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Phytochemical study and compounds evaluation of antioxidant activity phenolic of the essential oil of the fruit: *Citrus aurantium* spp. *Amara* (Bitter orange)

Sammama A[1*], Azoungad A[2], El yahyaoui O[3], Kerrouri S[4], Bouabid B[5], Ould Abdellahi L[6], Belfekih F[7], Kribii A[8], R Bengueddour[9]

[1-7, 9] Laboratory biochemistry, biotechnology, health and the environment, Department of Biology, Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco

[8] Laboratory of separation processes, team of chemistry applied to the environment, Department of chemistry, Faculty of Sciences, Ibn Tofail University Kenitra, Morocco

Abstract

The antioxidant compounds are the object of many projects; because in addition to their use as preservatives in food, by replacing synthetic antioxidants, they are intervene in the treatment of many diseases.

Bitter orange is a citrus of the Rutaceae family that includes several important fruits such as oranges, mandarins, limes, lemons and grapefruit. Citrus fruits have an important economic value for their essential oils. These are obtained as processing by-products of processed citrus fruits and are the most widely used in the world. Indeed, these citrus essential oils and their important components have gained acceptance in the food and pharmaceutical industries.

In the context of the discovery of new antioxidants from natural sources, we were interested in this work in the preliminary study by phytochemical screening and confirmation by analytical separation of a technique (TLC) micro-components extracts and quantification of total phenols, flavonoids and tannins by the Folin-Ciocaleu reagent, Aluminum trichloride and vanillin respectively. Finally, the evaluation of the antioxidant activity of the C. Aurantium fruit essential oil is carried out by the DPPH method and its extraction is carried out by hydro-distillation.

The results obtained showed the fruit richness in phenolic compounds mainly polyphenols (144.32±3.42 mg EAG/g of FVM) and flavonoids (39.21±1.00 mg EQ/g of FVM) thus, the existence of an antioxidant activity of the essential oil of the whole fruit of C. Aurantium (IC_{50}=15.2±0.11µg/ml) but less effective than that of vitamin C (IC_{50}=0.0027±0.26 µg/ml).

Keywords: *Citrus Aurantium*, phenolic compounds, DPPH, phytochemical screening, TLC and essential oil

1. Introduction

Different aromatic plants such as citrus fruits are characterized by the synthesis of odorous molecules that constitute what are known as essential oils (EO) or common essences.

Epidemiological studies have suggested beneficial effects of citrus fruits against many degenerative diseases [10, 42].

These positive influences on human health have significantly increased *citrus* consumption in recent years and it is estimated that world citrus fruits production reached 121 273.2 milliers tonnes in 2013-2014 session [20].

Citrus fruits are a good source of phenolic compounds, especially flavonoids (flavanones glycosides).

Currently, the extraction of these phenolic compounds of citrus has attracted a great scientific interest for their use such as natural antioxidants, mainly to prevent the oxidation of lipids [1, 46].

Indeed, in recent years, much research has focused on plants and their by-products to find natural, low-cost antioxidants that can replace synthetic additives that could be toxic and carcinogenic to the liver [30, 4].

The idea of this research was to discover the active ingredients in the citrus fruit studied and their antioxidant power and also to understand the extraction methods of these active ingredients.

We will try to highlight a proliferating species in our region, it is *Citrus Aurantium*, which we seek and assay a content of bioactive molecules especially phenolic compounds.

2. Materials and Methods

2.1 Vegetal material

2.1.1 Phytochemical screening

The plant material consists of the fruits of *C. aurantium*. These fruits are harvested in April 2015 in Kenitra. After washing under a continuous stream of water for 5 minutes, the fruits are cut into slices and the seeds are separated from them. Then, these fruits and seeds are dried and crushed using an electronic grinder and then stored carefully until they are used. The identification of the species is made by Professor Zidane L, the Biodiversity and Natural Resources Laboratory, Department of Biology in Kenitra Faculty of Sciences.

