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Photochemical Study and Biological Activity of Phenolic Compounds of four Varieties of common Wheat (*Triticum Aestivum)* and Barley (*Hordeum vulgar*) subjected to Water Stress.

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***Abstract*:** *Our work has focused on the quantitative and qualitative study of the polyphenols extracted from the aerial parts of four varieties: two of soft wheat (Triticum aestivum: MP, FA) and two varieties of barley (Hordeum vulgar: Jaidore, saida) both with and without water deficit Treatments (WD) and (NWD) respectively, and to test their antimicrobial activities (antibacterial: E.coli and Bacillus, antifungal: Fuzarium). The results of the quantitative analysis of ethanol extracts showed that the polyphenols content is considerable with the existence of an intra-varietal and inter-varietal variability. The variability is also distinguished (WD) and (NWD) treatment. The qualitative study of polyphenols begins with allocations between four solvents with different polarities .This leads to the obtaining of different phases. Their composition is identified by the UV spectrophotometer and the Thin Layer Chromatography. The obtained results indicate that the majority of flavonoids detected are flavonols and flavones type. The antimicrobial activity test revealed that the extracts of each variety had a considerable inhibitory effect on the growth of E.coli and Bacillus. In contrast, fungal activity has no effect on Fusarium.*

Key words: *soft wheat (Triticum aestivum), barley (Hordeum vulgar), polyphenols, water deficit, UV spectrophotometer - Visible, antimicrobial.*

1. Introduction

Cereals are the staple food in many developing countries, particularly in the Maghreb countries. In Algeria, Cereal products have a very strategic place in the food system and in the national economy. Today, wheat is part of the three major cereals with corn and rice, with about 600 million tons annually. It is the food, the most consumed by the people and it has an important nutritional power. Because of the importance of cereal-based products, several studies have shown that Plants produce secondary metabolites, in which all functions have not been identified, which are fundamental [1] in particular for the adaptation of plants to Environments. Like phenolic compounds, in plants are involved in the mechanisms of resistance to biotic and abiotic stresses and because of their effects [2] Recent studies have suggested the involvement of these compounds in the prevention and control of certain diseases. A great deal of knowledge from a scientific point of view, such as an increase in crop conditions such as doubling Flavonoid contents [3] and [4]. The objective of our study is to develop a photochemical study of a few Varieties of barley (Hordeum vulgar) and soft wheat (Triticum aestivum) in order to quantify and to qualify, the cereal content of phenolic compounds in the normal state without deficit And to test their antimicrobial activities in vitro, namely Antibacterial and anti fungal.

2. Material and methods

2.1. Plant Material

The study covered two types of cereals; two varieties of soft wheat (Triticum aestivum): Auric flaurance (FA) and Mexipake (MP) and two varieties of barley (Hordeum vulgar): Jaidore (J), and Saida (S). The preparation of the plant material is carried out under a greenhouse. The four varieties are harvested at the same Phenological stage with two different treatments without water deficit and with water deficit according to the precocity of the varieties. Seedlings are irrigated once a week during the early stages of plant life with 1/4 of the capacity in the field. At the four-leaf stage, the trial was divided into two treatments: the first pots were irradiated twice a week. While the pots remain, undergo the stop of watering by application of water stress. The four varieties were harvested during the upstream stage at two different WD and NWD states (the 13 / 04/2014 and the 20/04/2014) successively.

2.2. Phytochemical Study

2.2.1. Quantitative Study (determination of polyphenols)

The determination of the total phenols makes it possible to identify the content of the phenolic compounds in 1 g of plant material. The latter is ground in a water / ethanol mixture (50/50) and macerated for 24 hours according to the technique Liyana-Pathirana and Shahidi [5]. The content of the phenolic compounds of our extracts is estimated by the Folin ciocalteu method Adesegun and al [6], and determined by spectrophotometry according to the protocol of Miliauskas and al [7]. Quantification of phenolic compounds is calculated as a function of a linear calibration curve performed by a Gallic acid standard extract. [8] The results are expressed in milligrams equivalent Gallic acid per gram of the dry weight of the plant.

2.2.2. Etude Qualitative (Extraction, Identification and Separation of Phenolic Compound)

- **Extraction**

20g of vegetable material is cut into small pieces. Then macerated in a mixture of ethanol-distilled water (50/50). The ratio of vegetable material to hydro-alcoholic solution is 1/10 ml / g **[9]** The whole rests for 72 hours with renewal of the solvent 3 times every 24 hours with filtration. The extracts obtained are confronted by various organic solvents from the less polar to the more polar. The (DE) diethyl ether phase, ethyl acetate (DMA) phase, butanone phase (MEC) and the aqueous or residual phase (H 2 O). All the phases are evaporated to dryness at 50 ° C., except that the solvent in the diethyl ether phase evaporates in the open air **[10].** The residues were recovered by 5 ml of methanol.

