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(RESEARCH ARTICLE)

# Chemical composition, antioxidant and antimicrobial activities of *Capsicum annuum* var. annuum concentrated extract obtained by reverse osmosis

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## Abstract

The purpose of this study was to assess Chemical composition, total flavonoid and carotenoid contents with antioxidant and antimicrobial activities in crude and concentrated extracts of pepper fruit (*Capsicum annuum* var an.). The concentrated extract was obtained by reverse osmosis process. Radical scavenging potential was also determined using in vitro assay: 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals. The GC-MS analysis of concentrated extracts of *C. annuum* revealed the presence of major constituents such as lactic acid, valeric acid, 5-methoxy, butanedioic acid, phenylalanine, hexadecanoic acid, ethyl ester, 6-methoxy-hexane-2-ol, butane, 2,3diol, pentanoicacid,4-oxo-, 3-methyl-2-hydroxyl butanoic acid, benzeneacetic acid, 4-hydroxyl, 1,2-benzenedicarboxylic acid ester, 2,5-furandicarboxylic acid, 7-hydroxyl7-methyloctanoïc acid. Furthermore, the results indicated that the total flavonoid (3.7± 0.1g/L Eq Quercetin) and total carotenoid (54.33± 1.1 mg/100 mL of fresh extract) contents and the antioxidant activities ((83.44 ± 0.98%) in concentrated extract of *C. annuum* fruit were significantly ($p \leq 0.05$) higher than those recorded in crude extract. They also showed that the MIC values ranged from 10 to 20 µg/mL. This confirmed the existence of significant activity against the bacterial strains tested. Our results revealed that Gram positive and negative microorganisms were affected by the tested concentrated extract. Moreover, this study indicated that the concentrate of the *Capsicum annuum* fruit effectively shows the best ability to scavenge the free radicals. This concentrated extract also presents some antimicrobial activity against six microorganisms.

**Keywords:** *C annuum*; Concentrated extract; Reverse Osmosis; GC-MS; Antioxidant; Antimicrobial activity

## 1. Introduction

Pepper, specifically *Capsicum annuum an*. is the general name for plants coming from *Capsicum* species of Solanaceae family, native to southern North America and northern South America. It is an important agricultural crop, not only because of its economic importance, but also for the nutritional value of its fruits, mainly due to the fact that they are an excellent source of natural colours and antioxidant compounds [1-2]. Indeed, antioxidant nutrients play an important role in health maintenance. They neutralize harmful chemicals called "free-radicals" that cause cell damage in the body. Antioxidants have been strongly linked to the protection from numerous diseases, heart disease to cancer, eye disease to regulation of the immune system. Pepper is an important nutrient for human diet due to its contained vitamins and

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antioxidants. In particular, the antioxidant vitamins (vitamins A, C, and E), carotenoids are present in high concentrations in various types of pepper. Ascorbic acid (vitamin C) is the biologically active form of dehydroascorbic acid diseases. It is fundamentally provided by fruits and vegetables. Fresh pepper contains a higher amount of ascorbic acid than the other fruits and vegetable and it prevents some important illness such as cancer, anaemia, diabetics, and cardiovascular. It is also a good source of phenolic compounds such as flavonoids but the phenolics composition determined in pepper fruits is incomplete. Indeed, flavonoid compounds were known as antioxidant agent. This agent was benefit for people health. Several researches showed that high concentrations of flavonoids were significantly associated with reduced risk of cardiovascular disease through an improvement in vascular function and a modulation of inflammation [3-4]. Moreover, they could protect cell and tissue damage from free radicals by potent antioxidant capacity [5]. Flavonoids are phytochemical ubiquitous found in plants with a wide group of exploitable activities, including antimicrobial activity, antibiotic synergism and bacterial virulence removal [6]. Otherwise, hot peppers are the only plants that are able to produce capsaicinoids, responsible for their characteristic hot taste. The concentrations of these compounds depend on cultivar, maturity, growing conditions, and postharvest manipulation. Perera and Yen [7] reported that consumption of carotenoid rich foods reduces the incidence of several disorders such as cancers, cardiovascular diseases, age-related macular degeneration, cataracts, diseases related to compromise immune function, and other degenerative diseases. The importance of the carotenoid compounds in the diet has been recognized, not only as precursors of vitamin A but also as antioxidants in cell protection and in the prevention of degenerative diseases [8]. Carotenoids are fat soluble antioxidants found in many fruits and vegetables and are required for human epithelial cellular differentiation [9]. Furthermore, recent reports state that the *Capsicum* genus, among other plant genera, is a good source of antimicrobial and antifungal compounds [10].