2.1.2 The extraction of the essential oil of *Citrus Aurantium*

A biomass of 306 g of fresh plant material is subjected to a hydrodistillation pendant 4H, using a device of the clevenger

type. The essence thus recovered is kept at a low temperature (4 °C) away from light in a glass bottle, hermetically sealed to avoid any degradation. The hydrodistillation is carried out in the chemistry department «Laboratory of separation processes, team of chemistry applied to the environment.

2.2 Methods
2.2.1 Qualitative study of phytoconstituants
2.2.1.1 Phytochemical screening
Preliminary phytochemical screening aims to characterize the different chemical groups present in seeds and whole fruits. It was carried out on tube characterization reaction extracts, and by thin layer chromatography.

2.2.1.1.1 Tubes characterization reactions
This is a qualitative method regarding the implementation of the reactions tubes either by precipitation or by staining.

2.2.1.1.1.1 Characterization of Phenolic Compounds
Preparation of filtrate: an infusion is prepared from 5 g of powder sample added to 100 ml of boiling water and allowed to stand for 15 minutes; it is then filtered and adjusts with hot water up to 100 ml. The extract obtained will be used for the characterization of tannins and flavonoids.

a. Characterization of Tannins
Free tannins at 5ml are infused at 5% and 1 ml of $FeCl_3$ aqueous solution at 1 % are introduced into a tube. The development of a blackish or greenish blue color indicates the presence of tannins [41].Differentiation of catechin tannins and Gallic is done through:
* The reaction Stiasny: it consists of adding 30 ml of 5% infused with 15 ml of Stiasny reagent and then heated in a water bath at 90 ° C for 15 min. Obtaining a precipitate shows the presence of catechol tannins. Filter the filtrate saturated with sodium acetate, after adding 1 ml of 1% FeCl3. The development of a blue-black color signals the presence of gallic tannins not precipitated by Stiasny reagent [41].

b) Characterization of flavonoids
b.1 The reaction to cyaniding
1 ml infused at 5%, 1 ml hydrochloric alcohol and 1 ml of isoamyl alcohol are introduced into a tube by adding somemagnesium turnings. Crepitus reaction occurred for a few minutes [15].
The appearance of the isoamyl alcohol supernatant layer of a colouring:
- Orange-pink characterizes the flavones.
- Purplish-pink characterizes the flavanones.
- Red characterizes flavonones and flavanonols.

b.2 Anthocyanins
This test is performed by adding 5 ml of infused to 5%, 5 ml of sulfuric acid and 5 ml of NH 4 OH in a tube. In the presence of anthocyanins, the colour deepens by acidification and then turns purplish blue in basic medium [15].

b.3 Leucoanthocyanins
Cyanidin reaction is heated in a water bath for 15 minutes without the addition of magnesium shavings. In the presence of leucoanthocyane, it develops a cherry red colour with purplish or against the red-brown colour indicates the presence of catechol [15].

c) Characterization of Quinones
An amount of 0.5 g of the powder sample was introduced into 5 ml of petroleum ether and stirred for a few minutes; the resulting mixture was rested for 12 hours. After filtration, the extract is evaporated to the rota-vapor. The colour change of the aqueous phase in yellow, red or violet after adding a few drops of NaOH (1/10) is proving the presence of quinones [37].

2.2.1.1.1.2 Characterizationof Sterols and Terpenes
Preparation of the filtrate: 1 g of powder sample is digested in 20 ml ether, then stirred and left in the dark for 24 hours. After filtration, the filtrate was adjusted to 20 ml with ether.
The extract used in addition to the sterols and terpenes to the characterization of carotenoids and coumarins.
Sterols and terpenes are highlighted by adding 10 ml of the etheric extracted with 1ml of $CHCl_3$ and 1 to 2 ml of concentrated H2SO4 in a tube. The cookie contains only CHCl3 and the extract. The formation of a brownish red or purple ring at the two liquid contact zones, reveal their presence [7].

2.2.1.1.1.3 Characterization of carotenoids
After evaporation until dry, 5 ml ethereal extracts is added to 3 drops of a saturated solution of antimony trichloride in chloroform. The presence of carotenoids is indicated by a blue colour toned red [7].

