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Editorial Introduction to the First Issue of Algerian Journal of Biosciences

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Thanks to God and his awareness, the first issue of the **Algerian Journal of Biosciences (AJB)** was released. In this issue, the journal (**AJB**) presents to researchers and the readership a selected and specialized research list that touches several areas of life science.

The main objective of the publication of **AJB** is to strengthen the national and international scientific field and to provide an opportunity for Algerian and world researchers to disseminate their research, ideas and scientific activities. Achieving a high level of development and exploiting available resources are yielding economic, medical and food returns that are beneficial to society and the state.

This Journal **(AJB)** is dedicated to the dissemination of research in the fields of biological, agricultural and environmental science, which has been revised and reviewed by experienced and knowledgeable editorial members, as well as linguistic and scientific scrutiny by members of the review committee in various fields of science. With a view to scientific advancement, outstanding research is being published as a new beacon for scholars in various scientific disciplines around the world. It calls on those who wish to publish their research to comply with the publication quality standards, and we hope that the numbers coming from the magazine will be wealthier.

Finally, I extend my sincere thanks to all the members of the editorial and review committee who have invested their efforts in issuing and improving the quality and success of this Journal (**AJB**).

Algerian Journal of Biosciences

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Original Article

Characterization and acute toxicity evaluation of the MgO Nanoparticles Synthesized from Aqueous Leaf Extract of *Ocimum basilicum L*

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ABSTRACT

The aim of this study was to prepare magnesium oxide nanoparticles (MgONPs) using aqueous leave extract of *Ocimum basilicum L.* and to evaluate their acute toxicity. The characteristics of biosynthesized MgO powder was analyzed by UV–Vis spectroscopy, scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). The acute toxicity test of MgONPs was applied in Wistar albino rats with different concentration. Results showed that the broad bell-shaped spectrum band was obtained by UV–Vis spectroscopy indicates the formation of MgO. The SEM images provided further insight into the shape and size of MgO which to be ranging under 440 nm. Fourier transform infrared (FTIR) spectroscopy detected the vibration of the Mg─O bond that indicate the presence of magnesium oxide nanoparticles (MgO). In this study, the toxicity test showed no mortality or behavioral change in low dose of MgNPs (250 mg / kg b.w) but we observed that 50% of rats have died when treated with high dose of MgNPs (500 mg/kg b.w.). This study confirmed that aqueous extract of *Ocimum basilicum L.* has potential properties as biocatalyst for the biosynthesis of MgONPs without any toxicity under dose 250 mg/kg in rats.

1. Introduction

Nanotechnology has been a known field of research since the last century [1], which has the potential to advance scientific innovation while giving an enormous advantage to society [2]. Information technology is an exciting new field in science, with many possible applications in the field of medicine [3]. Today's nanotechnology harnesses progress Current in chemistry, physics, materials science and biotechnology to create new materials that have unique properties because their structures are defined on the nanometer scale [4]. The biosynthesis for obtaining nanoparticles using naturally occurring reagents such as vitamins, sugars, plant extracts, biodegradable polymers, and microorganisms as reductants and capping agents could be considered attractive for nanotechnology [5]. Recently, increasing interest in nanotechnology applications in various fields. can be observed. Due to the increasing range of applications [6], metallic nanoparticles are of particular importance because they often exhibit volume-dependent properties that differ from bulk materials. The progress made in time is evident from the development in technology that has revealed the ability of minerals to perform specific functions better than the shape of metals [7]. The application of nanotechnology in biology requires further studies for the development of new materials in the nanosized range [8]. Magnesium (Mg) is an essential mineral component of plants and non-toxic to living organisms [9]. Magnesium nanoparticles have received the attention of most scientists due to their low cost, ecofriendly and due to their great therapeutic usefulness as anti-cancer and anti-microbial activity. [10]. Plants are an essential source of many active molecules

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[11]. *Ocimum basilicum L*. is a traditionally important medicinal plant belonging to the family *Lamiaceae* and also an annual herb which is grown around the world several regions [12]. The aim of this study was to use aqueous extract of leaves *Ocimum basilicum* in order to prepare MgNPs and to evaluate the acute toxicity of MgONPs.

2. Materials and Methods

2.1. Chemicals

Magnesium Nitrate, sodium hydroxide and Sodium chloride and Ethanol were obtained from Sigma Aldrich.

2.2. Plant materials

The plant of *Ocimum basilicum* were collected in October 2019 at the full flowering stage, from El Oued region, Algeria. The leaves were washed with distilled water, then dried at room temperature, then grind to powder and stored at room temperature until use.

2.3. Preparation of plant extract

Aqueous extract was preparing by putting 10 g of dried leaves powder of *Ocimum basilicum L* with 100 ml of distilled water was boiled over low heat (50 C°) for 2 hours. After cooled and macerated to room temperature for 24 hours, then filtered through Whatman filter paper, the extract was then evaporated using a rotary evaporator according to the methods described by Derouiche et al. [13]. Which was used for the synthesis of and magnesium nanoparticles.

2.4. Biosynthesis of magnesium oxide nanoparticles

5 g of Magnesium Nitrate (Mg(NO3)2 · 6 H2O) was added to the solution of plant extract and heated at 80°C with continuous stirring for 4 hours. The Magnesium nitrate ions were reduced to Magnesia or Magnesium Oxide nanoparticles by using *Ocimum basilicum* leaves extract. The formation of Magnesium oxide nanoparticles (MgONPs) have been observed by color change of the solution from yellow to yellowish-brown color [14].

2.5. Characterization of the Mg nanoparticles

The MgO Nanoparticles prepared by the above method was characterized using UVD 3200 UV-Vis spectrophotometer. Furthermore, the morphology and size of Nanoparticles (NPs) was determined using scanning electron microscopy (SEM). The Fourier transform infrared spectroscopy (FTIR) analysis of plant extract and biosynthesized magnesium oxide nanoparticles was recorded under identical conditions in the range 400–4000 cm−1 resolution using FTIR spectrophotometer (vector 22, Bruker, Germany).

2.6. Acute toxicity test of biosynthesized Mg NPs

The test was performed using 12 healthy albino male Wistar rats aged 10 weeks old, weighing 213.5 ± 9.31 g. Animals had free access to water and standard diet. After the adaptation period, the animals were divided into three groups of four rats in each and the test MgONPs was injected intraperitoneally at a doses 0, 250 and 500 mg/kg b.w. Animals were observed after dosing at least once during the first 30 min, periodically during the first 24 h as described in study of Kaouachi and Derouiche [15]

3. Results and Discussion

Our study reported that the addition of *O. basilicum* (Fig.1) in the synthesis of MgNPs induced to changes the color from yellow to yellowish-brown color indicating the formation of MgO. Due to phytochemicals compounds present in the aqueous extract of O. basilicum such as alkaloids, carbohydrates, tannins, phenolic compounds, flavonoids and terpenoid [16] which reduced magnesium nitrate to MgO and formed a colloidal solution.

Figure 1. Leaves of *Ocimum basilicum L.*

3.1. UV–Vis analysis

UV–Vis absorption spectrum of Mg NPs is shown in Figure 2 Broad bell-shaped spectrum band was obtained at the wavelength 300 nm from UV–Vis analysis, confirming the formation of MgO The optical properties of metal nanoparticles strongly depend on the size, shape and interaction between the particles present on the surface of the nanoparticles [17]. Mg NPs is reported to exhibit a broad absorption peak in between 260-330 nm [18]. Nanoscale MgO possesses unique optical, electronic,

magnetic, thermal, mechanical and chemical properties due to its characteristic structures [19]. Magnesium oxide (MgO) is a category of the practical semiconductor metal oxides, which is extensively used as catalyst and optical material [20]. Aqueous extracts of *Ocimum basilicum* leaf was reported to exhibit carbohydrate and proteins, amino acids at highest concentration [21]. Therefore, the phytochemicals from *O. basilicum* perhaps reduce the Magnesium nitrate into Magnesium oxide nanoparticles through the bioreductional process.

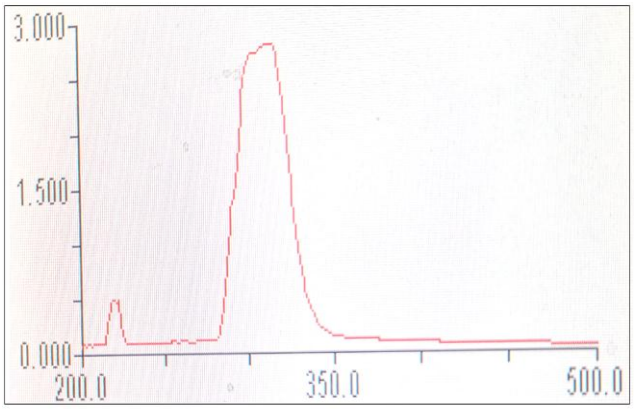

Figure 2. UV-Vis spectrum of MgO Nanoparticles

3.2. Scanning electron microscopy (SEM)

The result of Mg NPs size was showed through scanning electron microscopy (SEM) images (Figure 3). Scanning electron microscopy observation provided further insight into the shape and size of the synthesized nanoparticles [22]. In our results, SEM images indicate the size of some selected biosynthesized nanoparticles which was down to 440 nm this results according to the study of Sushma et al. [23], SEM analysis of MgO has showed the size of 50–400 nm with specific binding energies. The importance of determining the size of nanoparticles is that the MgNPs has a capacity for interaction with biological systems at the cellular level because the small size of nanomaterials favors their penetration into the cell. It is well established that nanomaterials have a greater capacity to penetrate cells [24,25].

