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POTENTIAL OF LENTIL POLYPHENOLS FOR ANTIOXIDANT, ANTIBACTERIAL, AND ANTIFUNGAL PROPERTIES (CORAL VARIETY)

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ABSTRACT

The objective of this study is to evaluate the content of total polyphenols, flavonoids and anthocyanins, to estimate the antifungal, antibacterial and antioxidant activity of the polyphenols extracted from the whole grains of dry *Coral* lentil, and to highlight the influence of cooking in water on the quantity and quality of phenolic compounds. This variety seems to be rich in phenolic compounds with a positive impact of cooking in water on the total polyphenol content.Concerning flavonoids and anthocyanins, cooking seems to have exerted a negative effect. An increase in antioxidant activity after cooking the grains was recorded.The results of the antibacterial activity showed that the most sensitive strains were the Gram-positive strains. The extract that showed maximum inhibition was raw coral.

For antifungal activity, *Alternaria* strains seem to be the most sensitive. Phenolic extracts from raw grains were found to be very active; at the concentration of 2mg/ml and phenolic extracts from cooked grains were found to be very active; at the concentration of 1mg/ml. The *Penicillium* sp strain has a random growth which prevented us from calculating their inhibition rates.The highest value of antifungal index 100 is marked for the phenolic extract of cooked coral lentil followed by raw coral lentil. The phenolic extract of cooked Coral lentil showed fungistatic activities on both strains at 1mg/ml and fungicidal activity on *Penicillium*,sp at 2mg/ml. The phenolic extract of raw Coral lentil showed both fungicidal and fungistatic activity on *Alternaria*,sp and fungistatic activity on *Penicillium*,sp at 2mg/ml.

1. Introduction

A consensus has recently begun to form on the importance of pulses from an agronomic, nutritional, economic, and territorial perspective. Actions to increase knowledge of the importance of pulses in family nutrition are critically needed given the shift towards more diversified and high-quality diets. 2016 has been designated as the International Year of Pulses by the United Nations, offering a rare chance to recognize the key contributions made by this plant family and how to strengthen them (Sieglinde *and al.*, 2018).

Legumes are regarded as a good source of polyphenols, however the amounts vary greatly between genera and species (Singh *and al.*, 2017).

Several research have characterized the phenolic makeup of Lens culinaris seeds, demonstrating the distinct differences between the compounds that make up the coat and the cotyledons. The lentil seed coat is extremely rich in catechins, procyanidin dimers and trimers,

and, in smaller concentrations, quercetin, myricetin, lutein, and apigenin glycosides, according to (Dueñas *and al.,* 2002).

The cotyledon mostly comprises low quantities of hydroxybenzoic and hydroxycinnamic acids. The cotyledon had two trans-p-coumaric acid esters, p-coumaroylmalic acid, and p-coumaroylglycolic acid, whereas the seed coat included the stilbene trans-resveratrol-5-glucoside (Djabali *and al*., 2021).

Lentils had not previously been linked to these compounds. Although cooking is required in order to ingest dried lentils, little research has been done on how cooking affects phenolic component quantity and profile as well as antioxidant, antibacterial, and antifungal activities. It is in this context that the main objectives of this study are set.

2. Materials and methods

2.1. Plant material

The dried lentils (Lens culinaris, Variété Corail) are locally produced and are characterised by an orange colour (Figure 1). They were purchased from a point of sale of dried vegetables for human consumption in the wilaya of Jijel (eastern Algeria).

Figure 1. Photograph of the lens variety studied.

2.2. Method

2.2.1. Preparation of the lens samples

Two batches of lentils were separated. The first batch consisted of 100g of dry (uncooked) lentils ground to powder (< 250 µm).

The second batch consisted of 100g of lentils cooked by boiling in a stainless steel pot with 600 ml of distilled water. The cooking was done covered to reduce the surface area in contact with the open air. The factors used in the cooking process (temperature and duration) were chosen with reference to preliminary analyses. The cooked lentils were drained, freeze-dried and ground to powder (< 250 µm).

The resulting powders from both batches were stored in hermetically sealed glass vials and deposited in the dark to avoid any deterioration of the samples.

2.2.2. Extraction and determination of polyphenolic compounds

2.2.2.1. Extraction

The extraction of total polyphenols from raw and cooked samples was carried out according to the protocol proposed by Mujica *and al*. (2009) , 1g of the powder from each sample was solubilised in 25ml of methanol acidified with 0.1% 2N HCl. The mixture was left for 2h at room temperature and then centrifuged at 1800g for 15min. The residue is re-extracted with 25ml of methanol and centrifuged again. At the end, the supernatants are combined and the dry extract is recovered after dry evaporation (variable time/45°C).

