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Phytochemical screening and identification of polyphenols, evaluation of the antioxidant and antibacterial activity of n-butanol extract of *Chrysanthemum fuscatum* Desf.

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Abstract

Two phases (R_2 solid and R_3 liquid) of butanol extracts from the plant *Chrysanthemum fuscatum* Desf (But-CFE) were obtained by maceration followed by liquid/liquid extraction by solvent polarity with a yield (3.47% and 4.22%). These fractions were also used to study their phytochemical screening and identification of polyphenols by planar chromatography (TLC and PC) and assay (The total content of polyphenols and flavonoids was estimated; fractions R_2 and R_3 contain (39.57 and 47.40) and (5.52 and 23.3) mg/g of polyphenols and flavonoids respectively). Evaluating antioxidant activity using four methods (DPPH scavenging, phospho-molybdate assay, iron reduction (FRAP) and electrochemical superoxide scavenging), the results of the study showed a clear activity in the inhibition of free radicals and it was found that there is a relationship between the proportion of flavonoids and polyphenols in the extracts and this inhibition. The application of the antibacterial activity against three species of Gram-positive and negative bacteria also showed the effect of But-CTE (R_2 and R_3) on certain bacteria such as : *Escherichia coli*, *Staphylococus aureus* and *Pseudomonas aeruginosa*; the diameter of the inhibition zone varies from 8 to 17 mm.

Keywords : *Chrysanthemum fuscatum* Desf; n-butanol extract; polyphenol; antioxidant; antibacterial

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1. Introduction

Cells naturally produce free radicals as part of their metabolic pathways through enzymatic and other non-enzymatic mechanisms whose activity alters the presence of antioxidants, but the imbalance between antioxidants and free radicals leads to the development of so-called oxidative stress (OSD), which is defined as the body's inability to defend itself against attack by active species [1-2]. Free radicals are defined as atomic or molecular chemical types that possess at the lower level an individual electron on the outside, which makes them very active and seek to capture an electron from neighboring molecules, leading to the occurrence of many diseases, including: mutations and cell death, autoimmune diseases, cancer diseases [3-4]. Antioxidants are defined as any compound that has the ability to retard, inhibit or destroy free radicals [5]. These substances are divided into two parts according to their sources. In industrial sector; it is widely used in food industries as preservatives such as BHA and BHT. As for the natural ones, they are found only inside the body mainly of an enzymatic nature found in the human body such as: deoxonase peroxide (SOD), catalase enzyme (CAT) and glutathione peroxidase (GPx), it is also found in plants such as antioxidant vitamins C, E, A in addition to some phenolic compounds and their derivatives [6-7].

Distributed in many tropical and subtropical countries, *Chrysanthemum fuscatum* desf is known for its high nutritional value, for its medical uses and for treatment in folk medicine such as diabetes and blood diseases [8-9]. Different parts of this plant contain a large amount of minerals and are considered a suitable source of protein, vitamins and beta-carotene, amino acids and various phenolic compounds. In this research, our objective was to estimate the two-phase free radical inhibitory potential of the n-butanol extract from the plant *Chrysanthemum fuscatum* by applying four methods, namely phospho-molybdate, FRAP, DPPH and electrochemical tests, as well as to estimate the antibacterial efficacy of this extract by applying it to certain types of Gram-positive and negative bacteria.

2. Materials and methods

Chemical products: 2,2-diphenyl-1-picrylhydrazyl (DPPH, 98%), DMSO and DMF were acquired from Sigma Aldrich, ascorbic acid from VWR Prolabo Chemicals, disodium phosphate from Carlo Erba Reagents, ferric chloride from MERCK, Sodium carbonate, monosodium phosphate and sulfuric acid from Fluka, trichloroacetic acid, potassium ferricyanide and ammonium molybdate from Biochem Chemopharma are all stepwise analytical reagents. Tetrabutylammonium tetrafluoroborate (Bu_4NBF_4) (electrochemical grade 99%).

2.1. Qualitative analysis

2.1.1. Extraction of vegetable matter

Crysunthium fuscatum Desf was harvested in 2017 in the region of Bir Alatter, Tebessa (north-eastern province of Algeria), the identity and systematics of the plant were confirmed by Mr. Halis Yousef, a researcher from the scientific and technical research center of arid zones of Touggourt. The parts of the plant studied were dried in the shade, then ground and stored in a closed container, away from light and humidity.