Despite the beneficial assets available to the pepper, various alterations compromise its quality. Relevant conservation operations to their main quality characteristics are therefore necessary. Otherwise, the lack of effective conservation of these seasonal products causes cyclical shortages in the year. These losses are estimated at 20 to 40% for these products [11]. Indeed, the effectiveness of preservation technique is measured in terms of the quality of product (hygienic, nutritional, sensory...). Membrane separation processes such as Tangential Microfiltration (MFT) and Reverse Osmosis (RO) have been developed and appear important to resolve this situation. Membrane technology in vegetable treatment has significant progresses in recent years. It is used to the conventional processes for juice concentration and clarification [12]. It has many advantages over traditional separation processes. In general, separation occurs at ambient temperature, with no phase change and without using a heat source, resulting in considerable energy savings and avoiding oxidation and degradation of thermolabile compounds [13]. Reverse osmosis (RO) was used for the concentration of fruit extract with promising considering the quality of the obtained products. Promising results were achieved with sugar solution concentration from 15 to 50° Brix. In this configuration, reverse osmosis occupies the first stage [14].

Although pepper fruit possesses a great edible plant biodiversity, there is a serious lack of information a on its concentrated extract by RO. Therefore, the present study has been undertaken to assess the chemical composition, total flavonoid and carotenoid contents in pepper concentrated extract obtained by RO. Moreover, the antioxidant activity was estimated by DPPH radical scavenging assay followed by evaluation of the antimicrobial activity against six common food borne pathogens. Thus, this concentrated extracts could be used as fundamental data for nutritionists or public health workers to recommend consumers the appropriate types of peppers *Capsicum* concentrated extracts for their health needs.

## 2. Material and methods

### 2.1. Plant material

Fruits of sweet pepper (*C. annuum var.an.*) were grown in an experimental field (INPHB Yamoussoukro, Côte d'Ivoire) under tropical climate conditions. The peppers were harvested at the maturity stage (fully red). After harvest, the fruits were immediately transported in the laboratory (LAPISEN, INPHB Yamoussoukro, Côte d'Ivoire). Then, the peppers were carefully selected to ensure that fruits free of defects were chosen.

### 2.2. Crude extract clarification and concentrate processes

#### *2.2.1. Extraction of crude extract*

The maceration and bioprocesses membrane separation was carried out according to method of Adjé *et al.* [15]. Fresh fruits were carefully washed and crushed. The maceration of pepper fruits was performed in two steps. Firstly, 5 kg of fresh plant material were macerated into 100 liters of aqueous ethanol (70:30 v/v) to obtain ethanolic extract. Aqueous

extracts were produced by the same weight (5 kg) in 100 liters aqueous solution. The maceration was slightly stirred to wet completely the crushed material and let for 24 h maceration time at room temperature. The crude extract was successively pre-filtered through a fine sieve (pore diameter of 1mm) and a nylon fabric lower porosity (pore diameter = 25 µm). Thus, crude extract (CE) is obtained.