The extraction yield is calculated by the following formula (Falleh *and al.*, 2008),

$$R(\%) = (M \text{ extract} / M \text{ sample}) \times 100 \quad \textbf{(1)}$$

2.2.2.2. Determination of total polyphenols

Total polyphenols were determined colorimetrically using the Folin-Ciocalteu method according to Djabali *and al*. (2020). The concentration of total polyphenols is calculated from the regression equation of the calibration curve established with gallic acid based on previous tests, and is expressed in µg gallic acid equivalent per gram of extract (µg GAE/g extract).

2.2.2.3. Determination of flavonoids

The aluminium trichloride (ALCL3) method was used for the determination of flavonoid content (Bakchiche and Gherib, 2014). 1.5ml of phenolic extract (1mg /ml) was added to an equal volume of ALCL3 solution (2%). The mixture was shaken vigorously and the absorbance was read at 430 nm after 30 minutes of incubation at room temperature. The flavonoid content was expressed as mg

quercetin equivalent per g sample (Huang *and al.,* 2004).

2.2.2.4. Determination of anthocyanins

The anthocyanin content was estimated by spectrophotometer using the differential pH method, using two buffer solutions, potassium chloride (pH 1.0; 0.2 M) and sodium acetate (pH 4.5; 0.4 M) (Lee *and al.,* 2005).

The absorbance of the extract was measured at 510 and 710 nm and then deduced by equation,

A= ([A510-A 710) pH 1,0-- [A510-A 710) pH 4,5) **(2)**

The anthocyanin content was determined according to the equation,

C = A. MW. FD. 4000 / E **(3)**

C, anthocyanin content (g ECy-3-Glu/g powder) ;

A, absorbance

MW, molecular weight of cyanidin-3-glucose (449.2g/mol);

FD, dilution factor ;

E, Molar extinction coefficient (26900) of cyanidin-3-glucose.

2.2.3. Evaluation of total antioxidant capacity

The total antioxidant capacity of the phenolic extracts was evaluated by the phosphomolybdenum method (Prieto *and al.,* 1999). A 0.3 ml volume of each phenolic extract (1000 ppm) was mixed with 3 ml of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes are incubated at 95°C for 90 minutes. After cooling, the absorbance of the solutions is measured at 695 nm against the blank. The total antioxidant capacity is expressed as milligram ascorbic acid equivalent per gram of dry matter (mg EAA/ g DM).

2.2.4. Determination of antimicrobial activity

The antibacterial activity was evaluated on four bacterial strains, Escherichia coli, Pseudomonas aerogenosa ATCC27853, Staphylococcus aureus ATCC29213 and Lactobacillus sp.

These strains were provided by the medical analysis laboratory BAKIEWA wilaya of Jijel.

The antifungal activity was tested on two fungal strains, *Penicillium sp* and *Alternaria sp.*The selected strains were chosen either for their capacity to contaminate foodstuffs or for their pathogenicity. These strains were provided by the microbiology laboratory of the University of Jijel.

2.2.4.1 Testing for antibacterial activity

The antibacterial activity of the extracts was determined by the agar diffusion method (Choi *and al.,* 2009). The bacteria to be tested were plated on petri dishes containing nutrient agar to obtain a young culture of the bacteria and isolated colonies. From these plates and with the help of a platinum loop, a few well isolated and perfectly identical colonies are taken and placed in 5ml of sterile physiological water. The bacterial suspension is well homogenised, and the optical density read at 625 nm is justified as (0.08 - 0.10). This density measured at 625 nm is assumed to be equivalent to 108 CFU/ml (Mohammedi, 2006). The inoculum is adjusted either by adding culture if it is too low or sterile physiological water if it is too high. Inoculation should take place within 15 minutes of inoculum preparation.

2.2.4.1.1. Preparation of phenolic extract concentrations

Dimethylsulphoxid (DMSO) is the preferred solvent for recovery of phenolic extracts recommended by most authors. It has been shown to have no potent inhibitory power (Toty *and al.,* 2013). Based on preliminary tests, one gram of solution with concentration ranging from 0.25mg /ml to 2mg/ml was prepared in DMSO. The discs are made from Wattman paper with a diameter of 6mm (0.28cm^2 surface area) by die cutting. Then, these discs are put in a test tube, autoclaved, and stored at room temperature (the test tube is hermetically sealed).

