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Qualitative study in vitro fruit and epicarpes Citrus Limetta Risso, Citrus Limon Burm and Citrus aurantiifolia (Christm.) Swingle Gharb of Morocco

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ABSTRACT

Several epidemiological studies have provided good evidence of the inverse relationship associated with the consumption of *citrus* fruits and chronic diseases. After many years of research in the role of phytomicronutrients such as phenolic compounds, carotenoids and alkaloids, they are now widely studied and appreciated to use in the control of these degenerative diseases. These positive influences on human health have significantly increased production and consumption of citrus in recent decades.

The lemon is a *citrus* fruit of the Rutaceae family which is rich in bioactive compounds. These could play a much more important protective role in the perspective of preventive nutrition. Indeed, these molecules have not revealed all their secrets until today.

This is qualitative study based on a phytochemical screening and analytical technique for separating the thin layer chromatography (TLC) extracts of fruits, épicarpes *limetta Citrus* , *Citrus limon* and *Citrus aurantiifolia* to highlight the existence of secondary metabolites that characterize them. The results of this study allowed us to reveal the existence of flavonoids, tannins, coumarins, alkaloids, carotenoids, quinones, sterols, terpenes, reducing compounds and iridoides. TLC tests confirm the presence of these secondary metabolites.

Keywords

Citrus; phytochemical screening; secondary metabolites; TLC.

INTRODUCTION

The worlds production of citrus fruits is about 122 million tonnes (Mt) coming in third place after bananas (125 Mt) and apples (70 Mt). In the Mediterranean area, this production is estimated of 18 Mt of which 80% are destined for the fresh fruit market. Moroccan *citrus* production occupies the fifth position in the Mediterranean with 1.3 million tons on average per year (Handaji et al., 2013). The main *citrus* grown for fruit production in Morocco are orange, mandarin, clementine, lemon and grapefruit. The major production areas in order of importance are the Souss Massa, the Gharb, the Moulouya, Tadla, Beni Mellal, the Haouz and Loukkos.

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The domestic and industrial sectors use specifically *Citrus*, especially for the production of juice, regenerate waste such as peels that can be a source of active biomolecules associated secondary metabolites that are known since ancient times for their pharmacological properties, biological and antioxidant, which are and remain the in vivo subject of much research as in vitro (Macheix et al., 2005).

The main intrest in this study is in the qualitative identification of secondary metabolites from a variety of *Citrus*, from three species (*Citrus limetta, Citrus limon, Citrus aurantiifolia*). Therefore, this study includes a screening of these phytochemical compounds order based primarily on specific colorimetric reactions, precipitation and chromatographic analysis in order to know their main components. This will allow us to identify some biological and antioxidant activities providing knowledge of the substances involved.

I. MATERIALANDMETHODS

I.1 Vegetal material

Harvesting of whole fruit to maturity, C. *limetta*, *C. limon* and *C. aurantiifolia* is performed during the months ofnovember to Marchin 2014/2015 randomly in the Kenitra region (West of Morocco). Whole fruits are washed, cut into slices and dried at room temperature and away from sunlight, in order to preserve the maximum molecules, then ground using an electric grinder and stored carefully. The epicarps are cut, dried and ground in the same way as the whole fruit. Species identification is made from INRA Kenitra.

I.2 Methods

I.2.1Phytochemical screening

Preliminary phytochemical screening aims to characterize the different chemical groups present in each species and its epicarp. It was conducted on extracts of tubes characterization reactions, and by thin layer chromatography.

I.2.1.1 Tubes characterization reactions

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

I.2.1.1.1 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 min, it is then filtered and adjust 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 (Timbo B , 2004).

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 (B Timbo , 2004).

b) Characterization of flavonoids

b. 1) Response to cyaniding

1 ml infused at 5%, 1 ml hydrochloric alcohol and 1 ml of isoamyl alcohol are introduced into a tube by adding some magnesium turnings. Crepitus reaction occurred for a few minutes (Diallo A, 2005a).

