Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(4):206-212

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

The Effect of Polarity of Solvent on the Polyphenol Content and on their Antioxidant and Antibacterial Activity of Meals of the Grains of *Nigella Sativa* L

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ABSTRACT

Polyphenols are bioactive molecules exhibiting a lot of scientific attention due to their multiple biological activities. In this study we compared the phenolic contents, the antioxidant activity and antibacterial activity of the Nigella Sativa cakes using three solvent system of different polarity. Polyphenolic extractions of the dried powder samples were performed using three systems solvents 50%, 60%, 80% methanol. The phenolic content, analyzed using Folin-Ciocalteu, samples ranged from 0,9918 ± 0,0576 to 1,7918 ± 0,0785 mg/ g dry weight, expressed as gallic acid equivalents (GAE). The concentrations of total flavonoids, detected using 2% aluminum chloride ranged from 0,2220 ± 0,0269 to 0,4423 ± 0,0092 mg/g of catéchine equivalent (mgEC) dry weight. The Evaluation of antioxidant power and reducing power was performed using the method of DPPH free radical scavenging And the phosphomolybdat assay. The extract phenolic of the fruit having a moderate antimicrobial activity against reference strains of pathogens (Staphylococcus aureus and Pseudomonas aeruginosa) with a zone of inhibition in the range of 8.36 to 9.32 mm. In addition, most phenolic extracts inhibits the growth of pathogens used in this study.

Keywords: Polyphénols; Flavonoids; Antioxidant activity; *Nigella Sativa* L.; Inhibition

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INTRODUCTION

Numerous studies have highlighted the role of oxidative phenomena in Initiation of diseases as diverse as arteriosclerosis, inflammatory disorders, heart, lung, cancers, and the aging process. Such diseases occur when the defense mechanisms against free radicals, which have the organization, are submerged. It is therefore necessary, at this moment, to help the body fight against these attacks. The use of grains or plants for their healing powers was created, transmitted and responded in the oldest known civilization. This is one of the manifestations of stress immemorial man to understand and use nature, responding to concerns of its oldest, the one born of sickness and suffering [1]. The consumer is looking for a healthy, natural food and prefers foods containing no synthetic additives. The plant extracts that provide an interesting antioxidant power are mostly rich in polyphenols [2]. However, these substances have anti-radicaux free activity, which is expressed both in terms of the protection of a food against oxidation than at protecting animal cells against aging and cancer However, these substances have anti -radicaux free activity, which is expressed both in terms of the protection of a food against oxidation than at protecting animal cells against aging and cancer [3]. *Nigella Sativa* is one of the most commonly used medicinal plants worldwide. The extracts from the seeds of this plant are widely used in traditional medicine for centuries against a multitude of ailments, including as an antidiabetic, antihypertensive and anti-inflammatory. Recent studies highlight promising and extraordinary healing properties (antitumor, hypoglycemic immunostimulatory anti-inflammatory and antioxidant [4]. In this work we tried to study the antioxidant and antibacterial activity of the methanolic extracts of the *Nigella Sativa* cake and to study the influence of the polarity of the solvent on this activity.

EXPERIMENTAL SECTION

Chemicals

Acide ascorbique (vitamine C) and sodium carbonate and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma. Folin–Ciocalteu reagent and sodium sulfate were purchased ammonium sulfate and galic acid were purchased from Aldrich. Authentic standards of phenolic compounds were purchased from Sigma.

Plant Material

Seeds constitute the part of the plant used in this study. They were cultured in the arabi saoudit the seeds were ground in a blender to obtain a powder.