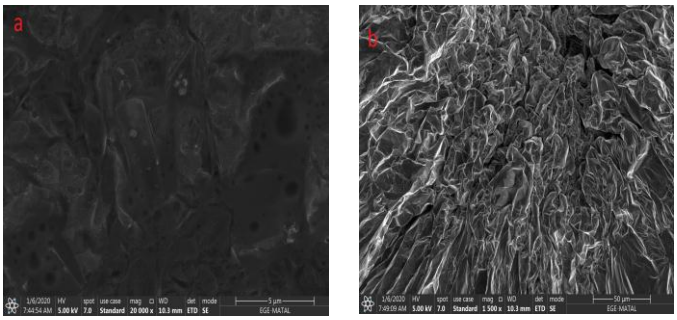

Figure 3. Scanning electron microscopy (SEM) of MgNPs measured in dimension 5µm (a) and in dimension 50µm (b)

3.3. Fourier infrared spectroscopy analysis

The functional group of MgO nanopowder was analyzed by FTIR spectrophotometer in the range 400–4000 cm-1 (Figure 4). FTIR spectra of the biosynthesized Mg NPs is shown a band 1644.80 cm-1 is ascribed to the stretching vibration of C=C in according with Solabomi et al., [10] that they found a band at 1633 cm-1. The peaks observed below 800 cm−1 confirmed the bond between magnesium and oxygen [26]. Also, the stretching vibration mode ~0600–850 cm−1 indicating Mg–O–Mg bonds [27]. Noori et al., (2019) found in their study that bands at 581 cm_1, 850 cm_1, and 890 cm_1 corresponded to stretching vibrations of the metal-oxygen bond, which corresponded to the presence of the MgO nanoparticles [28]. A broad band was observed ~3353 cm−1 due to O–H stretching vibration of water molecule which was in agreement with Balakrishnana et al., (2020) [27]. The prominent peak at 1382cm-1 is assigned to Mg–O vibration, almost the same result that we get a sharp peak on the wave number 1362.06 cm-1 [29].

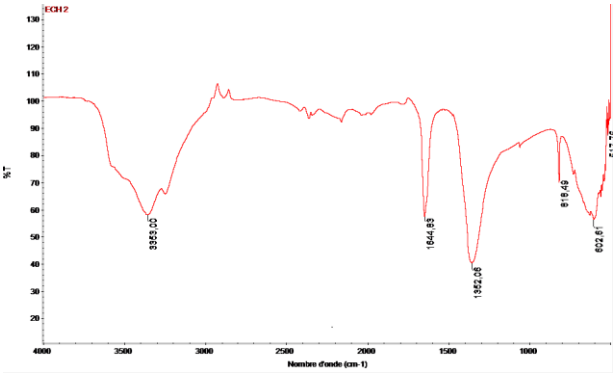

Figure 4. Infrared spectroscopy of magnesium nanoparticles

3.4. Acute toxicity test of biosynthesized Mg NPs

In this experiment the acute toxicity test was performed on albino Wister rats for 24 hours. Our magnesium nanoparticles were used with dose of 250 mg and 500 mg per kg of weight of rats. The results obtained during this test showed that no mortality was observed before 24 hours, which suggests the non-toxic effect of the magnesium nanoparticles at the law doses. The other physiological parameters of the rats were also determined during the experimental period and showed that treatment with the magnesium nanoparticles caused no symptoms or complications also no adverted effect in the rats during the treatment period in control group at dose (0 mg/ kg) and in the group at dose (250 mg/ kg), but in the group treated with dose of (500mg/ kg) we observed death rats in rate of 50% (table 1) which was in agreement with the study of Mazaheri et al., (2019) [30]. This study confirmed that Ocimum basilicum L. has a capability for the biosynthesis of Mg NPs. Moreover, the outcome of this in research determines the concentration of MgO effect and be appropriate for various applications, we found that treatment with the magnesium nanoparticles cause no toxic effect at low doses.

Table 1. Acute toxicity parameters of MgNPs in rats

Parameters	0 mg/ kg					250 mg/ kg					500 mg/ kg				
	0h	**3h**	**7h**	**14h**	**24h**	**0h**	**3h**	**7h**	**14h**	**24h**	**0h**	**3h**	**7h**	**14h**	**24h**
Death rats	0	0	0	0	0	0	0	0	0	0	0	50%	50%	50%	50%
Eyes	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Diarrhea	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N, Normal

Conclusion

This study proved the ability of *Ocimum basilicum L.* extract for the biosynthesis of Mg NPs which characterized by different methods; UV-VIS spectroscopy, FT-IR spectroscopy and SEM analysis. In addition, acute toxicity test assessment of the biosynthesized Mg NPs appeared its non-toxic effect especially when concentrations are low doses.

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Conflict of Interest

The authors declare that they have no conflict of interest

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Original Article

Evaluation of the anti-anemic activity of date syrup in Wistar rats

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ABSTRACT

The main objective of this study is to determine the effect of food intake of date syrup on the treatment of anemia.

First, we used 3 samples of dates from different regions for the preparation of the syrup. We have studied certain criteria (physical, chemical, biochemical, microbiological); where the results showed that the prepared date syrup has a good hygienic quality, and of remarkable nutritional quality due to its ideal content with many properties, such as carbohydrate and protein content.

To evaluate the anti-anemic properties of date syrup in rats, anemia is induced by a food having an iron deficiency. Date syrup, prepared from Djamaa dates, was administered by gavage of anemic rats at doses of 1000 mg / kg / day and 2000 mg / kg / day resulting in an increase in hemoglobin, red blood cell count, hematocrit and serum iron. Thanks to the results obtained, date syrup can be classified among the foods that help fight iron deficiency anemia.

1. Introduction

World production of dates is estimated at 7,416,000 tons. Algeria is one of the major date producers, occupying the fifth class in the world, the number of these trees is estimated at 13 million palms and 940 cultivars, with a total production estimated at 848,199 tonnes [**Error! Reference source not found.**]. Dates are the subject of significant commercial activity, especially the Deglet-Nour variety; it has a monopoly on national and international markets [2].

Indeed, the high content of sugar and nutrients, justify their use as raw materials in the manufacture of various food products with high added value such as date juice, jam, date syrup, date paste, date flour ... [3].

Date syrup is a product of high nutritional value; it is rich in carbohydrates, minerals, B vitamins, phenolic compounds and medium content of flavonoids. These antioxidants reduce the risk of degenerative diseases and certain types of cancer by reducing oxidative stress and inhibiting the oxidation of macromolecules. Given its richness in mineral salts, especially calcium and iron, date syrup can play an important role in the treatment of anemia [4].

Anemia is defined according to WHO as a pathological condition in which the hemoglobin content in the blood has become abnormally low following a deficiency of one or more essential nutrients. Anemia can be easily treated with a healthy diet. Although there are other types of anemia, which are serious and can also pose a threat to an individual's life [5].

Few studies have exploited the therapeutic effect of date syrup against different types of anemia, The present work aims to enhance a date by-product, with low market value, by manufacturing a syrup of biochemical and microbiological quality in accordance with standards; thus,

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use this syrup in the treatment of deficiency anemia.

2. Materials and Methods

2.1. Plant material, Collection and storage of samples

The plant material used in our study was the fruit of the date palm (*Phoenix dactylifera L*) of the variety "El Ghars". The dates were taken at the stage of complete maturation (Tamr stage). The choice of this variety is justified by its relative abundance on the national territory, taste quality and its wide consumption throughout the Algerian territory as well as its traditional therapeutic virtues, in particular represented by its richness in minerals.

The dates used in this work were harvested in October 2019 from three different palm groves in the Daïra of "Hassi Khalifa, El Oued and Djamaa" in El-Oued city; Algeria. In order to preserve the dates, we sorted the infested dates before packaging them in a plastic container until the air was expelled and then covered tightly; it is stored at room temperature. In this form, dates can be kept for three years [6].

2.2. Date syrup preparation

To have a good quality product, we have to start from a good quality raw material, so we started by sorting, washing and drying all the dates. We have carried out the following steps:

- ***Sorting:*** To eliminate of all immature dates, crushed dates or dates attacked by birds and insects. The sorting of dates was carried out entirely by hand [7].
- ***Washing:*** To remove soil particles, grains of sand, dust, plant debris, treatment products and pests. It was done with tap water. This is an essential step to have a product of good hygienic quality [7].
- ***Soaking:*** The dates were then subjected to drying by draining through a colander, followed by exposure to air and to room temperatures to remove excess water for one day [7].
- ***Pitting and cutting:*** To remove pits and done by hand in order not to interfere with the crushing process and to avoid damage by the pits. Dates were cut into small pieces to increase the contact surface with the water and to extract as much juice as possible [7].
- ***Juice extraction from dates:*** The extraction was carried out hot at a temperature of 85 C ° by the addition of 3 volumes of water (pH = 7.2) was used in one volume of date, mixing for half -hour after the mixture reaches the extraction temperature to facilitate the solubility of sugars in water by crushing the date pulp [4,8].
- ***Concentration of date juice:*** The concentration of the juice was carried out by direct heating at a temperature varying between 100 and 105 C ° to remove free water, for 2 hours [9].

2.3. Characterization of the hygienic quality of date syrups

The product obtained after extraction must undergo the following physicochemical, biochemical and microbiological analyzes:

- ✓ **Physicochemical parameters:** determination of pH, electrical conductivity, titratable acidity, soluble solids level, humidity level, ash content.
- ✓ **Biochemical parameters:** Determination of the total sugars content according to [10], of proteins according to [11].
- ✓ **Microbiological parameters:** in order to ensure that the prepared product has a hygienic quality which characterizes the risk to the health of the consumer and the commercial quality; namely the search and enumeration of: total mesophilic flora (Nutrient agar) at 37 ° C for 24 and 48 hours; total coliforms (VRBG agar) at 37^0C for 24 hours; enterobacteriaceae (Hektoen agar) at 37 ° C for 24 and 48 hours; staphylococcus (Chapman agar) at 37 ° C for 24 and 48 hours and yeasts and molds (Sabouraud agar) at 37 ^{0}C for 05 days.

2.4. In vivo study of the antianemia activity of date syrup

20 rats (adult male) were used in this experiment, their average body weight was 150 to ± 25g. During the period of this experiment, the rats were kept at a temperature of 25 ° C and a natural photoperiod, fed a standard well-balanced diet and drink tap water. They were treated in accordance with the principle set out in the manual on the care and use of experimental animals.

2.4.1. Induction of anemia in rats

After adaptation for 15 days, the rats were randomly assigned to the control group and the model groups with iron deficiency anemia. Five rats were randomly selected and given a normal diet as a control group. Others were given a low iron diet (coarse corn powder with an average iron content of 12mg / kg) for 4 weeks, with bleeding undertaken by puncturing the lateral tail veins three times a week (1, 0 - 1.5 ml of blood each time), to generate an animal model with iron deficiency anemia. While the rats in the control and model groups received deionized distilled water [12]. The rats were divided into 4 groups of 5 rats each, these are:

- ***Lot 01:*** non-anemic control;
- ***Lot 02:*** untreated anemic rats;
- ***Lot 03:*** anemic rats treated with date syrup at a dose of 1000 mg / kg of body mass per day by gavage, for 2 weeks;
- ***Lot 04:*** anemic rats treated with date syrup at a dose of 2000 mg / kg of body mass per day by gavage, for 2 weeks.