- **Separation: Thin layer chromatographic analysis**

The plates used are glass (20/20 cm and 20/10). The chosen adsorbent is silica gel for TLC. The system chosen for the three phases Ether diethyl ether, ethyl acetate and butanone is 50/20/25/2: distilled H2O / n butanol / ethanol / acetol. While the aqueous phase is carried out by the solvent system 50/20/25: Distilled H2O / n Butanol / EtOH. The eluent is poured to a height of 1 cm in a hermetically sealed elution tank until saturated with steam. The samples are deposited using a glass capillary pipette, where a 3 mm mark is marked with a pencil about 2 cm from the bottom side of the plate. The diameter of the produced job is dried quickly between each application. The plate placed vertically in the tank shall remain closed and shall not be moved. When the solvent front arrives about 1 cm from the upper end, the plate is removed from the tank. The level reached by the solvent is marked by a fine line. The plate is dried in the open air and reinforced by a dryer. The distances traveled by the different spots are measured by the frontal ratio (RF = Distance traveled by substance/ Distance traveled by the solvent front).

2.3. Biological Activity (Anti-Bacterial and Anti-Fungal)

The microorganisms tested in this study are the two bacteria Escherichia coli and Bacillus and the fungi Penicillium sp. In this experiment, 100 ml of ethanolic extracts of each variety of the flowering stage were used. After evaporation to dryness, the residues are recovered with 5 ml of ethanol. A sheet of Wattman paper is cut into 6 mm diameter discs sterilized in an autoclave at 120 ° C for 20 min. Then, they are soaked in six sterilized tubes each containing an ethanolic extract for each variety. The nutrient media used are respectively Potato

Detrox Agar (PDA) for Mushrooms and GN nutrient agar for bacteria. The same procedure is used for the sterilization of GN. The agar plates (GN and PDA) are melted and cast in half petri dishes. Once the agar plates are fully solidified, or a bacterial or fungal suspension of 10 µL is spread on the agar by a rake. The discs impregnated in the ethanolic extracts are half dried gently placed with a clamp on the suspension. The closed boxes are incubated respectively in ovens at 30 ° C. 72 hours for the fungi and at 37 ° C. 24 hours for the bacteria. The diameters of the zones of inhibition are measured with the aid of a caliper.

2.4. Statistic Study

The results obtained show the average of three replicates for the two phytochemical and biological studies. The statistical test performed is the two-factor and three-factor variance analysis, followed by a Newman-Keuls (NSK) average comparison test with a 95% confidence threshold performed by the Excel Stat version 2008 software.

3.Results and Discussions

3.1. Phenolic Compounds

The total phenolic compound content at the run-up stage in the four varieties at NWD varies from 2.88 ± 0.95 mg / g Gallic acid equivalent (eq GA) in the FA variety to 0.85 mg / g eq GA in the Saida variety. Jaidore and MP showed intermediate levels of 0.85 ± 0.95 to 1.62 ± 0.38 mg / g eq GA respectively. The maximum value is recorded in the FA variety, while the minimum value is recorded in the Saida variety. The two soft wheat varieties, show higher grades than the varieties of barley.

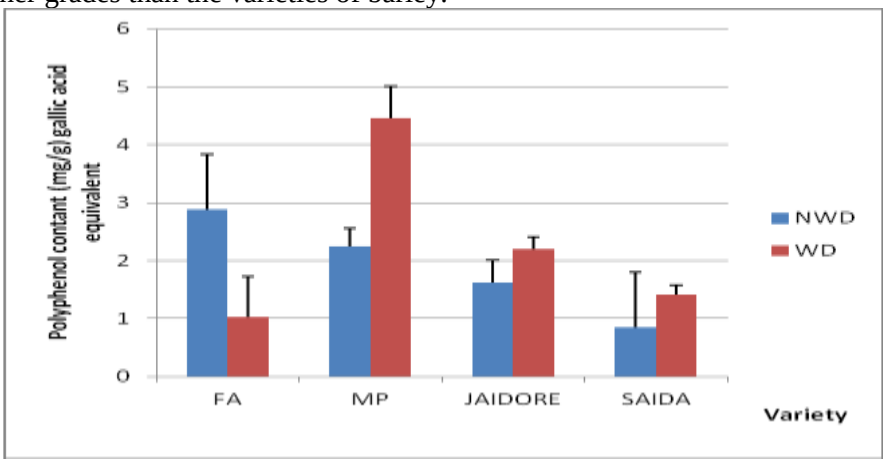

Fig.1: Total polyphenols content in the two varieties of soft wheat and two varieties of barley in NWD and WD treatments. A: Common wheat B: Barley C: Wheat and Barley