Preparation of Methanolic Extracts

In a beaker we macerated 5 g dry plant material well crushed seed meal of *nigelle sativa* L in the presence of 100 mL of different solvent systems (methanol / water: 50/50, 60/40, 80/20) for 72 hours. The mixture is filtered and the three extracts are evaporated under reduced pressure at 40°C to remove the alcohol (methanol), the aqueous phase obtained is washed three times with V (1/2) of hexane of 5 ml, for the removal of chlorophyll and carotenoid pigments and all polar products. A volume of 2 ml of ammonium sulphate (20%) and 2 ml of orthophosphoric acid (2%) are added to the aqueous phase. The polyphenols are extracted with ethyl acetate in a ratio of 1: 1 (v/v). The extraction is repeated three times for the mixtures respectively. The organic phase is dried by adding a sufficient amount of anhydrous sodium sulfate. The solvent is evaporated to dryness using a rotary evaporator (Büchi Rotavapor R-200). The residue is taken up in 10 ml of methanol and kept in a cool place.

Determination of Total Phenolic Content

The total phenol was assayed by a suitable method of Singlton and Ross (1965) with the Folin-Ciocaltu reagent [5]. A volume of 100 µl of each extract (diluted in methanol) was added to 500 µl of Folin-Ciocaltu reagent (10%). The solutions were mixed after 2 minutes and then 2 ml of the sodium carbonate solution Na_2CO_3 (2%) were added. The mixture is incubated for 30 minutes in the dark at room temperature. The absorbance of all the extracts was measured by the spectrometer (UV-Visible) at 760 nm.

Dosage of Flavonoids

The method of aluminum trichloride (AlCl3) is used to quantify the flavonoids in our extracts. Using catechin as standard, the flavonoid contents are expressed in milligrams of catechin equivalents (mg EC) per gram of dry matter [6]. A 1 ml volume of each extract and standard is dissolved in methanol with suitable dilutions added to an equal volume of an AlCl3 solution (2% in methanol). The mixture was stirred vigorously and then reading by spectrophotometry was carried out at 430 nm after 15 minutes of incubation.

Evaluation of Antioxidant Activity

DPPH Radical scavenging activity:

This test is carried out on all methanolic extracts. An aliquot of 100 µL of the sample at different concentrations. Are added to 1.9 mL of the DPPH solution (0.033 g/l). A negative control was prepared by mixing 100 µL of methanol with 1.9 mL Of the DPPH solution the reading of the absorbance is made against a white at 517 nm after 30 min Incubation in the dark and at room temperature. We used vitamin C (ascorbic acid) as a reference; the assay is repeated 3 times. The results are expressed as percent inhibition I% [7].

$$\mathbf{IP\% = [(Abs_0 - Abs_E) / Abs_0] \times 100}$$

IP %: Inhibition power

Abs_0: the absorbance of the control

Abs_E: the DPPH absorbance in the presence of the test compound at different concentrations after 30 min incubation.

The values of the necessary inhibitory concentrations for the reduction of 50 mol% of the radical Free DPPH (IC50) are calculated graphically (Figure 1) [8].

Phosphomolybdate Assay

The total antioxidant activities of various fractions of plant were evaluated by phosphomolybden complex formation method [9]. Briefly, A volume of 200 µl of each diluted extract is added to 2 ml of solution prepared (0.6 mol/L sulphuric acid, 28 mmol/L sodium phosphate and 4 mmol/L ammoniummolybdate) in sample vials. The blank solution contained 2 mL of reagent solution. The vials were capped and incubated in water bath at 95°C for 90 min. After the samples had been cooled to room temperature, the absorbance of mixture was measured at 695 nm against blank. The antioxidant activity was expressed relative to that of acid ascorbic. All determinations were assayed in triplicate and mean values were calculated.

Antibacterial Activity

Microbial strains tested:

The microbial strains used are *Staphylococcus aureus*, *Pseudomonas aeruginosa*.

Antimicrobial power study technique:

We assessed the susceptibility of these germs to Methanolic extracts of the tufts of the seeds of *Nigella Sativa* by the technique of antibio-aromatogram (Vincent's method) [10]. The culture medium used is Mueller-Hinton agar. The medium is poured on the whole surface of the box one faith the boxes are prepared in realizes Antimicrobial screening. The methanolic extracts are deposited in 3 ml volume on sterile Whatman paper discs 6 mm in diameter and have the places on the seeded agar plates. The sets are incubated at 35°C for 24 h [11].

