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ORIGINAL PAPER

In Vitro Anticoccidial Activity of Olive Pulp (*Olea europaea* L. var. Chemlal) Extract Against *Eimeria* Oocysts in Broiler Chickens

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Abstract

Aim The objective of the present study was to investigate in vitro anticoccidial effect of olive pulp (*Olea europaea L* var. Chemlal) extract on the destruction of *Eimeria* spp. oocysts isolated from infected chickens naturally.

Materials and methods The olive pulp (OP) powder was stirred manually in aqueous ethanol in preparation for extraction using the microwave-assisted extraction system. The identification of the phenolic compounds was obtained by ultra-high-performance liquid chromatography–mass spectrometry with electrospray ionisation (HPLC–ESI–MS). The treatment of *Eimeria* oocyst with OP extract and standard compounds (quercetin and oleuropein) leads to their lysis as shown by the release of substances absorbing at 273 nm.

Results Our results showed that the maximum number of reduced oocysts was recorded after 8 h of incubation of optimum OP extract, quercetin and oleuropein for different periods of time. Also, the number of *Eimeria* oocysts decreased considerably with increase concentrations after adding the optimum of OP extract in concentration ranging from 0.023 to 0.371 mg/ml. Positive correlation between the optimum OP extract concentrations and the number of *Eimeria* oocysts reduced was $R^2 = 0.959$. From this in vitro experiment, it can be concluded that the OP extract possesses an anti-*Eimeria* spp activity.

Conclusion To our knowledge, this is the first time that quercetin and oleuropein were tested to evaluate their anticoccidial activity. The findings of this study showed that phenolic compound of OP extract tested separately possesses anti-*Eimeria* spp. effect. Further studies should be carried out to test its in vivo efficacy of the OP bioactive compounds in broiler chickens.

Keywords *Olea europea* L. var. Chemlal · Co-products · Anticoccidial activity · Chickens · In vitro

Introduction

Coccidiosis is one of the most important diseases of poultry worldwide caused by protozoan parasites of the genus *Eimeria*. This infection causes an extensive destruction of the enterocytes [1] which results in reduced feed efficiency, body weight gain, and a temporary reduction in egg production [2]. The worldwide annual losses due to *Eimeria* infection were estimated to more than $3 billion [3].

It is very necessary to intensify control, especially in the current intensive farming conditions; because *Eimeria* oocysts are ubiquitous, easily disseminated in the broilers houses and their large reproduction potential [4]. Several factors can facilitate the disease development such as *Eimeria* virulence, high oocyst challenge, poor ventilation, high stocking density, low immune status of the host, bacterial enteritis, high humidity in litter and a lack of effectiveness of anticoccidial drugs. [5].

Since 60 years, anticoccidial drugs in feed of chickens have been used to control the coccidiosis [5, 6]. However, the main problem associated with their ineffective response is the development of resistance in *Eimeria* species of all synthetic available molecules [7–9]. In addition,

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the anticoccidial drugs in broiler meat may be potentially harmful to both the consumers and the environment [10, 11].

This situation encourages the use of herbal remedies in poultry diets instead of synthetic drugs. Some herbal extracts stimulate the immune system, enhance growth performance and can supply as well the anticoccidial effects [7]. Many studies have been carried out on medicinal plants and forages as alternative strategies to controlling avian coccidiosis and improving poultry performance worldwide [12, 13]. In Algeria, the olive trees represent a significant portion of the agricultural economy (32,300,000 trees) with production of 6,844,606 quintals of olive oil as the production is variable from 1 year to another [14]. According to the Provincial Direction of Agricultural Services, a large part of the olive production is concentrated in Bejaia province (600,551 quintals, 5,212,000 olive trees) which generated a huge quantity of residues called pomace. Pomace is a very promising source of valuable substances, as they contain a large number of bioactive compounds [15, 16]. These co-products become more and more usable in different fields, as animal feed, energy source and vegetation fertiliser [17–20]. However, to our knowledge, the anticoccidial activity of pomace olive has never been reported. The objective of the present study was to investigate in vitro anticoccidial effect of olive pulp (OP) extract on the destruction of *Eimeria* oocysts isolated from naturally infected chickens.

Materials and Methods

Plant Materials

The olive pulp (*Olea europaea* L., var. Chemlal) was collected in the traditional mill immediately after the olive pressing operation in the area of Bejaia (Algeria). Samples were dried for 17 days in the shade at room temperature until constant weight was obtained, and then crushed using a traditional grinder. The resulting powder was passed through a standard 250 µm sieve. Only the fraction with particle size ≤250 µm was collected, stored at +4°C in amber bottles and sterilised until used.

Extraction and Optimization of Total Phenolic Compounds (TPC)

Microwave-Assisted Extraction (MAE)

A domestic microwave oven (2450 MHz, Samsung Model NN-S674MF, Kuala Lumpur, Malaysia) was modified to extract the phenolic compounds from the olive pulp powder [21]. One gram of olive pulp powder was stirred manually in aqueous ethanol in preparation for extraction using the MAE system. The MAE parameters were microwave power (300–700 W), extraction time (30–120 s), liquid–solid ratio (10–50 ml/g) and ethanol proportion (20–50%). After that, the extract was filtered through a Buchner funnel lined with Whatman No 3 filter paper and the supernatant was collected in a volumetric flask. The extract was stored at +4 °C until used.

