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PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND ANTIOXIDANT POTENTIAL OF VARIOUS EXTRACTS OF DIETARY TURNIP (*Brassica Rapa L.*)

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Abstract: *In the present work, the aqueous and methanolic extract of the underground part (comestible part) of Brassica Rapa (Brassicaceae) was subjected to phytochemical and biological evaluation. The results show that the methanolic extract of B. rapa (MEBr) presents a higher yield than the aqueous extract of B. rapa (AqEBr) (24% vs 17%) respectively. The qualitative and quantitative phytochemical analysis revealed the presence of some chemical families. However, MEBr showed higher contents of polyphenols, total flavonoids and condensed tannins compared to AqEBr. The total antioxidant activity (TAC) of MEBr (5.32 ± 0.56 mg AAE/gr DE) was more powerful than that of AqEBr (2.68 ± 0.33 mg AAE/gr DE). According to the DPPH test, the IC50 values are (0.14±0.12 mg/ml vs 1.9±0.45 mg/ml) for MEBr and AqEBr respectively. On the other hand, the FRAP test confirmed that AqEBr has antioxidant activity with a maximum of 1.41mg/ml, which is still higher than that of MEBr 0.83 mg/ml. We conclude, that methanol was a more efficient extractor than water for B. rapa species, which allows a good yield, a high rate of secondary metabolites and a powerful antioxidant activity. B. rapa is a rich source of antioxidants that can eliminate free radicals and reduce the risk of chronic diseases.* Abbreviations: *MEBr: Methanolic Extract of Brassica rapa, AqEBr: Aqueous Extract of Brassica rapa, TAC: Total Antioxydant Capacity, DPPH: 2.2'-diphenyl-1-pycrilhydrazyl, FRAP: Ferric-Reducing Antioxidant Power Assay, IC50: Inhibitory Concentration 50.*

Keywords: *Brassica rapa, MEBr, AqEBr, phenolic compounds, phytochemical screening, antioxidant activity.*

1. Introduction

Frequent consumption of cruciferous vegetables (340 genus and about 3700 species), such as broccoli, cauliflower, cabbages, radishes, turnips and kale reported to reduce the risk of developing cardiovascular disorders, cancer, heart attacks , strokes and diabetes [1]. . Brassicas are the most important and widely consumed group of plants in the cruciferous family. In addition, Brassica vegetables can be stored in their natural form for a prolonged time, and are available all year around .The turnip (*Brassica rapa*) belongs to the *Brassicaceae* family (cruciferous vegetables). Among the different Brassica species, turnip is one of the first to be domesticated [2].

The main constituents of this species are: glucosinolates, isothiocyanates [3], flavonoids [4 -5], indoles [6], sulphur and phenolic compounds [7-8], carbohydrates

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[9], volatile compounds (mainly terpenes, esters, aldehydes and ketones) [10-11] , and some well-known antioxidants ; such as vitamin C , vitamin E and carotenoids, as well as antioxidant enzymes ;such as catalase, superoxide dismutase and peroxidase [12].

The bitterness of turnip is related to glucosinolate (isothiocyanate) degradation compounds [13]. Researchers have widely pointed out the therapeutic properties of turnip: hepatoprotective [9,14,15], antimicrobial, antitumor [16], antioxidant [11], anti-inflammatory [17], cardioprotective, lipid-lowering [18,19,20], anti-diabetic [21], nephroprotective [22] , and analgesic [23], as well as reducing obesity and metabolic syndrome.

In view of the therapeutic and antioxidant virtues of the turnip, a phytochemical, biological and antioxidant evaluation of the underground part of *Brassica rapa* was considered using two extraction methods (aqueous infusion and alcoholic maceration).

