**RESEARCH ARTICLE**

# Phytochemical and Antibacterial Evaluations of a Medicinal Plant, *Carthamus tinctorius* L Cultivated in the South West of the Sahara of Algeria in the Wilaya of Adrar

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**ABSTRACT**

This work was carried out on *Carthamus tinctorius*, on which we performed the extraction of secondary metabolites using cold maceration using a solvent consisting of methanol and acetone and water (7/7/6: V/V/V). We were able to determine the density of polyphenols, flavonoids and condensed tannins present in this medicinal plant. We also studied its antibacterial power tested on *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Enterococcus faecalis*, by a method called "disc diffusion method". The contents found are 0.25 mg CE/g DM in condensed tannins, 1.17 mg CE/g DM in flavonoids, and 7.18 mg GAE/g DM in polyphenols. The *E. coli* bacterium is the only one that was sensitive and whose zone of inhibition has a diameter equal to 14 mm. The minimum inhibitory concentration (MIC) of *E. coli* is equal to 100 mg/mL, and the minimum bactericidal concentration (MBC) is equal to 150 mg/mL; therefore the MBC/MIC ratio of the hydromethanolic extract of *C. tinctorius* is approximately 1.5, this enabled us to demonstrate that this extract has a bactericidal effect.

**Keywords:** Antibacterial power, *Carthamus tinctorius*, Condensed tannins, Flavonoids, Polyphenols.

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**Conflict of interest:** None

## INTRODUCTION

Since antiquity, man has provided himself with food and medicine through plants. Algeria is a very rich country with its varied natural plant cover due to its vast expenses, its many climates and its varied soils, there are many medicinal plants whose names differ from one region to another. In the Touat region, medicinal plants often spontaneous plants, and the inhabitants of this region use them up to date to treat themselves. Plants contain phytochemicals that belong to their secondary metabolisms. Secondary metabolites have antioxidant, anti-inflammatory, antimicrobial, anticancer, antihyperglycemic, antihyperlipidemic activities.[1] In the current research, we studied a medicinal plant, which is safflower of the dyers. Our work first aims at extracting secondary metabolites and evaluating them, and to evaluate the antibacterial power of safflower extract against: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis,* and *Enterococcus faecalis*.

## MATERIALS AND METHODS

The Adrar region is located in the southwest of Algeria, in the central Sahara, classified as an absolute desert.[2] It is divided into four geographical areas; the Gourara, Touat, Tidikelt, Tanezrouft.[3]

*Carthamus tinctorius* safflower is cultivated on all continents and grows naturally on waste and poor land.[4] It is a medicinal plant, herbaceous, annual, and 30 to 60 cm high.[5] The French name Carthame, Faux Safran, and the vernacular name in Adrar, Zaàfour. The family Asteraceae, the genus *Carthamus*, and the species *tinctorius*.[6] The harvests were carried out between January and March 2021. The plants picked were washed, and dried, then the flowers were ground to obtain a fine powder.

The humidity is determined by drying in an isothermal oven at 105°C at almost constant mass.[7] It is calculated using the formula:

$$H\% = [Mi-Mf/p] \times 100.$$

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The dry matter content is calculated with the formula:

Dry matter rate%=100 - Moisture rate%

The secondary metabolites were obtained by successive maceration and agitation for 2 hours each,[8] then filtered and degreased with hexane.[9] The extract is evaporated to dryness using a rotary evaporator. The extract obtained is used for the determination of the phenolic compounds and for the evaluation of the antibacterial power.

The dosage of polyphenols is based on the Folin-Ciocalteu coupling and the reduction of phosphomolybdic acid.[10,11] We obtained a dark blue color (Figure 1 and 2), and the polyphenol content according to the equation: Y= 9.137x + 0.038.

