



Phytochemical, Free Radical Scavenging and Antimicrobial Activities of the Maize Stigmas, Collected of Ain Mlila (East Algeria)

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ABSTRACT

The purpose of this study is to quantify the bioactive substances contained in the stigmas of Algerian corn in the area Ain mlila, the sample was subjected to a maceration in the methanol, and the content of total phenols and flavonoids using the Folin-Ciocalteu method and the trichloride of aluminum respectively showed that the results vary between 91.51 ± 2.83 to $313.58 \pm 0.01 \mu\text{g AGE/mg}$ of extract for the polyphenols and between 11.26 ± 2.64 to $101.59 \pm 0.02 \mu\text{g QE/mg}$ of extracts for the flavonoids. These results show the richness of the stigma of corn studied in phenolic compound. The anti-radical activity has proved that our extract is more active (84.57%) than that of ascorbic acid (83.4%) used as control. It is found that reducing activity is strongly related to the content of polyphenols with $R^2 = 0.9997$. Antibacterial activity was also tested on four bacterial strains (gram + and gram -) according to the disk diffusion method. No activity was reported on the reference strain *Escherichia coli* (ATCC 25921) and *Staphylococcus aureus* (ATCC 25923) and the two clinical strains tested.

Keywords: Maize Stigmas, Phytochemical Screening, Polyphenols, Flavonoids, Antioxidant Activity and Antimicrobial.

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1. INTRODUCTION

Medicinal plants have been used for centuries as a remedy for various human diseases. These plants owe their therapeutic power to substances, which they contain. For the evaluation of the biological activity of these plants, it is imperative to use appropriate biological tests and chemical screening methods (Tyihák et al., 2007). In most cases, the biological activity of secondary metabolites is recognized well before their chemical structures are determined (Sofowora, 2010). It is important to note, however, that the active nature of these compounds can produce beneficial, as well as adverse, effects on living organisms (Custodio et al., 2011; Benchikh et al., 2014; Dallali et al., 2018).

Algeria has a rich and diverse plant flora. Among the medicinal plants that make up the vegetation cover are stigma of corn, a part where female inflorescences have grown rapidly. Experts in phytotherapy recommend the use of these stigmas to treat certain pathologies such as cystitis, urinary retention and edema. This "hair" can also prevent the risk of diabetes.

The aim of our study was to perform the phytochemical analysis and to determine the contents of essential active compounds, polyphenols and flavonoids from the stigma of corn. In the present study we evaluated the antioxidant and antibacterial properties of the polyphenols of this hair.

2. MATERIALS AND METHODS

Plant material

The plant material consisted of stigma of corn which were collected in June 2016 from the Ain Mlila region of eastern Algeria (Latitude: $36^{\circ} 03' 12.42''$ N, Longitude: $6^{\circ} 36' 20.03''$ E). The plant materials were cleaned and dried in the shade in a dry and ventilated place at room temperature for 10 days. Then the plant material was turned to powder, weighed, and stored in clean glass containers.

Phytochemical Screening

The detection techniques used for screening active substances must be fast, simple, reproducible and sensitive to use only a small amount of plant material. These methods are therefore limited to the detection of some chemical groups. They are only indicative, and further confirmation by more precise and more selective methods is essential (Wagner and Bladt, 2001). Phytochemical Screening of the hair of maize was performed according to the protocol described in (Zellagui et al., 2012a) with some changes.

Extraction of polyphenols

The extraction of the polyphenols was carried out according to the protocol of (Alanis et al., 2005). Thus, for the extraction of

phenolic compounds, methanol, which has been recommended and often used, was chosen (Falleh et al., 2008).

60 g of the powder underwent maceration in 600 ml of 80% methanol. Simple manual stirring was carried out at the beginning to make sure that the entire surface of the powder was impregnated with the solvent. After an incubation period of 24 hours at ambient temperature, the heterogeneous mixture was filtered with a filter paper. The maceration was repeated three times, twice in the cold and once warm temperature. Thus, the three recovered extracts were combined and subjected to evaporation at low pressure at 60 °C with a Heidolph type rotator.