Determination of Total Phenolic Content

The content of the total phenolics of the OP extract was determined according to the Folin–Ciocalteu test [22]. The absorbance of the extract was compared to a calibration curve of gallic acid to estimate the concentration of total phenolic (TPC) in the sample. PTC was expressed as mg Gallic Acid Equivalents (GAE) per gram of powder on a dry weight (DW) basis.

Determination of Flavonoids Contents

Aluminium chloride colorimetric method was used for flavonoids determination [23]. Briefly, 1 ml of hydroethanolic extract solution was added to 1 ml of 2% methanolic $AlCl_3$_$6H_2O$ solution. The absorbance was measured with a spectrophotometer (UV–Vis SpectroScan 50) at 415 nm length wave after 10 min of equilibrium and then compared to a quercetin standard curve for the determination of the concentration of total flavonoids in the samples. The results were expressed in mg Quercetin/g Equivalent/g of powder on dry weight basis (mg QE/g DW).

Determination of Condensed Tannin (Proanthocyanidins) Contents

The method based on butanol/HCl dosage was performed with small modifications [24]. Two hundred and fifty microliters of extract were mixed with 2.5 ml of an acid solution of ferrous sulphate (77 mg of ferric ammonium sulphate (Fe_2 $(SO4)_3$) dissolved in 500 mL of (3:2 *n*-butanol:HCl)). After mixing and incubating at 95 °C for 50 min, the absorbance at 550 nm length wave was measured against a blank. Condensed tannins content (CTC) was calculated using the following formula:

$$\mathrm{CTC} = \frac{A_{550\ \mathrm{nm}} \times \mathrm{DF} \times \mathrm{MW}}{\varepsilon L},$$

where DF is the dilution factor; MW is the molecular weight of the cyanidin (287 g/mol), ε is the molecular extinction coefficient (34,700 l/mol/cm) and L is the spectrophotometer cell thickness (1 cm). The condensed tannin concentrations (mg/ml) were expressed as mg of cyanidin (CE) equivalents per g of extract dry weight (DW).

Determination of the Antioxidant Activity

Scavenging Activity Against the $ABTS^{\bullet+}$ Radical

The method of Re et al. [25] was adopted for the evaluation of the antioxidant activity of pulp olive extract. Briefly, a radical solution (7-mM ABTS and 2.45-mM potassium persulfate) was prepared in an ethanolic solution and left to stand in the dark at room temperature (27 °C) for 12–16 h before performing in the assay. This solution was then diluted with ethanol to get an absorbance of 0.700 ± 0.02 and equilibrated at 30 °C. The antioxidant activity (AOX) was calculated as the percentage of inhibition of absorbance at 734 nm length wave and the IC_{50} (µg/ml) was determined as follows.

$$\mathrm{AOX}\% = \left(A_{\mathrm{control}} - A_{\mathrm{sample}} / A_{\mathrm{control}}\right) \times 100,$$

where A_{control} is the absorbance of the blank control ($ABTS^{+\cdot}$ solution without test sample) and A_{sample} is the absorbance of the test sample.

Scavenging Activity Against the $DPPH^{\cdot}$ Radical

The OP extract was tested for the scavenging effect on the $DPPH^{\cdot}$ (1,1-diphényl-2-picryl-hydrazyl) radical using a colorimetric method [26]. 50 µl of hydroethanolic extract at different concentrations was added to 2 ml of DPPH stock solution (0.004%; w/v) and the reaction mixture was thoroughly mixed and incubated for 30 min at 37 °C. The absorbance of the resulting solution was measured at 517 nm length wave with a spectrophotometer. As a positive control, a synthetic gallic acid antioxidant was used and the IC_{50} was determined. The DPPH radical scavenging activity (S %) was calculated using the following formula

$$S\% = 100 \times A_{\mathrm{control}} - A_{\mathrm{sample}} / A_{\mathrm{control}},$$

where A_{control} is the absorbance of the blank control (containing all reagents except the extract solution) and A_{sample} is the absorbance of the sample.

Measurement of the Ferric Reducing Power (FRAP)

The ferric reducing power was determined as described by Oyaizu [27]. Briefly, 120 µl of OP extract solution at different concentrations was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 7.4) and 2.5 ml of potassium ferricyanide (1%). After an incubation of the mixture at 50 °C for 20 min, 2.5 ml of trichloroacetic acid (10%; w/v) was added, and the mixture was centrifuged at 3000 rpm for 10 min. A 2.5-ml aliquot of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1%; w/v); then, the absorbance was measured at 700 nm wave length. The higher the absorbance value, the stronger the reducing power.

