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Antibacterial and Antioxidant activity of Nigella Sativa

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ABSTRACT: Nigella sativa. Known by the common name "black cumin" is a medicinal plant from the Ranunculaceae family. Widely used in traditional medicine and as a food condiment in the Arab world. Basing on the seeds of this plant.
The Nigella Sativa extract prevented bacterial growth; yet their efficiency remains different. The effect of Nigella sativa extract, on Streptococcus faecalis, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa by the method of aromatogramme, shows that these bacteria are inhibited.
The extract of Nigella generated a zone of inhibition of 30, 25, 20, and 10mm respectively, Streptococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli.
Evaluation of antioxidant power, which was conducted using the method of trapping the free radical DPPH (1,1-diphenyl-2-picrylhydrazyl), said the methanol extract showed an antioxidant activity (IC50 = 12 256 mg / ml), higher than that recorded in the ascorbic acid (IC50 = 0.097 mg / ml).

KEYWORDS: Extract; Nigella Sativa; Streptococcus faecalis; Escherichia coli; Staphylococcus aureus; Pseudomonas aeruginosa; antioxidant; IC50; aromatogram.

1 INTRODUCTION

The Nigella is a plant of high notoriety, especially across the Arab-Muslim world where, for several centuries, it is often referred to as a cure. It is a dicotyledon belonging to the family of Ranunculaceae, used for thousands of years, as a spice for food preservation as well as a protective and curative against several health troubles. Also, it is known to haves veral medicinal properties intraditional medicine (Chopra R and al., 1956)

This plant has been widely studied; accordingly, many favorable biological properties have been reported as an Antioxidant (Suboh, S.M and all., 2004), Antimutagenic (Bourgou S and al.,2008), Hepatoprotective (Kanter, M and al.,2005), Anti-corrosion (Emad M and al.,2015). Also, Anti-inflammatory effects (Khadera, M and al.,2010), Antidiabetic (Louriga A.,1995) and Anti-iflammatory (Mohamed EL and al., 2006).

The main objective of this study was to evaluate in vitro antibacterial and antioxidant activity of the methanol extract of the seeds of Nigella sativa as the trapping method of free radical DPPH.

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2 MATERIALS AND METHODS

PLANT MATERIAL

The seeds of Nigella sativa were brought from Saudi Arabia, They were crushed in a blender and the powder obtained was preserved later until time of use.

EXTRACTION OF ESSENTIAL OIL

The extraction of the oil of Nigella sativa was performed according to the method of Soxhlet. Their soluble compounds were brought through the methanol and the extracts obtained were concentrated in a rotavapor under vacuum at 70 ° C. Then, stored in 4 ° in the dark.

THE DISC DIFFUSION METHOD

ANTIBIOGRAM

The antibiotics tested were: Ampicillin, Erythromycin, Tetracycline and Gentamicin. To achieve antibiotic susceptibility testing by the disk method, the bacterial culture is seeded on the surface of specially designed agar ; Mueller-Hinton (MH). Pre-impregnated disks with a known dose of an antibiotic are placed on the surface of the agar (Positive control). The antibiotic diffuses from the disk by creating a concentration keeper. Later, we determine the diameter of the inhibition zone to deduce the sensitivity of the characters or the resistance of the bacterial strain.

AROMATOGRAM

The tests are performed by the method of Vincent (Aromatogram), It consists of depositing to the surface of the agar medium the oil impregnated filter paper discs which is essentially pure, Mueller Hinton for bacteria, in Petridish planted before hand by flooding. An impregnated disc solvent (negative control) was placed in each petridish, the boxes are incubated after filing discs for 24 hours at 37 ° C. The results are expressed by measuring the diameter of the inhibition halos, in millimeters (mm).

MIC AND MBC DETERMINATIONS OF BACTERIA TREATED WITH ESSENTIAL OIL

The technical dilution in medium was used for the determination of the minimal inhibitory concentrations (MIC) as well as the bacterial minimal concentrations.

Essential oil is dispersed in the presence of 0.2% agar according to the method described by (Remmal and al.,1993). About 10 % of pure essential oil emulsion, a variable volume depending on the desired final concentration is removed. Then, it was mixed with the washed bacterial suspension, which was treated. Next, it was placed immediately at 37°C in a bath water with magnetic stirring during the whole time of incubation.

EVALUATION OF ANTIOXIDANT ACTIVITY

To study the antioxidant activity, we opted for the reduction method of DPPH (1,1-diphenyl-2-picryl-hydrazyl) described by Sanchez-Moreno (Sanchez-Moreno C et al., 2002).

For this reason, solutions with increasing concentrations are prepared by the dilution of the extract of the mothers solutions for various extracts and ascorbic acid (0 to 0.15 mg / ml) used as a standard. The DPPH solution is prepared by solubilization 6 mg of DPPH in 200 ml of methanol.