Statistical Analysis

All manipulations were performed in triplicate and the results are expressed on average ± SD using microsoft Excel 2007.

RESULTS AND DISCUSSIONS

Quantification of Total Polyphenols and Flavonoids

The results obtained for the determination of polyphenols and flavonoids are reported in Table 1. Values are the means of three repetitions plus or minus the standard deviation. The Folin-Ciocalteu reagent consists of a mixture of Phosphotungstic acid (H3PW12O40) and phospho-molybdic acid (H3PM012O40). It is reduced during the oxidation of phenols to a mixture of blue oxides of tungsten and molybdenum [12]. The color produced is proportional to the amount of polyphenols present in the plant extracts. From the results obtained (Table 1), we observed that the studied plant exhibited high estimated polyphenol contents in the studied part (tangles of the seeds) and recorded the best value (1.7918 ± 0.0785 Mg EAG/g MS) in the case of the 60/40 methanol/water system (Figure 1). The flavonoid assay was carried out using the aluminum trichloride colorimetric method. Aluminum trichloride forms a yellow complex with flavonoids which absorbs in the visible at 430 nm. We noticed a slightly low content of falavonoids. The concentration of flavonoids in the toroux extracts of the *Nigella Sativa* seeds ranged from 0.4423 ± 0.0092 to 0.4423 ± 0.0092 mg EC/g. The methanolic extract (60/40) containing the highest concentration of flavonoid (Figure 2). The lowest concentration of flavonoids was measured in the solvent system methanol / water 80/20 (0.2220 ± 0.0269 mg EC/g dry matter). The concentration of flavonoids in the extracts of the plant depends on the polarity of the solvents used in the preparation of the extract [13]. The type of standard used may also change the results [14].

Table 1: Total polyphenol and flavonoid content in methanolic extracts of seed cakes of the *nigella sativa*

System solvant (méthanol/eau)	Polyphénol (mg EAG/ g MS)	Flavonoïdes (mg EC/ g MS)
50/50	0.9918 ± 0.0576	0.2399 ± 0.0207
60/40	1.7918 ± 0.0785	0.4423 ± 0.0092
80/20	1.3368 ± 0.0872	0.2220 ± 0.0269

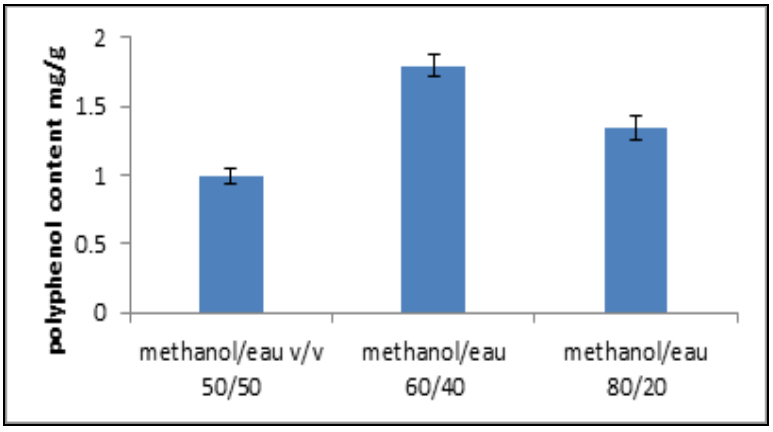

Figure 1: Comparison of total polyphenol content between methanol extracts of seed cakes of the *Nigella Sativa* L.