High-Performance Liquid Chromatography–Mass Spectrometry (HPLC–ESI–MS) Analysis of Phenolic Compounds

The identification of phenolic compounds of OP extract was obtained by ultra-high-performance liquid chromatography–mass spectrometry with electrospray ionisation (UPLC–ESI–MS) and quadrupole-time of flight detector (QTOF). The equipment was Xevo G2 mass spectrometer consisting of a hexapole, a collision cell and a time of flight analyser (QTOF) supplied by Waters (Milford, MA, USA). The electrospray probe was used in positive (ESI+) and negative (ESI−) modes as well as sensitivity analyser mode. The mass range considered was from 10 to 1000 Da. The corona voltage was 2.5 kV for (ESI+) and 0.5 kV for (ESI−). The sampling cone voltage was optimised between 20 and 50 V. Finally, 30 V was selected for the screening because more peaks were detected. Other MS parameters were as follows: the source temperature was 150 °C, the desolvation gas temperature 450 °C and the desolvation gas flow 650 l/h. MSE mode was selected for the acquisition, and collision ramp energy from 5 to 40 V was used. Mass Lynx v.4.1 software (Waters, Milford MA, USA) was used to analyse the samples and Croma Lynx (Waters, Milford MA, USA) was used to deconvolve the spectra. Quantitative data for pulp phenolic compounds were obtained by calibration curves obtained from known standards.

Determination of the Anticoccidial Activity

Eimeria Oocysts Isolation and Purification

Oocysts sample of *Eimeria* spp. was isolated from fresh faeces of broilers suffering from coccidiosis in Bejaia area (Algeria). The oocysts were sporulated by incubation in 2.5% $K_2Cr_2O_7$ solution in the presence of suitable humidity and temperature [28]. Sporulated oocysts were washed and counted using Malassez chamber. Mean number of oocysts per millilitre of sample was calculated. The identification of *Eimeria* species in chickens was made on the basis of some standard parasitological techniques [28]. The oocysts were identified according to size, shape, presence or absence of micropyle, time of sporulation, intestinal location and appearance and coarse characteristics of intestinal lesions. The percentage of each species in the mixed suspension was approximately 32.05% *E. acervulina*, 26.92% *E. tenella*, 15.35% *E. mitis*, 14.10% *E. brunetti* and 11.53% *E. maxima*.

The purification of the oocysts was carried out from one-litre phosphate-buffered saline (PBS, containing 8 g/l NaCl, 0.2 g/l KCl, 1.13 g/l Na_2HPO_4, $2H_2O$ and 0.2 g/l KH_2PO_4) with some modifications [29]. Neutral substrates containing antibiotics (Streptomycin 1 mg/ml and penicillin V 100 IU) were added to prevent any bacterial evolution and Fluconazole (17 mg/ml) was added as antifungal agent. The pH was adjusted to 7.4 and the solution was sterilised by membrane filtration through a 0.2-µm filter. The HBSS (Hanks' Balanced Salt Solution) medium was prepared in the laboratory (NaCI, 8.0; KCl 0,4; $CaCl_2$, 0.139; D-glugose, 1.0; Na_2HPO_4, 0,0478; KH_2PO_4, 0.06 and $MgSO_4$, 0.097 g/l in 1 l of distilled water). The solution was sterilised as well as that of the 0.2% agar.

Effects of the Pulp Extract and Standard Compounds on the Decrease of Oocysts Number

The activity of olive pulp extract and standard compounds (quercetin and oleuropein) (Fisher scientific, Fair Lawn, NJ, USA) was determined in triplicate by incubation at ambient temperature for 24 h [30]. The suspension solution was incubated at different periods of time: 0, 1, 2, 4, 6, 8 and 24 h. One millilitre suspension contains: 100 µl of washed suspension of *Eimeria* oocysts at 4.16×10^6 oocysts/ml; 700 µl of PBS; 200 µl of the optimum olive pulp extract. After incubation, the samples were centrifuged at 320*g* for 5 min and the absorbance of the supernatant was measured at 273 nm by spectrophotometer (Shimadzu, model: UV 100 Japan). Then, the percentage of destruction of sporulated oocysts was estimated. The LC_{50} value was then inferred from the regression curve. The number of oocysts was counted three times in a cell volume of 1 µl amounts to 4.16×10^4. The ethanol solvent was also used as a negative control.

Effect of the Diclazuril, Sulfaquinoxaline Sodium on the Decrease of the Oocysts Number

Diclazuril (Diclosol®, Avico, Arab Industry Veterinary Co, Amman, Jordanie) and Sulfaquinoxaline sodium (Coccidiopan®, Avico, Arab Industry Veterinary Co, Amman, Jordanie) were tested in triplicate (0.1, 0.3, 0.5, 0.7 and 1 mg/ml) using the microplate method described by Remmal et al. [31]. The number of oocysts was counted twice in a cell volume of 1 µl amounts to 2.32×10^4.

Statistical Analysis

A statistical analysis was performed using JMP® Software, version 7.0 (SAS Institute Inc, 2007). The results were expressed in mean ± SE. The values were statistically significant when the P value was ≤ 0.05. Inoculums suspension taken on time 0, 1, 2, 4, 6, 8 and 24 h on oocysts number was examined by the Student's t test. The lethal concentration is defined as the concentration that reduces the initial number of sporulated oocysts to 50%.