The variance analysis reveals a significant difference between the studied varieties. The Newman-Keuls test (SNK) classifies them into three groups: FA presents the first group. MP and Jaidore present the second group. The third group is presented by the Saida variety. FA > MP =Jaidore ≥ Saida ⇔2.88> 2.24 = 1.62 ≥0.85

On the other hand, in WD treatment, polyphenol contents varied among the four varieties of (46.28 ± 12.93) mg / g eq, as the maximum value in MP (2.2 ± 0.21) and as the minimum value in Variety FA at (4.46 ± 0.55) mg / g indicates an intermediate content of Jaidore (1.41 ± 0.16) and Saida compared to the levels recorded in the varieties studied in an FA variety, the total polyphenols decrease in the WD phase by 1/4 and half in the three varieties MP Jaidore Saida respectively compared to the NWD phase. But this content increases slightly in the MP variety of 1/6. The analysis of the variance represented has two factors revealed to be part of significant difference between the four varieties studied. However, it does not reflect any statistical significant difference between the two NWD and WD treatments. According to the Newman-Keuls test (SNK), two groups encompass the four varieties studied with a simple difference between the mean. MP> Jaidore ≥ Saida ≥ FA 4.46> 2.2 ≥ 1.40. The increase in the polyphenol content can be observed in the stressed state WD ≈NWD <=> 2.27≥ 1.89**,** But on the other hand there is a meaning between the variety MP Saida. MP≥ FA; Jaidore≥ Saida ⇔ 3.35 ≥1.94; 1.91 ≥ 1.13

3.2. Quantitative Analysis (Thin-film Chromatographic Analysis)

The observation of TLC plates is carried out in visible and UV light before and after the revelations. The use of different solvents with different polarities made it possible to separate the various methalonic extracts on TLC plates. The results are reported in the table. The aqueous extracts do not represent any spot during the migration.

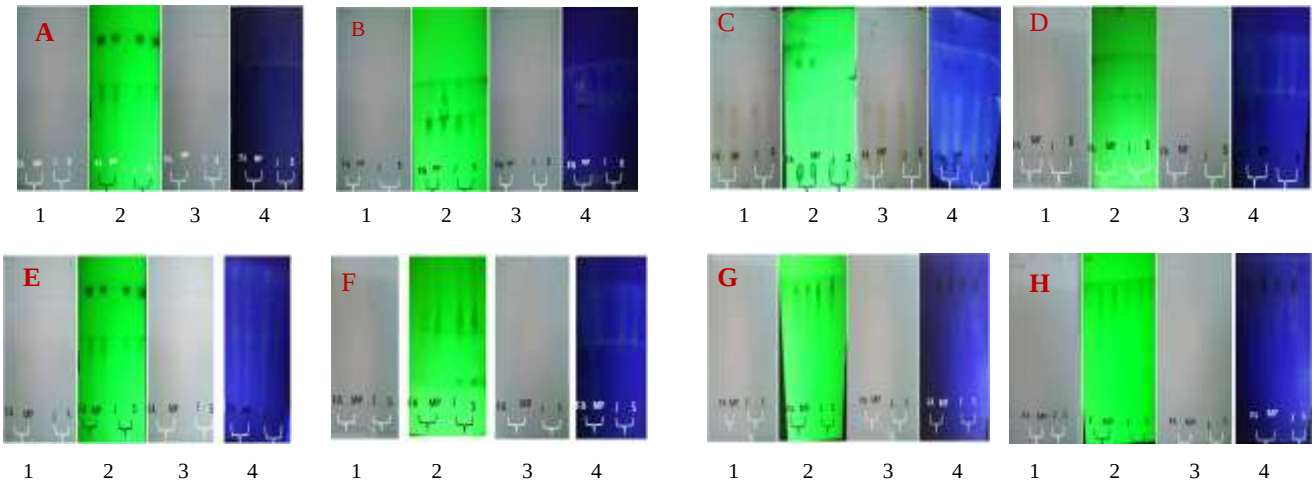

Fig. 2: The four varieties aqueous phase TLC in two treatments NWD and WD, in the system Toluene /MEC/ETOH/ Petrol Ether (4/3/3/5). A, C,E and G(NWD: ED, AC,MEC and aqueous phase); B,D,F and H(WD: ED, AC,MEC and aqueous phase) **1**: Visible, **2**: Under UV, **3**: Visible after pulverization with sulfuric acid 50%, **4**: UV after pulverization.