The appearance of the isoamyl alcohol supernatant layer of a colouring:

- Orange Rose characterizes flavones.
- Purple Rose characterizes flavanones.
- Red characterizes flavanones 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 (A Diallo, 2005a).

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 (Diallo A, 2005a).

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 (Ribéreau-Gayon and Peynaud, 1968).

I.2.1.1.2 Characterization of 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 (Badiaga, 2011).

I.2.1.1.3 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 (Badiaga , 2011).

I.2.1.1.4 Characterization of coumarin

After evaporation, 5 ml ether extracts, 2 ml of hot water and 1 mL of NH 4 OH at 25 % are added successively. The observation of an intense blue fluorescence under UV at 366 nm indicates the presence of coumarin (Badiaga 2011).

I.2.1.1.5 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.

I.2.1.1.6 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 (Bouhadjera , 2005).

I.2.1.1.7 Protein characterization

Biuret reaction: maceration is prepared by a 1g of powder samples in 10 ml of methanol for 24 h in the dark and then filtered. 2 -3 drops of an aqueous solution of CuSO4 to 2% are added to an aliquot of residue dissolved in 2 ml of 20 % aqueous NaOH. The appearance of a purple colour, sometimes with a reddish tinge, indicating the presence of proteins (Yves -Alain et al. , 2007).

I.2.1.1.8 Characterization derivative anthracene

a) Characterization of free anthraquinone

10 ml of chloroform and samples of 1g powder are heated for 3 minutes. After hot filtration, 1 ml of the extract was added to 1 ml of diluted NH4OH. After shaking, the more or less red colour indicates the presence of free anthraquinones (Mogode , 2005).

b) Anthracenes characterization combined

O -glycosides: A hydrolyzate is prepared from the residue samples exhausted with chloroform which are added to 10 ml of water and 1 ml of concentrated hydrochloric acid. After boiling for 15 minutes in a water bath, 5 ml of the hydrolyzate is added to 5 ml of chloroform. After decanting, 1 ml of diluted NH 4 OH is added to the organic phase. A more or less intense red color indicates the presence of genins O -glycosides (Mogode 2005).

C -glycosides: The sample solution is the aqueous phase obtained from the O- glycosides solution. 10 ml of water and 1 ml of FeCl 3 is added to this solution, then heated in a water bath for 30 minutes. After cooling, 5 ml of CHCl 3 are added to the solution. Drawing off the chloroform layer and adding 1 ml of diluted NH4OH ; the appearance of a more or less intense red color indicates the presence of genins C- glycosides (Mogode , 2005).

I.2.1.1.9 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 (Ilboudo , 2009).

I.2.1.1.10 Characterization of mucilage

1 ml of 10% aqueous decoction is added to 5 ml of absolute ethanol. Mucilage are characterized by the appearance of a flocculent precipitate (Badiaga , 2011).

I.2.1.1.11 drug characterization

0.5 g of powder and 5 ml of petroleum ether are introduced into a tube, and then heated at stirring for 15 min. After settling, the petroleum ether phase is evaporated to dryness in a water bath. With the addition of 3 to 4 drops of 5% KOH (alcohol) a purple color indicates the presence of narcotics (Diallo, 2005b).

I.2.1.1.12 Characterization of reducing sugar

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 (A Diallo, 2005b).

.I.2.1.1.13 Characterization of lipoids

20 g of powder are put into 150 ml of petroleum ether for 30 minutes. The filtrate obtained is evaporated to the hot plate to obtain an oily residue. This is added 3 drops of H2SO4. A strong violet or green color reflects the presence of lipoids (Rwandan Studies, 1977).

I.2.1.1.14 Characterization iridoides

1 ml of a decoction of the aqueous extract to 5% is added to 1 ml of concentrated hydrochloric acid. The formation of a black precipitate after heating characterizes the presence of iridoids (Paris and Moyse, 1965).