Results

The optimum of OP (*Olea europaea* L., var. Chemlal) concentration (22.30 mg/g; 0.743 mg/ml and 03 min) and the optimised parameters of the extract of OP are illustrated in Fig. 1. The identification of the phenolic compounds of the OP extract by ultra-high-performance liquid chromatography–mass spectrometry with electrospray ionisation (HPLC–ESI–MS) revealed the presence of biophenol molecules (Fig. 2). The quantitative data for the phenolic compounds of the extract were obtained by calibration curves from known standards.

The antioxidant capacities of the OP extract at different dilutions (1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and 1/128) and the standard compounds (quercetin, oleuropein) at different concentrations (0.05, 0.1, 0.3, 0.5, 0.7 and 1 mg/ml) were evaluated by DPPH, $ABTS^{+\bullet}$ and FRAP tests (Table 1). The IC_{50} determination shows a significant difference ($P < 0.05$) between the OP extract and the used standards. Quercetin has a pronounced antioxidant activity (0.102 ± 0.014 mg/ml) compared to oleuropein and OP extract (0.276 ± 0.037 and 0.509 ± 0.047 mg/ml, respectively).

According to our results, the OP extract and the standards compound tested at different concentrations (0.743 and 0.139 mg/ml, respectively) showed that the number of oocysts decreases after the treatment. However, the quercetin was most effective (45.38%), followed by oleuropein and the OP extracts (33.25% and 25.36%), respectively, after 8-h treatment. The lethal concentration LC_{50} of OP extract and diclazuril were 14.44 ± 1.206 and 0.5 ± 7711 mg/ml, respectively. The ethanol-treated *Eimeria* suspension (negative control) was significantly ($P \leq 0.05$) higher than the tested concentration of OP extract and the standards compound. The result of two anticoccidial effects (diclazuril and sulfaquinoxaline sodium) on the number of oocysts at 0.5 mg/ml concentration is shown in Fig. 5.

Figure 3a–c showed that a rate of 25.36, 45.38 and 33.25% of the oocysts number reduced has been recorded after 8 h of incubation with 0.745 mg/ml of the optimum OP extract, quercetin and oleuropein for different periods of time, respectively. This decrease in the number of oocysts causes a considerable release of 273 nm absorbing material from *Eimeria* oocysts that this depends on the concentration of the optimum OP extract, quercetin and oleuropein.

Figure 4 shows the number of *Eimeria* oocysts decreases considerably with increase in concent rations of OP extract in concentration ranging from 0.023 to 0.371 mg/ml. Positive correlation between the optimum OP extract

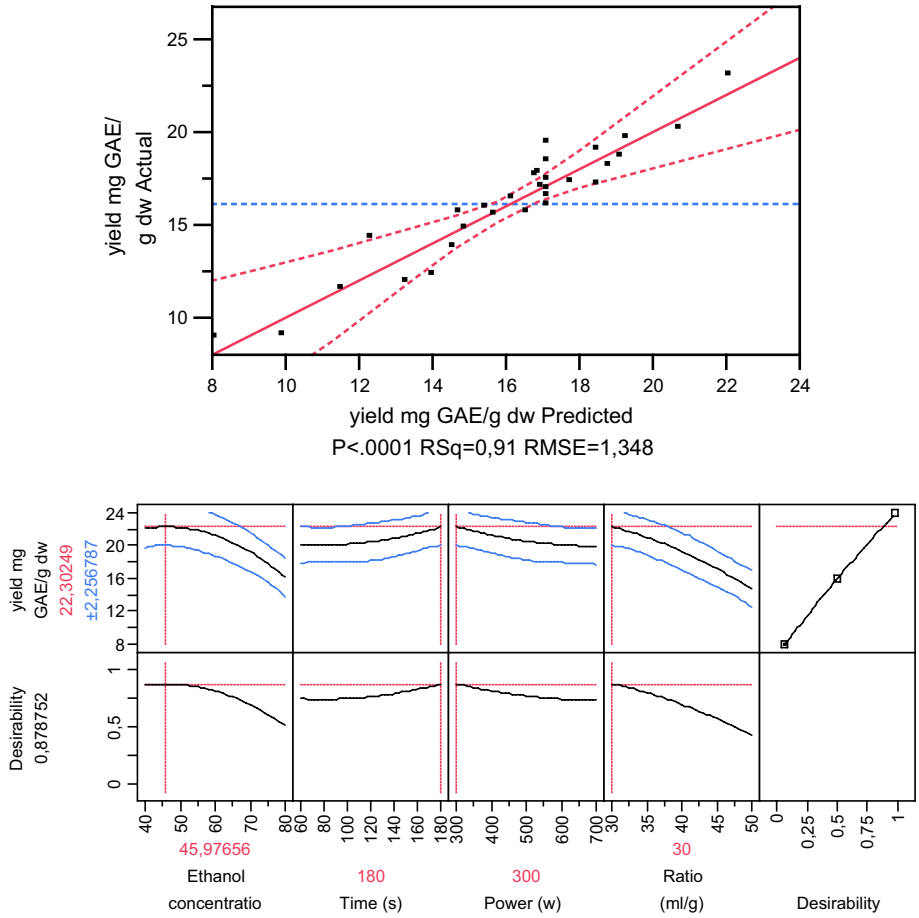

Fig. 1 Optimization of parameters of extraction of olive pulp (*Olea europaea* L., var. Chemlal) by microwave

concentrations and the number of *Eimeria* oocysts reduced was $R^2 = 0.959$. The result of two anticoccidial effects (diclazuril and sulfaquinoxaline sodium) on the number of oocysts at 0.5 mg/mL concentration is shown in Fig. 5.