2.2.1.1.1.4 Characterization of coumarins
After evaporation, 5 ml ether extracts, 2 ml of hot water and 1 ml of 25% NH_4OH are added successively. The observation of intense blue fluorescence under UV at 366 nm indicates the presence of coumarins [7].

2.2.1.1.1.5 Characterization of alkaloids
An amount of 2 g of sample powder is saturated in 15 ml of distilled water for 24 hours in the dark and then filtered. A few drops of Dragendorff reagent are added to 1 ml of the aqueous extract. The presence of alkaloids is marked by the formation of an orange precipitate.

2.2.1.1.1.6 Characterization of saponosides
2 ml of aqueous extract diluted to half in distilled water in a tube are stirred continuously for 15 seconds. The persistence of foam of at least 1 cm for 15 minutes indicates the presence of saponins [11].

2.2.1.1.1.7 Characterization of essential oils
The dichloromethane extract of 10 ml is evaporated to dryness. The product residue is dissolved in 3 ml of ethanol and then evaporated to dryness again. The feel of a fragrant smell indicates the presence of essential oils [22].

2.2.1.1.1.8 Characterization of mucilages
1 ml of 10% aqueous decoction is added to 5 ml of absolute ethanol. Mucilage are characterized by the appearance of a flocculent [7].

2.2.1.1.1.9 Characterization of reducing sugars

5 ml aqueous decoction are introduced into a tube and evaporated in a water bath until dry. To the residue is added 1 ml of Fehling reagent. Obtaining a brick red precipitate indicates the presence of reducing compounds [16].

2.2.1.1.2 Chromatographic Analysis (TLC)

Thin Layer chromatography (TLC): this is a qualitative method that is used to identify phytoconstituents contained in extracts.

2.2.1.1.2.1 Search for flavonoids, saponins and anthraquinones

Preparation of the filtrate a quantity of 1 g samples of the citrus is placed in 20 ml of 80% methanol. The solutions are subjected to stirring for 15 min, after sonication of 15 min, followed by filtration [18, 32].

2.2.1.1.2.2 Search for tannins and alkaloids

Preparation of the filtrate a quantity of 2g samples of Citrus is introduced into 15 ml of acetone and then subjected to a decoction for 1 h at 70 ° C. The acetone extracts are filtered hot and evaporated in an evaporator at 70 ° C. Then 10 mg of the crude extracts are dissolved in 1 ml of methanol [38].

2.2.1.1.2.3 Search for Coumarines

Preparation of the filtrate a quantity of 2 g samples of Citrus is placed in 10 ml of chloroform. The whole is heated for a few minutes and then filtered [37].

2.2.1.1.2.4 Search for carotenoids

Preparation of the filtrate a quantity of 1 g samples of Citrus is introduced into 5 ml of dichloromethane.

The solution is subjected to stirring for 1 hour. The Dichlorome̒ thaloniques extracts were filtered and concentrated in an evaporator at 40°C [40].

All extracts are deposited using a micropipette on silica gel plates DC- Fertigfolien ALUGAM SIL G / UV254. These are dried in an oven for a few seconds and then introduced into a vessel containing specific migration of solvents for each step of identification. After migration, the plates are removed, dried, examined under a UV lamp 254nm and UV 354nm an drevealed with a specific reagent for the identification of chemical components (Table 1).

Table 1: Migration and indicative of solvants used for the characterization of phytochemical constituents of fruits and seeds of *Citrus aurantium* by thin layer chromatography

Phytochemical Constituents	Extract	Migration solvents (ml)	UV Developers
Tannins	Acetone	Ethyl acetate: 40	Ferric chloride,
		Methanol: 8	Acetic acid,
		Distilled HO_2: 9	Distilled HO_2.
Flavonoids	Methanol	Ethyl acetate: 9	Reagent NEU
		Methanol: 1	
		Ammonia at 50%: 1	
Alcaloids	Acetone	Chloroform: 45	Reagent Dragendr off
		Diethylamine: 5	
Coumarins	chloroform	Ethyl acetate: 10	ammonia
		Toluene: 93	
Carotenoids	Dichloromethan	Diethyl ether: 60	-
		Petroleum ether: 40	
Saponoides	Methanol	Chloroform: 60	Antimony trichloride
		Methanol: 30	
		Distilled HO_2: 4	
Anthraquinones	Methanol	Ethyl acetate: 81	Potassium hydroxide
		Methanol: 11	
		Distilled HO_2: 8	