2. Materials and methods

2.1. Materials

2.1.1. Plant material

The choice of the plant drug was the *Brassica rapa* variety which is commonly called turnip and widely consumed by the Algerian population. Fresh *Brassica rapa* roots (rhizome) were collected in November 2018 from the local market of the Ghriss town located at 19 km from the Mascara province (Western Algeria). The Ghriss Plain is a part of the Oued Fékane watershed, which covers an area of 1,185 km^2 , located in north-west Algeria (Fig 1), between 35° 07' and 35° 31' N latitude and between 0° 0' and 0° 26' E longitude [24].

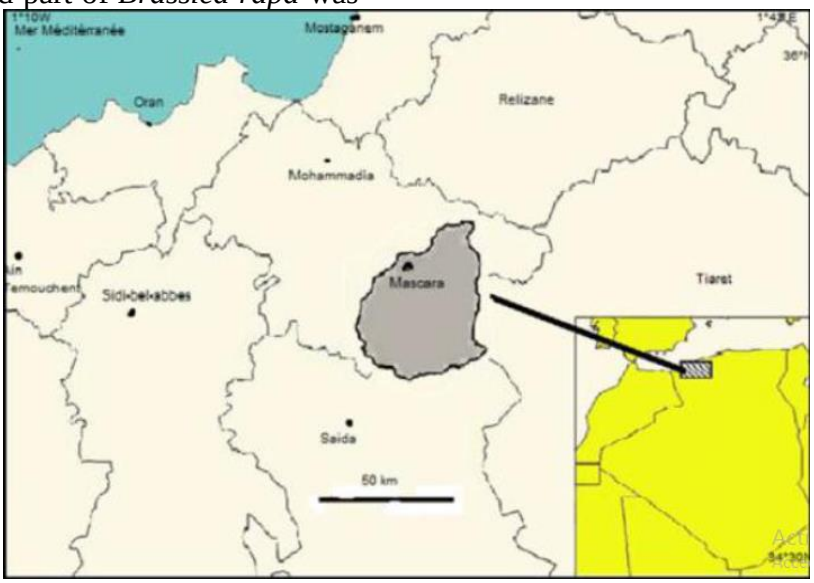

Fig 1: Location of the Ghriss plain [24]

In the laboratory, the *Brassica rapa* rhizomes were cleaned, peeled and ground and then dried at 38°C for 24 hours. After the drying process, the dried plant material was reduced to powder with an electric grinder (Fig 2) and stored in sealed and hermetically containers for later use**.**

Fig 2: *Brassica rapa* powder (original photo)

2.2. Methods

2.2.1. Moisture content

The moisture content is defined as the weight loss during drying. For this purpose, 200 g of the vegetable, cut into small pieces, were weighed in clean capsules and then placed in the oven at 105°C +/- 5°C for a period of 48 h +/- 1 h until the constant weight was obtained. Then allow the capsules to cool before weighing in a desiccator. Repeat this process several times until a constant weight was attained [28].

2.2.2. Ash content

The ash determination is based on the destruction of all organic matter under the effect of high temperature (500 ± 25°C). To perform this, the empty crucibles were weighed, 10 g of the sample was added to the crucibles, The empty crucibles were weighed and 10 g of the sample was added to them, then place them in a muffle furnace for 3-5h at 550°C. After removing from the oven, place these crucibles in a desiccator to cool. Weigh the cooled crucibles, then reheat the crucibles again for a half hour or more. Repeat this process until the weight remains constant [28].

2.2.3. Extracts preparation

To prepare the extract, two extraction methods were adopted: aqueous infusion and alcoholic maceration .For the aqueous extract, 10 g of plant powder are introduced into 50 ml of boiling water and allowed to infuse for 15 minutes. After cooling, the liquid was then filtered and rinsed with a little hot water to obtain 50 ml of filtrate [25]. For the alcoholic extract, the protocol of [26] was adopted, which consists of macerating (10gr) of the plant powder in 100 ml of methanol (80%). The extraction was repeated 3 times with renewal solvent. The extracts obtained were filtered and then evaporated using a rotavapor at 45°C. Two extracts were obtained: aqueous extract of *Brassica rapa* = AqEBr; and methanolic extract of *Brassica rapa* = MEBr. Both extracts were stored in sealed glass vials at ±4°C before testing and analysis.