2.4.2. Blood sampling and determination of anemic parameters

At the end of the experimental protocol, the rats were fasted for 12 h before blood collection. Blood samples

were taken by puncture of the lateral tail veins and blood was collected in EDTA tubes and dry tubes, for the purpose of performing laboratory-level bioassays.

The haematological analysis was carried out directly (after one hour of sampling) in order to avoid the autolysis of the cells and to obtain reliable results. The hematological analysis was determined on an automatic counter model BC 2800. This device is intended for the automatic haematological analysis which gives information on white blood cells, red blood cells, platelets, hematocrit (HCT), hemoglobin (HB), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration, and mean corpuscular hemoglobin content (TCMH) [13].

3. Results and Discussion

3.1. Characterization of the hygienic quality of date syrups

3.1.1. Physicochemical and biochemical parameters of date syrup

The results of the physicochemical and biochemical analysis are presented in the following table

Table 01: Physicochemical and biochemical parameters of date syrup

Parameters	Date syrup		
	Hassi Khalifa average ± SD	El Oued average ± SD	Djamaa average ± SD
pH	5.06 ± 0.10	4.99±0.11	4.99±0.19
E.C µs/cm	4.63 ±0.50	3.81±0.48	5.53±0.58
titrable acidity g/100g	0.64 ±0.07	0.59±0.48	0.68±0.04
soluble solids level^0 Brix	59.75 ± 0.12	60±0.50	68±0.0
humidity %	44.22 ±0.2	42.8±0.27	36.36 ±0.25
Dry matter%	55.78 ±0.27	57.18±0.27	34.07 ± 0.25
ash content %	5.3 ± 0.36	5.3±0.36	10.2 ±0.24
Proteins %	3.40 ± 0.08	2.30±0.080	7.30 ± 0.06
Total sugar %	49 ± 0.32	42±0.128	27 ± 0.12

Our results indicate that the physicochemical and biochemical quality of different samples used are almost identical, these values relate to the variety (*Ghars*) from different sampling regions.

The analysis of the results obtained indicates that the measured pH values show a slight difference between the three samples of date syrups. These results are lower compared to those obtained by [14], for the Ghars variety (pH 5.64), and higher compared to those obtained by [15].

The highest electrical conductivity content of date syrup is observed at the level of Djamaa date syrup at 5.53 ± 0.58 µs / cm. These results are comparable to those obtained by [9], for date syrup from the Ghars variety after an extraction with concentration by direct heating which show values of 5.51 µS / cm.

The lowest water contents were 36.36 ± 0.25% of the Djamaa syrup, followed by the syrup of El Oued (42.82 ± 0.27%) and Hassi Khalifa (44.22 ± 0.20%). Our results are different with those found by [16,3]. The variations in humidity levels are probably due to extraction methods, climatic conditions, storage and the type of date varieties used [17].

The syrup dry matter is inversely related to the water content. These results showed the lowest content of DM are observed in Djamaa date syrups (34.07 ± 0.25%), compared to that of Hassi Khalifa date syrup (55.78 ± 0.27%)) and date syrup of El Oued (57.18 ± 0.27%). Our dry matter content values are lower than those of [3] (84%) and [15] (77.88%).

The ash content of our samples is respectively 10.2 ± 0.2%, 5.3 ± 0.36% and 4.5 ± 0.15% for the syrup from Djamaa, El Oued, Hassi Khalifa. Our results are comparable to those recorded by [3] (6.8%). Likewise, they are higher than those mentioned by [15] by 0.96% for the Ghars variety.

The results obtained concerning the protein level seem higher compared to those advanced by [3, 18, 19]. According to these authors, date syrup contains 0.83%, 2.2%, 1.09% of protein, respectively.

The means of the total sugars contents of the three samples show that the date syrup from Hassi Khalifa has the highest content (49 ± 0.0325%), The values are lower than the value found by [16] (74%) and [20] (73%), [3] (79.45%) and [21] (80%).

These differences in biochemical parameters may be due to various factors such as the type of variety, the growing conditions of the variety, its stage of maturity, its geographical origin, the type of soil and the storage conditions of dates [22].

So from our results about physicochemical and biochemical quality, we can say that our samples of date syrup from different regions contain a considerable amount of the essential elements which qualifies it as good quality and conforms to the standards and previous studies.

3.1.2. Microbiological parameters of date syrup

The results of the microbiological analysis of different samples of date syrup are summarized in the following figure.The colonies appearing in the Nutrient Agar medium give us an idea of the total flora contained in our samples. Date syrup from Djamaa dates represents the highest load (2.4 Log CFU / ml) compared to those from the region of El Oued and Hassi Khalifa. According to [23], the total mesophilic aerobic flora can contain the germs: *Staphylococcus, Streptococcus, Lactococcus, Corynobacterium, Bacillus, Pseudomonas* and Acro*mobacter*.

The analysis of the results obtained shows that the number of Enterobacteriaceae, cultivated in Hectoen medium, in date syrup from Hassi Khalifa, El Oued and Djamaa are respectively 1.54 log UFC / ml, 0.91 log UFC / ml and 1.56 log UFC / ml, which are lower than the standards recommended for dry products by the order of July 2017 relating to the microbiological specifications of certain foodstuffs: international standards (<103 CFU / ml).

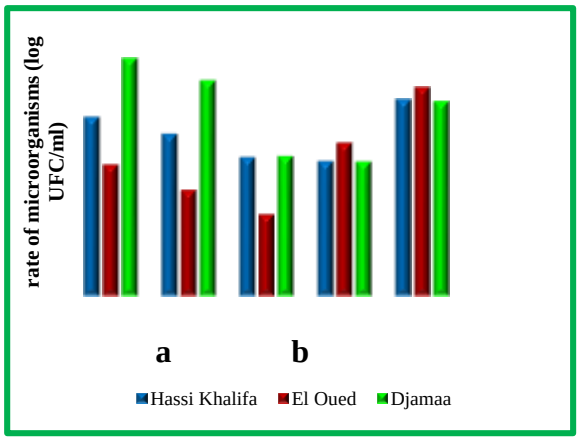

Figure 01: Enumeration of different groups in date syrups
a. Total mesophilic flora; b. Enterobacteriaceae; c. Staphylococcus; d. Yeasts and molds; e. Total coliforms

The results obtained from the research of *Staphylococcus aureus*, she specified that it is slightly present in El Oued syrup (0.91 log CFU / ml) compared to that of Hassi Khalifa syrup (0.54 log CFU / ml) and that of Djamaa (0.56 log CFU / ml). However, are lower than the international standards recommended for dry products (<103 CFU / ml). The other species of staphylococci generally produce smaller colonies, not causing the color change [24].

For total coliform bacteria, the analysis of the results shows that El Oued syrup contains 1.7 log CFU / ml and date syrup from Hassi Khalifa and Djamaa are 1.5 log CFU / ml, 1 which are lower than international standards (< 103 CFU / ml according to international standards).

The results of Yeasts and molds show 2.32 log CFU / ml (<104 CFU / ml according to the international standards recommended for dry products) in the sample of El OUED syrup, and 2.19 log CFU / ml for Hassi Khalifa syrup and 2.32 log CFU / ml in the sample from Djamaa.

The fermentation of lactose into lactic acid, due to the presence of coliforms in the VRBG medium, is revealed by a change in the color of the colonies to pink and brown caused by the precipitation of bile salts [24].

From the results obtained, it can be seen that the date syrup is of good hygienic quality. Our results are comparable to standards for concentrated products (3.103 for TMF and <103 for yeasts and molds).

These results can be explained by the physicochemical properties of date syrup rich in antioxidants (phenolic content is around mg / 100g Ms) which are responsible for the inhibition of the proliferation of these germs and by the pH which constitutes one of the main obstacles that the microbial flora must overcome to ensure its proliferation [9].

3.2. Invivo study of the antianemia activity of date syrup

The results relating to the determination of the haematological parameters (linked to anemia) of the rats treated in the present study are shown in the following figures (02 and 03).

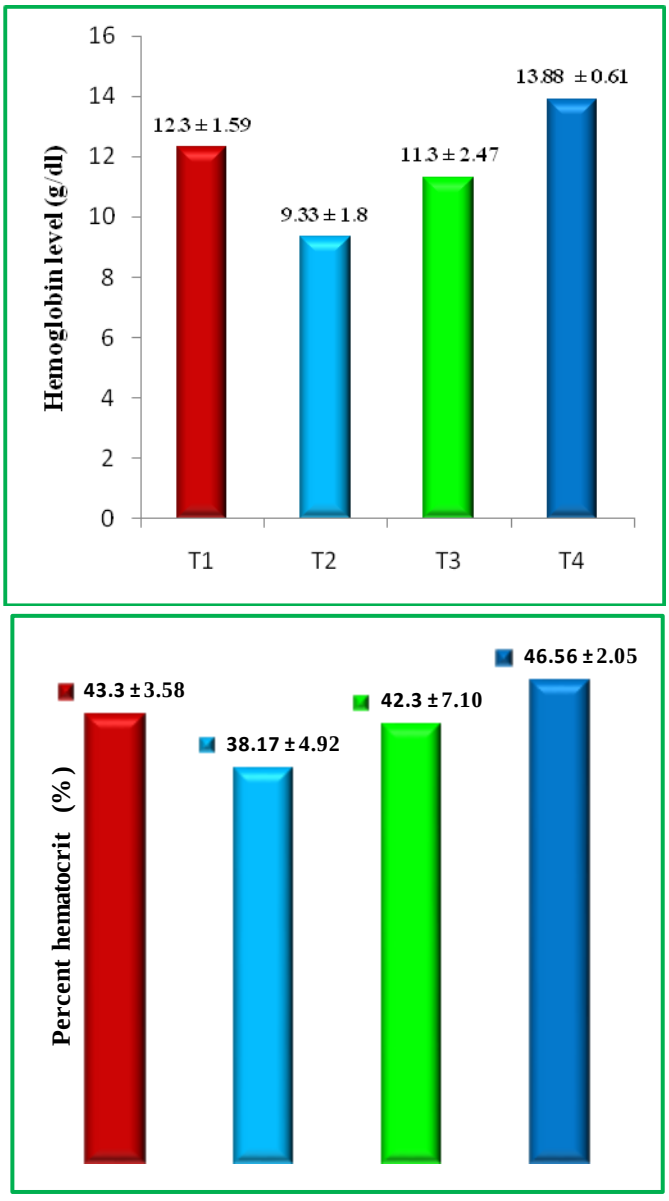

Figure 02: Hemoglobin level (g / dl) and Percentage of hematocrit (%) in the blood of various experienced rats White control rats (T1); Negative control rats (T2); Rats having received 1000 (T3) mg / Kg of date syrup, Rats having received 2000 mg / Kg of date syrup (T4).