2.2.2 Quantitative study of phenolic compounds in the essential oil of *C. aurantium*

2.2.2.1 Determination of total phenolics

The Folin-Ciocalteu (Sigma-Aldrich, Germany) method according to [37] of [37], simple and sensitive, and it is used to determine the total phenols. 500 µl of extract are added to 2500 µl of Folin reagent diluted 10-fold. In the last step, 1000 µl of 7.5% (w / v) sodium bicarbonate is added to the tubes. The absorbance is read at 765 nm after 2 hours of incubation at room temperature and in the absence of light. Gallic acid (Sigma-Aldrich, Germany) is used as a standard and the results are expressed in milligrams of gallic acid equivalent per gram of fresh vegetable matter (mg EAG/g FVM).

2.2.2.2 Determination of total flavonoids

The aluminium trichloride (Sigma-Aldrich, Germany) method described by [26] and cited by [8] is used to quantify flavonoids. 1000 µl of each sample (prepared in methanol and in distilled water concerning essential oil and hydrolate) with suitable dilutions, are added to1000 µl of the $AlCl_3$ solution (2% in methanol). After 10 minutes of reaction, the absorbance is read at 430 nm. The concentrations of flavonoids are deduced from the calibration range established with Quercetin (Sigma-Aldrich, Germany) and are expressed in microgram equivalent of Quercetin per gram of fresh vegetable matter (µg EQ/g FVM).

2.2.2.3 Determination of Condensed Tannins

Condensed tannins are determined by the acid vanillin method [21] (Sigma-Aldrich, Germany).

500 µl of extract are added to 2,500 µl of vanillin reagent (mixture with equal volume of 8% of HCl in methanol and vanillin 1% in methanol), after the tubes are maintained to 30 °C during 20 min with the bath Marie. The absorbance is read to 500 nm.
Catechin (Sigma-Aldrich, Germany) is used as a standard and the results are expressed in milligram of catechin equivalent per gram of fresh vegetable matter (mg CE/g FVM).

2.2.3 Evaluation of the antioxidant activity of the essential oil by test DPPH

The antioxidant activity is measured by the method DPPH = 1,1-diphenyl-2-picrylhydrazyl (Sigma-Aldrich, Germany). This activity is determined according to the method of [6]. The DPPH radical is dissolved in methanol at a concentration of 5.5 mg/ml and then sonicated for 3 minutes and kept at -4 ° C for 1 hour protected from light before use. To each extract (1 ml) is added 2.5 ml of DPPH solution and the absorbance is measured after 30 minutes at 515 nm. The blank is made from 2.5 ml of DPPH and 1 ml of methanol and measured the absorbance at t = 0. The DPPH trapping is calculated according to the following equation [29]:

$$IC_{50}\% = [1- (Abs\ (t = 30)/ Abs\ (t = 0))]*100$$

Where Abs (t = 0) = (absorbance of DPPH radical + methanol) at t = 0 min.
Abs (t = 30) = (absorbance of DPPH radical + phenolic extracts) at t = 30 min.

2.3 Statistical analysis

All data were reported as the means OD±SE. Statistical analysis was performed for the conducted in at least triplicate using one-way analysis of variance (ANOVA-Tukey) using SPSS 20 statistical software package. Results with $p < 0.05$ were considered to be statistically significant.

3. Results and Discussions

3.1 Qualitative study of phytoconstituants

3.1.1 Phytochemical Screening

The results of the phytochemical study on the whole fruit and seeds of *C. aurantium* are shown in Table 2 below.
The phytochemical screening carried out on the two samples made it possible to obtain the following results:

- Flavonoids exist in both parts of the fruit. They are in larger quantities in fruits (seedless) compared to their seeds. The cyanidin reaction confirms the presence of flavonones in large quantities in fruits compared to their seeds.
- In both parts, free tannins, carotenoids, essential oils and reducing sugars are present with very large quantities in fruits (seedless) compared to seeds.
- Coumarins and mucilages are important in pips compared to fruits (without seeds).
- Revealing saponins and sterols-terpenes are important in both samples.
- Finally, it should be noted that alkaloids are absent in both parts of the fruit.