2.2.4. Yield calculation

The yield of the two extracts was determined using the following equation:

$$R\ (\%) = (M1 / M2) \times 100 \quad (I)$$

R (%): yield in %; M1: mass of extract after evaporation of solvent; M2: mass of plant material used for extraction [27].

2.2.5. Phytochemical screening (qualitative)

Phytochemical tests consist in the identification of the different secondary metabolite families present in the edible part (roots) of *Brassica rapa* by qualitative characterisation, using the standard procedures as described by [29].The results were expressed according to the reaction type: Very positive: +++; Moderately positive: ++; Positive: +; Negative: -

Tannins

To a test tube containing 1 ml of plant extract, 1 ml of a diluted aqueous solution of 1% $FeCl_3$ was added. The blue-black and blue-green color indicates the presence of hydrolyzable tannins and condensed tannins respectively [29] .

Flavonoids
To 5 ml of each extract, 1 ml of isoamyl alcohol, a few magnesium chips and a few drops of hydrochloric acid are added**;** the appearance of a pink or red colour indicates the presence of flavonoids [29] .

Coumarins (UV Fluorescence)
To 2 ml of extract we added 0.5 ml of 25% NH_4OH. After mixture, the observance were made under UV at 366 nm. An intense fluorescence indicates the presence of coumarins[29].

Free anthraquinones (Bornträger test)
We Transfer 1 ml of each prepared extract into a test tube, then we added 1 ml of diluted NH_4OH and stirred. The more or the less red coloration indicated the presence of free anthraquinones [29].

Triterpenoids (Salkowski's test)
A volume of 2 ml of chloroform was mixed with 1 ml of plant extract, and 3 ml of concentrated sulphuric acid (H_2SO_4) was carefully added to the mixture, without stirring. The yellow color of the lower layer indicated the presence of triterpenoid [29].

Free quinones
The presence of free quinones was confirmed by adding a few drops of 10% NaOH to 5 ml of extract, the coloring turns to yellow, red or purple indicates the presence of free quinones [29].

2.2.4. Secondary metabolite quantification

Polyphenols (Folin-Ciocalteu)
The determination of total phenols was carried out according to the protocol established by [30], in a test tube 200 µl of the extract (1g dissolved in 10ml methanol), 1ml of 10-fold diluted Folin-Ciocalteu reagent, and 800 µl of the sodium carbonate solution Na_2CO_3 (7.5%) were added. After 30 min of incubation at room temperature, the absorbance was determined at 765 nm. A calibration range was established with gallic acid($y=0.592x+0.473$ $R^2= 0. 9489$) and the expression of the results were made in µg gallic acid equivalents per mg of dry matter (µg GAE/ mg DM).

Flavonoids (aluminium trichloride)
For the flavonoids determination, the aluminium trichloride colorimetric method was adopted [31]. A 500 µl of the extract was mixed with 1.5 ml of distilled water and then with 0.3 ml of a 5% sodium nitrite solution $NaNO_2$. After 5 min, 3 ml of a 10% $AlCl_3$ solution was added. Then, 6 min later, 1 ml of 4% NaOH was added. After a 5 min rest time, the reaction volume was homogenised with a vortex and the absorbance was measured at 510 nm. To establish the calibration curve, quercetin was used as a standard ($y=0.692x+1.471$ $R^2= 0.967$), the total flavonoid contents were expressed in µg quercetin equivalent / mg of dry matter (µg QE/ mg DM).

Condensed tannins (vanillin)
The amount of tannins was estimated using the vanillin method described by [32]. In test tubes 50 µl of plant extract and 750 µl of 4% vanillin solution were introduced and mixed with the vortex, then 750 µl of concentrated HCl were added and incubated for 20 minutes. The absorbance reading was obtained at 550 nm, the calibration curve was prepared under the same conditions using catechin as standard ($y=6.203x+0.394$ $R^2=0.9843$) and the results were expressed as µg catechin equivalent/mg of dry matter (µg CE/ mg DM).