The colorimetric determination of the flavonoids is obtained by the method using aluminum trichloride and soda (Figure 3, 4).[12,13]

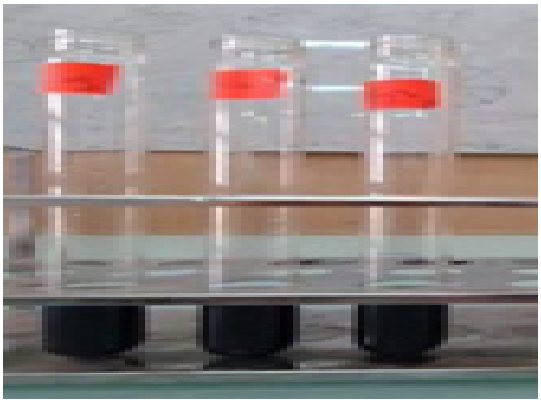

**Figure 1:** The dosage of polyphenols.

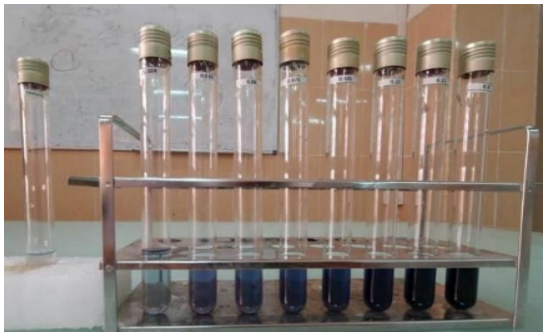

**Figure 2:** Gallic acid calibration range.

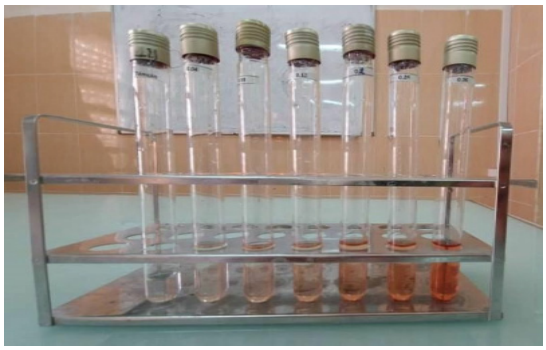

**Figure 3:** Catechin calibration range.

The determination of condensed tannins is based on the attachment of the aldehyde group of vanillin to carbon 6 of ring A of the catechin to form a red chromophore complex.[14-16]

The content is obtained according to the equation: Abs = 2.4405c + 0.0091

The antibacterial test is carried out on six pathogenic bacterial strains, namely: *E. coli*, *E. faecalis, P. aeruginosa, S. aureus, B. subtilis, B. cereus.*

The preparation of the inoculum is carried out using the revivification of the six bacterial strains to be studied. And to prepare *C. tinctorius* extract 100 mg of extracts are dissolved in pure dimethyl sulfoxide (DMSO) and 10 µL of the extract is applied to 6 mm discs of sterile filter paper.[17] The antibacterial activity of *C. tinctorius* was studied using five discs of filter paper placed in the petri dish, three of which are impregnated with extract, the fourth with pure DMSO solvent and the fifth with streptomycin.[18]

Statistical analyzes, all data from this study on the antibacterial activity of safflower were statistically analyzed. we used the post-hoc ANOVA and Tukey multiple comparison test.

The minimum inhibitory concentration (MIC) corresponds to the smallest concentration causing inhibition of the growth of a bacterial strain.[19] We prepared six concentrations: from 15 to 200 mg/mL, and after solidification of the Muller Hinton medium and extract, the bacterial strains are seeded.

The minimum bactericidal concentration (MBC) was obtained after subculturing. Transplantation involves only bacterial strains and concentrations without any bacterial growth in the previous test.