Table 2: Preliminary phytochemical screening of fruits and seeds of *Citrus aurantium*

Phytochemical constituents	*Citrus Aurantium. Amara*	
	Whole fruit	**Seeds**
- Free Flavonoids	+ + + + + Purplish-pink color	+ Purplish-pink color
Flavanones	_	_
Flavones	_	_
Flavonols–flavanonols	_	_
Leucoanthocyanins	+	_
Cathechols	_	_
Anthocyanins	_	_
- Free Tannins (Iron (III) chloride)	+ + + + (greenish)	+ + (greenish)
Catechetical Tannins	_	_
GallicTannins	_	_
Coumarins	+ (blue)	+ + + (blue)
Saponins	++	++
Alkaloids (Dragendorff)	_	_
Sterols and terpenoids	+ + + + + (Red-stranded brin)	+ + + (Red-stranded brin)
Carotenoids	+ + + + + (greenish-blue)	+ (Light green)
Mucilage test	_ (absence of precipitation)	+ + (flaky precipitation)
Essential oils	+ + + +	+ +
Reducing sugars	+ + + + + Red-brick precipitate	+ + Red-brick precipitate

Keys: (++++Strong positive reaction; +++Positive reaction; ++ moderate positive reaction; + weak positive reaction; - Negative reaction)

3.1.2 Chromatographic analysis (TLC)

The table 3 shows the groups of secondary metabolites that are contained in the samples studied.
After TLC and UV observation at 366 nm and reagent revelation of NEU, the tested whole fruit extract has various blue and yellow fluorescent constituents. On this basis, the yellow spots (Fr = 0.73), blue (Fr= 0.18, Fr = 0.95) are flavonoids of the whole fruit. In fact, the ammonia reveals the coumarins of the seeds at spots of Fr = 0.08 and Fr = 0.12. In addition the blue spots of Fr = 0.28 and Fr = 0.95 reveale dunder UV / 366nmet that is persisted after potassium hydroxide treatment, are anthraquinones of the whole fruit. On the other hand, in seeds are presented by a blue sport of Fr = 0.23.

The tannins of the whole fruit are revealed by ferric chloride, acetic acid and distilled water under UV / 366 nm by blue spots of Fr = 0.4, Fr = 0.73 and Fr = 0.84.
The detection of carotenoids by TLC is revealed under UV at 366 nm showed very visible blue spots of Fr = 0.14, Fr = 0.48, Fr = 0.69 and Fr = 0.86 in the whole fruit.
The chromatogram of the extract of whole fruit and seeds, following a UV extinction at 366 nm, shows the presence of blue spots, which confirms the presence of saponines triterpene when frontal reports are 0.59, 0.68, 0.77, 0.87 in the fruit and 0.71, 0.75, 0.79, 0.90 in *C. aurantium* seeds. On the other hand, the search for alkaloids was negative in both samples.
Finally, we can conclude that the results of the TLC confirm the presence of certain secondary metabolites in the two studied samples of *C.aurantium* and also reinforce what we obtained previously (Table 2).
Phytochemical examination indicates that *C. aurantium* (whole fruit) reveals various secondary metabolites. These results are in agreement with previous studies conducted on *C. aurantifolia*) [17, 34], *C. medicalinn* [31] and *C. maxima* [25] that also contain flavonoids, tannins, carotenoids, saponins and coumarins.
According to several studies, the phytochemical analysis of grapefruit seeds *citrus paradise* and *citrus limon* showed the presence of alkaloids, flavonoids, tannins, saponins, steroids, glycosides and essential oils [3, 33].
Our qualitative study has proven the enrichment of *C.Aurantium* by bioactive compounds which are the subject of several industrial researches (pharmaceutical, agro-alimentary, cosmetic...).