According to Table I, the number of spots in the WD treatment phase is greater than the WD treatment number. The Ether Diethyl phases showed a single spot with two colored spots, The Ether Diethyl phases showed one to two spots treatments to NWD treatments were phenolic acids, simple phenols and flavonoids. On the other hand, the extracts of the WD treatment contains three to four spots indicating that the plant synthesizes new molecules during stress. The ethyl acetate phases showed two to three spots to NWD treatments and 4spots of WD treatment. On the other hand, the Butanone phase records from two to four spots in the NWD and WD treatments. The aqueous phase repeated several times expresses no result. Maybe you have to choose another migration system.

The frontal ratios vary between 0.55 and 0.97 for NWD. They are very close to those of the WD stage which vary between 0.55 and 0.96 (Table 2).

From the results obtained, it can be assumed that the phases contain the eight types of flavonoids found by Yaou [11] and Lahouel [12]. It is observed that there are seven groups of phenolic compounds in the NWD treatment (1, 3, 4, 5, 6 and 7). And eight groups of phenolic compounds in the WD treatment (1, 3, 4, 5, 6, 7 and 8). The diethyl ether phase and the ethyl acetate butanone phase contain three different groups. The butanone phase is the richest in flavonoids because there are six groups of these compounds. It can thus be concluded that the two NWD and WD treatments contain mainly flavonols and flavones with different substitutions. Also, they are characterized by the presence of chalcones, flavanones and isoflavones are exclusively and mainly present in the ethyl acetate phases. These results are consistent with our work on cereals Chaib et al. [14].

TABLE I: The frontal reports of the tasks that appeared in the CCM WD AND NWD TREATMENT)

NWD					WD				
variety	Phase	N° Spots	R_f	Colors	variety	Phase	N° Spots	R_f	Colors
FA	ED	1	0.72	Green	FA	ED	1	0.55	Dark brown
							2	0.59	Green
							3	0.75	Yellow florescence
							4	0.90	purple
	AC	1	0.6	Brown		AC	1	0.53	Brown
							2	0.58	Green
							3	0.60	Yellow florescence
	MEC	1	0.57	Dark brown		MEC	1	0.67	Green Yellow
							2	0.72	Orange
							3	0.96	Light Blue
MP	ED	1	0.72	Green	MP	ED	1	0.56	Brown
							2	0.85	Green
							3	0.86	purple
	AC	1	0,65	Green		AC	1	0.60	Brown
		2	0,96	Fluorescent Yellow			2	0.65	Green
							3	0.75	Yellow florescence
							4	0.90	purple
	MEC	1	0.55	Dark brown		MEC	1	0.65	Yellow
		2	0.67	Yellow			2	0.67	Yellow florescence
		3	0.97	Orange			3	0.73	Orange
		4	0.98	purple			4	0.96	Light Blue
Jaidore	ED	1	0.71	Yellow	Jaidore	ED	1	0.57	Orange
		2	0.73	Fluorescent Yellow			2	0.60	Brown
							3	0.62	Yellow
							4	0.92	purple
	AC	1	0.65	Orange		AC	1	0.55	Green Earth
		2	0.67	Yellow florescence			2	0.63	Orange
		3	0.95	purple			3	0.77	Blue
							4	0.90	Yellow green
	MEC	1	0,58	Fluorescent Yellow		MEC	1	0.62	Yellow fluorescent
		2	0,65	Orange			2	0.66	Yellow orange
		3	0.97	Light blue			3	0.96	Dark blue
Saida	ED	1	0.71	Yellow	Saida	ED	1	0.60	Orange
		2	0.73	Fluorescent Yellow			2	0.92	Brown
							3	0.93	Fluorescent Yellow
	AC	1	0.65	Orange		AC	1	0.60	Green Orange
		2	0.67	Yellow fluorescent			2	0.65	Yellow green
		3	0.95	purple			3	0.75	Yellow
							4	0.90	Shiny Yellow

	MEC	1 2	0.64 0.97	Yellow fluorescent Light blue		MEC	1 2 3	0.65 0.69 0.96	Yellow Yellow fluorescent Blue

TABLE II: The RF intervals and the flavonoids contained for the three phases in twos treatments (NWD and WD)

