC₅₀=7.27 mg/mL). All the extracts showed a reducing capacity. Infusion exhibited the best reducing power (EC₅₀ = 11.29 mg/mL), compared to decoction (EC₅₀ = 39.32 mg/mL). Regarding antifungal activity, at 100 and 200 µg/mL, decoction and methanolic maceration resulted in 6 and 9 mm inhibition zone, respectively. In addition, at higher doses (800 and 2000 µg/mL), inhibition zone increased in a dose dependent manner for all the extracts. **Conclusions:** *Annona cherimola* Mill. could be an important source of bioactive molecules with antioxidant and antifungal activities.

* Corresponding author  Tel: +213 659319213

 bachirsb@yahoo.fr

1 INTRODUCTION

Annona cherimola Mill. (cherimoya) is a subtropical fruit tree that originated from frost-free valleys of the Andes at an altitude between 700 and 2400 m [1]. The species represents one of the most exploited and most important commercially from the Annonaceae species [2]. *A. cherimola* is utilized in the treatment and management of several diseases such as gastrointestinal disorders and ulcers [3], diabetes [4], nervous disorders [5], and even cancer [6].

The *A. cherimola*'s fruit pulp is a typical sweet fruit, fleshy and juicy, providing several important nutrients such as vitamins C and B (B1, B2, B3 and B6), iron, phosphorus, and calcium [7]. Recently, Albuquerque *et al.* [8] have analyzed the fruits pulp of 04 varieties and found that its energy content varied between 81 kcal and 102 kcal per 100 g of edible portion. In addition, the fruit is rich in water and has low total proteins and fats. Hence, the fruit pulp is

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considered by a lower energy and higher content regarding active molecules.

Several biological activities were attributed to *A. cherimola*. Falé *et al.* [9] demonstrated that decoctions of *A. cherimola* leaves inhibited the cholesterol biosynthesis through targeting the HMG-CoA reductase, and reduced the cholesterol absorption. An alkaloid extract (major compounds: norruciferine, anonaine, 1,2-dimethoxy-5,6,6a,7-tetrahydro-4H-dibenzoquinoline-3,8,9,10-tetraol, and, liriodenine.) of *A. cherimola* resulted in antidepressant-like effects in mice. Moreover, it enhanced the effectiveness of two antidepressant drugs: imipramine and clomipramine [10]. Furthermore, it has been demonstrated that *A. cherimola* exhibited a significant antiprotozoal activity ($IC_{50} < 30\text{g/ml}$) against *Entamoeba histolytica*, justifying its ethnomedicinal use in the treatment of gastrointestinal disorders [11]. Similarly, *A. cherimola* showed an interesting anti-*Helicobacter pylori* activity with a minimal inhibitory concentration $<15.6\ \mu\text{g/ml}$ [12]. The fruit was active against several species of enteropathogens too, such as *Escherichia coli*, *Salmonella* sp, or *Shigella* sp [13].

It has been reported that *A. cherimola* contains different phytochemicals such as flavonoids, tannins, phenolics, alkaloids, saponins and phytosterols [14]. Methyl butanoate, butyl butanoate, 3-methylbutyl butanoate, 3-methyl butyl 3- methyl butanoate, and 5-hydroxymethyl-2- furfural, were the predominant molecules in essential oils extracted from the bark [15]. The fruit contains Kaur-16-en-19-oic acid, as a major lipophilic compound besides fatty acids and sterols. Furthermore, the fruit is rich in flavan-3-ols: catechin, (epi)catechin- (epi)gallocatechin, (epi)gallocatechin, (epi) afzelechin-(epi) catechin, and procyanidin tetramer [16]. Although leaves are not edible, they are used in phytotherapy. Several bioactive alkaloids have been identified in this part of the plant such as two aporphine (anonaine and asimilobine), three oxoaporphine (lanuginosine, liriodenine and lysicamine) and two proaporphine (pronuciferine and stepharine) [17]. In another study, rutin was found to be the major compound isolated from decoctions of *A. cherimola* leaves' decoction, in addition to chlorogenic acid, rutin xyloside and kaempferol 3-O-rutinoside [9].