*2.2.2. Crude extract clarification process*

The clarification of crude extract was conducted in the microfiltration (MF) unit featured a ceramic multichannel membrane (P19-40, France) that had a total effective filtration area of 0.24 $m^2$ and an average pore diameter of 0.2 µm. Transmembrane pressure for feeding is set at 1.2 bar, and the CE was clarified against a transmembrane pressure of 0.6 bar. All trials were carried out with continuous crude product feed and permeate collection at flow. The feed-and-bleed procedure was also followed in the long term trials by implementing continuous extraction of retentate at flow. Thus, the clarified extract is obtained.

*2.2.3. Concentrate process*

The concentrate was obtained by reverse osmosis (RO). Reverse osmosis was performed with a composite polymer membrane of type SW 30-2540 (Filmtec) from Polymem (France). The characteristics of which were effective membrane area of 2.5 $m^2$. The operation conditions were 40 bar of transmembrane pressure and temperature of 30 °C. The extract circulated inside the fibers. The loop was continuously fed with cold clarified extract (20 °C). The both sample crude extract (CE) and concentrate (C) were lyophilized. Then, 5 g of each lyophilized extract are added to 20 mL of water and homogenized for 3 h at room temperature. Those ethanolic extracts (0.25 g/mL) were analyzed.

### 2.3. GC-MS analytical conditions of the concentrate

Within a three necked round bottom flask containing aqueous methanol (25 mL, 95%) and 25 mL of hydrochloric acid (3 M) was added to 0.5 g of freeze dried sample. The mixture was refluxed in a water bath at 90 °C for 2 h and cooled. In separator funnel containing the mixture, ethyl acetate (3 x 50 mL) was then added to recover the aglycones of O-glycosyl compounds. Anhydrous ($MgSO_4$) was added to remove moisture, filtered and evaporated of the solvent.

In general, the hydrolysis extract contain groups (phenols, alcohols and carboxylic acids) which can be derivatised to improve the chromatographic properties and separation on the GC-column. The most common derivatisation procedure of compounds containing-OH and-COOH groups are silylation. Among the many possibilities of silylating agents. Derivatisation experiments were performed by considering BSTFA. For the silylation procedure, a mixture of N.O-bis (trimethylsilyl) trifluoroacetamide BSTFA (0.5 mL) and dichloromethane (0.5 mL) were added to 10 mg of residue (extract with ethyl acetate after hydrolysis). The mixture was placed in a water bath at 70 °C for 1 hour. The silylated samples were injected into a GC-MS system (Shimadzu-QP2010SE) consisted of a gas chromatograph coupled with a mass spectrometer (quadrupole) in the EI (Electron Impact) brand fitted with a split/split less injector. Capillary column low bleed Zebron ZB-WAX (20 m × 0.18 µm) was used. The flow rate of carrier gas (helium) was maintained at 0.9 ml/min. The injector temperature was 280 °C. The own temperature increased from 70 to 270 °C with 4 °C/min and then held to 270 °C for 20 min. The detector temperature was 290 °C. Identification of components was based on comparison of their mass pectra with those of Wiley and NIST libraries and those described by Adams as well as on comparison of their retention indices with literature [9].

### 2.4. Determination of total flavonoid content

The total flavonoid content was determined according to the method of Ying and Wan [16]. 1 mL of extract aliquot was added to 0.3 mL of 5% sodium nitrite ($NaNO_2$) (w / v) and 0.3 mL of 10% aluminum chloride ($AlCl_3$) (w / v). After 6 min of incubation at ambient temperature, 2 mL of sodium hydroxide (NaOH) was added to the mixture. The final volume was brought up to10 mL by adding aqueous ethanol and then mixed. The obtained mixture was incubated at ambient temperature for 15 min and then the absorbance against blank was determined at 510 nm ((Spectrophotometer, Type JASCO UV-500, Japan). Quercetin was used as standard for the calibration curve. Total flavonoid content was calculated as gram quercetin equivalent per liter (gQE/L) fresh pepper extract. All samples were performed in triplicate.