The intervals of Rf			The flavonoids contained	
Treatments / Phases	NWD	WD	NWD	WD
Diethylic Ether	0.71-0.73	0.55-0.93	(6), (5), (7)	(1), (3), (5), (6)
Acétate d'éthyle	0.60-0,96	0.53-0.90	(1), (3), (5) (6), (7)	(1), (3), (4), (5) (6), (7)
MEC	0,55-0.97	0.62-0.96	(1), (4), (5), (7)	(4), (5), (6), (7), (8)

3.3. Biological Activity

The Jaidore extract had the strongest activity against the development and growth of E. coli on NWD treatment with an average diameter of the inhibition zones of 0.75 ± 0.57 mm followed by the Saida extract with an average of 0.65 ± 0.4 mm and lastly that of MP with a weaker effect of 0.35 ± 0.07 mm than the extracts of the other varieties. The extract of the FA variety exerts no inhibition against E.coli. On the other hand, in the WD treatment, the methanolic extracts of the four varieties exhibited the most vigorous activity with averages of the inhibition zones diameter of 1.28 ± 0.32 mm, 0.82 ± 0.07 mm, 0.75 ± 0.53 and 0.38 ± 0.54 mm in MP, S, J and F successively. Analysis of the two-factor variance shows a significant difference between the four varieties studied. However, it does not reflect any statistical significant difference between the two NWD and WD treatments. The Newman-Keuls test (SNK) classifies the studied varieties into four different groups. MP > S > J>FA ↔ 0.86 > 0.75 >0.65 > 0. 25, also the SNK test associates the two treatments in a single group. WD≈ NWD⇔ 0.76 ≈ 0.50. No antifungal activity is noticed for the fungus Penicillium.

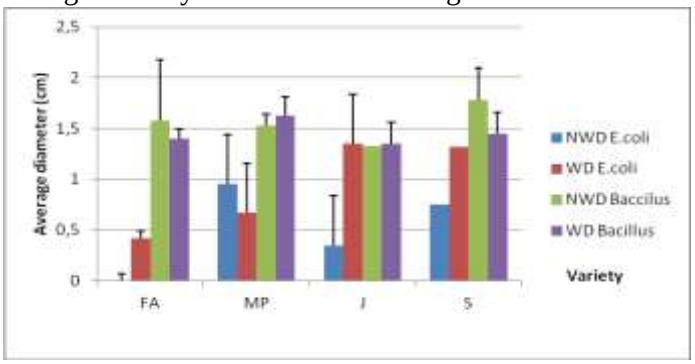

Fig.3: Disk development inhibition zones of the two E.coli bacteria and Bacillus to NWD and WD treatments by the extracts of the four varieties of soft wheat and barley from the ethyl acetate phase.

The MP extract had the strongest activity against the development and growth of Bacillus at NWD treatment with an average inhibition zones diameter of 0.95 ± 0.07 mm followed by the Jaidore extracts with an average of 0.8 ± 0.14 Mm, then FA with an average effect of inhibition of 0.75, lastly the saida variety with the smallest effect than the extracts of the other varieties 0.38 ± 0.05 mm..In contrast to the NWD treatment, the methalonic extracts of the four varieties have a remarkable effect with average inhibition zones diameter of 1.45 ± 0.21 mm in MP and 1.0 ± 0.14 mm in the remaining three varieties. The SNK test classifies the four varieties and combines the two treatments into one group. Comparing the effect of the two bacteria. The statistical study found a significant difference between the varieties studied and between the effects of the methanolic extracts of the ethyl acetate phase on the two bacteria. But shows no difference in effect between the two NWD and WD treatments. The SNK test associates the three varieties MP, S and J in a first group and the FA variety in a single

second group. In two groups and bacteria too. Only the two treatments fall into two different groups. MP ≈ S ≈ J> FA ↔ 0.97 ≈ 0.87≈0.74> 39; WD ≈ NWD ↔ 0.67 ≈ 0.81; Bacillus> E. coli ↔ 0.97 ≈ 0.69.

4. Conclusion

The photochemical analysis carried out on two kinds of cereals showed their richness in phenolic compounds for both NWD and WD treatments. The two varieties MP and Jaidore are rich in polyphenols better than the two varieties b Saida and FA. In this work, we detected some secondary metabolites of this plant. The two NWD and WD treatments show flavonols and flavones with different substitutions during the three phases. The butanone phase is the richest in flavonoids. The biological tests gave positive results for both bacteria. The Bacillus strain has a more detrimental effect than E.coli. But, no antifungal activity is noticed for the fungus Penicillium.

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