2.2.5. Antioxidant activity evaluation
In order to estimate the antioxidant activity of the two studied extracts (AqEBr and

MEBr); three different tests were used: TAC (Total antioxidant capacity) , DPPH (2.2'-diphenyl-1-pycrilhydrazyl) , FRAP (Ferric-Reducing Antioxidant Power Assay) .

Total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) of the plant extracts was evaluated by the phosphomolybdenum method. This method was based on the reduction of molybdenum Mo (VI) present as molybdate ions MoO_4^{-2} to molybdenum Mo (V) MoO^{+2} in the presence of the extract to form a green phosphate/Mo (V) complex at acidic pH. The stock solution (10 mg plant powder /10 ml methanol) was prepared. Then, 1 ml of Monosodium phosphate, 1 ml of sulphuric acid, 1 ml of molybdate and 500 µl of sample are introduced into a test tube, this solution was incubated at a temperature of 90°C for 90 min . The blank solution was prepared under the same conditions. The absorbance reading was obtained at 695 nm, the TAC was expressed in milligram equivalents of ascorbic acid per gram of dry matter (mg EAA/ g DM). [33].

DPPH test

DPPH radical scavenging activity was measured according to the protocol described by [34], in test tubes 30 µl of each extract (1mg/ml methanol) was introduced and 1.5 ml of the methanolic solution was added to DPPH (4 mg /100ml). After vortexing, the tubes were stored in the darkness at room temperature for 30 minutes.

The reading was performed by measuring the absorbance at 517 nm. The negative control consists of 1.5 ml of the methanolic solution of DPPH and 30µl of methanol. The positive control was represented by an ascorbic acid solution; we calculate the inhibition percentages by the following formula:

$$I\ \% = ((CA - TA)/CA) \times 100 \quad (II)$$

With: CA: the control absorbance; TA: the test absorbance.

IC_{50} calculation

IC_{50} (inhibitory concentration 50) is the concentration of the test sample required to reduce 50% of the DPPH radical. IC50s were calculated graphically as inhibition percentages according to different extract tested concentrations [35].

FRAP test

The protocol established by [36] was adopted, which consisted of removing 0.5 ml of each extract at different concentrations and adding 1.25 ml of a 0.2 M phosphate buffer solution (pH=6.6) and 1.25 ml of a 1% potassium ferricyanide $K_3Fe(CN)_6$ solution and incubated at 50°C for 20 min, The tubes were then cooled to room temperature. 2.5 ml of 10% trichloroacetic acid (TCA) were added to block the reaction, the tubes were centrifuged at 3000 rpm for 10 min. then the supernatant (1.25 ml) was added to 1.25 ml of distilled water, and 250 ml of a 0.1% iron chloride ($FeCl_3$) solution, the absorbance reading was made at 700 nm. The positive control was represented by an ascorbic acid solution.

2.2.6. Statistical analysis

Each value is the mean of three replicates. Values of different parameters were expressed as the mean ± standard deviation (SD).

3. Results and discussion

3.1. Moisture and ash content

According to our results the turnip has a moisture content of 74%. Our results were lower than those found by [37] which were 91.90%. According to [38], several factors (genetic, pedoclimatic, geographical, storage conditions, the plant's age,

vegetative cycle and the maturation stage can influence the plant's water content. According to our results, turnip roots have an ash content of 10%, which indicated that *Brassica rapa* is rich in mineral elements. In addition, the raw turnip contains a significant amount of potassium, 237 mg per 100 g [37].