The remaining compound attached to the wall of the evaporation flask were taken up with the recovered methanol, and then the obtained solution was left to stand until complete drying.

The yield is the ratio between the amount of the extract after evaporation of the solvent in mg and the quantity of the dry powdered plant sample in mg according to (Mahmoudi et al., 2012).

The determination of the total phenolic content

The total polyphenols were tested by the Folin-Ciocalteu colorimetric reagent according to the method referred by (Singleton and Ross, 1965).

A volume of 200 µl of each extract was mixed with 1 ml Folin-Ciocalteu (1 N). The solutions were mixed and incubated for 4 minutes. After incubation, 800 µl of the sodium carbonate solution (7.5% Na₂CO₃) was added. The last mixture was shaken and then incubated for 2 h at room temperature. Then, the absorbance of the resulting blue color was measured at 765 nm with a 1650PC UV-Vis spectrophotometry. The experiments were repeated three times and the results were reported on a standard curve and expressed in equal micrograms of gallic acid per milligram of extract (µg GAE/mg).

Determination of the total flavonoid content

The method of aluminum trichloride (AlCl₃) referred by (Boudiaf, 2006; Djerdane et al., 2006) was used to quantify the flavonoids in our extracts. The main reason for choosing this class of polyphenols lies in the fact that flavonoids are the most important polyphenols class, with more than 5000 compounds already written (Gomez-Caravaca et al., 2006).

1 ml of each sample and standard (prepared in methanol) were added to 1 ml of the AlCl₃ solution (2% dissolved in methanol). After 10 minutes of incubation at room temperature, the absorbance was measured at 430 nm. The experiments were repeated 3 times. The quantification of flavonoids was established as a function of a linear calibration curve ($y = ax + b$) performed by a quercetin standard at different concentrations under the same conditions as the sample. The results were expressed in micrograms of quercetin equivalent per milligram of extract (µg QE / mg).

Scavenging ability on the 2,2-diphenyl-1-picrylhydrazyl radical

In order to study the antiradical activity of our extract, we used the method based on the DPPH, according to the protocol of (Masuda et al., 1999). The DPPH solution was prepared by dissolving 3.94 mg of DPPH in 100 ml of methanol and then the mixture was incubated for 30 min. The concentrations of the extracts in the reaction medium were in 100, 10, 1, 0.1 mg/ml

order for the methanoic extracts and for the standard (ascorbic acid).

20 µl of the extract solutions were added to 2 ml of DPPH and the discoloration with respect to the negative control containing only the DPPH solution was measured at a wavelength of 517 nm for 5 min. The results can be expressed as the antiradical activity which is estimated according to the following formula:

$$\text{DPPH scavenging effect (\%)} = (A_0 - A_1) / A_0 * 100$$

In which A₀ is the absorbance of the DPPH; A₁ is the absorbance of the extract. The samples were analyzed in triplicate.

Screening of the antimicrobial activity

This activity was carried out within the microbiology laboratory of the Ain Fakroun Public Hospital, Oum el Bouaghi. The antimicrobial activity of the extracts was determined by the diffusion method in agar medium standardized by (NCCLS, 2003). Four bacterial strains were chosen for their pathogenicity and their frequent involvement in several infections. These were Gram-negative or Gram-positive strains, two reference strains got from the American Type Culture Collection (ATCC): *Escherichia coli* (ATCC 25921), and *Staphylococcus aureus* (ATCC 25923), and two clinical strains: *Staphylococcus aureus*, and *Escherichia coli*. We used Muller Hinton agar for the production of the antibiogram and for the study of the sensitivity of the bacteria to the different extracts of the stigmas.

Preparation of solutions

The extracts were taken up with Dimethylsulfoxide (DMSO). A series of concentrations including 8, 6, 4, 2, and 1 mg/ml was then prepared.

