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Phytochemical Screening and Assessment of The Antioxidant Activity of Bio-phenols of Olive Oil Mill Wastewater From The Cold Extraction of Olive Oil in Khenchela Region Eastern of Algeria

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Abstract

The objective of this study was to evaluate in vitro the antioxidant activity of polyphenols from olive oil mill wastewater in Khenchela region eastern of Algeria after conducting a phytochemical screening. The extraction of polyphenols was done by the maceration method. The quantitative estimate of the total polyphenols and flavonoids was evaluated by the colorimetric method. Antioxidant activity was evaluated in vitro using three tests DPPH, ABTS and FRAP and ascorbic acid as a reference antioxidant. The results obtained showed that the phytochemical screening carried out allowed us to highlight the presence of polyphenols, flavonoids, tannins and reducing sugars with large quantities, sterols and terpenoids in lesser amounts. The values of total polyphenols and flavonoids were sequential: (925.8 ± 8.1 µg GAE/mL) and (45.97 ± 9.5 µg QE /mL). The values of the antioxidant activity obtained from the three tests DPPH, ABTS, and FRAP were successively the followings: ($IC_{50:}$ 144.52 ± 1 µg/mL), (IC_{50}: 169.6 ± 35.3 µg/mL) and (IC_{50}: 248.13 ± 10.1 µg/mL). At the end of this study, it appears that OMW polyphenolic extract has an important antioxidant power.

Keywords: ABTS, DPPH, FRAP, OMW, Phytochemical Screening, Polyphenols.

1. Introduction

Olive oil production is a very common activity in Mediterranean countries including Algeria. These countries face a serious environmental problem because of their wastes, especially the effluents called olive oil mill wastewater (OMW) [1]. The cause of the polluting power of this waste is its polyphenol content. Currently, much of the attention in research is on the study of polyphenols that are considered natural antioxidant molecules [2]. The liquid by-product

produced during the olive oil production process is known as olive mill wastewater (OMW). Over 800 million olive trees are planted globally, with the Mediterranean accounting for 97 percent of all olive tree cultivation. Annual output of table olives and olive oil can exceed 10 and 2 000 million tons, respectively. In the Mediterranean basin, the production of OMW has reached 30 million tons per year [3,4]. In recent years, there has been an upsurge in interest in the recovery of OMW; examples include the manufacture of biofuel from OMW and the purifying of OMW from potentially dangerous chemicals. Among all the chemicals included in olive mill effluent, Phenolic Compounds are present in high amounts, ranging from 5 to 25 g/L. The study of these compounds is particularly intriguing because of their remarkable properties, which make them useful in a variety of industries, including cosmetics, food, and pharmaceuticals [5,6]. Indeed, phenolic compounds are one of the most significant and widespread classes of plant metabolites, and they have been researched for their antimicrobial, anticarcinogenic, and antioxidant activities [7]. For these reasons, extracting polyphenols from OMWW is an excellent technique to recover phenolic compounds and re-use them in a variety of applications. Several investigations on polyphenol recovery have been undertaken in order to create and refine the extraction technique of these chemicals [8].

The objective of this study was to evaluate in vitro the antioxidant activity of polyphenols from olive oil mill wastewater in Khenchela region eastern of Algeria after conducting a phytochemical screening.

2. Material and methods

2.1 Sampling

During the olive harvest season, fresh samples of OMW were taken in a modern cold extraction oil mill in Baghai Wilaya of Khenchela in the northeast of Algeria (35°31'19"N, 7°6'52"E, 886m a.s.l) (January 2019). The samples were collected from the liquid effluent collecting basin immediately after the olive press, stored in clean glass cans washed with the OMW to be analyzed, and then sealed without any alteration or treatment.

2.2 Phytochemical Screening

Phytochemical tests of the aqueous extracts were carried out using the classical techniques described by [9]. Polyphenol, flavonoids, tannins, saponins, sterols, terpenoids and reducing sugars were chosen for testing.

2.3 Polyphenol extraction

The protocol for extracting polyphenols from OMW is based on the method described by [10]. It is an extraction using maceration in a polar solvent (methanol) after a step of drying the OMW.

2.4 Quantitative study

The quantitative estimate of the total phenolic content (TPC) and total flavonoids content (TFC) was evaluated by the colorimetric method. TPC were determined by the reagent method of Folin Ciocalteau [11,12]. In an alkaline medium, polyphenols reduce Folin Ciocalteau's reagent to tungsten and molybdenum oxide of blue color. The intensity of this blue color reveals the level of total polyphenols in the mixture. Total Flavonoid Content (TFC) was performed by the method described by [13]. By means of a colorimetric test using aluminum chloride as a specific reagent. The principle of this method is based on the formation of a complex between flavonoids and aluminum chlorides.

2.5 Antioxidant activity

Antioxidant activity was evaluated in vitro using three tests DPPH, ABTS and FRAP and ascorbic acid as a reference antioxidant. DPPH test was evaluated following [14] method. $ABTS^{+}$ test was determined according to the method of [14]. FRAP test was determined according to the method of [15].

2.6 Statistical analysis

Data obtained was presented as (mean ± standard) deviation of three dependent determinations. Significant differences between means of total phenolic, total flavonoids and p values (< 0.05) was regarded as significant. Results of antioxidant activity were subjected to statistical analysis of variance (ANOVA) using ECXEL STAT (version 2014) package at $p < 0.05$ significant levels.