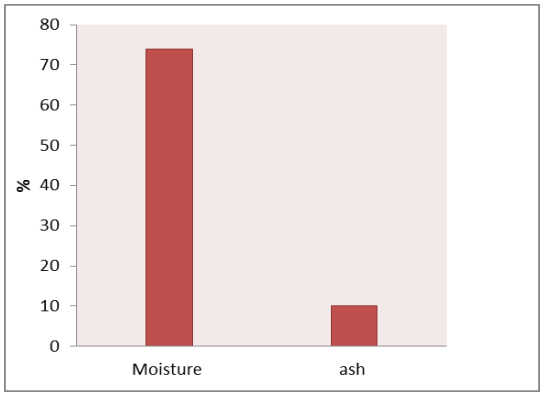

Fig 3: Moisture and ash levels of *Brassica rapa* roots

3.1. Extraction yields

After the extracts have been collected, the yields were calculated and represented in the (Fig 4)

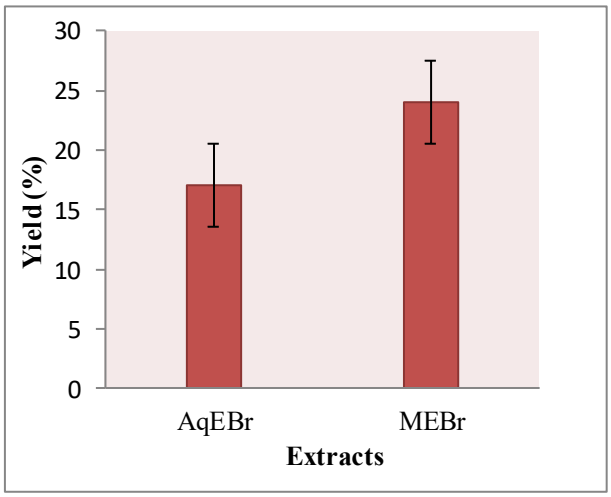

Fig 4 : Extraction yields of *Brassica rapa* roots

The highest yield was 24% for MEBr compared to 17% for AqEBr, this may be due to the differential solubility of the different phenolic compounds in the solvents and that this solubility is related to their degree of polymerisation, the interaction with other constituents and the used solvent type [27].

3.3. Phytochemical screening

The preliminary evaluation of the *Brassica rapa* phytochemical composition revealed the presence of some chemical groups (Table 1).

It can be seen that the two extracts MEBr and AqEBr are provided with different intensities of all the metabolites, except the anthraquinone class which is absent in both extracts. According to previous works, *Brassica* species are rich in phenolic compounds, flavonoids, hydroxycinnamic acid, coumarins and terpenoids, which are very beneficial for human health. These components exert an antioxidant activity by inhibiting carcinogenesis and the production of reactive oxygen species (ROS) [39]

In addition, *Brassica rapa* contains anthocyanins, responsible for red and purple pigmentation [40].

These data are comparable with our results, since the tests reveal the presence of flavonoids, coumarins and terpenoids.

On the other hand, our results are synchronized with those of [41], who confirmed the presence of flavonoids, sulphur compounds and anthocyanins in the *Brassica rapa* underground part.

3.4. Secondary metabolites

The total phenolic content (TPC), total flavonoid content (TFC) and condensed tannin content (CTC) were determined from the calibration curves of gallic acid, quercetin and catechin respectively. The TPC, TFC and CTC of the *Brassica rapa* .L tubers aqueous and methanolic extracts of tubers are presented in (Table 2).

Table 1:

Preliminary phytochemical analysis of *Brassica rapa*

N°	Phyto-chemical test		Extracts	
	Chemicals constituents	**Tests**	**MEBr**	**AqEBr**
1	Tanins	Ferric Chloride test	+	+
2	Flavonoids	Shinoda test	++	++
3	Coumarins	Fluorescence UV	++	++
4	Free anthraquinones	Borntrager's test	- -	- -
5	Terpenoids	Salkowski Test	++ +	+++
6	Free quinones	Sulfuric acid test	+	+++

Very positive: +++ ; moderately positive: ++; positive: +; negative: -

Table 2:

Total phenolic, flavonoids and condensed tannins contents of *Brassica rapa*

	TPC (*µ*g GAE/mg DM)	**TFC (*µ*g QE/mg DM)**	**CTC (*µ*g CE/mg DM)**
AqEBr	0.12± 0.0009	0.015 ± 0.0005	0.007 ± 0.0003
MEBr	1.48 ± 0.0009	0.15 ± 0.0005	0.11 ± 0.001