Preparation of the bacterial inoculum

Each strain was streaked onto the agar media to get isolated colonies. After 24 h incubation at 37 °C, 4 to 5 well-isolated colonies were selected and transferred with a platinum loop into a tube of physiological water solution to have an initial cell density or turbidity close to that of Mc Farland 0.5 (108 CFU/ml). This comparison was measured using a Mc Farland stallion.

Sterile Whatman paper discs no. 3 (6 mm in diameter) were impregnated with various extract solutions at increasing concentrations. Standard disks containing gentamicin, amoxicillin, penicillin and oxacillin antibiotics were used as positive controls. The petri dishes were then incubated for 24 h at 37 °C. The antimicrobial activity was tested by measuring the diameter of the growth inhibition zone surrounding the disk. Each experiment was carried out in triplicate.

3. RESULTS AND DISCUSSION

Phytochemical Screening

The results of phytochemical screening on stigmas showed the presence of all the major families of secondary metabolites such as volatile oils, sterols, triterpenes, flavone, anthracenoside aglycone, coumarins, polyuronides, tannins and alkaloids (table 1). On the other hand, we noted the absence of the saponins. The presence of these actives

compound indicates the importance of this stigmas in traditional and modern medicine.

Table 1: Phytochemical Screening of extracts of corn stigmas

Tests	Results
volatile oils	Water extract +
sterols and triterpenes	Extract n-hexane +
Flavone Alglycones	Water extract +
anthracenoside aglycone	Water extract +
coumarins	intense fluorescence under UV light +
polyuronides	methanol extract +
tannins	Water extract +
saponins	Water extract -
alkaloids	methanol extract +

+: presence, -: absence

Yield of phenolic compounds in extract

The obtained extract had a dark brown and pasty appearance (table 2).

Table 2: A yield of corn stigmas

Plant material	extract	(g)	Yield (%)
stigma	methanol	60	23.32

Content of Total Phenolic and Flavonoid Compounds

The results of total polyphenol essay in the corn stigma samples analyzed are reported in table 3.

Table 3: Total phenols and flavonoids content of stigma extract

methanol extract (mg/ml)	Total phenols (µg GAE/mg Ext)	Total Flavonoids (µg QE/mg Ext)
0.5	313.58 ± 0.01	101.59 ± 0.02
0.1	313.57 ± 0.0005	95.42 ± 5.53
0.01	104.31 ± 4.09	25.61 ± 9.81
0.001	91.51 ± 2.83	11.26 ± 2.64

It is important to note that the method used (choice of solvents) and the conditions under which the extraction was carried out (hot or cold), all affect the total content of phenols and flavonoids and thus affects the biological activities mediated by these metabolites (Lee et al., 2003; Belkhiri and Baghiani, 2017; Ouis and Hariri, 2017).

DPPH Free radical-scavenging activity

The antioxidant activity of the methanol extract of the stigmas and of the standard antioxidant (ascorbic acid) with respect to the DPPH radical was evaluated using a spectrophotometry after the reduction of this radical, accompanying its passage from the violet color (DPPH) to the yellow color (DPPH-H). This reduction capacity is determined by a reduction in the absorbance induced by anti-free radicals (Parejo et al., 2002; Bougandoura and Bendimerad, 2012; Kambale et al., 2013). Using the comparative histogram illustrated in figure 1, it is found that the percentage of inhibition of the methanol extract (84.57%) is increased to that of ascorbic acid (83.4%) only for the concentration 100 mg/ml, while the percent inhibition of the methanol extract is much lower than that of the standard antioxidant for all concentrations, respectively (0.1, 1, and 10 mg/ml).