Discussion

The use of plant extracts as remedy can attenuate the resistance of coccidia to medications and their impact on consumer's health, because they are not only natural products, but also include new therapeutic molecules for which no resistance has never been reported [32, 33]. This work was carried out to find a phytotherapic substance to help the control of *Eimeria* parasites in broiler chicken and to provide an alternative use of a plant extracts. To our knowledge, this is the first study to evaluate the effect of anticoccidial of the OP optimum (*Olea europaea* L., var. Chemlal) extract and their phenolic compounds directly on the viability of *Eimeria* oocysts collected from broiler chicken parasites in vitro. The results of this investigation have demonstrated that the OP extracts have a noticeably destructive effect on *Eimeria* parasite. Previous investigations have used in vitro test to screen the anticoccidial effect of some plant compounds in broiler chickens [34–38].

The study carried out by Hady and Zaki [12] demonstrated that *Artemisia annua* extract decreases the bloody diarrhoea in broilers experimentally infected by *E. tenella* compared to the infected control group. Other researchers reported that *Artemisia annua* and *Foeniculum vulgare* have an anticoccidial activity in chickens infected by *E. tenella*, and a significant reduction in faecal oocysts was recorded [39]. Likewise, *Artemisia herba-alba* anticoccidial activity was relied on decreasing excretion of *E. tenella* oocyst and bloody diarrhoea as reported by Messai et al. [40].

As described before in several studies, the phenolic components (flavonoids, flavonoids, phenolic acid, tannins, etc.) revealed a wide range of biological properties such as antibacterial, antiviral, anticancer, anti-proliferative and anti-inflammatory [41–45]. These compounds or secondary metabolites particularly possess also anti-protozoan activities, especially against *Plasmodium*, *Leishmania* and *Trypanosoma* spp. [46–50]. Then, the phenolic compounds are good candidate molecules due to a relatively low or no toxicity and because the presence of some flavonoids limits the resistance of protozoan to other drugs [51]. Moreover, the polyphenols from Palmae (*Cocos nucifera* L.) fibres