I.2.1.2 Chromatography Analysis

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

I.2.1.2.1 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 (Dohou et al., 2003; Okombe Embeya, 2011; Louiz et al., 2003).

I.2.1.2.2 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 (Sy et al., 2008).

I.2.1.2.3 Search for coumarin

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 (Ribéreau-Gayon and Peynaud, 1968).

I.2.1.2.4 Search for terpenoids

Preparation of the filtrate a quantity of 1 g samples of Citrus was added to 5 ml of hexane. The solution was sonicated for a few minutes and then stirring for 30 min and filtration (Randerath, 1971).

I.2.1.2.5 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 Dichlorométhaloniques extracts were filtered and concentrated in an evaporator at 40 ° C (Terrien and Fournier , 1998).

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 and revealed with a specific reagent for the identification of chemical components (Table 1).

I. RESULTS

The results of the phytochemical study, conducted on the whole fruit and the epicarp (peel) of *C. limetta* , *C. limon* and *C. aurantiifolia* are shown in Table 2 .

The phytochemical screening carried out on the three species has shown the following results:

The determination shows that flavonoids flavones and catechols are more abundant in the epicarp of *C. limetta* compared to whole fruit, which only contains flavonols and flavanonols. It is noteworthy that as flavones are present in the whole fruit and the epicarp of *C. aurantiifolia*. The whole fruit and the epicarp of *C. limon* reveal the presence of flavanones and leucoanthocyanins.

Regarding free tannins, they were detected in the six samples. The appearance of a blue-black coloration indicates the presence of Hardeners series of gallic acid in the epicarp of *C. limon*, *C. limetta*, *C. aurantiifolia* and the whole fruit of *C. limetta*.

The anthracene derivatives are composed of free and combined anthracene anthracene. The latter group consists of compounds -glycoside type present in *C. limon* and *C. aurantiifolia* (whole fruit and epicarp). The second type; C-glycosides is abundant in whole fruit and the epicarp of *C. limetta*.

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Table 1: migration and indicative of solvents used for the characterization of phytochemical constituents of three species of *Citrus* studied by thin layer chromatography.

Phytochemicalc onstituents	Extracts	Migration solvants	Developers UV	References
Tanins	acetone	Ethyl acetate : 40 Methanol : 8 Distilled HO_2 : 9	Ferric chloride, Acetic Acid, Distilled HO_2 .	(Sy et *al.,* 2008).
Flavonoids	Methanol	Ethyl acetate : 9 Methanol : 1 Ammonia at 50% : 1	Reagent NEU	(Dohou et *al.,* 2003).
Alcaloids	Acetone	Chloroform : 45 Diéthylamine : 5	Reagent dragendroff	(Sy et *al.,* 2008).
Coumarine	Chloroform	Ethyl acetate : 10 Toluene : 93	ammonia	(Ribéreau-Gayon et Peynaud, 1968).
Carotenoids	Dichloromethan	Ether diéthylique : 60 Ether de pétrol : 40	-	(Terrien et Fournier, 1998).
Saponoides	Methanol	Chloroform : 60 Methanol : 30 Distilled HO_2: 4	Antimony trichloride	(Louiz et *al.,* 2003).
Anthraquinones	Methanol	Ethyl acetate : 81 Methanol : 11 Distilled HO_2 : 8	Potassium hydroxide	(Okombe Embeya, 2011).
Terpenoids	Hexane	Benzen	Antimony trichloride	(Randerath, 1971).