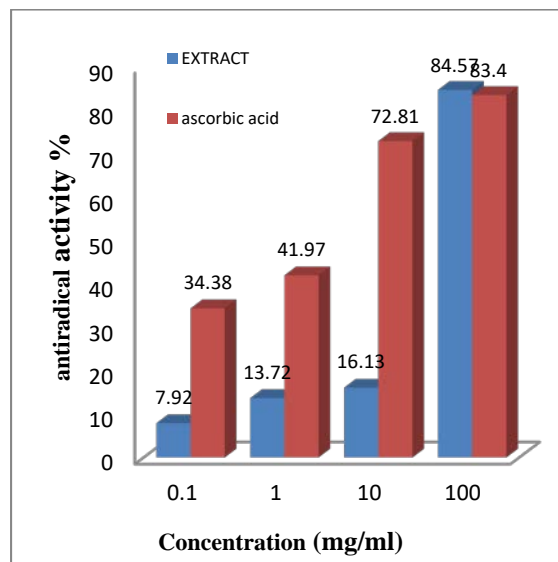


Figure 1: Comparative histogram of inhibition percent of methanol extract and ascorbic acid

Figure 1 shows that the percentage of antioxidant activity increases with increasing concentration either for vitamin C or for methanol corn stigma extract. It appears that the antiradical activity is highly dependent on the concentrations of the extracts. The more concentrated the extract, the higher the percentage of activity. The antiradical activity of the extracts is so relatively dependent on the content of total polyphenols and flavonoids (Naczek and Shahidi, 2004). Damak et al. (2008) reported that the total polyphenol concentration is much correlated with the antioxidant capacity typically evaluated by the DPPH test. Several factors influence the antioxidant potential and reduction kinetics, including reaction conditions (time, antioxidant ratio/DPPH, type of solvent, pH)

and phenolic profile in particular (Molyneux, 2004; Bouzid et al., 2011; Gresele et al., 2011).

Correlation between levels of total phenols, flavonoid, antiradical capacity:

The results of the total phenols, total flavonoids, and antiradical capacity used in the present study were compared and correlated with each other.

The curves of correlation are represented in the following figures (Figures 2, 3 and 4).

Significant positive correlations ($R^2 = 0.9901$) were observed between the contents of flavonoids and total phenolic content (Figure 2).

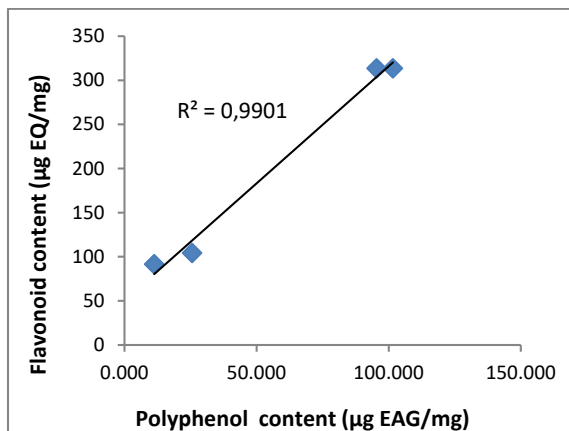


Figure 2: relationship between the total phenol and flavonoid contents.

It is noted that the reduced activity is strongly related to the content of polyphenols with ($R^2 = 0.9997$) and the flavonoid content ($R^2 = 0.9936$) indicating the significant contribution of phenols to these antioxidant assays. Therefore, the antiradical activity depends on the nature of the available phenolic compounds (Hong Chen and Tang Ho, 1997).

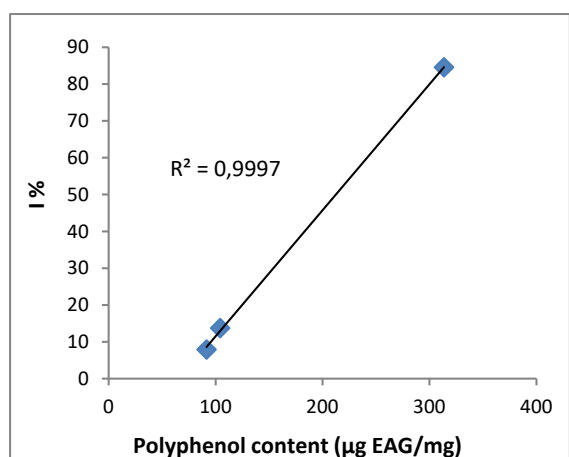


Figure 3: Relationship between the antiradical capacity and the polyphenol contents.