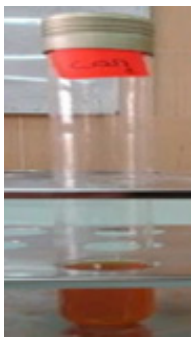

**Figure 4:** The dosage of flavonoids in crude extracts.

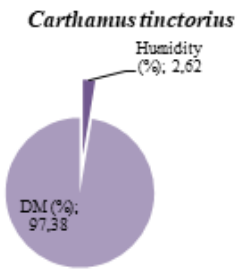


**Figure 5:** *C. tinctorius* moisture and dry matter percentage.

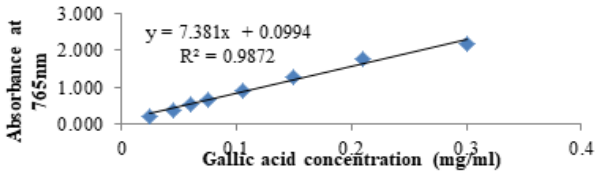


**Figure 6:** Polyphenol calibration the curve.

## RESULTS AND DISCUSSION

### Determination of Moisture Content

Desiccation of the *C. tinctorius* powder revealed that it contains an estimated moisture content of 2.62% (Figure 5). Indeed, the dry matter occupies a proportion of 97.38% in *C. tinctorius*.

### The Determination of the Rate of Polyphenols

The extract obtained by maceration of *C. tinctorius* was recovered in 20 mL of absolute MeOH (98%). This extract was assayed by colorimetry for polyphenols, flavonoids and condensed tannins. We calculated the proportion of polyphenols in *C. tinctorius*, with the linear regression equation of the gallic acid standard curve (Figure 6).

From the results reported in Figure 6, it turns out that the dosage of polyphenols showed that *C. tinctorius* has a polyphenol content of 7.18 mg GAE/g DM.

This value is highly superior to that of other authors who found a content equivalent to 0.24 mg GAE/g DM of the orange flowers of *C. tinctorius* and a tannin content of 5.19 mg CE/g DM.[20]

These variations in the total polyphenol content may be related to the distribution of polyphenols in plant parts as well as to the solubility of polyphenols in solvent.[21]

### The Determination of Flavonoid Level

We assay flavonoids by colorimetry, using aluminum chloride as a chromophore. The catechin calibration curve allowed us to assess the level of flavonoids in *C. tinctorius* (Figure 7).

According to the catechin linear regression equation, we were able to estimate, for *C. tinctorius,* a flavonoid content equal to 1.17 mg CE/g DM. The richness of the methanolic extract in flavonoids depends on the polarity of the solvents used in the preparation of the extract.[22]

### Determination of the Rate of Condensed Tannins

Condensed tannins are polymers of flavonoids, therefore catechin was chosen as a standard to measure condensed tannins in our samples.

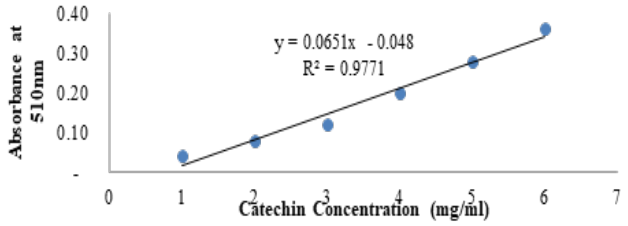


**Figure 7:** Flavonoid calibration curve

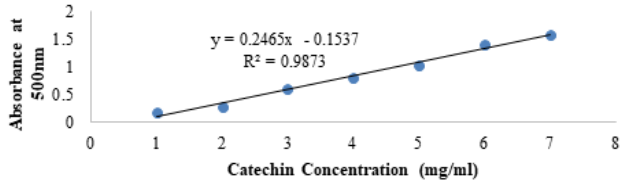


**Figure 8:** Condensed tannin calibration curve.

Thanks to the linear regression equation, we calculated the content of condensed tannins in *C. tinctorius*. It is evaluated at 0.25 mg CE/g MS (Figure 8).