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Species / Phytochemical constituents			*C. Limon*		*C. Limetta*		*C. Aurantiifolia*	
			Fruit	Epicarp	Fruit	Epicarp	Fruit	Epicarp
Tanins	Free tanins		++	++++	+++	++	++	++++
	Tanins cathéchiques		-	-	-	-	-	-
	Gallic tanins		-	++++	+	++	-	+
Flavonoïdes	Flavones		-	-	-	++++	++	+++
	Flavanones		++	++	-	-	-	-
	Flavonols, Flavanonols		-	-	+++	-	-	-
	Anthocyanins		-	-	-	-	-	-
	Leucoanthocyanin		++	++	-	-	-	-
	Catechols		-	-	-	++++	-	-
Anthracene derivatives	Free anthracene		-	-	-	-	-	-
	Anthracenes Combined	O -glycosides genins	++	+++	-	-	++	++
		C- glycosides genins	-	-	++	+++	-	-
Quinones			+	+++	+	+++	+	+++
Alcaloids			+++	+++	++	++	+++	+++
Sterols and terpenes			+	+	+	++++	+++	++
Reducing sugar			+++	++++	+++	++++	+	+
Saponosides			+	+	+	+	+	+
Coumarines			+++	++++	++++	++++	+++	++++
Caroténoids			++	+++	++	++	+++	++++
Proteins			+	+	+	+	-	-
Lipoides			-	-	-	-	-	-
Essential oils			++	+++	++	+++	++	+++
Iridoides			+++	+++	++++	+	+++	+
Mucilage			+++	-	-	-	++	++

Table 2: Preliminary phytochemical screening of the three species of *Citrus* studied.

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

In both species, *C. limon* (whole fruit and epicarp) and *C. limetta* (whole fruit and epicarp), reducing sugars are abundant relative to *C. aurantiifolia* (whole fruit and epicarp). Note as well the characterization of mucilage in *C. aurantiifolia* (whole fruit and epicarp).

The sterols and terpenes are reported in the epicarp of *C.limetta*, and *C. aurantiifolia* and whole fruit, but with a significant dominance in the whole fruit of *C. limetta*, *C. limon* and the epicarp of the latter.

The iridoides exist in all three species. They are in larger amounts in the whole fruit of *C. limetta*, *C. aurantiifolia*, *C. limon* and the epicarp of the latter relative to the epicarp of*C.limetta*, *C. aurantiifolia*.

Quinones are present in the epicarp of the three species, however they are almost absent in the whole fruit.

The presence of coumarins, carotenoids, alkaloids and essential oils is quite significant in six samples.

Search for drugs and lipoids were negative on the six test samples of *Citrus*.

The existence traces saponins and proteins for the six samples tested

The chromatographic analysis has highlighted a series of spots for certain phytochemical elements including flavonoids, tannins, coumarins, carotenoids and alkaloids in the whole fruit and the epicarp of *C.limetta*, *C. limon*and *C. aurantiifolia*. with regards to the saponins, terpenoids and the anthraquinones, they are less detected in the three species. These results confirm those of phytochemical screening (Table 3).

II. DISCUSSIONS

The phytochemical examination revealed a similarity of secondary metabolites in the three studied species of *Citrus* (fruit). These results met with those of *C. aurantifolia* (Dhiman et al, 2012;.. Rafi Khan et al, 2012), *C. medica* (. Nagaraju et al, 2012) and *C. maxima* (. KunduSen et al, 2011) which also contain alkaloids, tannins, steroids, carbohydrates, flavonoid, protein and saponin.

The phytochemical study of the epicarp of the three species of *Citrus* revealed that they are very rich in phytoconstituents. These results are comparable to those obtained in previous studies by Lawal et al., (2013), Kumar et al., (2012) and Oluremi et al., (2007) who reported the presence of tannins, alkaloids, flavonoids, reducing sugars, mucilages, phenolic compounds, saponins, steroids, terpenoids, coumarins and volatile oils.

These results are comparable with those obtained on *C. senensis linn* by Dhiman et al., (2012) and *C. reticulata* and *C. grandis* by Okwu et al., (2007) and by Sharma et al., (2008) *C. aurantium*.

This qualitative study showed high levels of *C. limetta*, *C. limon* and *C. aurantiifolia* bioactive compounds that are the subject of several industrial research (pharmaceutical, food, cosmetic….).