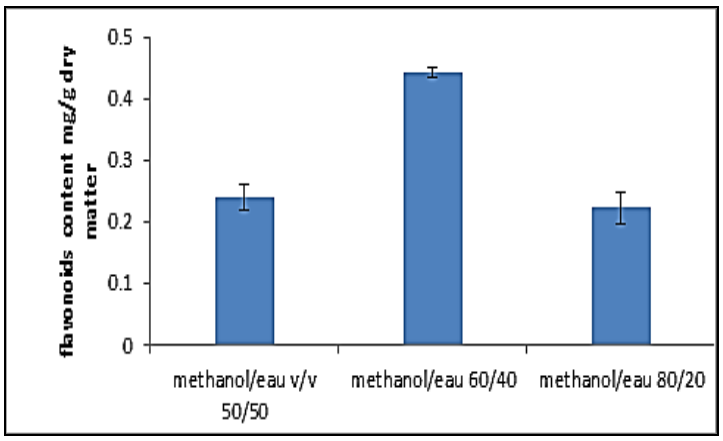

Figure 2: Comparison of the flavonoid content between the methanolic extracts of the cakes of the seeds of the *nigelle sativa* L.

Evaluation of Antioxidant Activity

DPPH Radical scavenging activity assay:

The antioxidant activity of a compound corresponds to its capacity to resist oxidation [15]. Many methods are currently used to evaluate this activity. The DPPH radical has been widely used for the study of the antiradical activity of the various natural extracts. The chemical compound 2,2-diphenyl-1-picrylhydrazyl was one of the first free radicals used to study the relationship Structure-antioxidant activity of phenolic compounds [16]. The absorbance was measured by spectrophotometry at 514 nm. From the values obtained, we calculated the inhibition percentages using the previously given formula. The values obtained made it possible to plot the curves represented in Figures 3-5, which show the variation of the percentage inhibition as a function of the concentrations of our extracts. The concentration corresponding to 50% inhibition (IC 50) was determined graphically. It is the ability to inhibit 50% of the free radical DPPH. The lower the value of IC50, the stronger the capacity for trapping the free radical of the extract tested [17] (Table 2).

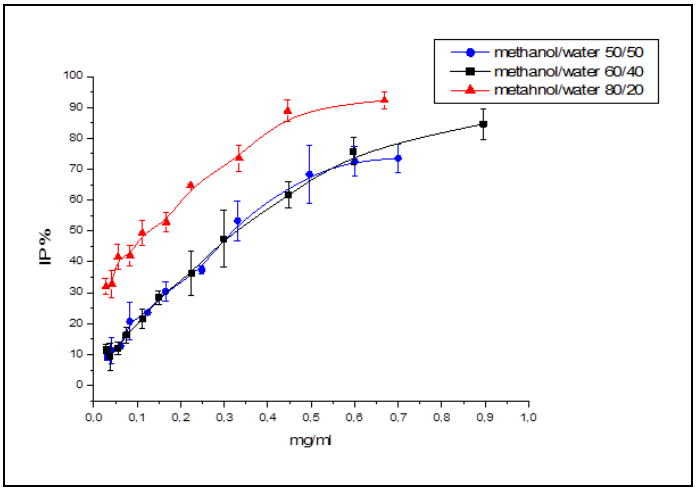

Figure 3: Variation of the inhibition power (IP%) as a function of the concentration of the methanol extract of the seed cakes of the *Nigella Sativa* L.

Table 2: The values of IC 50 in (µg/ml) of phenolic extracts

System solvant (méthanol/eau)	IC50 (µg/ml)
50/50	329.78 ± 15.69
60/40	516.42 ± 10.56
80/20	113.79 ± 12.85
Vitamine C	70.00 ± 2.58

From the results obtained we observed that the IC50 values varied from one extract to another my best activity Antioxidant is recorded in the case of the 80/20 methanol extract (IC50 = 113.79 ± 4.5 µg/ml), although the polyphenol content of the latter is the lowest compared to the other extracts. That is to say there is no correlation between the free radical scavenging capacities of the extracts measured by DPPH and the content of polyphenols; it depends on the nature of the molecule existing in these extracts of this part of the studied plant, unlike other studies that were done on other parts of the studied plant [18]. In general, the activity of the phenolic extracts studied is lower than that of vitamin C higher than other plants which have been studied by

other investigators [19]. It must be taken into consideration that the same phenolic compound mayHave different oxidation kinetics according to the test and the protocol used [20-22].