Few studies have evaluated the antioxidant activities of *A. cherimola* [18, 19]. To the best of our knowledge, very few studies have been carried out to measure the antioxidant activities of *A. cheriloma* fruit.

The present study aimed to characterize phytochemically the edible parts (fruits) of *A. cherimola* Mill. In addition, we assessed the antioxidant activities using different methods such as free radical scavenging and ferric reducing

antioxidant power. Besides, the antifungal activity of different extracts of *A. cherimola* has been investigated.

2 MATERIAL AND METHODS

2.1 Plant material

Commercial fruits of *A. cherimola* (from Ecuador) were bought from a market in France. Four fresh fruits at full ripeness, 7–11 cm diameter, 10–18 cm height, were peeled and seeds were removed from the pulp. Fruit pulp (1.2 kg) was cut into small pieces (about $1\ \text{cm}^3$) for further experiments. All the experiments were carried out in Algeria.

2.2 Extraction

The methanolic extract was obtained by soaking fruit pulp (200 g) with 400 ml of methanol for 24 hours. The mixture was then filtered through Whatman filter paper and the filtrates obtained were dried at $45\ ^\circ\text{C}$ for 72 h. To obtain the aqueous maceration extract, 200 g of the fruit pulp were macerated in 400 ml of distilled water for 24 hours. The extract was filtered and dried in an oven at $45\ ^\circ\text{C}$ for 72 h. Decoction extract was prepared by boiling 200 gram of the fruit pulp in 800ml of distilled water. Likewise, 200 gram of fruit pulp were put into a Beaker, containing 800 ml boiling distilled water, with continuous stirring for 30 min to obtain the infusion extract.

The extraction yield, expressed in % i.e. g of extract/100 g of dried material, was calculated using formula (1):

$$\text{Yield (w/w \%)} = (\text{Weight of dried crude extract/Weight of pulp used}) \times 100 \dots\dots\dots (1)$$

2.3 Phytochemical screening

The phytochemical screening of the extracts of *A. cherimola* pulp was performed using standard procedures described by Trease and Evans [20].

2.4 Determination of Total Phenolic Content

Total phenolic content was obtained using the Folin–Ciocalteu's reagent as described by Benarba and Meddah [21]. Absorbance was measured at 760 nm using UV spectrophotometer (Shimadzu, Japan). Gallic acid was used to generate the calibration curve, and the total phenolic content was expressed as mg gallic acid equivalent (GAE)/g dry weight of the extract. Values were determined in triplicate.

2.5 Determination of total flavonoid Content

The total flavonoid content of *A. cherimola* extracts was determined by the aluminum chloride colorimetric method as previously described by Zhishen *et al.* [22]. Catechin served as a positive control, and absorbance was

read at 510 nm. The total flavonoid content was expressed as mg catechin equivalents (CE)/g dry weight of extract. Values were determined in triplicate.

2.6 Determination of condensed tannin content

The condensed tannin content of *A. cherimola* extracts was determined using the vanillin-HCl method modified by Lin and Tan [23]. Samples were mixed with 5 mL vanillin-HCl (8% conc. aq. HCl and 4% vanillin in methanol). The calibration curve was obtained using standard solution of catechin in the concentration range 05-40 µg/ml, and the condensed tannin content was expressed as mg catechin equivalents (CE)/g dry weight of extract. Values were determined in triplicate.

2.7 Antioxidant activity

2.7.1 Evaluation of total antioxidant capacity (TAC)

The phosphomolybdenum assay was used to evaluate the total antioxidant capacity as described by Grochowski *et al.* [24]. 0.3 mL of each extract was added to 3 mL of the molybdate reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). A calibration curve was prepared for a methanolic solution of ascorbic acid (1000, 500, 250, 125, 62.5 and 31.25 µg/ml). The absorbance was determined at 695 nm and the total antioxidant capacity was expressed as mg equivalent of ascorbic acid.

2.7.2 Free radical scavenging of DPPH radical

The free radical scavenging activity of *A. cherimola* extracts was determined, using DPPH. 1.5mL of methanolic solution of DPPH was added to 750µL of each extract (at different concentrations). After 30 minutes, in dark, absorbance was measured at 517nm against a blank (the reaction mixture without DPPH). The inhibition percentage for scavenging DPPH radical was calculated according to formula (2):

$$[(A1 - A2) / A1] \times 100 \dots\dots (2)$$

A1: the absorbance of the reaction mixture without extract;
A2: the absorbance of the reaction mixture with sample.