Each value was expressed as means ± Standard deviations for triplicate experiments. CE: Catechin equivalent; DM: Dry Matter; GAE: Gallic acid equivalent; QE: Quercetin equivalent; TFC: Total flavonoid content; TPC: Total phenolic content; CTC: Condensed tannins content

According to our results, the *Brassica rapa* concentration of bioactive compounds (TPC, TFC, CTC) varied depending to the extractor type, of which extraction with methanolic maceration is more efficient than aqueous extraction .These results are similar to several works, including those of [42], who confirmed that the methanolic extract of *B. rapa* (root) had values of 0.3 µg GAE / mg and of 0.041- 0.085 µg rutin equivalent / mg of Fresh weight in polyphenols and flavonoids respectively. In addition, according to [37], the TPC of turnip was at 1.5 µg / mg.

On the other hand, our results are considerably lower than those found by [43], who detected a TPC of 2.1 - 25.9 µg GAE / mg of dw with 70 % ethanol. According to [44], the *B. rapa* subsp. *rapifera* .L aqueous extract contains 5.64 µg/mg of total phenols. According to the work of [45], the *Brassica rapa* subsp. *rapifer* aqueous extract of showed an amount of phenolic and flavonoid compounds with values of 9.41 ± 0.18 *µ*g GAE/mg and 1.01 ± 0.09 *µ*g QE/mg, respectively. The content of phenolic compounds varied significantly between the different parts of the turnip.

For example, turnip green was revealed to contain 51.71 µmol/ g dw of phenolic compounds compared to 38.99 µmol/g dw in turnip top .

The family, amount and concentration of different bioactive molecules in turnips are dependent on the development stage, as well as on biotic and abiotic factors. For example, norisoprenoids, terrpenes and aldehydes are highly concentrated at the germination stage (9-day-old turnip sprouts); whereas, at the maturity stage there is a decrease in sulphur and nitrogen compounds [11].

Furthermore, herbivory pressure and high humidity always increased the abundance of secondary metabolites [46]. However,

the choice of extraction solvent, pH, light and heat can influence the content of phenolic compounds [47].

Several studies have confirmed the richness of turnip in glucosinolates, isothiocyanates, flavonoids, volatile substances [3, 4, 11, 48]. Flavonoids are major components in turnips, present as glycosides. Among the 35 flavonoids reported in this plant, kaempferol, quercetin and isorhamnetin are the most common aglycones [49].

Flavonoids play an important role in UV protection, pigmentation and disease resistance. This explains their high concentration in the leaves and fruit epidermis. Flavonoids detected in turnip include 27 flavonols, two flavanones and six chalcones [50, 51] .

The presence of tannins suggests the ability of our plant to play a major function as an antioxidant agent [52]. This variation can be explained by the fact that the extraction of condensed tannins, depends on their chemical nature, the solvent used and the operating conditions

3.5. Antioxidant activity

3.5.1. Total Antioxidant Capacity (TAC)

The reducing capacity of a compound can serve as a significant indicator of its potential antioxidant activity [53]. The quantitative estimation of total antioxidant capacity was calculated according to the equation ($y = 0.175x+0.125$. $R^2= 0.9975$).

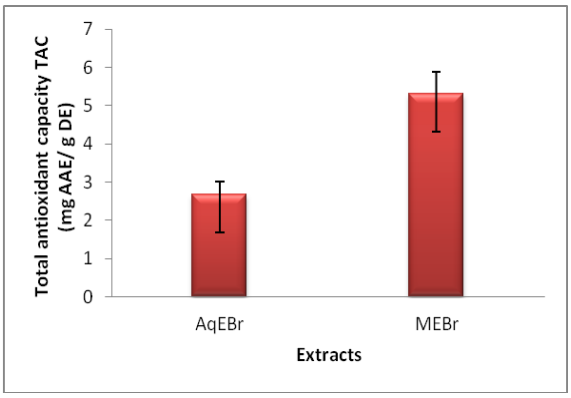

Fig 5: Total Antioxidant Capacity (TAC) of different *Brassica rapa* extracts.