### The Antibacterial Evaluation of *C. tinctorius* Extract

The disc diffusion method is used to get the results. This method is used to highlight the sensitivity or not, to extract and when a zone of inhibition appears.[23] The measurement of the inhibition diameter is carried out with a simple ruler. The results represent the average of three repetitions. The presence of a zone of inhibition demonstrates the existence of an obvious sensitivity of the strains studied with respect to the extract tested.[24] The susceptibility of a bacterial strain can be classified according to its inhibition diameter. The results show that for *C. tinctorius*, there is only the bacterial strain *E. coli* (6), which was sensitive, and the diameter of the zones of inhibition is equal to 14 mm (Table 1). The other strains were not sensitive to the *C. tinctorius* extract (not sensitive).

From the results reported in the table above, we find that *C. tinctorius* extract possesses antibacterial power with an inhibition diameter that exceeds 10 mm.

These results show us that our antibacterial evaluation is consistent with that of another author, who demonstrated that the chloroform extract of *Carthamus caeuleus* causes zones of inhibition that oscillate between 9 and 18 mm in diameter against several strains such as *E. coli*.[25,26] Our antibacterial

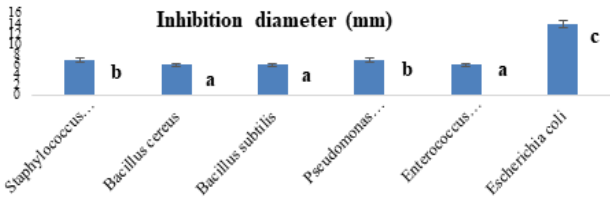


**Figure 9:** The antibacterial effect of *C. tinctorius* extract.

**Table 1:** Diameters of the zones of inhibition of the *C. tinctorius* extract with respect to the six bacterial strains studied.

| *Strains* | *Diameter of inhibition zone* (mm)* | *Diameter of inhibition zone (the antibiotic) (mm)* |
|---|---|---|
| *E. faecalis* | 06 | 07 |
| *S. aureus* | 07 | 34 |
| *E. coli.* | 14 | 35 |
| *B.subtilis* | 06 | 27 |
| *B. cereus* | 06 | 2 |
| *P. aeruginosa* | 07 | 22 |

*: The average of three repetitions

**Table 2:** The type of inhibition of the *C. tinctorius* extract, against the bacterial strain *E. coli*.

| *Extract of the studied plant* | *MIC (mg/mL)* | *MBC (mg/mL)* | *MBC/MIC* | *Type of the inhibition* |
|---|---|---|---|---|
| *C. tinctorius* | 100 | 150 | 1,5 | bactericidal |

evaluation results are likely due to the polyphenols present in our extract.

Indeed, polyphenols have proven to be good antibacterial substances.[27,28] Results obtained with other studies of *C. tinctorius* yielded larger zones of inhibition against *E. coli*, and demonstrated the efficacy of safflower against different bacterial strains. The antimicrobial activity of safflower extracts has also been shown to be due to phenolic compounds.[29-32] The antibacterial effect of safflower is an inactivity of bacteria due to the disorganization and disruption of the membrane layer of bacteria by the polyphenols and consequently, the flow of the different constituents of the bacteria to the outside of these cell walls. Thus, polyphenols also interfere with metabolic enzymes and cause inactivation.[33] For these strains, complete growth inhibition was noted with the essential oil extracted from safflower leaves and roots.[34]

We have represented the mean diameters of the zone of inhibition by the letters a, b and, c. The inhibition means of the bacterial strains tested with the same letters are not significantly different at $p = 0.05$ (Figure 9).

### The Determination of Minimum Inhibitory and Bactericidal Concentration

To determine the MBC and the MIC, we retained the only bacterium sensitive to *C. tinctorius*, whose inhibition diameter varies from 10 to 14 mm, namely *E. coli*. We prepared the concentrations: 200, 150, 100, 50, 25, 15 mg/mL.[35]

The MIC and MBC obtained from the extract are presented in Figure 10 and Table 2. For *E. coli*, the MIC obtained is equal to 100 mg/mL (Table 2).