The liquid-solid extraction was prepared by mixing acetone/water (80/20), we soaked 100 g of the plant powder in 1 L of solution for 24 h three times respectively. After filtration, we evaporated to dry the filtrate using a rotary evaporator. We performed a liquid/liquid extraction with solvents of decreasing polarities (petroleum ether, chloroform, ethyl acetate and n-butanol). For the n-butanol extract (But-CFE), we obtained a heterogeneous solution after fractional crystallization by acetone-butanol (95/5) which was filtered liquid phase (R_3) and recrystallized to obtain a solid phase (R_2). To calculate the yield of extractions, we applied equation (1)

$$R\% = \frac{m}{m_0} \times 100 \ldots \ldots \ldots (1)$$

Where: R%: extraction efficiency; m: amount of extract and m_0: the quantity of dry plant

2.1.2. Phytochemical screening

a. Characterization of flavonoids : A solution was prepared from 10 g of dry plants soaked in 150 mL of 1% HCl solution for 48 hours; the solution was filtered and used for the characterization of different types of flavonoids [10].

[1] Flavonoids: We take 10 mL of the previous solution and titrate it with a 2 N ammonia solution (NH_4OH) where we follow the pH until we obtain a basic solution, the appearance of a light yellow color is a characteristic of flavonoids

[2] Free flavonoids: A free flavonoid has been identified by adding 5 mL of filtrate to 2.5 mL of amyl alcohol; after stirring, a yellow color is observed in the alcoholic phase, indicating the presence of free flavonoids

[3] Flavonoid glycosides : We take the alcoholic phase obtained in the last test, evaporate it and dissolve the residue in 3 mL of hydrochloric acid HCl (1%), heat the solution in a water bath for 2 minutes, then add 2.5 mL of amyl alcohol and a small quantity of magnesium Mg, the red color indicates the presence of flavonoid glycosides [11].

b. Characterization of alkaloids : 10 g of plant powder soaked in 150 mL of HCl solution (1%) for 48 hours after filtration of the solution and titration with NH_4OH (2N) until pH = 9 and then extraction of the solution with chloroform; the organic phase was evaporated and the residue dissolved in 2 mL of HCl (1%). By adding three drops of Mayer reagent (1.35 g $HgCl_2$ and 5 g KI dissolved in 100 mL dist. water) a white precipitation is obtained, which indicates the presence of alkaloids [11].

c. Characterization of cardenolides : 1 g of plant powder, soaked in distilled water for 20-30 minutes, after filtration we carry out an extraction process (liquid-liquid) with 10 mL of a mixture of chloroform and ethanol. The organic phase is evaporated and the precipitate obtained is dissolved in 3 mL of glacial acetic acid, and by adding a drop of iron trichloride ($FeCl_3$), followed by drops of sulphuric acid (H_2SO_4), the observation of the bluish green color in the acid phase indicates the presence of cardinolides.

d. Tannin characterization : 10 g of vegetable powder is soaked in ethyl alcohol (50%) for 30 min and then the solution is filtered, adding a drop of ferric chloride solution ($FeCl_3$) to the filtrate a green color is observed indicating the presence of tannins [11].

e. Characterization of sterols and polyterpenes : A 5 g sample of plant powder, soaked in 20 mL of chloroform for 30 minutes after filtration, we put the filtrate obtained in a test tube and carefully add 1 mL of sulfuric acid (H_2SO_4) to the wall of the tube. A green color appears in the layer between the two phases, which after some time changes to red, indicating the presence of unsaturated sterols and terpenes [12].

f. Characterization of saponins: 2 g of plant powder has been shaken in 80 mL of hot water, if foam is produced and persists for 15 minutes; this indicates the presence of saponins [11].

g. Steroid characterization: A maceration of 5 g of powdered plant material in 20 mL of ethyl alcohol (70%) for 30 min, after filtration we evaporate the filtrate and dissolve the precipitate in 20 mL of chloroform and divide it into two test tubes: In the first tube we add 1 mL of acetic acid and then carefully 1 mL of sulfuric acid, the absence of a green color indicates the presence of unsaturated steroids. In the second tube we add sulfuric acid; the presence of yellow color changed to red indicates the presence of steroid derivatives.