3. Results and discussion

3.1 Phytochemical Screening

The results obtained are illustrated in (Table 1). It showed that the phytochemical screening carried out allowed us to highlight the presence of polyphenols, flavonoids, tannins and reducing sugars with large quantities, sterols and terpenoids in lesser amounts. Similarly, we noted the total absence of saponins. They were similar to those obtained from [16,17]. These phenolic components (polyphenols, flavonoids, tannins, and reducing sugars) exhibit pharmacological properties such as antioxidant, antibacterial, anti-inflammatory, and wound healing [18].

Table 1. Phytochemical screening of OMW

Chemical groups	OMW
Polyphenols	+++
Flavonoids	+++
Tannins	+++
Saponins	-
Reducing sugars	+++
Sterols	+
Terpenoids	+

+++: present in large quantities, +:less presence, -: absence

3.2 Quantitative study

The values of total polyphenols and flavonoids of olive oil mill wastewater are given in Table 2. TPC was measured from the fitting curve using gallic acid as a standard ($y = 0.0048 x + 0.0041$; $R^2 = 0.99$), TFC was determined using quercetin as a standard ($y = 0.0423 x + 0.052$; $R^2 = 0.99$). They were sequential: (925.8 ± 8.1 µg GAE/mL) and (45.97 ± 9.5 µg QE /mL). Our results are almost similar to those obtained by [17]. Many factors influenced the variation of OMW phenolic content, including olive cultivar, olive oil extraction processes, physicochemical properties of OMW samples, fungal and bacterial flora in OMW and storage conditions [19, 20].

Table 2. The values of total polyphenols and flavonoids of OMW

TPC (µg GAE/mL)	TFC (µg QE /mL)
925.8 ± 8.1	45.97 ± 9.5

3.3 Antioxidant activity

The results obtained from the DPPH, ABTS, and FRAP tests were presented in (Fig. 1).

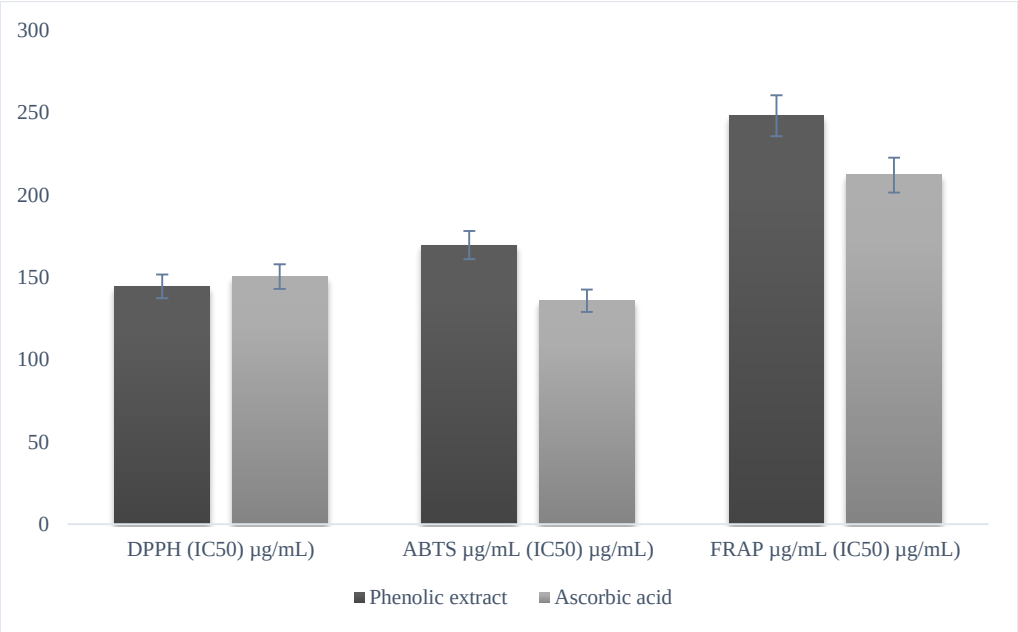

Fig 1. DPPH, ABTS and FRAP assays of the phenolic extract of OMW

It has been noticed that the phenolic extracts of OMW represent a very strong reducing power with IC50 values comparable to that of ascorbic acid. The values of the antioxidant activity obtained from the three tests DPPH, ABTS, and FRAP were successively the followings: (IC_{50}: 144.52 ± 1 µg/mL), (IC_{50}: 169.6 ± 35.3 µg/mL) and (IC_{50}: 248.13 ± 10.1 µg/mL). These results are similar to those obtained by [20]. The presence of polyphenols, which are known to be effective sensors or "scavengers" of free radicals, contributed to the antioxidant capacity of OMW phenolic extracts. The results of our free radical scavenging experiments using OMW phenolic extracts are supported by previously published research that show the significant antioxidant action of phenolic extracts. The antioxidant activity of phenolic extracts can be attributed to the presence of simple phenols, phenolic acids, etc.) [20, 21].

4. Conclusion

At the end of this study, it appears that the quantitative assays reveal an appreciable amount of polyphenols and flavonoids in OMW. In addition, the polyphenolic extract of OMW has an important antioxidant power. Therefore, the recovery of these phenolic molecules from the effluents of olive oil production could solve a major environmental problem and enhance them as a natural antioxidant in the pharmaceutical and food industries.

6. References

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