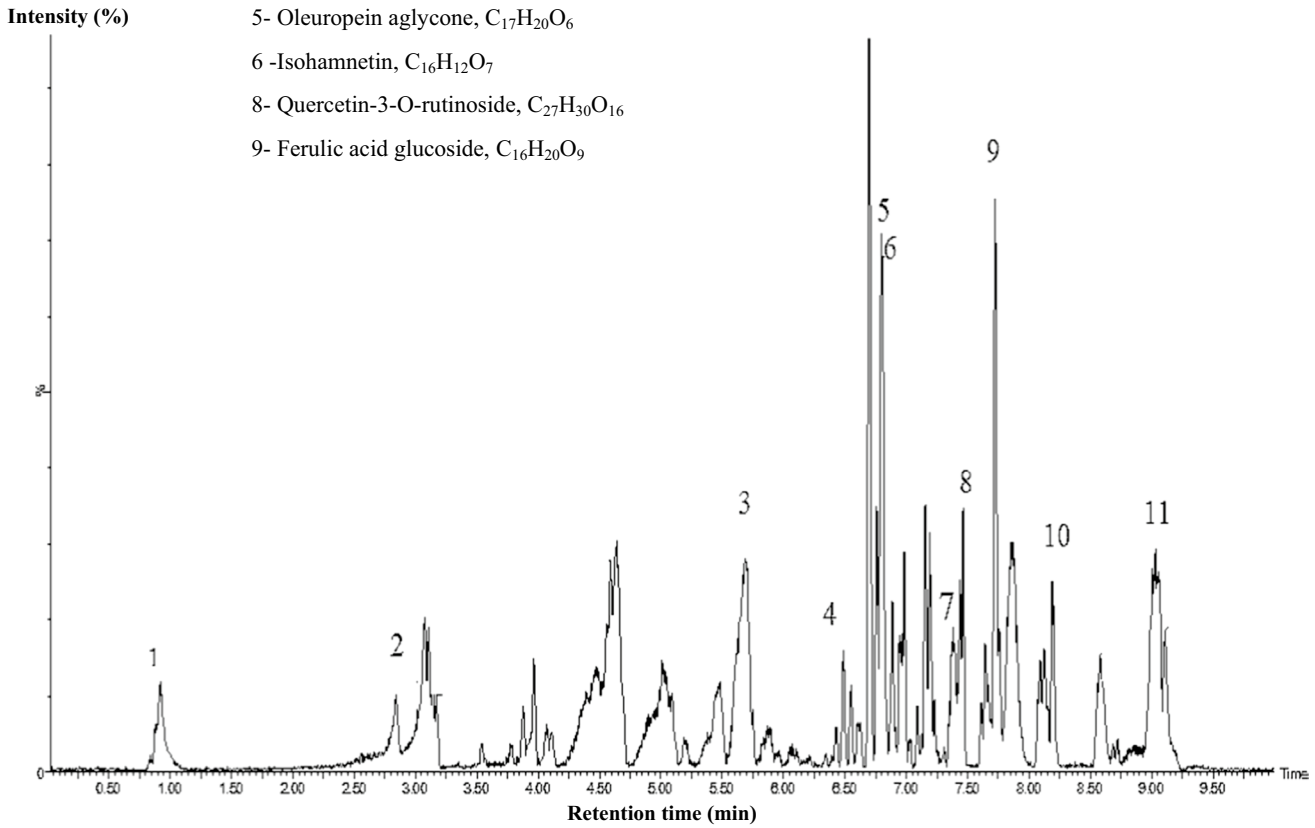

Fig. 2 HPLC–ESI–MS chromatograms of the phenolic profile of the olive pulp extract (*Olea europaea* L., var. Chemlal)

Table 1 DPPH, $ABTS^{+\bullet}$ and FRAP tests of phenolic compounds of the olive pulp extract (*Olea europaea* L., var. Chemlal), quercetin and oleuropein

Compounds	IC_{50} Concentration (mg/ml, ± SD)		Inhibition (%)		FRAP (OD, 1 mg/ml)
	DPPH	ABTS	DPPH	ABTS	
Quercetin	0.10 ± 0.01	0.12 ± 0.03	76.52	65.64	2.08
Oleuropein	0.23 ± 0.01	0.28 ± 0.0	59.2	58.72	1.18
Hydroethanolic extract of pulp	0.51 ± 0.05	0.54 ± 0.05	25.36	23.54	1.76

IC_{50} inhibitory concentration 50, *SD* standard deviation, *OD* optical density, *DPPH* 1,1-diphényl-2-picryl-hydrazyl, *ABTS* 2,2′-azino-bis (3-éthylbenzothiazoline-6-sulphonique

showed antiproliferative activity against *Leishmania amazonensis* [52].

In the present study, the OP extract used as natural anticoccidial product is very interesting because the results of the characterization by HPLC show that the OP extract contains large concentrations of polyphenolic compounds or biophenoles (Caffeoylputrescine, apigenin 7-O-glucoside, oleuropein aglycone, quercetin 3,7,-O-glucoside, 3,4-DHPEA-EA, quercetin-3-O-rutinoside, diligustilide, ferulic acid glucoside, 4-*p*-coumaroylquinic acid, deacetoxyoleuropein aglycon). In addition, the major compounds of OP extract are flavonoids called again bioflavonoid. It should be noted that these flavonoids are recognised archetypal antioxidants such as oleuropein (OE), hydroxytyrosol (HT), tyrosol (T), coumaric acid, ferulic acid, caffeic acid, quercetin, etc. and had interesting biological activities [53]. In addition, Ghanbari et al. [54] confirmed that the different parts of olives and their by-products have a valuable bioactive profile as well as medicinal and functional proprieties.

The OP extract at different concentrations showed a significant antioxidant activity in agreement with those reported by Morelló et al. [55] and Moudache et al. [56, 57]. The results of present study correspond also with those published previously, which demonstrated that the antioxidant activity of plant extracts contains quercetin and oleuropein [58–63]. Antioxidant activity is often accompanied by antiviral and antibacterial activities of the phenolics compounds. In addition, it was shown that the antioxidant activity of flavonoids is determined by the presence of free hydroxyl groups and their mutual location [64].

The results of DPPH, $ABTS^{+\bullet}$ and FRAP tests of the optimum and the standard compounds (quercetin,