20 ml of the supercooled nutrient agar is poured into Petri dishes. After solidification of the culture medium, 100µ1 of the bacterial suspension to be tested is spread on the surface. The discs impregnated with the different

extracts, are then gently placed on the surface of the agar. The Petri dishes are first left for 1 h at room temperature for pre-diffusion of the substances, before being incubated at 37°C in the oven for 24 h (Mohammedi, 2006).

On removal from the oven, the absence of microbial growth is indicated by a translucent halo around the disc, identical to sterile agar, whose diameter is measured; (including the 6mm disc diameter). Antibacterial activity is determined by measuring the diameter of the inhibition zone around each disc.

2.2.4.1.2. Determination of the minimum inhibitory concentration

The minimum inhibitory concentration (MIC) is the lowest concentration for which there is no growth visible to the naked eye after an incubation time of 18 to 24 h. It was determined by observing the growth-induced cloudiness of the test germs in each tube (Toty *and al.,* 2013).

2.2.4.1.3. Determination of bacteriostatic and bactericidal activity

A sample from the zone of inhibition is transferred to a tube containing heart-brain broth which is then incubated at 37 °C for 18 hours. The tubes are examined with the naked eye, a cloudy medium indicates bacteriostatic activity; while a clear medium indicates bactericidal activity of the extract (Laouer *and al.,* 2003).

2.2.4.2 Testing for antifungal activity

2.2.4.2.1. Determination of the inhibition rate

Phenolic extracts solubilised in DMSO were successively diluted to 0.25-0.5-1-2 mg/ml. These concentrations were chosen on the basis of preliminary tests. 1 ml of each phenolic extract, of each concentration, is added to tubes containing 19 ml of sterile potato dextrose agar (PDA) medium, still liquid. The mixture is homogenised and brought to 45°C (Subrahmanyam *and al.,* 2001). It is then immediately poured into 90 mm petri dishes (20ml/plate) (Satish *and al.,* 2010). After agar solidification, the petri dishes are divided into two parts (corresponding to the number of strains to be tested) and inoculated with a mycelial disc, 6 mm in diameter taken from the young culture of the fungus. The PDA without extract served as a control for each strain (Khallil, 2009).

The final phenolic extract (CF) concentrations used were calculated from the following equation (Mohammedi, 2006).

$$CF = Ci/20 \quad \textbf{(4)}$$

With,

CF, final concentration of the phenolic extract in lml of PDA;

Ci, initial concentration of phenolic extract solubilised in DMSO.

The strain was incubated for 7 days at 30°C (Mohammedi, 2006). The percentage inhibition of mycelial growth, compared to the control, was calculated by the following formula ,

$$PI(\%) = (A-B)/A \times l00 \quad \textbf{(5)}$$

Where,

PI(%), Inhibition rate expressed as a percentage;

A, Diameter of colonies in the "positive control" dishes;

B, Diameter of colonies in the boxes containing the grain extract (Bajpai *and al.,* 2010).

2.2.4.2.2. Determination of the antifungal index

The concentration that inhibits 100% mycelial growth is expressed as the antifungal index (AI l00). The AI l00 values were calculated graphically, where the abscissa is represented by the concentration of the phenolic extract and the ordinate by the percentage of inhibition of mould growth (Chang *and al.,* 2008).

2.2.4.2.3. Liquid dilution method

This technique consists of two steps, the first to determine the minimum inhibitory concentrations (MICs) and the second to determine the fungicidal (FC) and fungistatic (FCS) concentrations.

2.2.4.2.4. Determination of minimum inhibitory concentrations

After sporulation of the selected fungal strain, the spores of the young culture are recovered by adding 10 ml of sterile distilled water under agitation (Solis-Pereira *and al.,* 1993). Afterwards, the absorbance of the fungal suspension is evaluated at 625 nm; in order to

standardise the spore suspension at 106 spores/ml (Hossain *and al.,* 2008). It is estimated that an absorbance between 0.08 and 0.1 corresponds to a concentration of l06 spores/ml (Braga *and al.,* 2007). Phenolic extracts from raw and cooked lentils, solubilised in DMSO, were added to potato dextrose broth (PDB) at a rate of lml in 9 ml. The PDB is then diluted successively beforehand to give the dilutions 0.25-0.5-1-2 mg/ml. These concentrations were chosen after preliminary tests.