Experimental data brought out evidence of the impact of nutrients and food constituents on a variety of biological targets (Top 2000 Public Health Committee, Gerber et al., 2002). In fact, these data carried on *citrus* fruits have shown that they have a significant antioxidant potential and they represent an important source of phenolic compounds, mainly phenolic acids and flavonoids (Ramful et al, 2010;. Barreca et al. 2010; Jayaprakasha et al., 2008; Kelebek et al, 2008).. In addition, they possess properties: anti-carcinogenic and anti-estrogenic (Havsteen 2002; Middleton et al., 2000), inhibitors of enzymes and antimicrobial (Williams et Grayer 2004), antiviral (Havsteen 2002; Middleton et al. 2000), cytotoxic and antitumor (Crozier 1997), antifungals (Swiader and Lamer-Zarawska 1996), antioxidants (Duthie and Dobson 1999; Morel et al, 1998) and anti-inflammatory (Hiermann et al, 1991).

Notably, coumarins are considered antifungals (Sardari et al., 1999), antibacterial (Kayser and Kolodziej 1997. Kwon et al, 1997)., Antiviral (. Fuller et al, 1994), antimalarials (Yang et al, 1992), anti-inflammatory (Garcia-Argaez et al., 2000; Hiermann et al., 1998;. Lino et al, 1997), anti-tumorals (Fujioka et al., 1999; Seliger Kofinas et al, 1998) and anticoagulants (Egan et al., 1990).

Furthermore, the tannins are endowed with several biological activities; antibacterial (Garcia-Argaez et al., 2000; Asad et al., 1998), antiviral, anti-diarrhea and astringent and healing (Di Carlo et al., 1999), anti-inflammatory (Lino et al., 1997), antihypertensives (tachen et al., 1993), anticoagulant (Hiermann et al., 1991).

The presence of other bioactive compounds in citrus assigns them with health benefits (Hsiou et al., 2000), alkaloids which possess various biological activities (Milcent and Chau 2003).

As for terpenes, they are used as additives in food and cosmetics industries (Tsao and Coats 1995) and several of them have biological activities: antimicrobial, insecticides, anti-carcinogenic, anti-inflammatory (Murakami et al., 2004 ; Griffin et al, 1999), anesthetics and antihistamines (Velickovic et al, 2003), neuroprotective (Hyun et al, 2007).