Red blood cell, hemoglobin, and hematocrit levels were lower in anemic rats than in normal mice. The level of red blood cells, hemoglobin, and hematocrit increased significantly after consuming date syrup. The level of haematological parameters in the high dose group was significantly higher than in the 1000 mg / Kg / d group, which indicates that the high dose 2000 mg / Kg / d was more effective in relieving the symptoms of anemia. Our results are close to those of [12]; who observed a decrease in the number of hemoglobin and hematocrit with the administration of an iron deficient diet and bleeding for 4 weeks in rats.

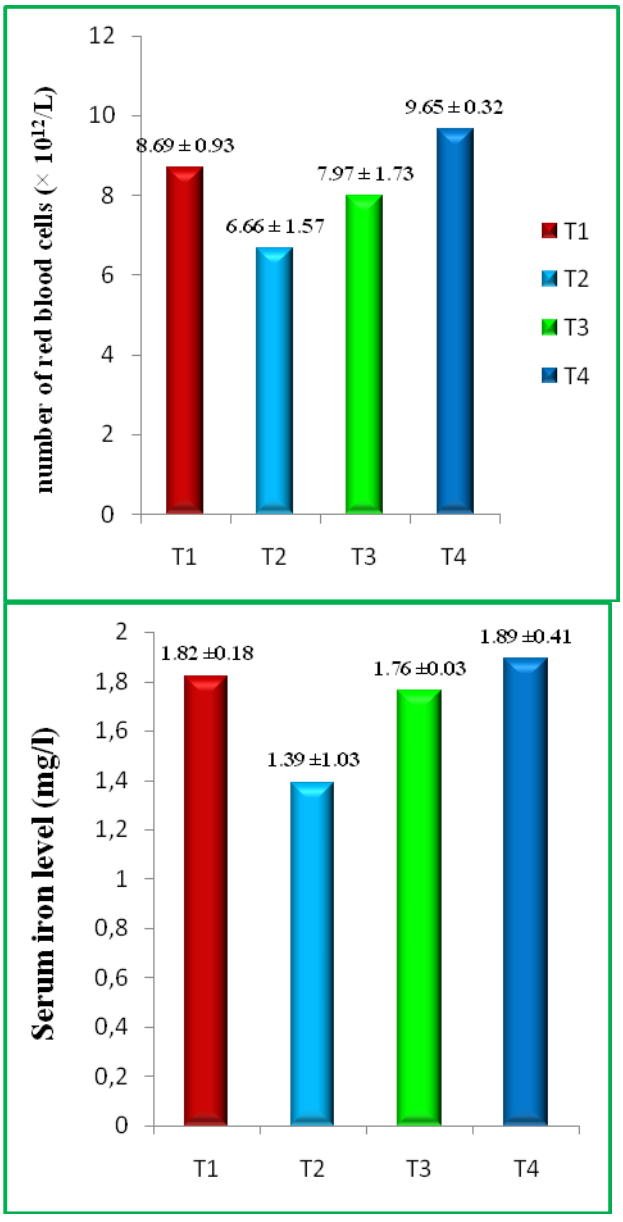

Figure 03: Number of red blood cells (* 1012 / L) and Serum iron concentration in the blood of various experienced
White control rats (T1); Negative control rats (T2); Rats having received 1000 (T3) mg / Kg of date syrup, Rats having received 2000 mg / Kg of date syrup (T4).

Measurement of serum iron levels is useful for the clinical diagnosis of iron deficiency anemia. Serum iron is the amount of rotating iron that needs to be transported. Our observation showed that iron deficiency anemia significantly reduced serum iron levels in rats. On the other hand, treatment with date syrup may increase the iron level in the blood of iron deficient rats. The serum iron level of the rats in the high dose group (2000 mg / Kg / d) showed significant difference compared to the control group but was much higher than that of the 1000 mg / Kg / d group. The results indicated that date syrup at a high dose has a significant effect on improving iron deficiency anemia.

[25, 12] also obtained similar results in reduction of serum iron level with administration of iron deficient diet and bleeding for 4 weeks in rats. Iron is necessary for the synthesis of hemoglobin, so it is reasonable to assume that any iron deficiency will result in a slower rate of hemoglobin synthesis. The results showed a marked decrease in hemoglobin and serum iron in mice fed an iron-deficient diet [12].

The results show that date syrup is of great importance in the therapeutic effect of iron deficiency anemia. The first reason perhaps lies in the fact that this drink has a high bioavailability of iron ”and that the presence of proteins, carbohydrates and fats and elements such as Zn, Fe and Ca and the presence of abundant amounts of vitamin A contributes to the synthesis of hemoglobin. Dietary modification may play a central role in the treatment of anemia [26].

Patients with nutritional anemia due to iron deficiency should be educated about foods rich in iron. Foods like green leafy vegetables, tofu, red meats, raisins, and dates contain a lot of iron [27].

Dates exhibit anti-cancer properties and are also a good source of natural antioxidants. Dates can improve hemoglobin (Hgb) levels by increasing erythrocyte production [28]. [29] found that the use of dates in the treatment of deficiency anemia in children is beneficial and the iron content.

[30] found that date extract increases the level of Hgb, which indicates that dates are rich in iron and the presence of proteins, carbohydrates, fats and elements such as Zn, Fe, Ca and the presence of abundant amounts of vitamin A helps in the synthesis of Hgb.

This suggests that nutritional correction plays a central role in controlling anemia. Considering the many properties of dates and its various ingredients, it is suggested that this cheap, healthy and affordable fruit should be considered in the primary school feeding program [28].

Conclusion

The syrups produced in our study have a good hygienic quality. Their nutritional quality is also appreciable since their low carbohydrate content is between 27 - 49%; protein is between 2.30 and 7.30%. In addition, our samples are of microbiological quality in accordance with standards and their consumption does not present any contamination problem.

Administration of date syrup by gavage at doses of 1000 mg / kg / day and 2000 mg / kg / day significantly increased the hemoglobin level and the number of red blood cells, hematocrit of serum iron per day 16. The dose of 2000 mg / kg / day allowed complete recovery of the hematological parameter and the serum iron level of the rats on day 16 compared to the control rats. Date syrup is of great importance in the therapeutic effect of iron deficiency anemia, due to its richness in iron and other properties contribute to the synthesis of hemoglobin.

Our results confirm and validate the traditional therapeutic indication of date syrup in the absorption of anemia. This

syrup could, according to our experimental conditions, therefore be recommended to reduce anemia complications.

Acknowledgements

This paper and the research behind it would not have been possible without the exceptional support of the Pasteur Institute in Algiers, of the medical analysis laboratory of the Mother-Child Hospital in "El Oued" and Saad Amamra in "Debila". We thank all these services for their help in ensuring the breeding and treatment of rats, as well as carrying out the necessary analysis.

Conflict of Interest

The authors declare that they have no conflict of interest in this work

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Original Article

Effects of extraction methods on total polyphenols, free radical scavenging and antibacterial activity of crude extracts of *Cleome arabica* L. growing in Oued Souf region

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ABSTRACT

This study aims to phytochemical study, antiradical scavenging and antibacterial Activity of the extracts of *Cleome arabica* L. from the region of Oued Souf (East South Algerian).
The crud extracts two methods obtained methanolic were maceration (EM) and ultra-sound (EU). The yields were: 8.95% and 9.60%, respectively. The quantitative estimation of total polyphenols is 11.57 mg EAG/g Ex (EM) and 13.46 mg EAG/g Ex (EU). The flavonoids contents in (EM) and (EU) are respectively 5.48 and 6.19 mg EQu/g Ex. the anti-free radical activity in the extracts at (concentration 0.1mg/ml) showed a great capacity to scavenge of the DPPH• radical and the percentage of inhibition was 41% in (EM) and 60.67 % in (EU). The results of the antibacterial activity of two bacterial strains: *Escherichia coli* and *Staphylococcus aureus*, revealed that Cleome arabica L. has a significant effect on the two strains with inhibition zones variable from 0 to 12 mm for *Escherichia coli* and 0 to 13 mm for *Staphylococcus aureus*.

1. Introduction

Cleome arabica L. is herb common in desert and rises in the Mediterranean sea on stony slopes and sandy ravines up to 2300m altitude [1]. In the Sahara, is found on rock, sand and gravel, is the only species of the Capparidaceae family in the region of Oued Souf -East South Sahara Algerian [2]. Perennial plant 30 cm tall, with upright and branched stems; small leaves hairy and trifoliate, the flowers have petals whose color turns yellow to dark purple, the fruit is a hairy pod 2 to 5 cm long [3], with a foul odor, toxic and has hallucinogenic effects [4].
According to [1], the camels, goats and sheep refuse this plant; the natives use it as a diuretic and against rheumatism [2], also is used in traditional medicine by the Nomads as analgesic of neuralgic pains [5].
This study aims to phytochemical study, antiradical scavenging and antibacterial activity of the extracts of *Cleome arabica* L., collected in the region of Oued Souf (East South Sahara Algerian).

2. Materials and Methods

2.1. Plant material

The *Cleome arabica* L. was collected in November 2014 from the Oued Souf region (East South Algerian). The plant is then dried, crushed, stored and protect from light

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and moisture.

2.2. Preparation of extracts

We prepared the extracts of plant material with two extraction methods:

2.2.1. Maceration

50g of plant material with 500 ml of methanol for 24 h, after filtration, the macerated is evaporated in Rota-vapor at 55°C [6].

2.2.2. Extraction with ultra-sound

According to [7], 200 ml of methanol is added to 20 g of plant material then take mixtures to ultra-sound Type JP Selecta (3.1A; 720 W) under the conditions: 30°C for 30 min, the extract is evaporated in Rota-vapor.

2.3. Estimation of total polyphenols

The total phenols content are determined according to the method described by [8], 0.2 ml of the extract was mixed with 1 ml of Folin-Ciocalteu reagent (10%). Then 0.8 ml of a solution of Na_2CO_3 (7.5%) was added to the mixture. The mixture is incubated at room temperature, protected from light, for about 30 minutes. The absorbance is measured at 760 nm. The results are expressed in mg equivalent Gallic acid/g Ex).