A 10 µl spore suspension of the fungal strain to be tested was inoculated into test tubes containing PDB medium at different concentrations; these tubes are incubated for 7 days at 30°C. In parallel, one tube containing PDB medium was inoculated only with the fungal spore suspension, to serve as a control. The minimum concentrations for which no obvious growth was observed are defined as minimum inhibitory concentrations (Bajpai *and al.,* 2008).

2.2.4.2.5. Determination of fungicide and fungistatic concentrations

For the tubes in which no growth is observed, the experiment is continued in petri dishes. Each dish containing 20ml of sterile PDA is inoculated with 1 µl of each tube showing complete inhibition of fungal growth. Growth is monitored for 1 to 4 days at 30°C. When there is no growth resumption; the concentrations are called fungicidal (CF) (Zarrin *and al.,* 2010) and the concentrations for which growth occurs are called fungistatic (CFS) (Bajpai *and al.,* 2008).

2.5. Statistical processing

For each parameter, the means plus or minus the standard deviation of three trials as well as the graphical representations were carried out by Excel 2007. The results obtained were processed by analysis of variance (ANOVA) using the IBMSPSS2016 software at a significance level of 0.05.

3.Results and discussions

3.1. Contents of polyphenolic compounds

The contents of polyphenolic compounds in raw and cooked lentils are recorded in table 1.

Table 1. Polyphenolic compound contents of raw and cooked lentils

Polyphenolc compound	Raw lentils	Cooked lentils
total polyphenol (mg GAE/g)	2.53±0.2[a]	2.62±0.19[a]
Flavonoids (mg QAE/g)	1.40±0.024 [a]	0,13± 0.01 [b]
Anthocyans (g ECy-3-Glu/g)	4.08±0.11[a]	2.35±0.04[b]

Online, the same letter means no significant difference (p≤0 .05).

3.1.1. Total polyphenol content

It should be noted that cooking did not have a significant impact on the total polyphenol content obtained, despite a slight increase compared to the raw state. Referring to the bibliography, variable polyphenol contents in raw lentils have been reported. The values recorded in the present study are significantly different from those cited by Ladjal Ettoumi and Chibane (2015) (6.21 µg EAG mg -1 DM), Zhang and al. (2015)(5.04 - 7.02 µg GAE/mg DM for red lentils and between 4, 56 and 8.34 µg GAE mg-1 DM for green lentils) and those reported by Djabali *and al.* (2020) (95.51±4.01 and 172.36 ± 2.78 µg EP mg $^{-1}$DM the *Ibla* and *Metropole* lentil varieties respectively.

The higher amounts of polyphenols recorded after cooking may be due to a facilitated solubilisation and extraction of these compound after tissue embrittlement by the thrmic treatment. Dewanto *and al.* (2002) attribute this increase to the facilitated release of phenolic compounds previously bound to cellular constituents during cooking.

In addition to cooking, there are other factors that can influence the total polyphnol content. Zielinski (2002) explains that variation in phenolic content is attributed to many factors including genotype, agronomic practices, maturity level at harvest, storage locations and post-harvest climatic and geographical conditions. Similarly according to Hegedűsová (2015), the difference in total polyphenol content could be due to the ecotype, the geographical region where the plant grows and even the method of extraction of these compounds.

3.1.2. Flavonoid content

Raw lentils recorded a mean flavonoid content of 1.40±0.024 mg QAE/g. A significant decrease in the amount of flavonoids is marked after cooking. Indeed, referring to the literature, lentils are not only an excellent source of macronutrients, but also contain phytochemical compounds, which can be classified into phenolic acids, flavanols, flavonols, soyaponins, phytic acid and condensed tannins (Yanping *and al.,* 2012). Profound changes in phenolic equipment also occur when plant organs are subjected to technological processes to transform them (bleaching, cooking, etc.) (Macheix *and al.,* 2005).

Kebe (2014) explains these losses by a migration mechanism in the cooking water, mainly for cafeoylquinic acid and small molecules of flavan-3-ols.

3.1.3. Anthocyanin content

Cooked lentils had a significantly lower average anthocyanin content than raw lentils. Richardson and Finley (1985) report that temperatures above 100°C can cause the degradation of most of the anthocyanins.