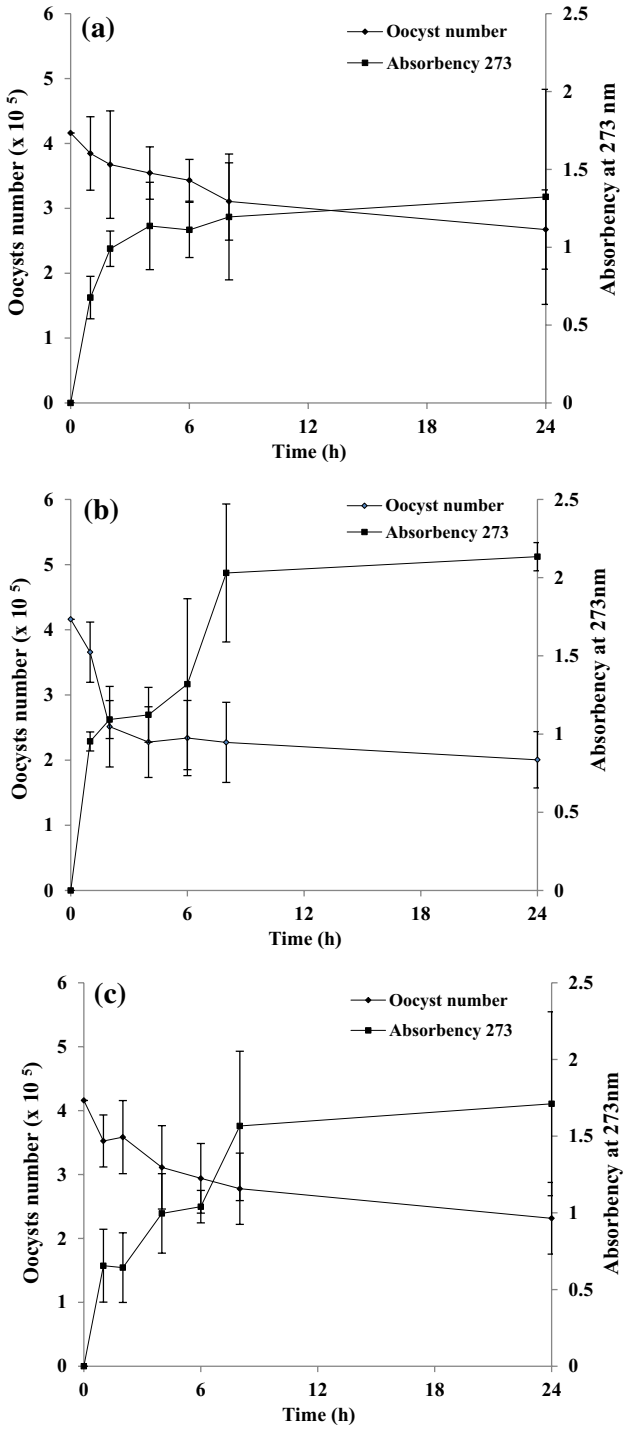

Fig. 3 Kinetics of the decrease of the oocysts number and 273 nm wave length absorbing material release from *Eimeria* oocysts treated by optimum of olive pulp extract (*Olea europaea* L., var. Chemlal) (**a**), quercetin (**b**) and oleuropein (**c**)

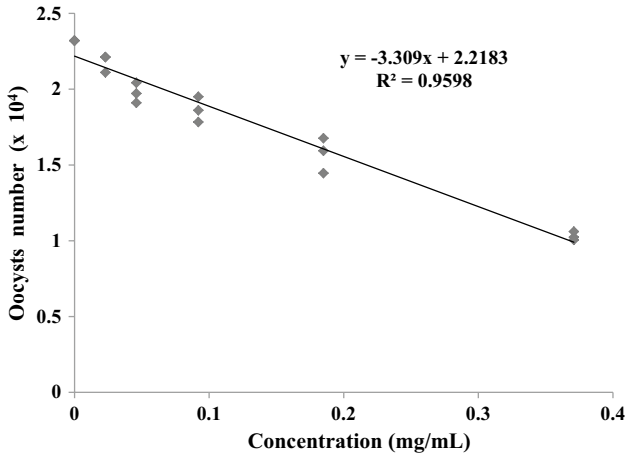

Fig. 4 The correlation between the optimum of olive pulp extracts (*Olea europaea* L., var. Chemlal) concentrations and the number of *Eimeria* oocysts

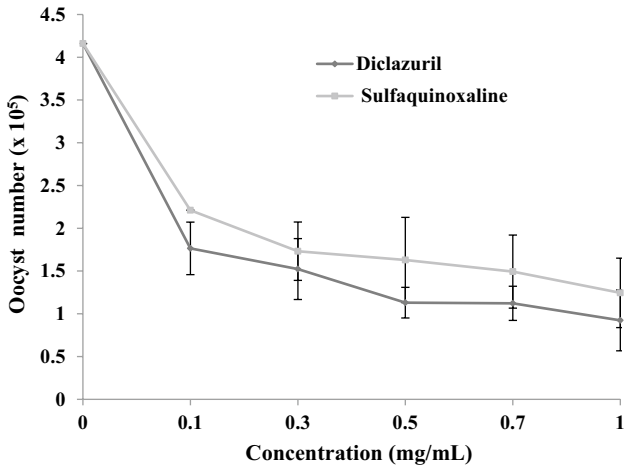

Fig. 5 Effect of diclazuril and sulfaquinoxaline concentrations on oocysts number

oleuropein) were revealed by the antioxidant capacities. However, the difference of quercetin, oleuropein and the optimum of OP extract values could be attributed to a polar character which can contribute to the potentialisation of power to reduce the free radicals [44, 65]. According to Miguel [66], some flavonoids having multiple hydroxyl (OH) groups act as pro-oxidants. Several studies have shown that the reducing power of phenolic compounds can be considered as an indicator potential antioxidant effect [67, 68]. The results of this study correspond also with those published previously, which demonstrated the antioxidant activity of the olive oil co-product [69]. This effect could due attributed to the hydrogen donor ability to form stable free radicals, thus preventing the oxidation and their propagation. In addition, the mixtures of different phenolic compounds present in the olive oil co-product extracts have greater antioxidant activity than the individual compounds because of their synergy [70].