2.1.3. Chromatography

In this study we use two chromatography methods, paper chromatography (PC) using Wattman 03 paper as the fixed phase and two mobile phases, the aqueous phase (acetic acid 20%) and the organic phase (butanol/acetic acid/water 4:1:5). Thin Layer Chromatography (TLC): using silica gel as the fixed phase and (toluene/acetone/chloroform 8:7:5) as the mobile phase. The characterization of the spot was performed using a UV lamp (245 and 365 nm) and NH_3 vapor, the R_f value being calculated [13-15].

3. Quantitative analysis

3.1. Evaluation of secondary metabolites

a. Estimation of total phenolic content (TPC): The total phenolic content of two phases R_2 and R_3 of the But-CFE extract was estimated by using reagent; folin-ciocaltau [16] and gallic acid as a positive control. The reagent changed its color from yellow to blue by reduction, the results being expressed in mg gallic acid equivalent per gram of dry plant powder. 0.1 ml of each extract mixed with 0.5 mL folin-ciocaltau reagent (10%) after 5 min, 2 mL sodium carbonate (20%) was added; the mixture was stirred and reacted for 20 min in the dark. The absorbance was measured at 760 nm and for three times the total phenolic content (TPC) was calculated using equation (2):

$$\mathrm{TPC(mg/g)} = \frac{A}{K} \times F \times \frac{V}{P} \dots\dots\dots (2)$$

Whereas: C: total phenolic compounds (mg / g); A: absorbance value at 760 nm; K: the trend of the standard curve for gallic acid; F: coefficient of dilution of extracts; V: the dissolved volume of the extract; P: the primary mass of the dry matter (g)

b. Estimation of total flavonoid content (TFC): The total flavonoid content in both R_2 and R_3 phases of the But-CFE fraction was estimated using the colorimetric method by aluminum chloride [17], the result was expressed in mg quercetin equivalent per gram dry weight of the plant. 1.5 mL of aluminum chloride ethanolic solution ($AlCl_3$ 2%) was added to 1.5 mL of extract after 30 min incubation in the dark at room temperature, absorbance measured at 420 nm, the operation repeated 3 times. The flavonoid content was calculated by equation 3:

$$\mathrm{TFC}\left(\frac{mg}{g}\right) = \frac{A'}{K'} \times F' \times \frac{V}{P} \dots\dots\dots (3)$$

Where A': absorbance at 420 nm; k': slope of the quercetin curve, F': extract dilution coefficient; TFC: the quantity of flavonoids (mg/g), V the volume in which the phenolic extract is dissolved, p the initial mass of the sample in (g).

3.2. Application of extracts to biological activity

3.2.1. Antioxidant activity

a. Free radical scavenging effect: The free radical scavenging effect of DPPH was evaluated by the method of Zhou and Yu (2004) [18] with ascorbic acid as a control solution, at a different concentration than in the towing phase. Two phases R_2 and R_3 of But-CTE were prepared from each of them, we added 0.15 mL to 3 mL of DPPH solution in ethanol (0.1 mM), the mixture was shaken and left for 30 minutes in the dark and the absorbance was measured at 517 nm with the spectrometer (spectroscan 80D/80DV UV/visible), the percentage absorption was calculated by equation 4:

$$I\% = \frac{A_0 - A_{extr}}{A_0} \times 100 \dots\dots\dots (4)$$

Were A_0 is the absorbance of control solution (blank+0.15 ethanol and DPPH), A_{extr} is the absorbance of samples.

b. Determination of ferric reducing power (FRAP): Ferric reducing power determined by the Oyaizu method [19] with ascorbic acid as a positive control. A phosphate buffer solution of pH 6.6 is prepared from 12.75 mL (Na_2HPO_4, $12H_2O$); 0.05 M and 3.25 mL (NaH_2PO_4, $2H_2O$; 0.02 M) and made up to 100 mL with distilled water and the pH is controlled to 6.6 by adding HCl solution. 1 mL plant extract is mixed with 2.5 mL buffer solution and 2.5 mL potassium ferricyanide $K_3(FeCN_6)$ (10%), the mixture is incubated at 50°C for 20 min, after which we add 2.5 mL trichloroacetic acid (10%), then we take 2.5 mL from the mixture and add it to 2.5 mL distilled water and 0.5 ml ferric chloride (10%). Absorbance was measured at 700 nm and the results were expressed as the mM equivalent of the ascorbic acid in equation (5):

$$\mathrm{AEAC} = \frac{K}{K'} \dots\dots\dots (5)$$

Where AEAC is the antioxidant efficiency equivalent to ascorbic acid; K: the slope of extract plot; K': the slope of control (ascorbic acid) plot

c. Phosphomolybdate (Mo (VI)) assays (TCA): The reducing power of phosphomolybdate determined using the method of Prito An; al (1999) with ascorbic acid as a positive control [20]. 1 mL of each extract added to 1 mL of phosphomolybdate (prepared from 0.6 M sulphuric acid and 28 mM Na_3PO_4 and 4 mM ammonium molybdate $(NH_4)_2MoO_4$).