The values obtained in the present study are different from those reported by Oomah *and al.* (2011) (0.20 mg ECy-3-Glu/g for red lentils). Through these results, it appears that the anthocyanin content varies significantly between varieties. Horbowics *and al*. (2008) add as other factors the extraction solvent, the extraction method, the standard used, the temperature and light, the cultivation methods (grafting, fertilisation,...) or more broadly the production methods (open field, soilless,...).

3.2. Total antioxidant activity

The total antioxidant activity values recorded for raw and cooked lentils are significantly different. They are equivalent to 9±0.24 mg AAE/ g DM and 11.67±0.088 mg AAE/ g DM respectively. Cooking positively affected the antioxidant activity.

Our results are in agreement with those of Gharachorloo *and al.* (2012) who showed a significant increase in antioxidant activity of lentils after cooking.

Throughout the literature search, very large differences are noted regarding this correlation.

Some studies showed a good correlation between IC_{50} and polyphenol and flavonoid content, while other studies did not (Athamena *and al.*, 2010; Mariod *and al.,* 2010).

On the other hand, it is well established that antioxidant activity is positively correlated with the structure of polyphenols. Generally, polyphenols with a high number of hydroxyl groups present the highest antioxidant activity (Heim *and al.,* 2002) due to their power to donate more atoms to stabilize free radicals (Torres de pinedo *and al.,* 2007). Thus, the antioxidant effect is not only dose-dependent but also structure-dependent (Rodriguez-Bernaldo *and al.*, 2009).

3.3. Antimicrobial activity

3.3.1. Antibacterial activity

The results of the antibacterial activity are shown in Table 2.

From these results, it appears that the phenolic extracts of raw and cooked lentils have a good antibacterial activity against the four tested strains. On the other hand, whatever the bacterial strain considered, phenolic extracts are active when concentrated.

Several in vitro and in vivo studies have been focused on the evaluation of the antimicrobial properties of phenolic extracts. At present, this effect is certain and has been demonstrated by numerous experimental studies. Studies of the inhibitory power of

flavonoids on bacterial growth have shown that many flavone compounds (apigenin, kaempferol and others) have a significant effect on different gram-negatif (*Escherichia coli)* and gram-positif (*Staphylococcus aureus*) bacterial strains (Ulanowska *and al.,* 2007).

Table 2. Diameters of the inhibition zones (mm).

Concentration of the phenolic extract (mg/ml)		Bacterial strains			
		E. coli	*P. aeroginosa*	*Lactobacillus*	*S. aureus*
Lentils cooked	2	24 ± 0.2	26 ± 1	25 ± 0.25	31.2 ± 0.12
	1	12 ± 0.52	24.5± 0.5	15 ± 1.2	2 0 ± 0.1
	0.5	11.5± 1	15 ± 1.5	12 ± 0.33	18.5 ± 0.5
	0.255	10 ± 2.1	7 ± 0.1	11 ± 1.4	15 ± 1
Lentils raw	2	22.5±0.25	32.5 ± 2.5	40 ± 0.5	21 ± 0.2
	1	11 ± 0.3	25.5 ± 1.5	27 ± 0.1	16.5 ± 1.5
	0.5	10 ± 1.2	23 ± 1	19 ± 0.1	12 ± 0.5
	0.255	9 ± 3	6.5 ± 0.3	12 ± 0.3	8 ± 0.2

Polyphenols, especially flavonoids and tannins, are known to be toxic to microorganisms. The mechanism of toxicity may be related to the inhibition of hydrolytic enzymes (proteases and carbohydrolases) or other interactions to inactivate microbial adhesins, transport and cell envelope proteins (Cowan, 1999).

Table 3. Minimum inhibitory concentration

Strain bacterial	Extract phenolic	Concentration (mg/ml)					
		2	1	0.5	0.25	0.125	0.0625
E. coli	*Raw lentils*	-	-	-	CMI	+	+
	Cooked lentils	-	-	-	+	+	+
P. aeroginosa	*Raw lentils*	-	-	CMI	+	+	+
	Cooked lentils	-	-	CMI	+	+	+
L. aureus	*Raw lentils*	-	-	-	-	CMI	+
	Cooked lentils	-	-	-	CMI	+	+
S. aureus	*Raw lentils*	-	-	-	-	CMI	+
	Cooked lentils	-	-	-	CMI	+	+

(+), cloudy, (-) does not change.