2.4. Dosage of Flavonoids Content

The flavonoids were estimated using method citing in [9], 0.5 ml of extract with 0.5 ml of $AlCl_3$ (2%). After 1h at room temperature, the absorbance was measured at 420 nm. The flavonoid content was estimated as (mg Quercetin equivalent /g Ex)

2.5. Anti-free radical scavenging

In this study, we used the DPPH• free radical to evaluate the scavenging activity of the plant's crude extracts.

The Anti-free radical activity of different extracts was measured by the method described by [10], 1ml of extract with 1ml DPPH (0.1 mM). The tubes are incubated at 37°C for 15 min. The absorbance is estimated at 515 nm. The following formula determines the inhibition:

% DPPH radical scavenging = [(Ac – As)/Ac] x100.

Where Ac is the absorbance of the control and As is the absorbance of the sample.

2.6. Antibacterial activity:

2.6.1. Source of pathogens and cultures medium

One Gram negative bacteria (*Escherichia coli* ATCC 25922) and one Gram positive bacteria (S*taphylococcus aureus* ATCC 25923) obtained from the Pasteur Institute, Algiers. Nutrient agar Mueller Hinton was used as a growth medium for investigated microorganisms.

2.6.2. Antibacterial activity

The agar diffusion method determined the antibacterial activity of extracts and antibiotic (AMC_{30}: Amoxyclav 30µg/disk) [11].

and 1mg/ml diluted in DMSO) are then deposited on the agar surface previously seeded with bacterial suspension (10^6 CFU/ml) in exponential growth phase. The Petri dishes were incubated at 37°C for 18-24 h. The inhibition of microbial growth is determined by measuring each disk's zones of inhibition diameter (mm) [12].

3. Results and Discussion

3.1 The Yield, Content of total polyphenols and flavonoids

The yield of extraction (Fig. 1) was registered as the maximum value in the extract of the Ultra-sound method (EU) and the minimum in the extract of the Maceration method (EM).

The total phenols and flavonoids of methanolic extracts from *Cleome arabica* L. are presented in (Fig. 1). We observed the relationship in the quantitative content of the polyphenols and the flavonoids.

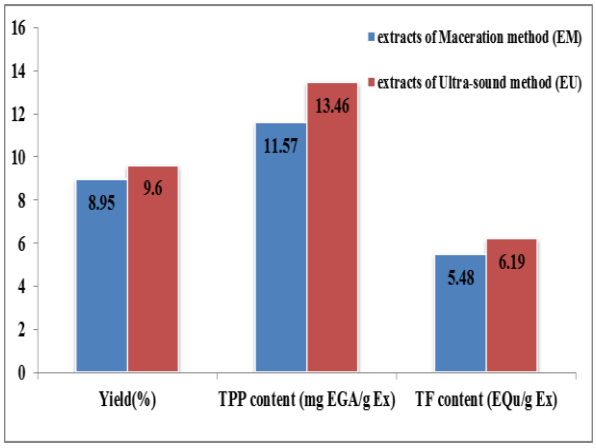

Fig 1. The yield (%), the content of total polyphenols and flavonoids of different extracts methanolic of *C. arabica* L.

3.2 Antibacterial activity

The antibacterial activity of extracts *Cleome arabica* L. are summarized in (Table 1 and Fig. 2).

Table 1. Diameter of inhibition zones (mm) of different concentrations of extracts of *Cleome arabica* L.

	Concentrations (mg/ml)	*Escherichia coli* (ATTC 25922)	*Staphylococcus aureus* (ATTC25923)
extract (EM)	1	12	11.5
	0,75	9	9.5
	0,5	8.5	8
	0,25	8	0
extract (EU)	1	8	13
	0,75	0	9.5
	0,5	0	9
	0,25	0	8
Antibiotic Amoxiclav	30µg/disk	44	45.5

The comparison of the activities of different extracts of *C. arabica* L. reveals a high sensitivity of the tested germs, especially with: EU extract (13 mm in C: 1 mg/ml), also showed very expressive inhibition diameters (44-45.5 mm) with antibiotic tested (Amoxiclav).

Escherichia coli is very sensitive to different concentrations of the EU extract (8 mm with a concentration of 1 mg/ml. Also, we did not notice the effect of the EU extract with this strain.

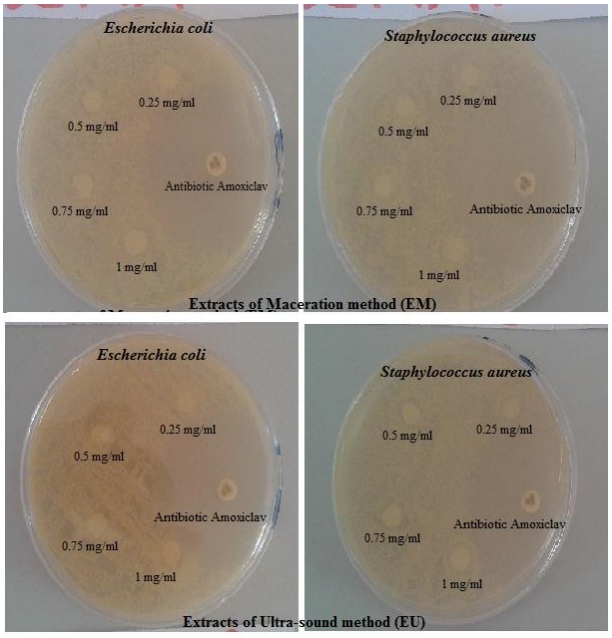

Fig 2. Antibacterial activity of different concentrations of extracts of *C. arabica* L. against strains bacteria.

3.3 DPPH˙ radical scavenging

The results of radical scavenging activity of extracts at concentration 0.1 mg/ml showed a great capacity to scavenge of the DPPH• radical and the percentage of inhibition was 41% in extracts of the Maceration method (EM) and 60.67 % in extracts of the Ultra-sound method (EU). The results indicate that extracts of *C. arabica* L. have a reduced potential with radical scavenging activity.

The variability observed in the values of yield, polyphenols, and flavonoid contents could be attributed to the difference in the samples studied [13].

In the result of anti-free radical activity, it was clearly noticed superiority extracts of the Ultra-sound method to extracts of the Maceration method. This was due to antioxidation's activity, which was closely linked to phenolic compounds' structure and quality than the concentration and quantity of these compounds within the plant tissue [14]. The strong effect of antioxidants in some ethanol extracts samples could be explained by the difference in antioxidant activity between samples for different behavior to give a proton and an electron between samples [15].

The results of present investigation indicated that the antibacterial activity varies with methods extraction used.

The observed differences in medium sensitivity the different concentrations of extracts between Gram-positive and Gram-negative bacteria can probably be attributed to the structural and compositional variations in the nature of the cell wall between the two groups [16].

This inhibitory effect of *C. arabica* L. extracts might be due to the action of special organic compounds such as flavonoids [17, 18].

4. Conclusion

This work is comparison to phytochemical study, antiradical and Antibacterial Activities of the extracts of *Cleome arabica* L. The crude methanolic extracts were obtained by two methods Maceration (EM) and Ultra-sound (EU). The contents of total polyphenols and flavonoids are proximities in two methods of extraction. The anti-free radical activity showed a great capacity to scavenge the DPPH• radical, and in the antibacterial activity revealed that *Cleome arabica* L. extracts have a significant effect on the two strains used with inhibition zones variable.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Original Article

The Effects of the Fertil Verde Fertilizer on the Growth and Yield of Chili Pepper (*Capsicum annuum* L) in Southern Algeria

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ABSTRACT

A field experiment was conducted on a private farm in El Oued State of Algeria to study the efficiency of using Fertil Verde as a fertilizer by foliar spry on hot pepper seedlings. The treatment by Fertil Verde increased all growth. The increase was significant in the number of main and secondary stems, the size of the leaves, and the weight of the dry leaves. While, the weight of the wet leaves did not significantly increase.
Moreover, the use of Fertil Verde has led to a significant increase in the yield qualities of the plant: the weight and shape of the fruits, fresh yield of the plant, the content of pigment, and the size of the plant.
Thus, the combination of fertilizing is common and Fertil Verde is a promising low-cost option in the production of high yields.

1. Introduction

Hot peppers (*Capsicum annuum*) are members of the Capsicum genus and the solanaceae family that includes tomato and potato plants. They are thought to be native of the southern part of North America [1, 2, 3]. Nowadays their cultivation has spread to all continents and serves mainly for food and pharmaceutical uses [4, 5, 6]. The continuously growing population puts pressure on agriculture to produce more crops, so there has been keen interest in raising production regardless of the quality, which has led to an increase in the use of chemical additives which exacerbated and increased the harmful effects on health and the environment, and the toxic effects of pesticides [7, 8]. As a result of the negative effects of chemical additives concerns have shifted in many countries to encourage organic agriculture whose products are characterized as clean and free from the residual effects of pesticides and chemical fertilizers. Organic agriculture is one of the modern applied agricultural environmentally friendly technologies. It has grown and developed very widely in recent years as a result of the rising need to sustain agriculture [9, 10] and preserve the fertility and productivity of soil. Several studies confirmed that biological fertilization leads to an increase in the production of some vegetable crops such as tomato, reduces the use of mineral fertilizers to 25-50 % compared to the control [11], and increases the yield of potato tubers by 17.3 %. The addition of organic fertilizers leads to a significant increase in the yield of many crops [12].
The use of organic fertilizer has been reported to improve the flavor and quality of vegetable crops. Organic manure derived from various green waste contains variable amounts of major nutrients and is a valuable source of

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plant nutrients. Among various sources of organic matter [13, 14, 15] Fertil Verde has been recognized as having considerable potential as a medium for the growth of plants and amendment of soil. There is growing interest in the potential of organic manure. As the cost of inorganic fertilizers is very high and they are not always available on the market, farmers fail to apply inorganic fertilizers to the crop field in optimal time. Peppers are still usually grown with the conventional applications of inorganic fertilizers and pesticides [1]. The objective of the experiment was to discover the effects of Fertil Verde on the growth and yield of *Capsicum annuum.*

2. Materials and Methods

The place and the experience design

This study was carried out during the 2019/2020 season on a farm by the peasant investor of Ghemam Amara Mohammed Khazzani in the town of Hassi Khalifa, EL-OUED in southern Algeria. It is characterized by sandy soils.