2.7.3 Ferric ion reducing antioxidant power assay (FRAP)

The ferric ion, reducing antioxidant power, was evaluated as described by Oyaizu modified by Baghiani *et al.* [25]. 2.5 ml of each extract, at various concentrations, were mixed with 2.5 ml of 200 mmol/l sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. After incubation at 50°C for 20 min, 2.5 mL of 10% trichloroacetic acid (w/v) were added and the mixture was then centrifuged at 650 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml deionized water and 1 ml of 0.1%

of ferric chloride. The absorbance was measured at 700 nm.

2.8 Antifungal activity

The antifungal activity of the extracts was carried out based on the disc diffusion method against *Candida albicans* that was obtained and identified from the hospital of Mascara city (West Algeria). *C. albicans'* cells were obtained from a 24 h culture on YPG broth. Inoculum suspension of 10³ CFU/mL was prepared in sterile distilled water from 24 h-old culture grown on YPG broth. Sabouraud's dextrose agar plates were inoculated with prepared suspension. Sterile blank discs of 6 mm diameter were impregnated with different concentrations of the extracts (100, 200, 400, 800, and 2000 µg/mL) and placed on the plates. The diameters of growth inhibition zones were measured after 5 days incubation at 35°C.

3 RESULTS

3.1 Extract yields

The obtained results showed that the extraction yield of infusion, decoction, methanolic maceration, and aqueous maceration of *A. cherimola* fruit pulp was found to be 10.5%, 5%, 7.5% and 7%, respectively. The results revealed that extraction by infusion was more effective compared to decoction, methanolic maceration, and aqueous maceration.

3.2 Phytochemical Screening

Table 1 shows that phenols, tannins, flavonoids, and saponins were present in all extracts. However, gallic tannins were solely present in the infusion and decoction extracts and were not detected in both maceration extracts. Catechic tannins, anthraquinones, steroids, anthocyanins, and betacyanins were not detected in all extracts of *A. cherimola* pulp.

Table 1: Phytochemical screening of *A. cherimola* pulp extracts

Phytochemicals	Infusion	Decoction	A.M	M.M
Phenols and tannins	++	++	++	++
Gallic tannins	+++	++	-	-
Catechic tannins	-	-	-	-
Flavonoids	++	+++	+	+++
Saponins	+	+	+	+
Anthraquinone	-	-	-	-
Steroids	-	-	-	-
Anthocyanin and Betacyanin	-	-	-	-

A.M: Aqueous maceration; M.M: Methanolic maceration; (+): presence (-): absence

3.3 Total phenolic, flavonoid and condensed tannin contents

As shown in table 2, the highest total phenolic content was found in the aqueous maceration extract. Decoction extract showed the lowest phenolic content. The total phenolic content was 2.85 ± 0.50 mg GAE/g and 2.83 ± 0.72 mg GAE/g in infusion and methanolic maceration, respectively.

Table 2: Total phenolic, flavonoid and condensed tannin contents in *A. cherimola* fruit extracts

Extract	Total phenolic content ^a	Total flavonoid content ^b	Total condensed tannin ^b
Infusion	2.85 ± 0.50	2.56 ± 0.11	6.62 ± 0.35
Decoction	2.50 ± 0.23	0.84 ± 0.06	1.80 ± 0.05
M.M.	2.83 ± 0.72	0.61 ± 0.09	1.44 ± 0.05
A.M.	3.68 ± 0.95	0.62 ± 0.02	2.00 ± 0.01

^a: (mg gallic acid equivalent/g extract) ^b: (mg catechin equivalent/g extract)
A.M: Aqueous maceration; M.M: Methanolic maceration

Flavonoid content (TFC) values were expressed in catechin equivalent as milligrams per gram of the extract (mg CE/g extract). Our findings showed that infusion extract had the highest TFC (2.567 ± 0.11 mg CE/g). TFC levels for the decoction, aqueous, and methanolic maceration were 0.84, 0.61, and 0.62 mg CE/g, respectively.