3.3.1.1. Minimum inhibitory concentration

Table 3 shows the results of the MIC estimation. It can be seen that the phenolic extracts have a good susceptibility against all bacterial strains following the recorded MIC values (0.125- 0.5mg/ml)

From these results we can see that all strains were completely inhibited at 0.5mg/ml, however at 0.0625mg/ml all strains were resistant.

3.3.1.2. Determination of bacteriostatic and bactericidal activity

The results of the bacteriostatic and bactericidal effect are shown in Table 4.

These results show that the effect of the different phenolic extracts on the different strains is bacteriostatic. On the other hand, it is bactericidal for high concentrations which shows a dose-response antibacterial activity.

Table 4. Bacteriostatic and bactericidal concentrations

Strain bacterial	Extract phenolic	Concentration of phenolic extract (mg /ml)			
		2	1	0.5	0.25
E. coli	Raw lentils	-	+	+	+
	Cooked lentils	-	+	+	+
P. aeroginosa	Raw lentils	+	+	+	+
	Cooked lentils	+	+	+	+
Lactobacillus	Raw lentils	-	-	+	+
	Cooked lentils	-	+	+	+
S. aureus	Raw lentils	-	-	+	+
	Cooked lentils	-	+	+	+

(+), cloudy, (-) does not change.

3.3.2. Evaluation of the antifungal activity of polyphenolic extracts

3.3.2.1. Determination of the inhibition rate

The measurement of the inhibition rates (%) allowed us to classify the fungal strains according to their degree of sensitivity to each concentration tested in figures 02 and 03.

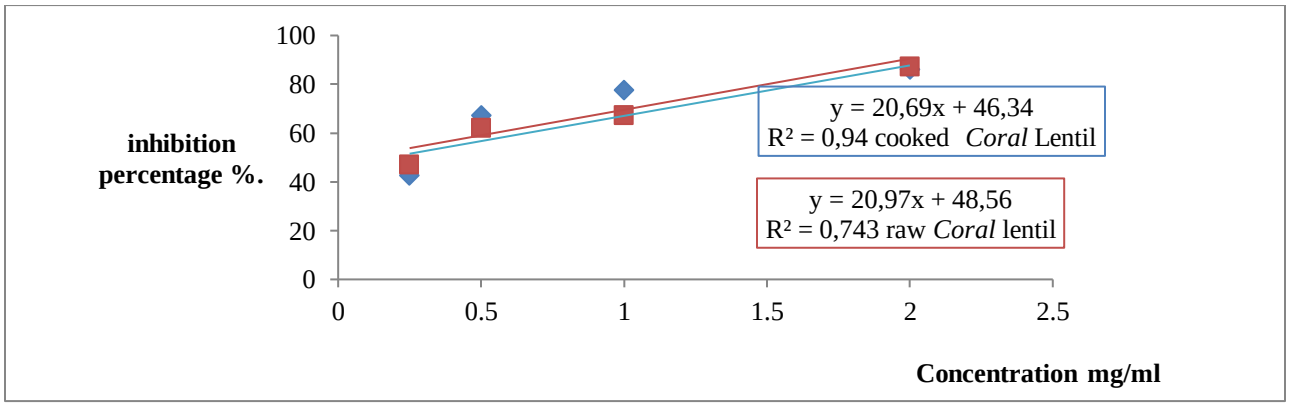

Figure 2. Inhibition rates of phenolic extracts of cooked and raw lentils against *Alternaria sp.*