The experiment was conducted on an area of 160m^2. The experimental design was the Complete Type Randomized (R C B D). Two treatments with three replications of each treatment were used in this study. There were 30 pepper shrubs on every block which measured 0.75 x 6 m (with the surface of 4.5 m^2) with 1m gaps between the blocks.

We used the organic manure Fertil Verde. The treatments were including:

T1 - no manure (control)

T2 - Fertil Verde

Soil analysis and irrigation water

A homogeneous sample of the soil was taken from mixing samples of each experimental plot. Chemical analysis was carried out at the Fatilab laboratory (quality control and compliance analysis). Mechanical and physical analyses were carried out at the Public Works laboratory for the south in El Oued according to [16]. The chemical, mechanical, and physical properties of the soil are given in Table 1. Water for irrigation was analyzed at the laboratory of the Algerian Water Corporation. The chemical analyses of the irrigation water are presented in Table 2.

Table 1. Physiochemical analysis of the soil

Parameter	Soil
Sand (%)	97.2
silt (%)	2.8
clay (%)	/
pH	7.2
EC mm hos	1.86
$NO3^+$ppm	13.29
NH_4^+ ppm	25.35
NH_3 ppm	23.9
P ppm	1.5
K ppm	51.05

Table 2. Chemical analyses of the irrigation water

Parameter	Eau
pH	6.79
EC mm hos	2.69
NO_3^+ ppm	70.47
NH_4^+ ppm	12.11
Ca^{++} ppm	701.4
Mg^{++} ppm	87.5
Na^+ ppm	412.5
K^+ ppm	30.4
So_4^{--} ppm	1319.6
HCo_3^- ppm	262.3
Cl^- ppm	535.34

Plant material

Hot pepper seedlings (*Capsicum annuum* L) were sown in the experimental area in mi February 2019. The organic fertilizer was chicken and sheep manure. The recommended inorganic chemical fertilizers were added to all the treatments (plots) through drip irrigation.

Hot Peppers is one of the distinguished cultivar groups of *Capsicum annuum*. It is a member of the Capsicum genus and the solanaceae family that includes tomato and potato plants.

Chilli peppers (*Capsicum annuum* L*)* are produced in significant quantities in the valley region where seedlings are planted in February and harvested from May to October. This pepper is a slow growing short-term standing densely branched perennial flowering singly with elongated fruits usually upright, measuring up to 5 cm x 3 cm, green to yellowish green when immature, red when mature, with smooth walls and extremely pungent [17].

Fertil Verde manure

Fertil Verde is a concentrated organic liquid extracted from Leonardite, produced by the Italian company Bio-Alternativa, the fertilizer is used in small quantities during all stages of the plant 0.4 l/ha. rich in humic acids, fulvic acids, amino acids, vitamins, and trace elements dissolved at the molecular level in specially prepared water. It is a compound used to improve the properties of soil and plants. Can be used with all types of fertilizers in all climate zones and all types of soil. Table 3 illustrates its characteristics (https://fertil-verde.bio/en/products.html).

Figure 01: Fertil Verde fertiliser Product photo

Table 3: The characteristics of Fertil Verde

Parameters, measure	Test units	Test results
*p*H		7.5
Humified organic matter chemically active	%	88.2±0.1
(from total organic matter)		
Organic matter (on dry matter)	%	67.1±0.1
Total Humic acids	grams/liter	140.0±1.4
Total Fulvic acids	grams/liter	79.9±3
Organic carbon	%	30.1±0,01
Phosphorus (P_2O_5)	grams/liter	1.25±0.08
Nitrogen	grams/liter	24.7±0,02
Potassium, K_2O	grams/liter	103±1
Density	grams/cm3	1.113

Properties measurements

- properties the Growth of Chilli Peppers

- The leaves area (cm^2)
- Wet and dry weight of leaves (g)
- Number of main stems
- Number of secondary stems
- The status of stems

- The yield properties

- Wet and dry weight of green and red fruit (g)
- Yield ton/hectare
- The color level of the green fruits
- The shape of the fruits

Statistical analysis

Data were subjected to statistical analysis by the T test and 5% level of probability was used to compare the means of treatment.

3. Results and Discussion

The Effects of Fertil Verde on the Growth of Chilli Peppers

The focus of the study was to investigate the effects of the source of fertilization on hot pepper crops to find suitable alternatives to the conventional nutrient solutions. We tested the response of the pepper plant to fertilization by Fertil Verde in the growth parameters.

The application of Fertil Verde manure had a significantly positive effect on the plant height, number of branches, number of secondary stems. status of the stems, leaf area, and the weight of the dry leaves. It did not affect the weight of the wet leaves.

Pepper plants treated by Fertil Verde yielded the highest growth in the number of main and secondary stems, the size of the leaves, soft and dry weight of the peppers. They were substantially higher than those of the control treatment.

The results showed statistically significant differences by applying the T test in Excel. The calculated t values were 2.66, 2.77, 1.25, 2.32, 4 for the growth parameters (leaf area, weight of the dry leaf, weight of the wet leaf, number of branches, and number of secondary stems) respectively. See Table 4.

The results also showed that the number of branches increased by 100 %, and the difference between the weight of the dry leaf and the number of secondary stems was 40 % over the control plants. The difference was 25 % in the weight of the wet leaf and the size of the leaves. Table 4.

Table 4: The effect of Fertil Verde on the growth parameters of hot pepper (*Capsicum annuum* L)

Properties	T1 Control	T2 Fertil Verde	T_{stat}	$T_{critical}$	$P_{(0.05)}$
Number of main steams	3	6	2.32	2.13	0.04
Number of secondary stems	7.33	12.33	4		0.008
the leaves area (cm2)	22.71	30.5	2.66		0.028
dry weight of the leaves(g)	0.12	0.2	2.77		0.025
The wet weight of the plant leaf (g)	0.63	0.8	1.25		0.14

Visual Observation

Application of Fertil Verde resulted in darker green leaves of the peppers (*Capsicum annuum* L).

Most of the main and secondary branches erected by plants were treated with compost in addition to their dark green fruits. Figure 2

This finding is in accordance with the observations of [10, 18] in the contribution of organic fertilizers added by sprinkling or sprinkling the soil in improving the number of main and secondary stems.

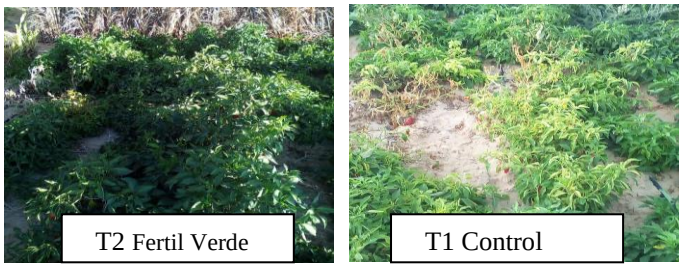

Figure 2: The effect of spraying with Fertil Verde on the color and status of the hot pepper (*Capsicum annuum* L).

Application of Fertil Verde was also responsible for variation in the growth characteristics (Significant Tcal $P < 0.05$) where the Fertil Verde fertilizers have a regulatory ability to release nutrients resulting in an increase in the growth characteristics of the plant. This means that Fertil

Verde was able to provide enough nutrients for the appropriate growth of hot pepper plants.

It seems likely that some growth promotion is due to plant hormone-like activity related to microflora associated with Fertil Verde and to metabolites produced as a consequence of secondary metabolism.

The most important effect of Fertil Verde on vegetables is its plant growth-promoting quality and reported improvement of chemical and biological properties of the soil. The use of Fertil Verde can also result in better initiation of roots, increased root biomas, and general development.

Vermicompost has previously enhanced growth variables such as the size of leaves and the weight of dry roots in species such as marigold, cornflower, tomato [19], and pepper [20, 21].

II- The effects of Fertil Verde on the weight of chilli pepper fruits

The results in Table 5 show that the application of Fertil Verde results in higher yield, more intense green color of the fruits, and heavier average weight of the fruits. Combined application of organic manure with usual fertilization significantly surpassed the application of the usual fertilization alone.

This promising treatment significantly promoted the yield per hectare to 60.96 t with each individual plant producing 1.53kg. Production per hectare in the control treatment was 39.23 t and 0.98 kg per plant. The increase of the yield of the recommended treatment over the control treatment reached 55 %. The results also showed that the addition of fertilizer reduced the deformation of fruits, especially twisting of the pods. We recorded 80 % of straight fruits when adding Fertil Verde as against 60 % when treating with the usual fertilization. Figure 3

The mean values of the weight of the fruits were higher with Fertil Verde (12.6 g) as against 10.86 g in the control treatment. Table5

Organic manures play a role in higher yields and improving the quality of fruit. This is attributed to the increase in the release of most nutrients. The results of [22, 23] confirmed the beneficial effects of using organic manure on growth and fruiting of crops.

This is in line with studies by other researchers who reported improvement in yield and the quality of fruit [24, 25].

Table 5: The effect of Fertil Verde on yield parameters of hot pepper (*Capsicum annuum* L)

Yield parameter	T1 Control	T2 Fertil Verde	T_{stat}	$T_{critical}$	$P_{(0.05)}$
Fresh yield (kg h-1)	39.5	60.96	3.6	2.13	0.011
Fresh yield (g plant-1)	0.98	1.53	-3.7		0.01
fruit weight g	10.86	12.6	1.03		0.18
straight fruits %	60	80	/	/	/

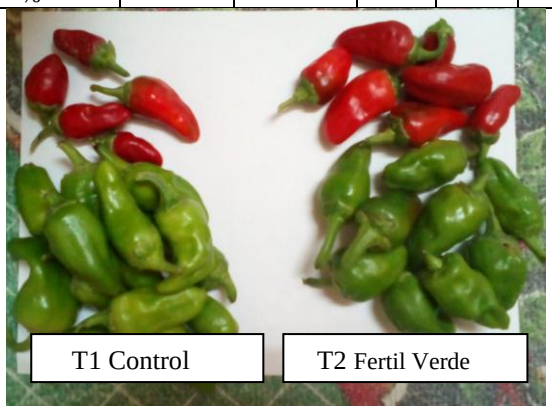

Figure 3: The effect of spraying with Fertil Verde on the shape of the hot pepper fruits

The increase in the average weight of the fruit and the yield of the fertilizer treatment may be due to the increase in plant growth as a result of the role of the natural organic metabolite in the activation of photosynthesis and its influence on permeability. Cell membranes, increased respiration rate, protein synthesis, and enzyme activation in the processes of biological metabolism lead to an increase in the productivity of the chili pepper plant, which is in agreement with the results of [26, 27].