d. Oxygen voltammogram: In an electrochemical cell of 25mL, we then place the superoxide radical anion generated by commercial molecular oxygen dissolved in DMF containing 0.1 M of Bu_4NBF_4 at room temperature 28°C. The scanning rate is maintained at 100 mV/s. The applied potential range was -1600 mV to 0.0 V relative to the ECS. Voltammogram of Qurceutin (positive control): A solution of 2 mg/mL quercetin (a standard compound) in DMF was prepared, and using a 1 mL syringe, the solution is gradually added in different volumes of 0.1 to 1 mL so that the concentration changes in the cell with each addition, and we plot the cyclic voltammogram curves for each addition under the same conditions: Potential; E € [0, -1600], sweep rate 100 mV/s and temperature is 28°C.

Voltammograms of the extract (But-CFE): In the same way and under the same conditions as quercetin, the two phases (R_2 and R_3) of the But-CFE extract were added to the superoxide radical dissolved in DMF (20 mg/mL) and the voltammograms were recorded. The trapping capacity of the superoxide radical was calculated using equation (7):

$$I\% = (1 - \frac{I_{pa}^{s}}{I_{pa}^{0}}) \times 100$$

Where I_{pa}^{0}, I_{pa}^{s} are the anodic pic current of $O_2^{\cdot -}$ in absence and in the presence of CFE extract and from plots of I% versus C (concentration of extract) [21]. We can calculate the IC_{50} which is the latest concentration that scavenge 50% of peroxide radical.

3.2.2. Antibacterial activities

The in vitro antibacterial activity of the two d-phases (R_2 and R_3) of But-CFE extract was evaluated by the disc diffusion method using Mueller-Hinton agar. The samples were prepared by dissolving 300 mg of the n-butanol fraction of CFE in 1 mL of DMSO as a stock solution, the discs containing 10 µL of each concentration of But-CFE. Growth inhibition zones were measured at different concentrations after 24-hour incubation at 37°C. The microorganisms used were: Gram positive (*Staphylococcus aureus* H3300) and two Gram negative bacteria (*Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC9027) [22]. In the same manner and under the same conditions as the two phases of the But-CFE extract, five antibiotics (aztreonam, cefalexin, cefotaxime, ciprolofxami and vancomycin) medical antibiotics were added and registered their inhibition zones. All data obtained in this study were analyzed using Origin 8.0 Pro software.

4. Results and discussion

4.1. Qualitative analysis

4.1.1. Phytochemical screening

Phytochemical screening allows us to make a first identification of the natural compounds existing in the plant, phytochemical screenings of *C. fuscatum* have been carried out and the results are presented in Table 1. Through the results, the preliminary tests showed the presence of the majority of metabolites such as flavonoids, tannins, terpenes. Then, we proceeded to a liquid-liquid extraction with different polarities of the solvents.

4.1.2. Extraction yield

The results are shown in Figure 2, where the extraction yield ranges from 0.3% for the acetate extract to 10.95% for the aqueous phase, but for the R_3 extract it is 3.74%, while for R_2 it is 4.22%.

4.1.3. Results of chromatography

Chromatography is known as the most fundamental and widely used method to separate natural compounds, because of its simplicity and speed, and it is a method that gives us an overview of the content of the whole extract [2, 3], this method depends on the distribution of the extract between two phases stationary and mobile.

After numerous content separation tests the two phases of the But-CFE extract and using a UV lamp (254 nm, 356 nm) for NH_3 detection, we obtain the results which are presented in figure 3 and table 3. After comparison the results with standard [7] flows distinguish the products.