To test the decrease potential of the OP extract on *Eimeria* parasite in broiler chicken, the extract optimal concentration (22.3024 mg/g GAE of dry matter: 0.743 mg/ml) was used to define the time necessary for the oocysts maximum reduction. The results of anticoccidial effect have been evaluated from the lysed oocysts number after treatment depending on time. This observation is based on the alteration state of oocysts treated surfaces and the cells fragmentation. Note that the positive control is also taken into account which gives a strong support for the tested extract. The data of this study showed a correlation of OP extract concentrations with the reduced oocysts numbers. This destructive effect of oocysts is a result of intracellular content release such as aromatic amino acids and nucleotides that are expressed by UV absorption substances after treatment [31]. The results of this study also correspond to those previously published, which showed that the increase in the concentration of plant extracts leads to an increase in anticoccidial activity [30, 31]. Regarding the concentration of extraction solvent at 45.97% ethanol (Fig. 1) of optimum extract, neither destruction of oocysts nor deleterious effect were recorded. In contrast, Gadelhaq et al. [71] reported that a significant effect of inhibition of sporulation and oocysts deterioration occurred at high ethanolic concentrations (50 and 70%).

This anticoccidial activity could be attributed to an individual or a combined effect of the bioactive compounds [72]. These constituents could play many beneficial-associated properties such as antioxidant and anti-inflammatory effects associated with compounds rich in bioactive elements (e.g., polyphenolic) [73, 74]. Our in vitro results could be explained by the in vivo positive effects of the olive pulp extract on the chicken production reported by Sayehban et al. [20]. The results of this study correspond to those previously published, which demonstrated that the anticoccidial activity might be due to the bioactivity constituents such as ascorbic acid, flavonoids, phenol compounds and carotenoids [75, 76].

Many investigations have established relationships between the activity, the chemical structure and the mode of action of flavonoids as well as the absorption level and their bioavailability [5, 77–79]. In this research, it may be assumed that the different proportions of OP extract, quercetin and oleuropein are related to factors cited previously. In addition, the hydrophilic and hydrophobic nature of bioactive (quercetin and oleuropein, respectively) may be the major cause of anticoccidial activity. According to Tasdemir et al. [80], quercetin showed an in vitro leishmanicidal activity with IC 50 of 1.0 µg/ml. The insertion of two OH functions improved considerably the leishmanicidal in vivo activity with 15.3% of inhibiting infection, while other flavonoids were completely inactive.

It has been demonstrated that the olive pulp of the family *Oleaceae* has higher antioxidant activity, mainly residing in the hydrophilic nature [55], in contrary to oleuropein. This could be explained by the fact that oleuropein is to some extent polar and thus, it would rapidly diffuse through the bilayer of the intestinal epithelial cell membrane [81]. On the other hand, Gourama and Bullerman [82] showed that oleuropein has an influence on the development and sporulation of *Aspergillus parasiticus*. It is important to underline that those natural antioxidants containing soluble lipid seem to be more effective due to their penetration into the cell, which could affect the intracellular of *Eimeria* [83].

In the present investigation, the difference in proportions (oocysts destroyed) could be explained by the difference of polarity and the hydrophilic or lipophilic nature. Also, other studies showed the beneficial effect of plant extracts against multiple or monospecific infections with either *E. tenella* or *E. acervulina* [84, 85]. This is supported by Allen and Danforth [86], who demonstrated that the antioxidant compounds are well known to have a cellular protective action against oxidative stress and reduce the severity of *E. tenella* infections by altering the degree of intestinal lipid.

According to Peek and Landman [87], the use of Diclazuril (0.5 mg/ml) showed a significant reductive efficiency on sporulated *Eimeria* oocysts after 24 h of incubation compared to our results, while the sulfaquinoxaline showed a slight reduced oocysts number. Indeed, in agreement with our observations, many investigations demonstrated that the in vitro anticoccidial effect of diclazuril is high [88, 89]. This difference may be attributed necessarily to pharmacological properties of drugs and their action on coccidia development. Diclazuril lethal effect against both asexual and sexual stages of *E. tenella, E. necatrix* and *E. acervulina*, the gametocytes of *E. brunetti*, and the zygote of *E. maxima* was documented [90].

Conclusion

From this in vitro experiment, it can be concluded that the OP (*Olea europaea* L., var. Chemlal) extract possesses the ability to destroy *Eimeria* spp. collected from naturally infected broiler chickens. To our knowledge, this is the first time that quercetin and oleuropein are tested to evaluate their anticoccidial activity. The findings of this study showed that phenolic compound of OP extract tested separately possesses anti-*Eimeria* effect. Further studies should be carried out to test the in vivo efficiency of the OP bioactive compounds in broiler chickens.

Author contributions NID carried out the experimental work and wrote the manuscript. CN participated in biochemistry analysis of extract plant and reviewed the manuscript. MA, MG and KM contributed in technical assistance of plant extraction. AA designed, supervised the

experimental study and reviewed the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no potential conflict of interest.

Ethical approval Ethics committee approval was received for this study from the scientific committee of Faculty of Life and Nature Sciences, University A. Mira, Bejaia, Algeria.

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