This might be explained by the fact that the organic manure application rate resulted in higher nutritional content in crop vegetative and generative organs. Furthermore, sustained a steady, orderly, and smooth supply of nutrients that might create favorable conditions for plants.

4. Conclusion

The addition of Fertil Verde to traditional fertilization yielded better characteristics in the growth of chili peppers, which made it highly efficient in the production and quality of fruits as production increased by 55 percent.

Also, the outcome of the experiment indicated that there were significant interaction effects on many of the parameters considered.

the growth parameters (the plant height, number of branches, status of the stems, leaf area, and the weight of the dry), This resulted in improved production characteristics and fruit quality (higher yield, more intense

green color of the fruits, heavier average weight of the fruits and twisting of the pods).

Further studies are required and highly recommended to understand the deep effect of application of the Fertil Verde manure on chili pepper production and other yields.

Appendix

Appendixes, if needed, appear before the acknowledgment.

Conflict of Interest

The authors declare that they have no conflict of interest

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Original Article

Antimicrobial and antioxidant activity of methanol extract of *Echinophora spinosa* L. from Jijel, Algeria

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ABSTRACT

In this study, the antioxidant and antimicrobial activities of *Echinophora spinosa* were investigated. Antimicrobial activity of methanol extracts obtained from *Echinophora spinosa* was examined using the disc diffusion method. Antioxidant activity of the methanol extracts was examined using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test. Methanol extract of ripe fruits of *E. spinosa* showed highest total phenolic content (69.17 ± 1.2 µg GAE/mg extract) and the major flavonoid contents (12.122 ± 0.44 µg QE/mg extract) was found in leaves of the plant. In disk diffusion antimicrobial assay, *E. spinosa* manifested broad spectrum of activity. The largest capacity to neutralize DPPH radicals was found for ripe fruits methanol extract of *E. spinosa* plant. The results shows that the various parts of *E. spinosa* extracts promising antioxidant and antimicrobial activities have potential bioactivities due to high content of phenolic and flavonoid compounds.

1. Introduction

The genus *Echinophora* L. (Apiaceae) in the Mediterranean area is represented by seven species [1,2]. In North Africa, only *Echinophora spinosa* L. was reported from Algeria and Tunisia as a very rare taxon [3,1].

Echinophora spinosa L. is a psammophilous species growing on maritime sands. The plant is edible with a pleasant taste: thornless young and tender leaves are used for salads and the roots as carrots [4].

The plants genera *Echinophora* species are also used in folk medicine to heal wounds and to treat gastric ulcers due to its antifungal, carminative, and digestive properties [4]. *E. spinosa*, though other members of this genus have been used as wound healing, antispasmodic, and digestive agents [5,6].

From a phytochemical composition of *E. spinosa*, only a few works have been conducted on the phytochemical profiles of this species gathered in the Mediterranean area [7,4,8,9,6].

Antibacterial properties of essential oil of *E. spinosa* have been demonstrated against *Clostridium difficile*, *C. perfringens*, *Enterococcus faecalis*, *Eubacterium limosum*, *Peptostreptococcus anaerobius* and *Candida albicans* [4]. In addition, Pavela et al., [9]; Pavela et al., [6], mentioned that the essential oil of leaves and ripe fruits of *E. spinosa* are rich in phenolic compounds and exhibited insecticidal activity.

The aim of our work was to investigate the chemical composition of the methanol extract of leaves and ripe fruits of *E. spinosa* collected from Jijel (Algeria), and to evaluate the antioxidant and antimicrobial activities.

2. Materials and Methods

2.1. Collection of plant material

The plant material (leaves and ripe fruits) of *Echinophora spinosa* L. (Figure 1) was collected from Jijel, Algeria

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(Figure 2), during the month of May and June, 2018. The identity was subsequently confirmed by Dr. BOUNAR Rabah, University of M'sila. The fresh material was kept in perforated poly bags and immediately brought to the laboratory.

Fig 1. *Echinophora spinosa* L. plant.

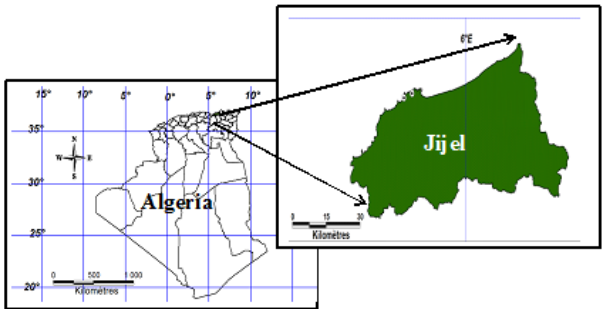

Fig 2. The study area.

2.1. Preparation of plant extracts

Plant leaves and ripe fruits of *E. spinosa* were washed thoroughly with distilled water and shade-dried at room temperature. The dried leaves and ripe fruits were uniformly ground using an electric grinder. The powdered plant material (200 g) was extracted for 2 days in 1 L 100% methanol. The separated extracts were then filtered through Whatman No. 1 filter paper and the methanol filtrate evaporated to dryness using a rotary evaporator at room temperature (30 °C). The thick extracted mass was then dried at room temperature, and the dried extract stored in an air-tight container at 4 °C until further use [10].

2.2. Determination of plant extract yield (%)

Yield percentage (*w*/w) from the dried extracts was calculated by formula (1):

$$Yied\,(\%) = \left[\frac{W1}{W2}\right] \times 100 \qquad (1$$

Where W1 is the dry weight of extract after evaporating the solvent and W2 is the weight of the soaked plant powder.

2.3. Determination of total phenolic content

The total phenolic content in methanol extract of leaf and ripe fruits of *Echinophora spinosa* were quantified by using a Folin-Ciocalteu colorimetric method spectrophotometrically as described by Khouchlaa et *al*., [11] with slight modification. Gallic acid was used as standard (concentration: 5.00–40 µg/mL) while *E. spinosa* whole plant extract was 100 µg/mL. Firstly, 1 mL of the extract or standard gallic acid solution was used in screw cap tube and 5 mL of Folin-Ciocalteu reagent was added. Then, 2 mL (2 %) of anhydrous sodium carbonate (Na_2CO_3) was added followed by 30 in incubation at 30 °C. The vehicle solvent was used as blank solution. UV absorbance was taken with a UV–VIS spectrophotometer at 760 nm. The amount of total phenolics was calculated as gallic acid equivalent (GAE) in mg per g of dry weight extract.

2.4. Determination of total flavonoid content

Total flavonoid contents in methanol extract of leaves and ripe fruits of *Echinophora spinosa* were quantified using the aluminum chloride ($AlCl_3$) colorimetric method [12], was used to determine total flavonoid contents, using quercetin as a standard. The absorbance was measured at 415 nm and total flavonoid contents were expressed as quercetin equivalents in milligrams per gram sample (average of the triplicate analysis). Crude extracts that have been attuned to come under the linearity range and different dilution of standard solution of Quercetin (20–100 µg/ml).

2.5. Antimicrobial activity assay

The plant extracts were tested for antimicrobial activity using the disk diffusion method. The antimicrobial activities of the methanol extracts of leaves and ripe fruits of *E. spinosa* were evaluated against microorganisms from the American Type Culture Collection (ATCC), namely two strains of Gram positive bacteria: *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923; three strains of Gram negative bacteria: *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 35659 and *Pseudomonas aeruginosa* ATCC 27853; one strain of yeast: *Candida albicans* ATCC 24333 and one strain of filamentous fungi: *Aspergillus niger* ATCC 16404.

2.5.1. Determination of antibacterial activity

The Disk diffusion assay was done in accordance with the National Committee for Clinical Laboratory Standards guidelines for bacteria [13]. Antibacterial activities were evaluated by measuring the diameters of zones of

inhibition in mm against the test organism. This assay was done in triplicate.

2.5.2. Determination of antifungal activity

Antifungal susceptibility testing by disk diffusion was done as per Clinical and Laboratory Standards Institute (CLSI) guidelines for filamentous fungi [14] and National Committee for Clinical Laboratory Standards guidelines for yeasts [15]. The antifungal activity was observed with inhibited growth of the microorganisms giving a clear, distinct zone of inhibition around the discs. The diameter of zone of inhibition was measured. The data was obtained from three individual experiments.

2.6. DPPH radical scavenging assay

The antioxidant activity methanol extract of leaves and ripe fruits of *E. spinosa* was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay [16]. Briefly, serial dilutions were carried out with the stock solution (1 mg/mL) of the extracts. Diluted solutions (1 mL of each samples) were reacted with 1 mL of a freshly prepared DPPH (2,2-diphenyl-1-picryl hydrazyl) methanol solution (80 µg/mL) for 30 min in the dark at room temperature. Absorbance values of these solutions were determined with a spectrophotometer at 517 nm. Methanol was used as a blank. butylated hydroxytoluene (BHT) was used as a positive control. The experiment was carried out in triplicate. The percent (%) inhibition of DPPH radical was calculated by the following formula (2):

$$Percent\quad DPPH\quad Scavenging = \left[\frac{A_C \times A_S}{A_C}\right] \times 100 \quad .....2)$$

where A_C is defined as the absorbance of the control reaction (comprising all reagents without the test compound (Sample)) and A_S is the absorbance of the test compounds. The antiradical activity was expressed as IC_{50} (µg/mL), the extract dose required to cause a 50% decrease of the absorbance at 517 nm. A lesser IC_{50} value corresponds to a greater antioxidant activity.

2.7. Statistical analysis

The results were analyzed by one way ANOVA. Duncan's multiple range test was used to identify significant differences among the mean (SAS 9.0). Difference among means at 5% level ($p < 0.05$) was considered statistically significant.

3. Results and discussion

3.1. Extract yield

Extraction yield of the samples expressed as percentage of dry weight plant material is presented in Table 1, were varied in different parts of plant. Methanol extract of leaves of *E. spinosa* presented the highest amount of extraction yield (17.75 % *w*/*w*), whereas methanol extract of ripe fruits the lowest (7 % *w*/*w*). The increase in the extraction yield of methanol extract of leaves might be due to the conditions for harvesting the plant. However, it is difficult to compare these results with those of the bibliography, because the extraction yield is only relative and seems to be linked to the extraction methods applied, to the genetic properties of the species used, the geographical origin and the conditions for harvesting the plant material [17].