The results presented in Figure 3 and Table 2 shows that *C. fuscatum* Desf is enriched with flavonoids, and that the bibliography [23] suggests the presence of:

- Blue sky: isoflavanon without free (OH) in position C_5
- Green or yellow green: Orone without (OH) in position C_4 or flavanone without (OH) in position C_5, flavanone with free (OH) in C_3 with presence or absence of free (OH) in position C_5
- Yellow lighting: Flavanole with free (OH) in C_3 with presence or absence of free (OH) in C_5 position

4.2. Quantitative analysis

4.2.1. Determination of phenols (TPC) and flavonoids (TFC)

The amount of phenols is between 39.5 and 47.4 for each of the liquid butanol extract R_3 and solid butanol extract R_2, respectively. The amount of flavonoids is between 5.5 and 23.3 for the solid butanol extract and the liquid butanol extract, respectively.

4.2.2. Application of extracts to biological activity

4.2.2.1. Assessment of antioxidant activity

a. DPPH radical scavenging activity: The DPPH molecule is a stable free radical due to the non-positioning of the free electron in the molecule, a property that allows it not to form a dimmer as is the case with most free radicals. The DPPH molecule is characterized by a violet color that is absorbed in an ethanol or methanol solution at nm 517. When this molecule is found with a concentration of HA capable of providing hydrogen, this results in a decrease and loss of the purple color [24]. In order to study the antioxidant activities of a sample by inhibiting free radicals, a color change of DPPH from purple to yellow is detected [25]. The DPPH radical scavenging activity for R_2 and R_3 was estimated by spectrophotometry by monitoring the reduction of free radicals, for this we used a UV-Visible spectrophotometer (SpectroScan 80d/80dv) to measure the absorbance at 517 nm; the results are presented in figure 4. According to the IC_{50} value, one sample proves the higher free radical scavenging activity and indicates that a lower amount is sufficient to decrease the DPPH concentration by 50%. According to (Figure 4, c), extract R_2 had the lowest IC_{50} of 0.39 mg/mL, indicating the highest antioxidant activity, followed by R_3 with an IC_{50} = 2.611 mg/mL.

Ascorbic acid, the standard antioxidant used to compare the results, has potent antioxidant activity with an IC_{50} = 0.039 mg/mL. These results show that R_2 extract has good antioxidant activity which is 10 times lower than that of ascorbic acid. These results are much lower than those of a study of the same type of plant where the value of root inhibition of DPPH was 0.004 mg/mL, due to the difference in the geographical location of the plant and the difference in the secondary metabolic compounds contained in each plant [26].

b. Phosphomolybdate assay (TAC): This is a spectral method for quantifying antioxidant capacity through the formation of a phosphomolybdenum complex, as it is based on the return of molybdate Mo(VI) (MoO_4^{2-}) from plant extracts containing the antioxidant compounds to bluish-green Molybdenum Mo(V) (MoO^{2+}) [27]. From Figure 5 (a) and (b), we can calculate the TAC values from equation 6; the result has been shown in the diagram (Figure 5, c). From the result, we see that all extracts have antioxidant capacity and differ from one extract to another, where we recorded the highest TAC value for extract R_2 (55.207 mM) and the lowest TAC value for extract R_3 with equal TAC (27.617 mM).

c. Iron Reducing Power Test (FRAP): This test is based on the return of triple iron (Fe^{3+}) present in the $K_3Fe(CN)_6$ complex to ferrous (Fe^{2+}). The reaction is detected by the yellow-triple iron transition to the bluish green of the ferrous. The absorbance is measured by the UV-visible device at wavelength 700 nm [28]. From Figure 6; (a) and (b) and equation (2), we obtain the values of AEAC which are plotted in Figure 6; (c). With these results, we can observe that the two fractions of the But-CFE extract have a variable reduction capacity, ranging from 72.035 to 351.76. We can observe that the AEAC value of R_2 was the highest, confirming its phenol content [29-30] (see Table 2 which is responsible for the antioxidant activity in the plant).

d. Electrochemical essays

[1] Oxygen voltammograms: Oxygen/superoxide voltammograms were recorded for the first time in the absence of the compounds, to determine the anodic peak current Ipa0; this value corresponds to the concentration of $O_2^{\bullet-}$ at the surface of the electrode. The radical $O_2^{\bullet-}$ was generated as a function of the reduction reaction (Figure 7. Peak B). The presence of this radical is easily detected by its anodic current measured during the reverse sweep according to the oxidation reaction (Figure 7, peak A)

[2] Voltammograms of the two phases of the But-CFE extract: The inhibition property of superoxide free radicals was studied using cyclic voltmeters with quercetin as a control; Figure 8 shows the voltammograms of the free radicals in the presence of different concentrations of quercetin, and it can be noted the decrease of the anodic peak with the increase of the quercetin concentration; this decrease is due to the reaction with the free peroxide radical