Regarding total condensed tannin, the infusion extract had the highest content with a concentration of 6.623 mg CE/g extract, whereas, lower values were found for decoction, aqueous and methanolic maceration (1.803, 1.442, and 2.001 mg CE/g extract, respectively).

3.4 Antioxidant activity

3.4.1 Total antioxidant capacity

The total antioxidant capacity for the different extracts of *A. cherimola* pulp was evaluated by phosphomolybdate method [26]. The results are expressed as mg/ml of ascorbic acid equivalents (AAE). As shown on figure 1, decoction exhibited the highest antioxidant activity since its IC_{50} (12.61 ± 0.76 mg/ml AAE) was the lowest among all extracts. The aqueous maceration exhibited the less important antioxidant activity ($IC_{50} = 21.98 \pm 1.09$ mg/ml AAE). Hence, the antioxidant capacity, of all the extracts, could be ranked in the following order: decoction; infusion; methanolic maceration; and aqueous maceration.

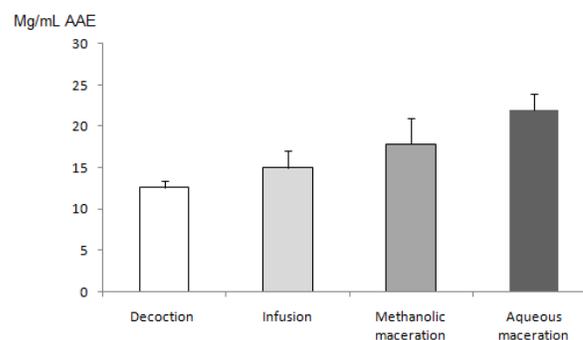


Figure 1: Total antioxidant capacity of *A. cherimola* pulp extracts

3.4.2 DPPH Radical Scavenging Activity

The antioxidant activities of the *A. cherimola* extracts were examined using DPPH. The antioxidant activities of extracts were evaluated using the percentage scavenging activities (Figure 2) and IC_{50} values (mg/ml) (Table 3).

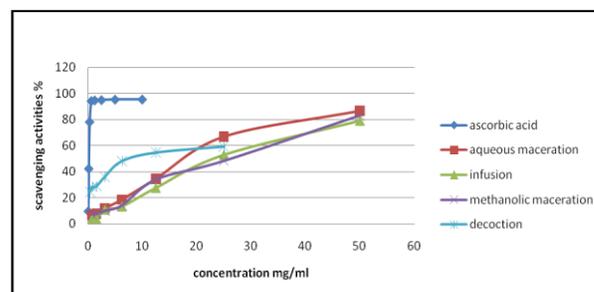


Figure 2: DPPH free radical scavenging activity

Table 3: IC_{50} values of DPPH-scavenging activity of *A. cherimola* pulp extracts

	A.A.	A.M	M.M	Infusion	Decoction
IC_{50} (mg/mL)	0.20 ± 0.01	26.80 ± 0.09	22.33 ± 0.05	28.09 ± 0.12	7.27 ± 0.08

A.A: Ascorbic acid; A.M: Aqueous maceration; M.M: Methanolic maceration; (+): presence; (-): absence

Our results demonstrated that both extracts possessed antioxidant activity. A promising scavenging activity was observed for decoction. Regarding IC_{50} values, decoction seems to possess the best antioxidant activity ($IC_{50} = 7.27 \pm 0.08$ mg/mL). In spite of that, infusion, methanolic maceration, and aqueous maceration exhibited poor antioxidant activities with IC_{50} of 28.09 ± 0.12 , 26.80 ± 0.09 and 22.33 ± 0.05 mg/mL, respectively. Indeed, the lower the IC_{50} of the extract, the higher will be its antioxidant activity and vice versa [27].

3.4.3 Reducing power

Concerning evaluation of the reducing power of the *A. cherimola* extracts, results are presented on figure 3.