3.2. Total phenolic content

The total phenolic content of *E. spinosa* was evaluated as expressed by gallic acid equivalents per mg of extract. The value was obtained from regression equation of the calibration curve (y = 1.07x + 1.09; r^2 = 99.82). There was a significant difference in total phenolic content in all parts of *E. spinosa* (Figure 3). Highest phenolic content (69.17 ± 1.2 µg GAE/mg extract) was observed in methanol extract of ripe fruits while lowest content (53.75 ± 0.58 µg GAE/mg extract) was found in methanol extract of leaves. The total phenolic contents in essential oils of different parts of *E. spinosa* were also previously reported; however, since those results are in a methanol extract, it is difficult to compare them with the obtained in this work [6,3,8,5].

3.3. Total flavonoid content

The aim of this study is to highlight the differences in secondary metabolite contents between the methanol extract from leaves and ripe fruits of *E. spinosa* in term of flavonoid contents (Figure 4).

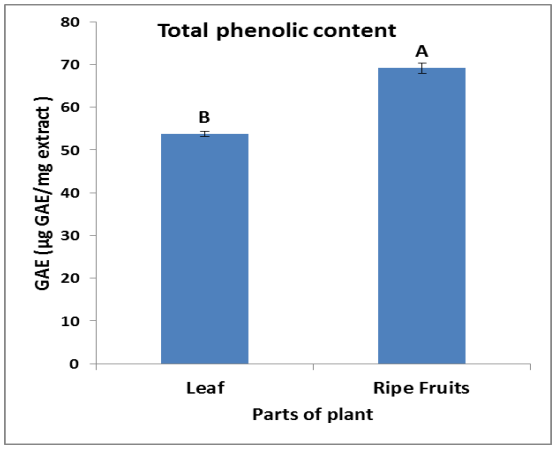

Figure 3. Total phenolic content in methanol extracts of leaves and ripe fruits of *Echinophora spinosa*. Data are mean ± SD ($n = 3$). Columns in figure that are headed with the different letter are significantly different ($P < 0.05$) according to the Duncan's multiple range test.

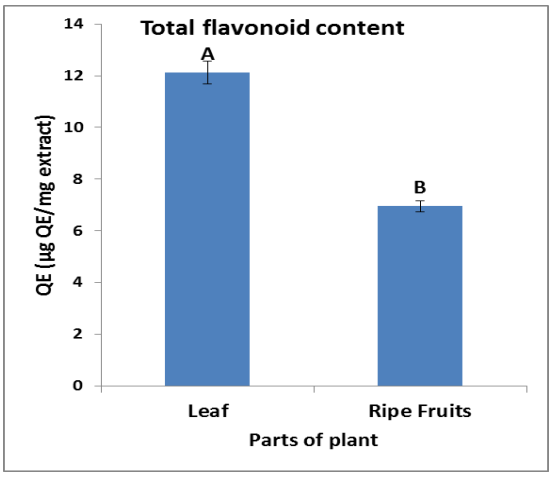

Figure 4. Total flavonoid content in methanol extracts of leaves and ripe fruits of *Echinophora spinosa*. Data are mean ± SD ($n = 3$). Columns in figure that are headed with the different letter are significantly different ($P < 0.05$) according to the Duncan's multiple range test.

Difference in total flavonoid content among leaves and ripe fruits of *E. spinosa* was statistically significant ($p < 0.05$).The highest flavonoids content was detected in methanol extract of the leaves (12.122±0.44 µg QE/mg extract) and the lowest content was seen in methanol extract of the ripe fruits (6.95±0.22 µg QE/mg extract) in (Figure 4). Several agents such as plant age, pretreatment of plants, parts of plant and extraction method also are effective on the contents of phenolic and flavonoids compounds [18,19]. The flavonoid plays an important role in the defense of plants towards pathogens, parasites, diseases, and predators [20]. Plants rich in secondary metabolites such as phenols and favonoids are rich in antioxidant activities. Flavonoids are the most important phenolics which are responsible for various biological activities [21].

3.4. Free Radical Scavenging Activity

The DPPH test was widely used as a fast, reliable and reproducible parameter to investigate *in vitro* the total antioxidant activity of plant extracts [20]. Radical-scavenging activity of the methanol extracts from the leaves and a ripe fruits of *E. spinosa* was evaluated by DPPH radical assay. In the present study, the antioxidant potential of the methanol extract of leaves and ripe fruits as well as BHT was explored in a dose-dependent (0.1–1.2 mg/mL) manner and the concentration with a 50% radical scavenging percentage (IC_{50}) was calculated by the diagrams as shown in Figure 5.

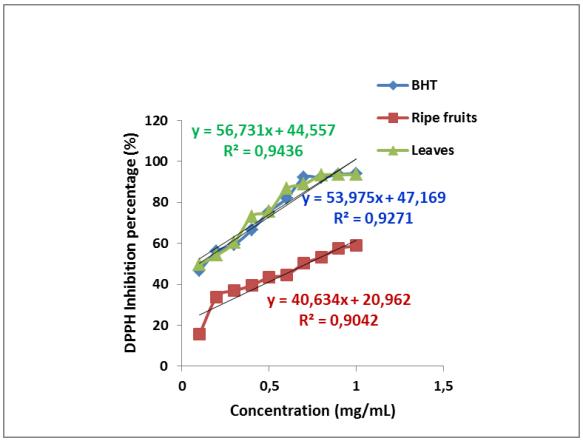

Figure 5. DPPH free radical scavenging activity (%) of BHT and of methanol extract of ripe fruits and leaves *Echinophora spinosa*.

The concentrations that led to 50% inhibition (IC_{50}) are presented in Table 1. Then, the antioxidant activities were compared with that of BHT. After analyzing the percentage of inhibition, the IC_{50} was calculated for each sample. The obtained results indicate that the methanol extract of ripe fruits from *E. spinosa* with an IC_{50} value of 0.087 ± 0.003 µg/mL proved to be an effective free radical scavenger than BHT and methanol extract of leaves of *E. spinosa*. This suggests that the plant extract contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. These results were consistent with the findings of many studies who described such correlation between total phenolic content and antioxidant activity besides indicated that the antioxidant effect of extracts, is due to the contribution of phenolic compounds in these extracts [22], and flavonoids [23].

Table 1. DPPH radical scavenging assay (IC_{50}) and extract yield of methanol extracts of *Echinophora spinosa*.

	Extract yield (%)	IC_{50}
Leaves	17.75	0.728 ± 0.028a
Ripe fruits	7	0.087 ± 0.003b
BHT	-	0.095 ± 0.001b

Data are mean ± SD (n = 3). Columns in table that are headed with the different letter are significantly different (P < 0.05) according to Duncan's multiple range test. BHT used as a positive control.

3.5. Antimicrobial activity of extracts

The results of agar disc diffusion are presented in Table 2. Both methanol extracts of *E. spinosa* showed antimicrobial effects against studied pathogens except for *S. aureus*, and *P. aeruginosa*. Antibacterial activity of methanol extracts of leaves and ripe fruits of *E. spinosa*

was tested against Gram positive and Gram negative bacteria. It was found that the methanolic extract of ripe fruits of *E. spinosa* showed the highest antibacterial activity against *B. subtilis* (30.5 ± 0.50 mm), *E. coli* (23.67 ± 1.53 mm) and *P. mirabilis* (20.50 ± 0.50 mm) as compared to methanol extracts of leaves and Gentamycin (Table 2). The antifungal activity of methanol extracts of leaves and ripe fruits of *E. spinosa* were assessed against two fungal species, *C. albicans* and *A. niger* in terms of zone of inhibition. The results indicated that both plants extracts showed antifungal activities at variable degrees against tested organisms (Table 2).

Table 2. Antimicrobial screening test of methanol extract of *Echinophora spinosa* against some microbial strains.

Part of plants	Inhibition zones (mm)						
	Gram positive bacteria		Gram negative bacteria			Pathogenic fungi	
	B. subtilis	*S. aureus*	*E.coli*	*P. mirabilis*	*P. aeruginosa*	*C. albicans*	*A. niger*
Leaves	13.67 ± 1.5c	00±0.00b	13.50±0.87c	9±1.00b	00.00±0.00b	7.67±0.29c	2,0±0.50c
Ripe fruits	23.67±1.53 a	00±0.00b	30.50±0.50 a	20.50±0.50a	00.00±0.00b	19.67±1.53b	4,67±0.58b
Gentamycin (5 µg)	20.17±0.29b	18.50±0.50a	27.33±1.15b	19.00±1.00a	21.67±0.58a	_	_
Amphotericin B (20 µg/mL)	_	_	_	_	_	27.83±0.29a	19.33±1.15a

Values in the table are means of three independent experiments and error bars indicates standard deviation of the mean. Letters show significant deference using Duncan's test ($p < 0.05$).

The methanol extract of ripe fruits and leaves of *E. spinosa* are found to have antimicrobial properties along with higher quantities of phenolics and flavonoids [24].

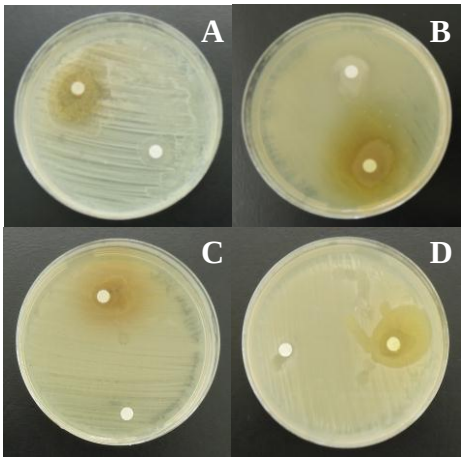

Fig 6. Zone of inhibition of different extracts of *Echinophora spinosa* on different microbial strains (mm): A) *C. albicans;* B) *P. mirabilis;* C) *S. aureus;* D) *E. coli.*

4. Conclusion

This study suggested that methanol extract of *E. spinosa* have significant effect in free radical scavenging and antimicrobial activities. The ripe fruits of methanol extract from *E. spinosa* have strong antioxidant and antimicrobial activities than the leaves methanol extract as they contain phenol and flavonoids which are known as potent antioxidants and antimicrobial agents. According to obtained results, it can be recommended that the ripe fruits of *E. spinosa* who was a relationship with the best phenolic and less flavonoids compounds quantity, as well as antimicrobial and antioxidant activities. The results provide evidence that supports the traditional uses of *E. spinosa* and can be applied for further pharmacological and phytochemical investigations.

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