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Phytochemical constituents	FR(Rf)								
	C. Limon			*C. Limetta*			*C. Aurantiifolia*		
	Whole	Epicrp	Colour	Whole	Epicarp	Colour	Whole	Epicarp	Colour
Tanins		Rf1=0.916 Rf2= 0.178	Blue	Rf1= 0.916 Rf2= 0.119 Rf3= 0.071	Rf1= 0.916 Rf2= 0.119 Rf3= 0.071	Blue	Rf1= 0.916 Rf2= 0.178 Rf3= 0.119 Rf4= 0.071	Rf1= 0.964 Rf2= 0.916 Rf3= 0.214	Blue
Flavonoids	Rf1= 0.063 Rf2= 0.15 Rf3= 0.85	Rf1= 0.063 Rf2= 0.15 Rf3= 0.85 Rf4= 0.925	Yellow Red Blue Blue	Rf1= 0.063 Rf2= 0.15 Rf3= 0.85	Rf1= 0.063 Rf2= 0.15 Rf3= 0.85	Yellow Red Blue	Rf1= 0.063 Rf2= 0.15 Rf3= 0.85 Rf4= 0.925	Rf1= 0.063 Rf3= 0.15 Rf2= 0.1 Rf 4= 0.85 Rf5= 0.925	Yellow Red Blue Blue Blue
Alcaloids	Rf1= 0.964	Rf1= 0.964	Blue	Rf1= 0.988 Rf2= 0.952 Rf3= 0.095	Rf1= 0.988 Rf2= 0.952 Rf3= 0.714 Rf4= 0.594 Rf5= 0.345 Rf6= 0.297 Rf7= 0.095	Blue	Rf1= 0.952 Rf2= 0.595 Rf3= 0.095	Rf1= 0.952 Rf2= 0.595 Rf3= 0.154 Rf4= 0.095	Blue
Coumarines	Rf1= 0.826 Rf2= 0.866	Rf1= 0.826 Rf2= 0.866	Blue	Rf1= 0.826 Rf2= 0.16	Rf1= 0.826 Rf2= 0.333 Rf3= 0.186 Rf4= 0.426	Blue	Rf1= 0.826 Rf2= 0.866 Rf3= 0.24 Rf4= 0.333	Rf1= 0.826 Rf2= 0.16 Rf3= 0.24 Rf4= 0.333 Rf5= 0.866	Blue
Anthraquinones	Rf1= 0.214	Rf1= 0.214	Yellow	Rf1= 0.214	Rf1= 0.214	Yellow	Rf1= 0.214	Rf1= 0.214	Yellow
Saponoids	Rf1= 0.843	Rf1= 0.843	Yellow	Rf1= 0.843	Rf1= 0.843	Yellow	Rf1= 0.843	Rf1= 0.843	Yellow
Terpenoides	Rf1= 0.214	B1= 0.214	Blue	Rf1= 0.214	Rf1= 0.214	Blue	Rf1= 0.214	Rf1= 0.214	Blue
Carotenoids	Rf1= 0.487 Rf2= 0.634 Rf3= 0.853 Rf4= 0.914	Rf1= 0.487 Rf2= 0.634 Rf3= 0.853 Rf4= 0.914 Rf5= 0.768 Rf6= 0.365	Blue Blue Blue Yellow Yellow Blue	Rf1= 0.487 Rf2= 0.914 Rf3= 0.853	Rf1= 0.487 Rf2= 0.914 Rf3= 0.853	Blue Yellow Blue	Rf1= 0.487 Rf2= 0.634 Rf3= 0.853 Rf4= 0.914 Rf5= 0.768	Rf1= 0.487 Rf2= 0.634 Rf3= 0.853 Rf4= 0.914 Rf5= 0.768 Rf6= 0.304 Rf7= 0.182	Blue Blue Blue Yellow Yellow Blue Yellow

Table 3: Front Reports (FR) of the three species studied based on phytochemical constituents.

CONCLUSION

According to current theories of nutrition, one of the secrets of good health is to absorb more antioxidants that support healthy aging of various organs of the body. This is one major reason why a Mediterranean diet rich in fresh fruits and vegetables is recommended.

Ingestion of secondary metabolites through fruits and vegetables could allow our bodies to strengthen its defenses against daily oxidative processes that threaten our cells, even if the mechanisms involved probably far exceed the direct reduction of reactive oxygen species by these secondary metabolites such as polyphenols.

Currently, the health benefits of citrus fruits have been mainly attributed to the presence of bioactive compounds. Indeed, this work is a phytochemical study of the powder and whole fruits of épicarpes *C.limetta*,*C. limon* and *C. aurantiifolia*. It has allowed us to highlight some secondary metabolites by qualitative characterization reactions such as phenolics (tannins and flavonoids, coumarins, quinones), sterols and terpenes, reducing compounds (reducing sugars and mucilage), alkaloids, anthracene derivatives combined, essential oils, carotenoids, iridoides, the saponins and proteins.

Hence their extensive use in many industrial applications in the food industry (beverages, confectionery, condiments, ice cream) , cosmetics (perfumes, soap, massage oil) and pharmaceutical respectively. Uses in paints, insecticides, plastics and textiles are also mentioned by several studies. In addition, citrus byproducts (epicarp) are a good source of secondary metabolites and their extractions considerable scientific interest.

Indeed, in recent years, much research has focused on the by-products for extracting natural antioxidants referred to replace synthetic antioxidants.

Finally, additional research is needed to quantify these compounds in our samples and studying their antioxidant and biological activities is essential.

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