### 2.5. Determination of total Carotenoid content

Total carotenoid content was quantified according to the method of Rodriguez-Amaya and Kimura [17]. 10 g of each sample (CE or C), lyophilized previously, were dissolved in 20 mL acetone and the supernatant decanted. The sample was filtered and washed with 30 mL acetone. Then, the acetone was evaporated. This procedure was repeated four times until no residual solvent remained. The obtained dry sample was dissolved in 60mL petroleum ether. The mixture was filtered, transferred quantitatively to a 100 mL volumetric flask and volume was adjusted with petroleum ether. 2 mL

of the obtained mixture were collected in a test tube with 8 mL petroleum ether. Absorbance was read at 450 nm (Spectrophotometer, Type JASCO UV-500, Japan) and Total carotenoid content was calculated with a $\beta$-carotene curve.

### 2.6. Evaluation of the antioxidant activity via DPPH radical scavenging assay

Antioxidant activity of *C. annuum* extract was measured as scavenging free radical potential in methanolic solution of DPPH (1,1-diphenyl-2-picrylhydrazyl), according to the method of Schmeda-Hirschman [18]. To 2.4 mL from 0.25 g/mL of each sample of crude extract (CE) and concentrate (C), 0.8 mL of 0.1 mM DPPH (methanol) was added, vortexed and followed by incubation at room temperature for 10 minutes. The control sample was prepared without any extract or fraction. The decrease in absorbance in presence of DPPH was measured at 517 nm by using Spectrophotometer (Type JASCO UV-500, Japan). Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the control.

$$\%\text{DPPH inhibition activity} = [A_0 - A_1/A_0] \times 100 \quad [1]$$

Where: $A_0$ is the absorbance of the control and

$A_1$ is the absorbance of the sample.

Ascorbic acid was used as a positive control. Samples were analyzed in triplicate.

### 2.7. Antimicrobial assay

#### *2.7.1. Microbial strains*

The microbial strains used belong to Gram positive bacteria (*Staphylococcus aureus* UFPEDA02*, Enterococcus faecalis* ATCC6057, and *Bacillus subtilis* UFPEDA86) and Gram negative bacteria (*Escherichia coli* ATCC25922, *Pseudomonas vulgaris, Pseudomonas aeruginosa* UFPEDA416*)*. The samples were acquired from the Antibiotics Department of the University Federal of Pernambuco, Recife, Brazil.

#### *2.7.2. Determination of minimal inhibitory concentration (MIC)*

The broth micro-dilution assay was performed according to CLSI reference methods M7-A6 for bacteria (2003). Ninety-six-well microplates were used to obtain the MIC value of concentrated extract from *C. annuum* against microorganisms. The MIC was determined by measuring each well with a microplate reader (ASYS UVM 340, Cambridge, UK). The MIC was defined as the lowest sample concentration that inhibited bacterial growth compared with the optical density of the controls. Chloramphenicol (50 µg/mL) was used as a positive control for all of the strains.

### 2.8. Statistical analysis

All analyses were performed in triplicates. Results were expressed by means of ± standard deviation (SD). Means were separated according to student test ($p \leq 0.05$), with the help of the software Statistica 7.1 (StatSoft Inc, Tulsa USA Headquarters).

## 3. Results and discussion

### 3.1. Chemical composition of the concentrate

The GC-MS analysis of concentrated extract of *C. annuum* revealed the presence of 28 compounds representing different classes (Table 1, Fig 1.). The constituents such as lactic acid, valeric acid, 5-methoxy, butanedioic acid, phenylalanine, hexadecanoic acid, ethyl ester, 6-methoxy-hexane-2-ol, butane, 2, 3 diol, pentanoic acid, 4-oxo-3-methyl-2-hydroxyl butanoic acid, benzeneacetic acid, 4-hydroxyl, 1, 2-benzenedicarboxylic acid ester, 2,5-furandicarboxylic acid, 7-hydroxyl 7-methyloctanoïc acid, were found to be important in the concentrate. The compounds in GC–MS analysis were identified on the basis of comparison of the retention time (RT) and mass spectra with the references present in the NIST mass spectral library. In this servey, most of the identified volatile compounds were hydrocarbons, fatty acids, fatty esters and some novel constituents such as Phenylalanine. Many of these identified compounds have already been reported to be pharmacologically active. Indeed, hexadecanoic acid is known to have potential antibacterial and antifungal activities [19].