Table 3: Front Reports (FR) fruits and seeds of *Citrus aurantium* based on phytochemical constituents

Les constituants Phytochimiques	Fruit Without Seeds		Seeds	
	Fr	color	Fr	Color
Tannins	0.40	Blue	_	_
	0.73	Blue	_	_
	0.84	Blue	_	_
Flavonoids	0.18	Blue	_	_
	0.73	Yellow	_	_
	0.88	Blue	_	_
	0.95	Blue	_	_
Coumarines	_	_	0.08	Blue
	_	_	0.12	Blue
Alkaloids	_	_	_	_
Carotenoids	0.14	Blue	_	_
	0.48	Blue	_	_
	0.69	Blue	_	_
	0.86	Blue	_	_
Saponins	0.59	Blue	0.71	Blue
	0.68	Blue	0.75	Blue
	0.77	Blue	0.79	Blue
	0.87	Blue	0.90	Blue
Anthraquinones	0.28	Blue	0.23	Blue
	0.95	Blue	_	_

3.2 Quantitative study of phenolic compounds in the essential oil of C. aurantium

3.2.1 Characterization of the essential oil obtained by hydrodistillation

After extraction, we determined the organoleptic characteristics of our essential oil and compared with those of the AFNOR [5]. The results are shown in the table 4 below.

Table 4: Organoleptic characteristics of hydrodistilation extracted of OE of *Citrus Aurantium* Seedless Fruits

Organoleptic Characters	Aspect	Color	Odor
Essential oil	Liquid	Pale Yellow	Strong lemon and fresh
Essential oil AFNOR	Liquid	Translucent slightly tinged with yellow	Strong lemon and fresh

3.2.2 Essential oil yield of *Citrus aurantium*

The yield of essential oil fresh (seedless) whole fruits of *Citrus aurantium*, calculated on the basis of the dry matter is found of the order of 0.15%. While, the results obtained by studies conducted in Tunisia [12] and Iran [36], indicate that the essential oil of *C.aurantium* has high yields of 0.46% and 0.7% respectively. Let us note that these variations of yield can be due to several factors notably the interaction with environment (climate, soils...), the extraction technique, the period and the medium of harvest, the cultural practices and the age of the material vegetable [9, 2, 12].

3.2.3 Determination of total phenols, flavonoids and condensed tannins

The results are expressed in terms of equivalents of the specific standards by using the calibration curves (figure 1) by using the equations mentioned in the table 5.

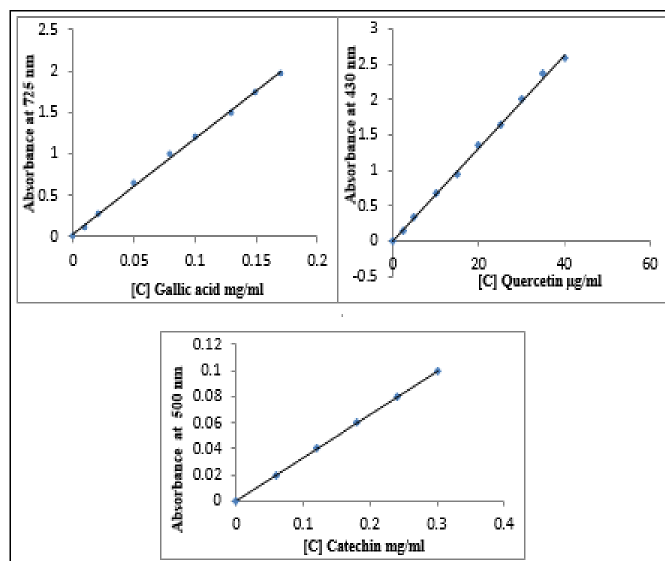

Fig 1: Calibration Curves of the gallic acid (polyphenols), quercetin (flavonoids) and catechins (condensed tannins)

Table 5: Equations of calibration curves of gallic acid (polyphenols), quercetin (flavonoids) and catechins (condensed tannins)