[3] Figure 9 shows the voltammograms for free radicals in the presence of different concentrations of R_2 and R_3 phases of the But-CFE extract. Using the results of the curves in Figure 8 and equation (7), we plotted a curve of the variation in the rate of inhibition in terms of concentrations of R_2 and R_3 phases of the But-CFE extract (Figure 10). The IC_{50} value for the two fractions R_3, R_2 was extracted. The values are shown in Figure 11 with the IC_{50} value of the quercetin (control solution)

[4] From the results presented in Figure 9, it can be seen that the addition of the two extracts to the medium results in a constant decrease in the current density of the anode I_{pa}. For the R_2 extract, the anode current decreased from 182.84 to 55.047µA/cm^2, and for the R_3 extract, the anode current density decreased from 181.36 to 30.50 µA/cm^2. The decrease in anodic current density indicates the decrease in the concentration of radicals around the electrode surface, which means an interaction between the free radical $O_2^{\bullet-}$ and the content of But-CFE extract which has an antioxidant capacity that could be a polyphenol according to Carine Bourvellec et al [31]. The IC_{50} values (Figure 11) indicate that the lowest value was recorded for the R_2 extract (0.21 mg/ml), showing that it has a high capacity to inhibit the free radical $O_2^{\bullet-}$ and this is due to the richness of the extract in phenolic compound (it can be observed that in Table 2), while the IC_{50} value for R_3 was 0.24 mg/mL and for quercetin (the control) the value was 0.23 mg/mL, which is higher than the R_2 value (lower in trapping capacity than R_2). In all the antioxidant assays, it can be observed that R_2 extract has a significant antioxidant power due to the richness of flavonoids and phenolic compounds that has an effective property against cancer and as an antioxidant [32].

4.2.2.2. Antibacterial testing

The antibacterial effect of the R_2 and R_3 fractions of the But-CTE extract were studied on three types of bacteria: Two gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and one gram-positive bacteria (*Staphylococcus aureus*), the result was presented in Table 4.

Antibiograms: Five antibiotics (aztreonam, cefalexin, cefotaxime, ciprolofxami and vancomycin) were recorded and the results of their antibiogram tests against the three types of bacteria in Table 5, to be used as a standard for R_2 inhibition. From the results presented in Table 4, we find

that the R_2 fraction has significant efficacy on some bacteria; *Escherichia coli* and *Staphylococcus aureus* showed significant sensitivity with an inhibition diameter of 10.6 and 14.64, respectively, at a single concentration of 300 mg/ml, hence the conclusion of the MIC of R_2; MIC € [1.50-3.00] mg/10ul. And for R_3, we note that no efficacy is observed, and compared to the results of the antibiotics in Table 5 (the antibiotic susceptibility of the five medical antibiotics; aztreonam, cefalexin, cefotaxime, ciprolofxami and vancomycin) showed a high sensitivity compared to extracts with an inhibition diameter of 13.07 to 35.69 mm.

5. Conclusions

In this study, we devoted ourselves to the study of the extract of n-butnol (R_2, R_3) from the aerial part of *Chrysanthemum fuscatum* Desf; among the criteria that led to the selection of this plant, there is the geographical origin and the traditional use of this plant, which affects the natural composition of the plant. Our research focuses on the identification of the active compounds (the different polypenolic compounds), the results of the qualitative analysis; separation by CP and TLC chromatography. For the qualitative analysis; phenol and flavonoid content (The determination of total polyphenols (TPC) by the Singleton and Ross method using the Folin-Ciocalteu reagent and total flavonoids (TFC) by the aluminum chloride method Ordonnez). Evaluation of antioxidant and antimicrobial activity for samples of R_2 and R_3 phases of the butanolic extract of the plant (the total antioxidant capacity (CAT) of the extracts is evaluated by the phosphomolybdenum method, DPPH test that we use (2,2-diphenyl-1-picrylhydrazyl). The determination of antioxidant capacity by the FRAP method is performed as follows. The FRAP method is based on the reduction reaction of ferric iron (Fe^{3+}) present in the ferrous complex $K_3Fe(CN)_6$ (Fe^{2+}) by an antioxidant and the inhibition of superoxide by cyclic voltammetry), as well as the content of the two But-CFE extract samples varied, with the phenol content being more effective for antioxidant activity and the flavonoid content being more effective for antimicrobial activity.

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