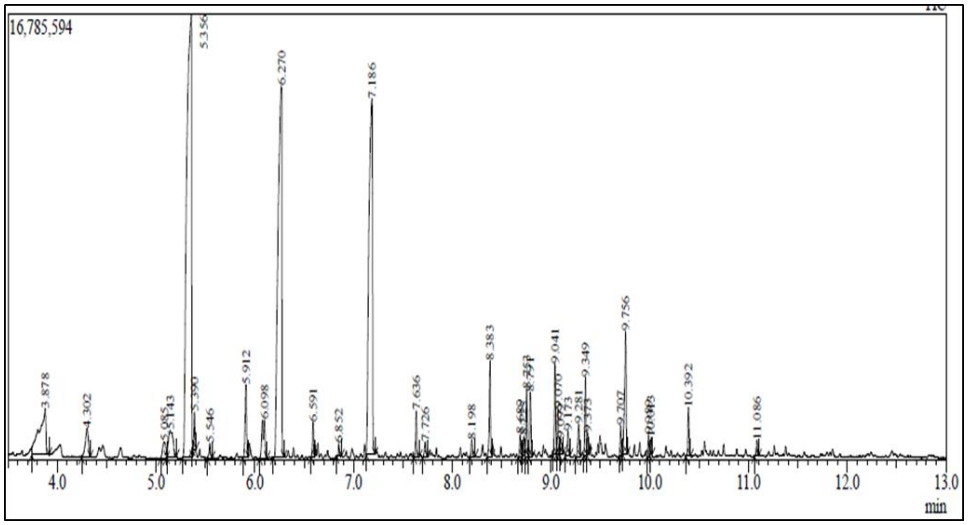


**Figure1**GC-MSchromatogram related to compounds of *C. Annuum* concentrated extract

**Table 1**Chemical composition of concentrated extract of Fresh *Capsicum* fruits analyzed by GC–MS

| Nº | Nameofcompounds | Molecular formula | Retention time (min) | Peakarea (%) |
|---|---|---|---|---|
| 1 | Phenylalanine | $C_9H_{11}NO_2$ | 3,878 | 4.51 |
| 2 | 6-Methoxy-hexane-2-ol | $C_8H_{18}O_2$ | 4,302 | 1.25 |
| 3 | 3-phenyl Butane-2-ol | $C_{10}H_{14}O$ | 5,085 | 0.75 |
| 84 | Butane, 2,3 diol | $C_4H_{10}O_2$ | 5,143 | 1.73 |
| 5 | Lactic acid | $C_3H_6O_3$ | 5,356 | 30.90 |
| 6 | ethyl malonate | $C_5H_4O_4$ | 5,390 | 0.52 |
| 7 | Acetamide | $CH_3CONH_2$ | 5,546 | 0.30 |
| 8 | Pentanoic acid, 4-oxo- | $C_5H_8O_3$ | 5.912 | 1.67 |
| 9 | 3-methyl-2-hydroxyl Butanoic acid, | $C_5H_{10}O_3$ | 6.098 | 1.56 |
| 10 | Valeric acid, 5-methoxy | $C_5H_{10}O_2$ | 6,270 | 20.49 |
| 11 | 2-Hydroxyisocaproicacid | $C_6H_{12}O_3$ | 6.591 | 0.64 |
| 12 | alpha.-D-Galactopyranose | $C_6H_{12}O_6$ | 6.852 | 0.17 |
| 13 | Butanedioic acid | $C_4H_6O_4$ | 7.186 | 18.56 |
| 14 | Benzoic acid | $C_7H_6O_2$ | 7,726 | 0.36 |
| 15 | 2-Hydroxy-4-methylpentanoic acid | $C_6H_{12}O_3$ | 8,198 | 0.29 |
| 16 | Benzeneacetic acid, 4-hydroxyl | $C_8H_8O_3$ | 8,383 | 1.78 |
| 17 | D-Erythro-Pentopyranose, 2-deoxy- | $C_8H_8O_3$ | 8,689 | 0.39 |
| 18 | 1,2-Benzenedicarboxylic acid, ester | $C_{24}H_{38}O_4$ | 8,753 | 1.15 |
| 19 | 2,5-Furandicarboxylic acid | $C_6H_4O_5$ | 8,791 | 1.20 |
| 20 | Hexadecanoic acid, ethyl ester | $C_{18}H_{36}O_2$ | 9,041 | 1.72 |
| 21 | Pentanoic acid, 4-methyl- | $C_6H_{12}O_2$ | 9.070 | 0.65 |
| 22 | Benzeneacetic acid, | $C_8H_8O_2$. | 9.099 | 0.38 |
| 23 | 2-deoxyD-Erythro-Pentopyranose | $C_5H_{10}O_4$ | 9.281 | 0.75 |
| 24 | 7-hydroxyl 7-methyloctanoïc acid | $C_{15}H_{34}O_3$ | 9.349 | 1.29 |
| 25 | phtalic acid | $C_{24}H_{38}O_4$ | 9.373 | 0.32 |
| 26 | 2,5-Furandicarboxylic acid | $C_6H_4O_5$ | 9.707 | 0.45 |
| 27 | 2,5-Furandicarboxylic acid | $C_6H_4O_5$ | 9.989 | 0.36 |
| 28 | Hexadecanoic acid, ethyl ester | $C_{18}H_{36}O_2$ | 11.08 | 0.30 |