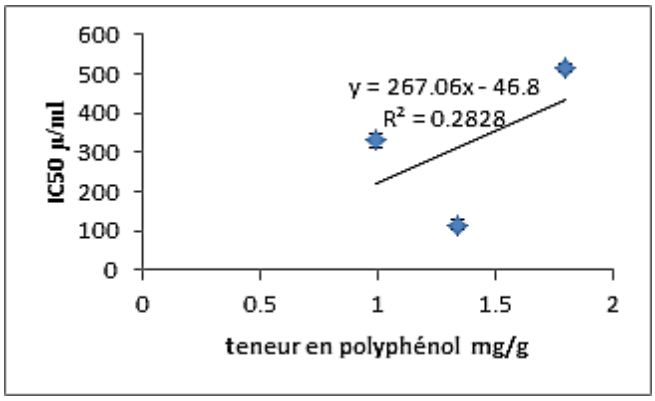

Figure 4: Negative correlation between DPPH radical trapping, expressed as IC50, and polyphenol content

Phosphomolybdate Assay

The evaluation of the antioxidant activity of our extracts is done with respect to the vitamin C used as antioxidant in the agri-food industry. It is necessary to demonstrate the effectiveness of this antioxidant of reference to this test. By plotting a calibration curve, we plotted the curves representing the variation of the reducing power expressed in absorbance as a function of the inverse of the number of dilution for each extract (Figures 6 and 7). From these graphs it is evident that for each extract when the concentration increases the antioxidant activity increases, the extract of the solvent system 80/20 represents the strongest reducing power compared to the other solvent systems studied. The results (Table 3) showed that the 80/20 extract represents the VCEAC value equal to 10.44 ± 1, 1358 And this is the highest value and that the other 50/50 and 60/40 systems represent the values of VCEAC 7,5433 ± 1,2661 and 8,72 ± 0,8554 successively. The phosphomolybdate method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and Formation of a Mo (V) green complex with Absorption at 690 nm [21].

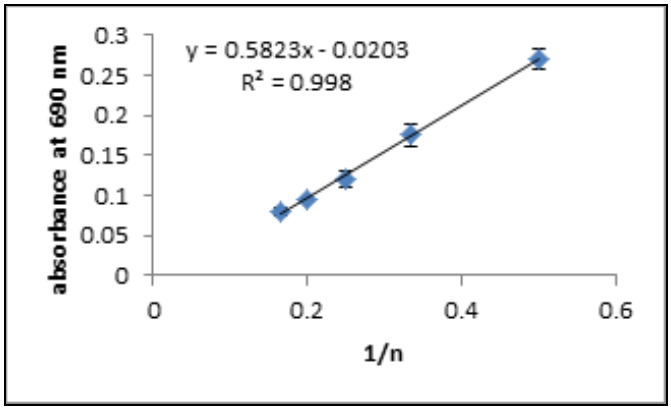

Figure 5: The variation of the absorbance as a function of the concentrations of the phenol extract of the 50/50 methanol/water solvent system in the phospholomolybdate test

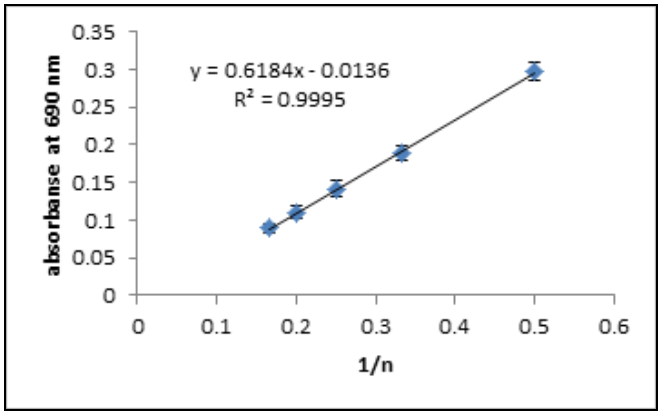

Figure 6: The variation of the absorbance as a function of the concentrations of the phenol extract of the 60/40 methanol/water solvent system in the phospholomolybdate test