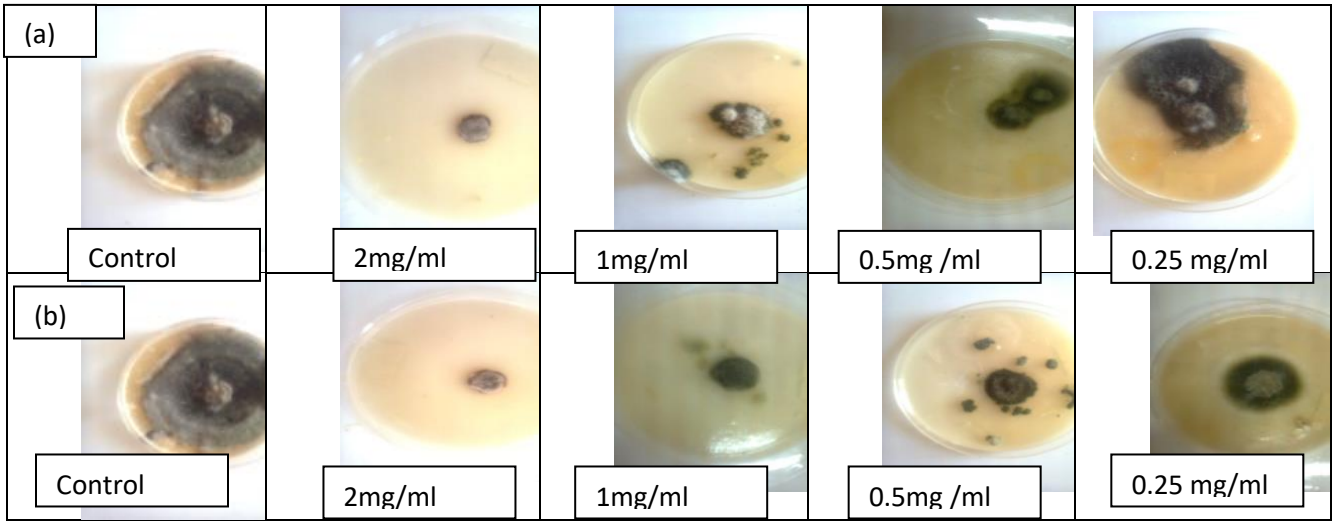

Figure 3. Inhibition effect of phenolic extracts against *Alternaria sp*. strain (a), Cooked lentils, (b), Raw lentils

The phenolic extract of raw coral lentil was found to be very active; at the concentration of 2mg/ml and at the concentration of 1mg/ml for the phenolic extract of cooked coral lentil. Both phenolic extracts were found to be active at concentrations of 1mg/ml and 0.5mg/ml (inhibition rate 50-74%).

The two phenolic extracts at a concentration of 0.25mg/ml are moderately active (inhibition rate 25- 49%).

The *Penicillium sp* strain has a random growth which prevented us from calculating their inhibition rates, the results obtained are shown in Figure 4.

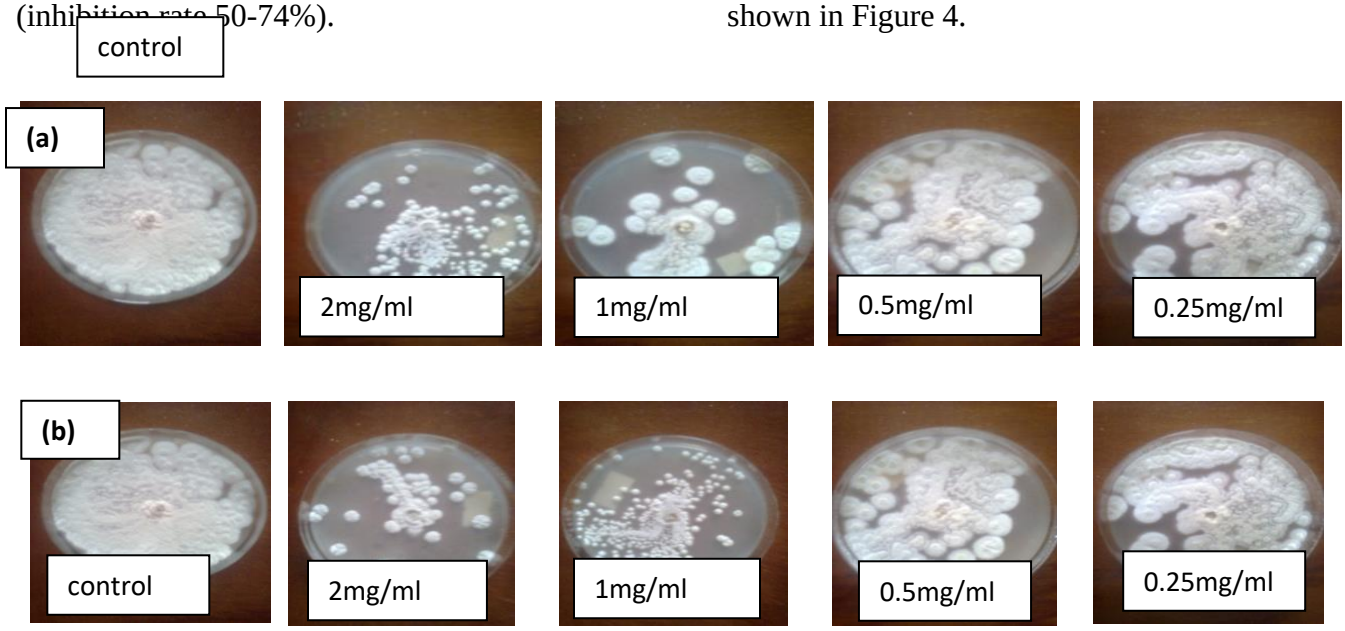

Figure 4. Inhibition effect of phenolic extracts on *Penicillium sp.* (a), Cooked *Coral* Lentil (b), Raw *Coral* Lentil.