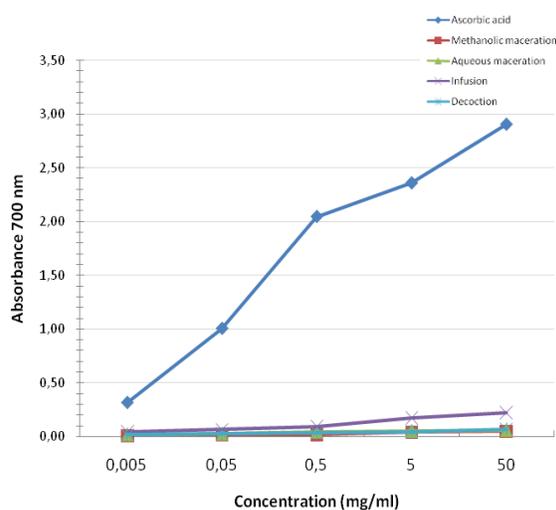


Figure 3: Reducing power of *A. cherimola* pulp extracts compared with ascorbic acid

Table 4: EC₅₀ values (mg/mL), reducing power assay

	A.A.	A.M	M.M	Infusion	Decoction
EC ₅₀ (mg/mL)	1.12	49.20	47.01	11.29	39.20
	± 0.06	± 0.01	± 0.01	± 0.10	± 0.01

A.A: Ascorbic acid; A.M: Aqueous maceration; M.M: Methanolic maceration; (+): presence; (-): absence

All the extracts showed a reducing capacity (Table 4). This reducing power was less than that of standard (ascorbic acid, EC₅₀ = 1.12±0.06 mg/mL). Moreover, infusion exhibited the best reducing power (EC₅₀ = 11.29±0.10 mg/mL) when compared to decoction (EC₅₀ = 39.32±0.01 mg/mL). Both methanolic and aqueous macerations displayed the lowest reducing power as their EC₅₀ (49.20±0.01 and 47.01±0.01 mg/mL, respectively) were four-folds that of infusion.

3.5 Antifungal activity

In the present study, antifungal activity of *A. cherimola* was performed using disc diffusion method. As shown on figure 4, at 100 and 200 µg/mL decoction and methanolic maceration resulted in 6 and 9 mm inhibition zone, respectively. On the other hand, no antifungal activity was exhibited by both infusion and aqueous maceration at these concentrations. When the extracts were used at higher doses (800 and 2000 µg/mL), inhibition zone

increased in a dose dependent manner for all the extracts. The strongest antifungal activity was exhibited by decoction, methanolic maceration, and infusion.

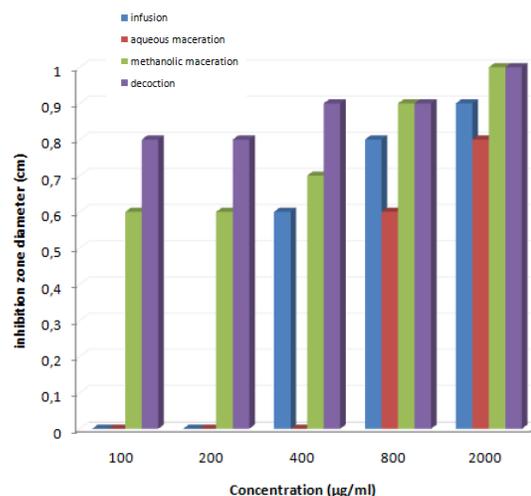


Figure 4: Inhibition zone diameter of antifungal susceptibility of *A. cherimola* pulp extracts

4 DISCUSSION

The present study has been undertaken to investigate the phytochemical profile, and assess the antioxidant activity and antifungal effect of four extracts of *A. cherimola*.

The phytochemical screening of *A. cherimola* extracts revealed the presence of phenols, tannins, flavonoids, and saponins. These phytochemicals can act individually or in synergism and could explain different biological activities characterizing the plant such as antimicrobial, antioxidant, antiinflammatory, antifungal, antitumoral, anxiolytic, and anti-*Helicobacter pylori* [28].

The obtained results showed that *A. cherimola* extracts are relatively rich in total phenolic and flavonoid compounds. Our findings are consistent with those previously reported by Barreca *et al.* [19] revealing that the fruit is rich in flavanols and procyanidins dimers and trimers. García-Salas *et al.* [29] reported the isolation of 21 phenolic compounds from *A. cherimola* pulp. The major compounds were flavan-3-ols: (epi) catechin and procyanidin.