The method comprises mixing, in a tube, 2 ml of the solution of DPPH freshly prepared with 100 ml of each solution. A negative control is prepared in parallel with methanol. The all is incubated in the dark for 30 minutes and the absorbance is measured at 517 nm.

The evaluation of the antioxidant activity is expressed as a percentage inhibition of the DPPH radical according to the following relationship (Djeridane A et al., 2006).

$$\% IP = (Abs_{blanc} - Abs_{échantillon}) Abs_{blanc} \times 100$$

Where **%IP** is inhibition percentage and **Abs** is the absorbance.

This formula allows to draw the straight line which represents the variation of percentage inhibition for the different concentrations of each sample (y = ax + b). Hence, it is possible to deduce the concentration that can reduce 50% of DPPH for the studied sample and ascorbic acid. This concentration which is generally referred to as IC50, is calculated using the following equation:

IC50= 50-ba

Where IC50 is the concentration required to reduces 50% of DPPH , « a » is the slope of straight and « b » is the intercept of the straight. The IC50 value was determined graphically by linear regression.

3 RESULTATS & DESCUSSION

ANTIMICROBIAL ACTIVITY

The extracts of the seeds of Nigella sativa have a broad inhibition spectrum towards the bacterials trains investigated. The essential oil has a high antimicrobial activity against Gram positive and Gram negative germs (Aljabre et al., 2005).

In our work the Nigella extract has produced a zone of inhibition of 30 ,25,20,10 mm, respectively on Streptococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli.

Tab 1: Diameters of the inhibition zones antibiotics and oil Nigela sativa (in mm)

	Antibiotic in (mm)				Extract in (mm)
Strains	**AMP**	**CN**	**TE**	**E**	**NS**
Escherichia coli	20S	25S	30S	10R	10
Pseudomonas aeruginosa MC1	36S	30S	37S	11R	25
Streptococcus feacalis	30S	24S	38S	30S	30
Staphylococcus aureus	39S	35S	34S	40S	20

Ampicilline(AMP), Gentamicine(CN), Tétracycline(TE), l'Erythromycine(E), Nigella sativa (NS)

The results are rated according to standards (R: resistant strain, S: susceptible strain, I: intermediate strain) (Robin et al., 2010).

Some studies indicate that Nigella sativa oil has a significant antimicrobial activity against Listeria monocytogenes. The effective inhibitor of the Nigella sativa oil on L. Monocytogenesis, twice more important than that of gentamicin. (Manoj et al., 2005).

Beside the antibacterial activities and antioxidant, the Nigella sativaoil, also, has an antiviral effect vis-a-vis the herpes viruses: murine cytomegalovirus (MCMV) (Salem, 2005).

The determination of MIC showed various levels of action according to the strain. In general, the pathogenic germs used in this bioassay were sensitive to the essential oil of Nigella sativa, with MIC ranging from 0.125% to 0.008 % contrary to MBC which vary between 0.250% and 0.015%.

Tab 2 :CMI and CMB of the methanol extract against microorganisms

Essential Oil / **Strains**	**MIC %**	***MBC %***
Escherichia Coli	0.125	0.250
Pseudomonas aeruginosa	0.063	0.125
Streptococcus feacalis	0.008	0.015
Staphylococcus aureus	0.031	0.063

Table 2 shows the minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of the antibiotics, of the essential oil on Streptococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli.

The table analysis shows that the MIC and MBC of bacteria towards the essential oil are much higher than those for bacteria in positive Gram and negative Gram. This difference can be explained only through the structural difference of the wall of these different categories of bacteria.

CHEMICAL ANALYZES

THE OUTPUT

The extract obtained from Nigela sativa is of greenish color with a strong odor, from Nigella sativa powder 60g to about 13.37% is obtained in the extractable organic matter by methanol.

THE ANTIOXIDANT ACTIVITY

The antioxidant activity of the methanol extract of Nigella Sativa and standard antioxidant (ascorbic acid) vis-a-vis the DPPH radical were measured using a spectrophotometer following the reduction of this radical. The latter is accompanied by its passage from the color violet (DPPH) to yellow (DPPH-H) measured at 517nm. This reduction in capacity is determined by a decrease in absorbance induced by anti-radical substances (Majhenic L et al., 2007).

The values obtained have allowed us to trace tests curves (absorbance in relation to concentrations) for ascorbic acid and extract Nigela Sativa. These curves, having an exponential shape, are represented by two linear portions: a descending line representing the reduction in DPPH radical and a horizontal one indicates that the reduction of DPPH was complete.

Figure 1 shows the DPPH reduction curve depending on the concentration of ascorbic acid and the table shows the IC50 of the extract of NigelaSativa. These results show that ascorbic acid has an anti-radical activity very powerful with IC50 (Djeridane A et al., 2006) equal to 0.097 mg / ml. This value is close to that obtained by Bentabet N. et al. Which is of the order of 0.08 mg / ml.