	Gallic acid	Quercetin	Catechins
The Equations	Y=11.492x+0.0301	Y=0.0662x-0.0025	Y=0.3333x
Coefficient of Determination	R^2=0.9982	R^2=0.9984	R^2=1

The results of the assays (figure 2) show that EO has a fairly high polyphenol and flavonoid content of 144.32±3.42 mg EAG/g Fresh Plant Material (FVM) and 39.21±1.00 mg EQ/g (FVM) thus, that the hydrolate shows a value of 4.75±0.26 mg EAG/ml and 0.27±0.01 mg EQ/ml respectively. This implies that during the hydrodistillation of seedless fruit, the hydrolate requires a small amount of polyphenols, but which can be probably reducing sugars, waxes or hydroxyl groups.
In parallel, the essential oil shows a fairly low condensed tannin content of 11.48±1.16 mg EC/g (FVM) whereas, the hydrolate registers a value of 0.16±0.02 mg EC/ml, this value is very negligible compared with that present in the OE. This oil has a high content of polyphenols followed by flavonoids and then by the tannins.
At the conclusion of these quantitative characterization results, the fruit of *C. aurantium* through these constituents in polyphenols, flavonoids and tannins is a promising source of bioactive compounds beneficial to human health (an antioxidant role).

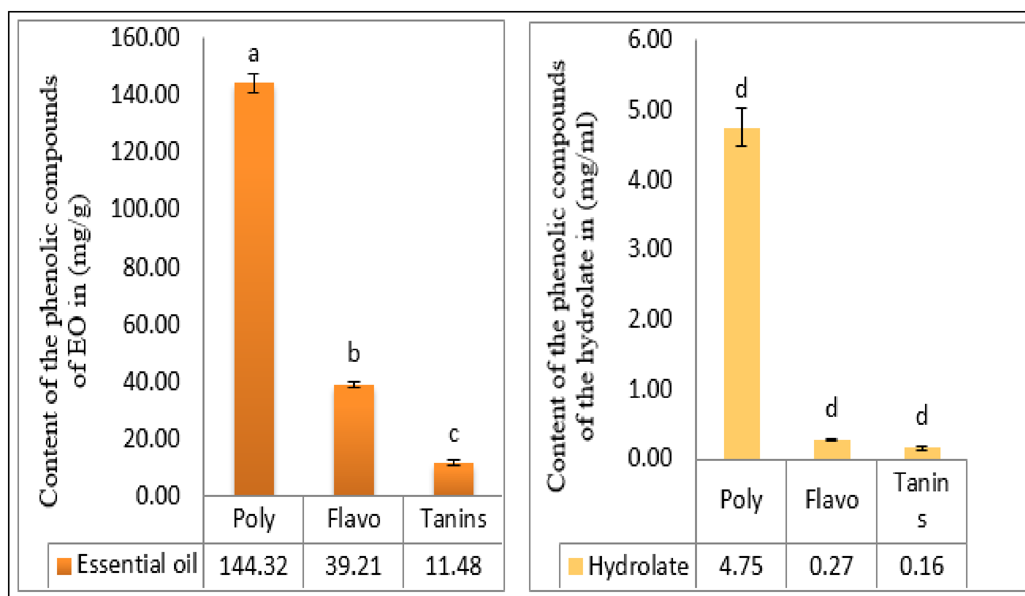

Fig 2: Determination of the content of phenolic compounds of essential oil and hydrolate of C.aurantium obtained by hydrodistillation. All the data are represented in mean±standard deviation, n=3.

3.3 Evaluation of the antioxidant activity of the essential oil by test DPPH

The antioxidant activity is determined by the DPPH test because this method is simple, reproducible and widely used in the food industry [14].
The antioxidant activity of *C.aurantium* essential oil from the Kenitra region is evaluated by the DPPH reduction method by comparing it with ascorbic acid as the reference antioxidant (Table 6)
In the present study, the essential oil of *C.aurantium* and vitamin C were able to reduce the free radical DPPH. IC_{50} is inversely related to the antioxidant capacity of a compound because it expresses the amount of antioxidant required to decrease the free radical concentration by 50%. The lower is the value IC50, the greater the antioxidant activity of a compound.
The results obtained have shown that the essential oil of IC_{50}=15.2±0.11 µg/ml has a lower antioxidant power than that of ascorbic acid (Vit C) whose IC_{50}=0.0027±0.26 µg/ml (table 6).
Studies on the antioxidant effect of the EO of bitter orange peel indicate that the concentration required for the neutralization and stability of 50% of the DPPH concentration is in the range of 800 µg/ml [44]. The latter is much higher than that of whole fruit (fruit without seeds). This probably shows that the pulp portion is rich in antioxidants.