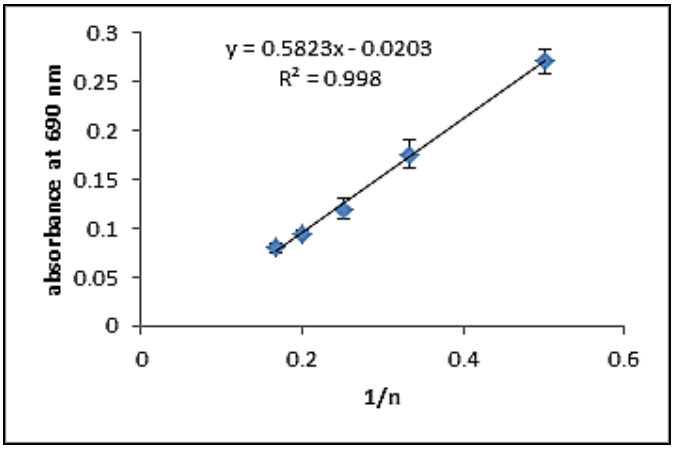

Figure 7: The variation of the absorbance as a function of the concentrations of the phenolic extract of the 80/20 methanol / water solvent system in the phospholomolybdate test

Table 3: The values of VCEAC of phenolic extracts

System solvant (méthanol/eau)	VCEAC (mM)
50/50	7.5433 ± 1.2661
60/40	8.72 ± 0.8554
80/20	10.44 ± 1.1358

Antibacterial Activities Assay

All discs are left dried for 24 h at room temperature before use. The results obtained from Table 4 show the high sensitivity of the *staphylococus aureus* bacteria to all the extracts tested. The radius of the antibacterial activity varies from (15.5 ± 1.5 mm) to (17.5 ± 1.5 mm) the best value of the sensitivity radius and recorded in the phenolic extracts of the 80/20 methanol / water solvent system. For *pseudomonas aeruginosa* bacteria which to a gram negative character the sensitivity is average it varies from (11 ± 1.2 mm) to (17.5 ± 1.4 mm). The average value recorded for the phenolic extracts of the 50/50 and 60/40 methanol/water solvent systems may be due to the nature of the external wall of the bacteria studied (*pseudomonas aeruginosa*). The results obtained in this test confirm the results obtained in the other chemical tests. It can be said that the antibacterial activity of the extracts does not depend on the polyphenol content, but depends on the nature of the molecules existing in the extracts.

Table 4: The zone of sensitivity of the bacteria to the methanol extracts expressed in (mm)

Gram	A bacterial strain	Sytsem solvent	Inhibition diameter [d]=[mm]
Gram(+)	***Staphylococcus aureus***	**50/50**	16.00 ± 2.3
		60/40	15.50 ± 1.5
		80/20	17.50 ± 1.5
Gram(-)	***Pseudomonas aeruginosa***	**50/50**	11.00 ± 1.2
		60/40	12.5 0 ± 2.3
		80/20	17.50 ± 1.4

CONCLUSION

In this work we studied the influence of the polarity of the solvent on the extraction of the polyphenol and the antioxidant and antibacterial activity of the latter. The results obtained show that the 60/40 methanol/water solvent system represents the best system for extracting the best amount of polyphenol (1.7918 ± 0.0785) and falvonoids (0.4423 ± 0.0092). (1.7918 ± 0.0785) and falvonoids (0.4423 ± 0.0092) DPPH and the phosphomolybdate reduction correspond to the phynolic extracts of the 80/20 solvent system. The same for antibacterial activity the inhibition effect of the bacterial strains of the extracts Phenolics of the cereals of the grains of the nigelle sativa is recorded in the system case Solvent 80/20 from these results it can be said that the Polarity of the solvent Influx on the content of polyphenols and the nature of polyphenol molecules extract.

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