In comparison with the ascorbic acid, the essential oil has the lowest antioxidant power with IC50 = 12.256 mg/ml.

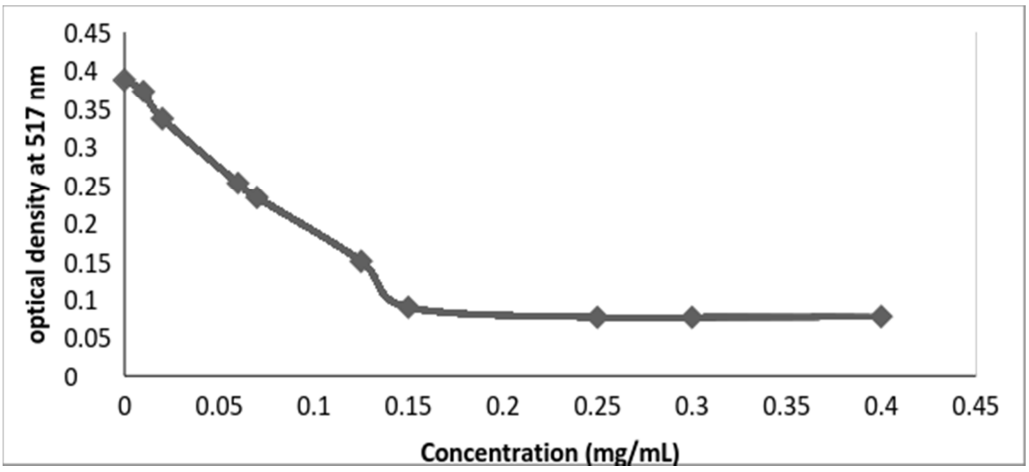

Fig1: DPPH reduction curve in relation to the concentration of ascorbicacid.

According to the results got, the methanol extract has a moderate antioxidant power, their EC50 is 12.255 mg / ml (Figure 4) which is much higher than that of ascorbic acid whose value is of the order 0.097 mg / ml (Figure 5).

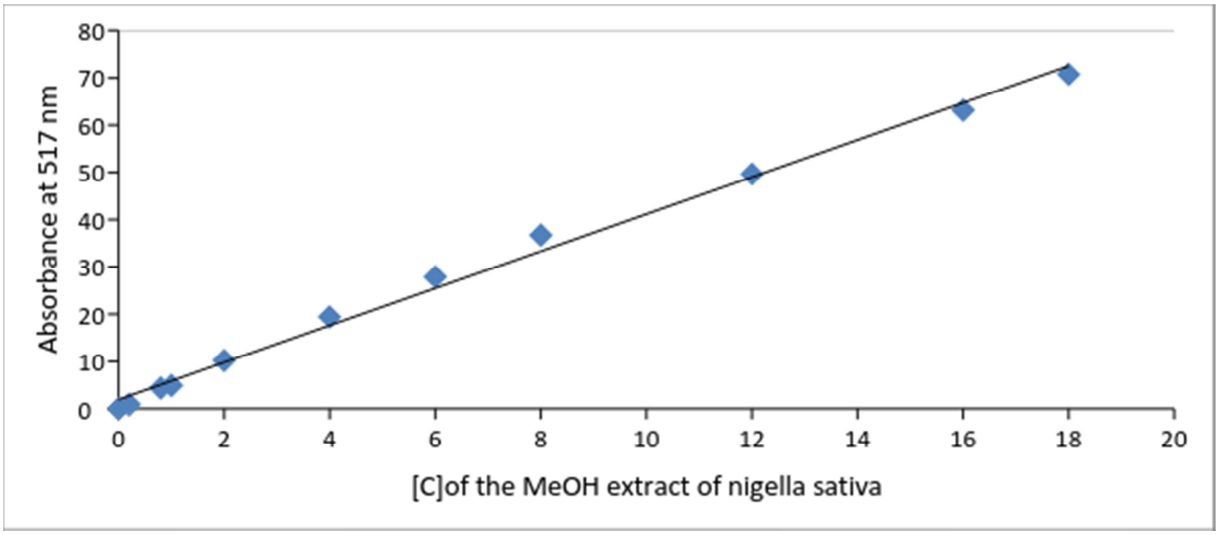

Fig2: The reducer Power of the methanol extract of the seeds of Nigella Satival.

Several studies on Nigella Sativa show that it is rich in phenolic compounds species. The latter is responsible for many biological activities including antioxidant activity. Also, it is anticancer and antimicrobial (Fabienne, O.L 2013).Antimicrobial activity.

4 CONCLUSION

The study of the antioxidant activity of the extract from the Nigella Sativa species, according to the method of entrapment of the free radical DPPH has showed that the methanol extract has a moderate antioxidant activity. This extract may, therefore, be an alternative to some synthetic additives.

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