RT- Retention time

It is a saturated fatty acid. Many fatty acids have antibacterial and antifungal properties and could modulate immune responses by acting directly on cells [20]. Hexadecanoic acid and methyl ester have antioxidant and anticancer properties respectively [21]. 2,5-Furandicarboxylic acid (FDCA) also shows antibacterial activity and is the main ingredient of antimicrobial food additives and some antibacterial herbs [22]. Screening studies on FDCA-derived anilides showed their important anti-bacterial action.

The diacid itself is a strong complexing agent, chelating ions such as $Ca^{2+}$, $Cu^{2+}$and $Pb^{2+}$. It is used in medicine to remove kidney stones [23]. Hydroxy-isocaproic acid (HICA), also known as leucic acid or DL-2-hydroxy-4-methylvaleric acid, is an end product of leucine metabolism in human tissues such as muscle and connective tissue. According to the clinical and experimental studies, HICA could be considered as an anti-catabolic substance [21]. It is a physiological agent which is normally present in the human body in small amounts. Otherwise, it may be noted that this pepper species is rich in secondary metabolites, similar to other medicinal plants [21]. Among the secondary metabolites identified in *Capsicum annuum* exercising a wide range of biological activities on humans, may be listed: pentanoicacid, 4-oxo; acetamide, ethylmalonate, valeric acid, 5-methoxy. These compounds were tested for their antimicrobial, anti-inflammatory, antioxidant, anti-cancer and hepatoprotective activities [24].

Moreover, acetamide derivatives have excellent antibacterial and antifungal activities [25]. The organic acids which include lactic acids don't only lower the pH, thereby affecting the growth of pathogen, but they can also be toxic to the microbes [26].The presence of bioactive phyto-constituents is crucial in the well-known pharmacological activities shown by concentrate.