It can be seen that the TAC of MEBr is higher compared to AqEBr with values of (5.32 ± 0.56 vs 2.68 ± 0.33 mg AAE/gr DE) respectively (Fig 5). This suggests that the antioxidant activity increases with increasing polarity of the extracting solvent [43]. According to [54], the extraction time also participates in the increase of phytochemical content and antiradical activity .

3.5.2. DPPH evaluation

From our results, it can be seen that the inhibition percentage of DPPH free radical increases with increasing the extract concentration. However, MEBr demonstrated a strong antioxidant capacity compared to ascorbic acid. On the other hand, AqEBr showed a moderate anti-radical activity (Fig 6).

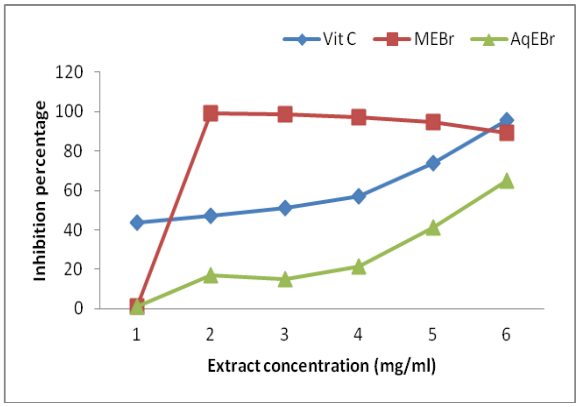

Fig 6: Antiradical activity (DPPH test) of *Brassica rapa* methanolic and aqueous extracts

Several researchers have widely pointed out the in vitro and in vivo antioxidant activity of turnip [9, 42, 55]. In addition, ethyl acetate extract from turnip roots exhibited higher free radical scavenging and lipoperoxidation inhibitory activity than other solvents (n-hexane, chloroform) [42]. However, [55] reported a inhibition percentage of DPPH radical with 11.11-86.3% using ethanol (70%) as an extractant solvent. On the other hand, [42] revealed a medium-low rate with 13-26%

of the methanolic extract of the *Brassica rapa* underground part.
In addition, further studies have revealed that turnip flower buds have the highest antioxidant capacity compared to other edible parts (leaves, stems and roots) with the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging test [8,56] .
According to [57], the anti-free radical activity is dependent on the number, position and nature of substituents on the B and C rings (hydroxyl, metaxyl, glycosyl groups) and the degree of polymerization of phenolic compounds.

IC_{50} (DPPH test)

According to our results, the methanolic extract showed the highest antiradical activity with an IC_{50} of 0.14±0.12 mg/ml compared to the aqueous extract with an IC_{50} of 1.9±0.45 mg/ml.

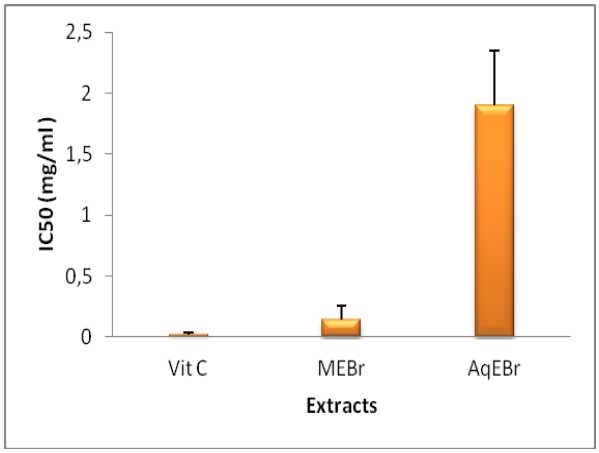

Fig 7 : IC_{50} of different *Brassica rapa* extracts and ascorbic acid

The results of [42] are synchronized with our, which they found that IC_{50} of ethanolic extract (70%) of the *Brassica. rapa* subsp. *rapifera* .L edible part was 0.23-2.00 mg/ml. According to 8 , flower buds showed an IC_{50}value of 0.94 mg/ml, followed by leaves and stems IC_{50}=1.12 mg/ml, and then roots had the lowest antioxidant capacity with an IC_{50} value of 2.88 mg/ml.