Table 6: Antioxidant activity of the essential oil of *citrus aurantium* and ascorbic acid by DPPH assay. All the data are represented in mean±standard deviation, n=3.

Samples	Equation	R^2 values	IC_{50} values µg/ml
Essential oil	y = 163.09x + 47.521	R^2 = 0.9405	15.2±0.11[a]
Ascorbic acid	y = 420.95x + 48.939	R^2 = 0.9938	0.0027±0.26[b]

A study was carried out in Morocco [19] on the analysis of the essential oil of C. aurantium peels by gas chromatography, was identified fifteen constituents. These main constituents represent 99.86% of the total chemical composition. This essential oil is dominated by monoterpene hydrocarbons with limonene (90.90%), myrcene (1.51%) and β-pinene (1.41%) as main components, followed by oxygenated hydrocarbons of monoterpens (2.52, 0.88, 0.24, 0.17 and 0.10%), then sesquiterpene hydrocarbons (0.25 and 0.11%). these results are concomitant with a study conducted in Tunisia [23] is found twenty-seven components, accounting for 99.48% of the total oil. This bark essential oil was dominated by monoterpene hydrocarbons (93.49%) and limonene was the main constituent (90.25%), followed by α-terpinene (1.10%). Linalool was the main oxygenated monoterpene (1.56%) of the latter.

It appears that this antioxidant activity may be due to the presence of certain potentially active compounds (polyphenols) in the essential oil. The main role of these compounds as free radical reducers is emphasized in several reports [45]. Limonene, which is a major component of the bitter orange essential oil, has a strong antioxidant activity [24].
It is not only the major components of EO that are responsible for this antioxidant activity, but there may also be other minority compounds that can interact in a synergistic or antagonistic way to create an effective system against free radicals [28, 38].
On the other hand, the use of natural antioxidants is of great interest in the food industry because their possible use as a natural additive has emerged from a growing tendency to replace synthetic antioxidants with natural antioxidants because most antioxidants currently used as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) may be cytotoxic as well as increase the development of cancer cells [43, 24].
In conclusion, our results confirm that C. aurantium oil can be used as a potential natural antioxidant in the food industry.

4. Conclusions
During the present study having for objective the research of the natural compounds with therapeutic interest of the plant species chosen, we opted for Citrus Aurantium chosen on the basis of its wide distribution in the Kenitra region and its misuse.
The latter was the subject of a phytochemical screening to highlight the different phyto-constituents present in the fruit of this plant. The seedless fruit of C. aurantium is rich in flavonoids, tannins, polyterpene and carotenoids while its seeds are rich in coumarins and mucilages.
The yield of the essential oil extracted is 0.15% for fresh fruit, it is relatively high compared to some plants of the citrus genus.
The essence of the C. aurantium plant extracted by hydrodistilation is obtained with a quantitative yield and comparable to previous work. The application of spectrophotometry as a means of analysis allowed us to quantify the active phenolic compounds present:

- 11.48±1.16 mg EC/g for the tannins.
- 39.21±1.00 mg EQ/g for the total flavonoids.
- 144.32±3.42 mg EAG/g for the total phenols.

The antioxidant activity of the essential oil is evaluated by the anti-ridicalizing test which consists in estimating the trapping capacity of the free radical DPPH (IC50=15.2±0.11 mg/ml) while this essential oil is endowed with a very important antioxidant power. It would be interesting to conduct a more in-depth study on this plant and other medicinal plants in order to isolate, purify and identify the active ingredients with antioxidant activities.

5. References

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