### 3.2. Antimicrobial activity

Antimicrobial activity of ethanolic extract of *C. annuum* fruit was tested against six microorganisms. These were two (03) Gram negative bacteria (*P. aeruginosa P. vulgaris* and *E. coli*) and three (03) Gram positive bacteria (*E. faecalis*, *B. subtilis* and *S. aureus*). The results of the minimal inhibitory concentration (MIC) examined against different pathogenic microorganisms are shown in Table 2. They indicated that the obtained MIC values confirmed the existence of significant activity against the bacterial strains tested in our study, with MIC values ranging from 10 to 20 µg $mL^{-1}$. Our results showed that Gram positive and negative microorganisms were affected by the tested concentrated extract. Similar study was performed by Careaga *et al*. [27], who investigated the antimicrobial effect of *Capsicum* extract on *P. aeruginosa* inoculated in minced beef and observed a reduction of *P. aeruginosa* growth, with a bacteriostatic effect.

A previous study carried out by Molina-Torres *et al*. [28] required MIC values of 300 µg/$mL^{-1}$ and 25 µg $mL^{-1}$ to inhibit *E. coli* and *B. subtilis* growth, with capsaicin. Our findings were agreement with the findings of the previous study for *S. aureus* [29]. One the other hand, they were disagreement with the observations for *E. coli* [30]. Furthermore, the antimicrobial activity of highly polar ethanol extracts of *C. frutescens* pepper was already reported against a number of microorganisms [31, 32]. The significant antimicrobial activities may be attributed to the compounds identified in the GC–MS analysis such as lactic acid (30.90%), valeric acid (20.49%) and 5-methoxy butanedioic acid (18.56%) which were found as the major constituents [33]. The isolation of these antimicrobial agents from capsicum fruit could lead to an important change in the area of food safety and may be used in the prevention of food-borne diseases.

**Table 2**Minimal inhibitory concentration (MIC) of ethanolic extracts to six microorganisms

| Microorganisms | | MIC (µg/ml) |
|---|---|---|
| Gram positive bacteria | *Enterococcus faecalis* | 15 |
| | *Bacillus subtillis* | 20 |
| | *Staphylococcus aureus* | 20 |
| Gramnegativebacteria | *Pseudomonas vulgaris* | 10 |
| | *P. aeruginosa* | 15 |
| | *Escherichia coli* | 16 |

### 3.3. Total flavonoid and carotenoid contents and antioxidant activities

Total flavonoid and total carotenoid contents with the antioxidant activities in crude extract (CE) and concentrate (C) of *Capsicum annuum* fruit, are shown in table 3. The results indicated that obtained total flavonoid and total carotenoid contents and antioxidant activities in crude extract of *C. annuum* differed significantly ($p \leq 0.05$) from those found in

concentrate(C) of *C. annuum* fruit. Indeed, they revealed that the flavonoid content in concentrate (3.7 ± 0,10 g/L Eq Quercetin) was significantly (p≤0.05) higher than that observed in the crude extract (1.11 ± 0.04 g/L Eq Quercetin). Considering the nature of solvent, our study showed that ethanol was able to extract a higher proportion of flavonoid and carotenoid compounds from *C. annuum*. Similar observation was shown by Chinn *et al.* [34], who noted that the choice of solvent should be made according to the degree of solubility of pigments and that is a major factor influencing the molecule extraction process. Otherwise, total carotenoid content in concentrate (54.33 ± 0.05 mg/100 mL of fresh extract) differed meaningfully (p≤0.05) from that obtained in crude extract (35.6 ± 0.3 mg/100 mL of fresh extract). The differences in carotenoid content may be due to the influence of genotype and maturity stages and processing.