3.3.2.2. Determination of the antifungal index (AI_{100})

The IA_{100} determined graphically are summarised in Table 5. The *Alternaria sp* strain tested does not have the same IA_{100}, it varies according to the variety and the concentration of the phenolic extract used. The highest IA_{100}

value is marked for the phenolic extract of cooked lentils. The IA_{100} of the strains belonging to the genus *Penicillium* was not determined because of the dispersion of the spores, therefore we could not measure their diameters.

Table 5. Antifungal indexes (IA100) of phenolic extracts of lentils

Fungal strains	A I_{100} (mg/ml)	
	Phenolic extract cooked *Coral* lentil	Phenolic extract Coral lentil Raw
Pénicillium sp	ND	ND
Alternaria sp	*2.59*	*2.45*

ND, not determined

3.3.2.3. Minimum inhibitory concentrations (MIC)

The minimum inhibitory concentrations (MICs) of the lentil phenolic extracts in liquid medium are presented in Table 6. The phenolic extract of cooked lentils showed a MIC of 1mg/ml on both genera (*Alternaria sp* and *Penicillium*). The phenolic extract of raw lentils showed a MIC of 2mg/ml on the genus *Penicillum* sp and 0.5mg/ml on the genus *Alternaria sp.*

Table 6. Minimum inhibitory concentrations (MICs) of phenolic extracts from lentils

Strain	MIC of *Coral* lentil phenolic extracts (mg /ml)	
	cooked	raw
Pénicillum sp	1	2
Alternaria sp	1	0.5

3.3.2.4. Fungistatic and fungicidal concentrations

The fungistatic (CFS) and fungicidal (CF) activities are presented in Table 07.

Table 07. Fungistatic (CFS) and fungicidal (CF) activities in mg/ml of lentil phenolic extracts on both strains.

Strain	Phenolic extract cooked *Coral* lentil		Phenolic extract of raw *Coral* lentil	
	(CFS)	(CF)	(CFS)	(CF)
Alternaria ,sp	1	2	0.5	2
Penicillium,sp	1	ND	2	ND

ND, not determined

The phenolic extract of cooked lentils revealed fungistatic activities on both strains at 1mg/ml and fungicidal activity on *Penicillium sp.* at 2mg/ml.

Regarding the phenolic extract of raw lentils, subcultures revealed both fungicidal and fungistatic activity on *Alternaria sp.* (CFS=0.5mg/ml and CF=2 mg/ml) and fungistatic activity on *Penicillium sp.* at 2mg/ml.

Benmeddour *and al.* (2014) reported that the inhibitory action on moulds may be due to the formation of hydrogen bonds between the hydroxyl group of the phenolic compounds and the active sites of target enzymes. Cushnie and Lamb (2005) found that phenolic compounds showed inhibitory activity against *Penicillium digitatum* and *Penicillium italicum.*

According to Vicente *and al*. (2003), natural compounds are classified according to their mechanisms of action, covering inhibitors of cell wall component synthesis, lipid synthesis and protein synthesis.

4. Conclusions

Polyphenols are compounds of interest, the content of which is highly influenced by internal and external factors, including heat treatment. In this study, the applied heat treatment had a different influence on the content of polyphenols, flavonoids and anthocyanins. The antibacterial activity of the phenolic extract against four bacterial strains was demonstrated by the disk diffusion method. The zones of inhibition indicate that all strains are sensitive to phenolic extracts. The MICs obtained indicate that the phenolic extract showed good susceptibility against all bacterial strains. For antifungal activity, the measurement of inhibition rates (%) allowed us to classify the fungal strains according to their degree of sensitivity to each concentration tested. The MICs obtained vary according to the fungal genera. The subcultures carried out after obtaining the MICs made it possible to observe varied activities of phenolic extracts on the two strains.

It is desirable to continue this study with the quantitative and qualitative characterisation of the polyphenolic profile, the use of other extraction methods, the study of the effect of other cooking methods in order to draw more reliable conclusions.

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