Even though decoction was not found to be the richest extract in phenolic compounds, it exhibited the most important antioxidant activity. Likewise, our findings revealed that infusion exhibited the strongest reducing power among the analyzed extracts. This may be due to the presence of some molecules with strong antioxidant effect and/or reducing power. In consistence with our findings, it has been found that *A. cherimola* juice had the

highest antioxidant activity (98.085 Trolox Eq/100 g) when compared to the plant's skin and flesh. Interestingly, the antioxidants of the plant were absorbed by Burkitt's Lymphoma and colon cancer cell lines both under normal and oxidative stress conditions [18]. The obtained results in the current study agree with those showing an important antioxidant activity of methanolic and ethanolic extracts of *A. cherimola*, attributed to its flavonols and procyanidin [30]. The fruit is rich in flavan-3-ols (catechin and epicatechin) known to be powerful antioxidants preventing several diseases such as cardiovascular diseases and cancer [31]. Furthermore, their antioxidant activities and biological effects are enhanced owing to their good bioavailability [32].

These phenolic compounds possess several phenolic hydroxyl groups with the capacity to scavenge free radicals and exhibit antioxidant activity. It has been found that hydroxylation of both rings B and A in the flavonoid skeleton could explain and / or enhance the antioxidant activity [33]. It is believed that phytochemicals of the *A. cherimola* fruits with antioxidant activity could be responsible of its ethnomedicinal utilization against several diseases such as cancer [34]. Indeed, procyanidin was demonstrated to present important antioxidant activities [35-38]. In addition, the phytochemical screening showed that gallic tannins were only detected in the infusion and decoction. Gallic tannins from the fruits have been shown to be strong antioxidants both *in vitro* and *in vivo* [39-41].

Candida species, considered as opportunistic pathogenic fungi, cause several systemic infections in humans. These fungi develop drug-resistance and result in treatment failures [42]. Therefore, search of anti-*Candida* molecules from natural sources such as plants has been undertaken. In recent years, different herbal products and molecules exhibiting anti-*Candida* activities, have been reported [43-45]. *Candida albicans* is responsible of severe invasive infections characterized by high mortality rates achieving more than 65% even with antifungal drugs [46]. In the present study we assessed the antifungal activity of *Annona cherimola* pulp extracts against *Candida albicans*. Our results showed that at 100 and 200 µg/mL, only decoction and methanolic maceration exhibited antifungal effect. At 800 and 2000 µg/mL, the extracts experienced a dose-dependent increase of the antifungal activity. Decoction, methanolic maceration, and infusion resulted in the highest antifungal activity as revealed by the inhibition zone. Similar results were reported previously. Navarro-García *et al.* [47] demonstrated that *A. cherimola* seeds extracts exerted an important antifungal effect against *C. albicans*. Among the studied extracts, the methanolic extract was the most effective. The antifungal

activities of medicinal plants are usually attributed to their secondary metabolites such as flavonoids or tannins, known to be natural antifungal agents [48]. The antifungal effect of the studied *A. cherimola* extracts might be attributed to flavonoids, found in all the extracts. In fact, flavonoids are produced in plant after a microbial infection which could explain their antimicrobial activity demonstrated in several studies [49].

5 CONCLUSIONS

Our findings revealed the presence of various phytochemicals with well documented biological activities, including phenols, tannins, flavonoids, and saponins. Furthermore, our results showed that *A. cherimola* extracts are relatively rich in total phenolic and flavonoid compounds. The highest total phenolic content was found in the aqueous maceration. In addition, infusion showed the most important total flavonoids content.

Regarding the antioxidant activity, both extracts possessed an activity as assessed with DPPH. The strongest scavenging activity was observed for decoction. Similarly, both extracts exhibited reducing power. The best reducing power was attributed to infusion. Both methanolic and aqueous macerations possessed the lowest reducing power. Interestingly, all the studied extracts exhibited an antifungal activity against *Candida albicans*. The strongest antifungal activity was exerted by decoction, methanolic maceration and infusion.

The antioxidant and antifungal activities may be attributed to the presence of phenolic compounds such as flavan-3-ols: (epi)catechin and procyanidin.

Further investigations should be carried out to identify the active component of the extract and to confirm the mechanism of action and may shed light on the use of *A. cherimola* as a useful dietary supplement.

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