As for the antioxidant activities, significant variations between crude extract and concentrate were observed. They were found to be high (83.44 ±0.98%) in concentrated of *Capsicum annuum* fruit. Our results suggest that the concentration procedure was efficient. Reverse osmosis (RO) is a cold-operated process that presents an interesting alternative to heat treatments for highly heat sensitive bioproducts, in particular to preserve their original functionalities and activities. RO is used to preserve bioactivities have to be kept [35]. The results were agreement with those obtained by Cao and Prior [36], who identified bell-pepper with strong antioxidant activity using oxygen radical absorbance capacity (ORAC) assay. They indicated correlation between antioxidant activity and this compound. According to Khokhar and Apenten [37], the antioxidant activity could be influenced by the presence of hydroxyl group. Indeed, the antioxidant activity is also very dependent on the number and position of the hydroxyl group presence in the molecules [38]. It appeared a strong correlation between the both parameters (flavonoid (r=0.82) and carotenoid (r=0.91)) and antioxidant activity in concentrated extract. The report of Esmaeili *et al*. [39] corroborated correlation between the flavonoid and carotenoid contents and antioxidant activities. The strong correlation between the flavonoid content and antioxidant activity was shown by Kuna *et al*. [40], who found a correlation coefficient of 0.86. Some works confirmed correlation between the phenolic compounds content and the antioxidant activity [41]. On the other hand, others studies didn't find correlation between the both parameters (flavonoid and carotenoid contents) and antioxidant activities or low correlation [42]. This observed in crude extract. Otherwise, flavonoids are the main class of chemical compounds found in with antioxidant activity [43]. Recent studies showed that flavonoids contributed significantly to the scavenging activity of vegetable [44, 45]. Indeed, Flavonoids are naturally occurring in plants and are thought to have positive effects on human health. Studies on flavonoidic derivatives revealed a wide range of antibacterial, antiviral, anticancer and anti-allergic activities [36]. Moreover, flavonoids had highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals implicated in several diseases [37]. Our results corroborated the findings in the literature for other extracts of plant products [47]. They suggested that phenolic acids and flavonoids may be the major contributors for the antioxidant activity.

**Table 3**Total flavonoid and carotenoid contents with antioxidant activities in crude and concentrated extracts from pepper (*Capsicum annuum* var an) fruit

| **Parameters** | **Samples** | |
|---|---|---|
| | **Crude extract** | **Concentrated extract** |
| Total flavonoid content (g / L Eq Quercetin) | 1.10 ± 0.04[a] | 3.70 ± 0.10[b] |
| Total carotenoid content (mg/100mL of fresh extract) | 35.60 ± 0.03[a] | 54.33 ± 1.10[b] |
| Antioxidant activity (%) | 59.53 ± 0.54[a] | 83.44 ± 0.98[b] |

All analyses were performed in triplicates and the values in the table are the mean ± standard deviation. On the same line, the means followed by a similar letter aren't significantly different (p ≤ 0.05) according to the Student's test.

## 4. Conclusion

This study showed that concentrated extracts of *C. annuum* contained the major constituents such as lactic acid, valeric acid, 5-methoxy, Butanedioic acid, Phenylalanine, Hexadecanoic acid, ethyl ester, 6-Methoxy-hexane-2-ol, Butane, 2,3diol, Pentanoic acid, 4-oxo-, 3-methyl-2-hydroxylButanoic acid, Benzeneacetic acid,4-hydroxyl, 1,2-Benzenedicarboxylic acid ester, 2,5-Furandicarboxylic acid, 7-hydroxyl7-methyloctanoïc acid. The studied concentrate from the *Capsicum annuum* fruit is good sources of flavonoids and carotenoids. Furthermore, these parameters contents and antioxidant activity was found to be higher in concentrate than those obtained in crude extract. The study also showed that Gram positive and negative microorganisms were affected by the tested concentrated extract*. Capsicum* fruit used as concentrated extracts, increase nutritional value in different foods and diets. Concentrate of fresh peppers fruits generally may provide the types of nutritional and health benefits associated with the consumption of pepper

fruits extract. Development and consumption of *Capsicum* pepper extract with high antioxidant activity may help in decreasing the incidence of certain types of diseases in humans. On the other hand, we found the best antimicrobial activity against the bacteria tested with the *Capsicum* extract obtain by reverse osmosis.

These findings suggest that future investigations could be performed in order to evaluate the stability of compounds of concentrate for preservation time of their potential health and food-protecting benefits or to identify other possible biological and industrial applications.

## Compliance with ethical standards

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*Disclosure of conflict of interest*

Authors declared that there is no conflict of interest.

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