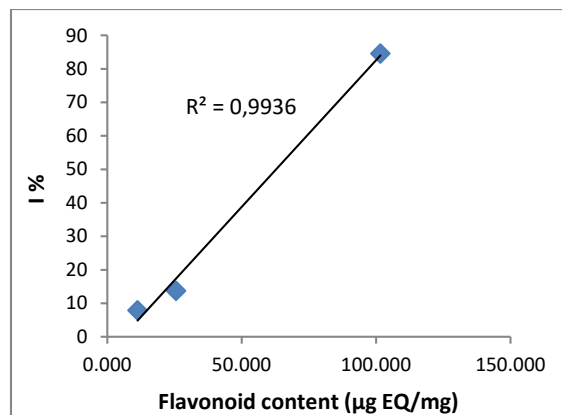


Figure 4: Relationship between the antiradical capacity and the flavonoid contents.

Antimicrobial Activity

Sensitivity results to stigma extracts

The four strains tested seem to be resistant to the different concentrations of the studied extract, as no zone of inhibition is observed around the disks for all the strains tested. It was found that this extract is inactive on the bacterial strains. However, it should be emphasized that the ideal efficacy of an extract may not be due to a major active ingredient, but to the combined action (synergy) of different compounds that produce this extract (Essawi and Srour, 2000).

Results of the antibiogram

The antibiotic is used to measure the ability of an antibiotic to inhibit bacterial growth.

Four standard antibiotics (amoxicillin, gentamicin, penicillin and oxacillin) were tested on bacterial strains, including *Staphylococcus aureus* gram (+) and *Escherichia coli* gram (-).

Table 4 shows the values in mm of the zones of inhibition for different strains studied.

Table 4: Antibiogram of the germs studied in the presence of different antibiotics

Antibiotics Strain	Diameters of the inhibition zones (mm)			
	Gentamicin	Amoxicillin	Penicillin	Oxacillin
<i>Escherichia coli</i> (ATCC 25922)	23± 2.89	17± 2	R	R
<i>Staphylococcus aureus</i> (ATCC 25923)	20± 2.55	32± 3.4	R	R
<i>C. Staphylococcus aureus</i>	17± 1.66	R 20± 2.45	R 8± 0.21	R
<i>C. Escherichia coli</i>	18±2.07	R< 6± 0.1	R	R

R: resistant, C: clinical strains

The diameter of the inhibition zone differs from a bacterial strain to another and of an antibiotic to another.

It is observed that the different strains of bacteria studied react differently to the antibiotics tested, even if they are two strains of the same bacterial species. For example, *Escherichia coli* ATCC 25921 has an inhibition zone of 17 mm diameter for Amoxicillin, while clinic strain of the same species is resistant against the same tested antibiotic. In addition, the reference strain, *Staphylococcus aureus* (ATCC 25923) is sensitive to

Amoxicillin with a zone of inhibition of 32 mm Diameter, while clinical strain of the same species was resistant to amoxicillin, which shows the character mutagenic potential of these strains which allows them to acquire resistance to antibiotics.

It seems that the two reference strains studied, *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25921), as well as the clinical strains were resistant to antibiotics Oxacillin, and penicillin.

It has also proved that the reference strain *Escherichia coli* (ATCC 25921) and the clinical *Escherichia* tested are very sensitive to gentamicin with zones of inhibition varying between 23 and 18 mm of diameter, respectively.

Thus, it appears that *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus aureus* clinic strains are sensitive to gentamicin with zones of inhibition of 20 and 17 mm diameter, respectively.

4. CONCLUSION

The present work focused on research of natural substances in the stigmas of corn with antioxidant and antimicrobial activities. We can confirm that the stigmas of corn contain a considerable amount of total phenols and flavonoids. It is considered a promising source of antioxidant agents, but the absence of the antibacterial activity can be explained by the nature of the compounds present in this plant.

Conflict of interest statement

The author declares that he has no conflict of interest.

5. ACKNOWLEDGEMENT

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