3.5.3. FRAP evaluation

The reduction of Fe^{+3} is often used to study the ability of a substance to release electrons. This property constitutes an important mechanism of antioxidant action [58]. Our results revealed that AqEBr has the highest antioxidant power compared to MEBr with maximum values of (1.41 mg/ml vs 0.83 mg/ml) respectively. However, ascorbic acid showed the highest antioxidant power with a maximum of 1.63mg/ml (Fig 8).

Antioxidant activity depends not only on the concentration of phenolic molecules but also on the structure of these molecules. Low or high antioxidant capacity may be due to the solvent's polarity, which modified the capacity to dissolve a selected group of antioxidant compounds, thus influencing the antioxidant activity evaluation [59]. The temperature strongly determined the level of enzyme activity involved in the polyphenol synthesis. Therefore, different thermal conditions can induce a significant change in the final polyphenols concentration. Other environmental factors could probably play a role in the process of polyphenol synthesis such as CO_2 content (C/N interaction) or the level of oxidative stress [60].

4. Conclusion

From the results presented, it appears that methanol is a more efficient extractor than water, which MEBr exhibited a higher antioxidant capacity and richness in secondary metabolites than AqEBr .Therefore, the selection of an appropriate solvent-based system remains one of the most important steps, in the optimization of the polyphenols, flavonoids and other antioxidant compounds extraction, for a better active ingredients valorization. Furthermore, the results obtained in this

work are very promising; they indicate that the turnip *Brassica rapa* can be a easily available food source of biologically active compounds.

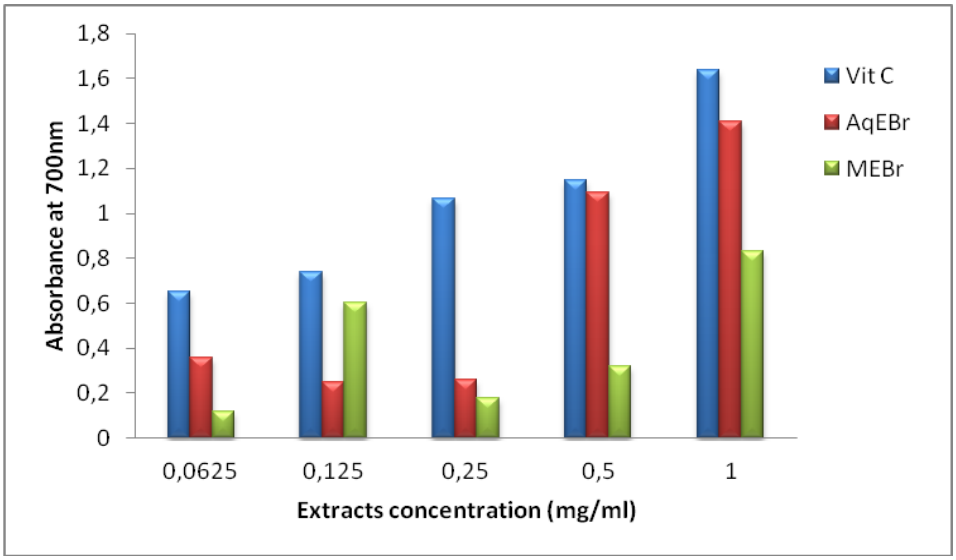

Fig 8: Antiradical activity of *Brassica rapa* methanolic and aqueous extracts

5. Acknowledgments

The authors declare that they have no conflicts of interest in relation to this article.

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