In another study the antibacterial effect of *C. tinctorius* against *E. coli* gave an inhibition diameter of 3 mm. In another study, the MIC of *C. tinctorius* against *E. coli* was equal to 25 mg/mL for an aqueous extract and equal to 12.5 for a methanolic extract, whereas for *S. aureus* and *P. aeruginosa*, the MICs with a hydromethanol extract were 12.5 and 6.25 mg/mL, respectively. These results are clearly different from our results obtained against *S. aureus* and *P. aeruginosa*. This is probably because we used an extract at a concentration equal to 100 mg/mL instead of 200 mg/mL, used in these cited studies. Another study also demonstrated the possibility of using these antibacterial extracts simultaneously in combination with other antibiotics against *S. aureus* and *E. coli*. According to this study, using these plant extracts with antibiotics does not weaken their antimicrobial action.[36] Another study on *C. tinctorius* obtained a (MIC) against *S. aureus* equal to 30 mg/mL. [37]

For *E. coli* the MBC is equal to 150 mg/mL (Figure 10 and Table 2).

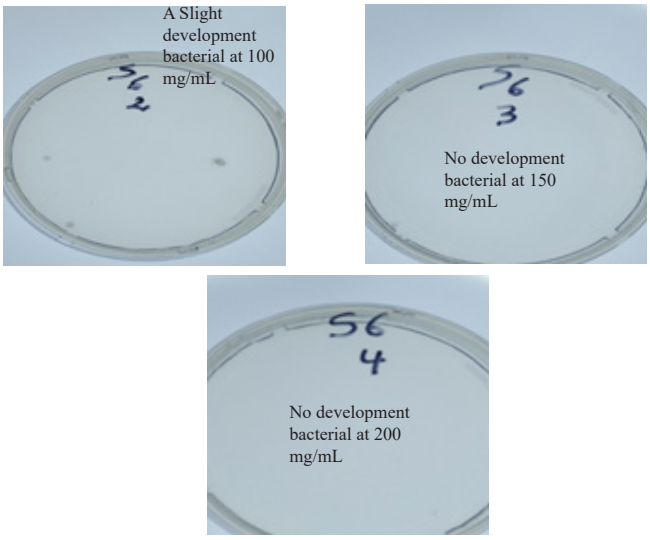


**Figure 10:** Minimum Bactericidal Concentration (MBC) of *C. tinctorius* extract, against *E. coli.*

### The Type of *C. tinctorius* Inhibition Extract, against *E. coli*

To classify the different types of inhibitions obtained, if the MBC/MIC is less than four or equal to four, this substance has a bactericidal effect. If the ratio is greater than four, then we speak of a bacteriostatic effect.[38] For *E. coli*, the MIC is equal to 100 mg/mL, and the CMB is equal to 150 mg/mL. The MBC/MIC ratio is equal to 1.5; therefore, the hydromethanolic extract of *C. tinctorius* has a bactericidal effect (Table 2).

There is consistency between our results and those obtained by other authors, who tested the antibacterial effects of *C. tinctorius* oil against *E. coli*, *E. cloacae*, *S. aureus*, and *S. agalactiae*. Indeed they have obtained very significant antibacterial effects of bacteriostatic and bactericidal type.[39] Some authors have observed certain antibacterial activities of hydromethanolic extracts of *C. tinctorius* against *B. mycoides, B. subtilis, B. cereus.* The antimicrobial effects obtained are of the ''bactericidal'' type. Aqueous extracts of *C. tinctorius* had the strongest bactericidal type effects. [40]

At the end of this work and the results obtained on the extract of the medicinal plant *C. tinctorius,* we can say that this work is part of the national effort to conserve medicinal plants and promote local traditional medicine and research. New natural substances and chemical compounds from plants. It seems to this effect that safflower is one of the medicinal plants that can enable us to achieve these objectives. Safflower is not the only medicinal plant in this case. That is why it would be interesting to pursue and study other plants existing in the Adrar region to identify and highlight the other secondary metabolites and their effectiveness in enhancing existing treatments in the treatment of various human diseases.

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