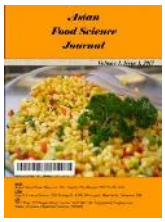

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Sensory Characteristics and Antioxidant Activities of the Spice from the Fruit Pulp of *Coelocaryon oxycarpum*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JNS and JTG designed the study and managed the literature searches. Then, authors JNS and CGD performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to assess the sensory characteristics and antioxidant properties of a spice from the fruit pulp of *Coelocaryon oxycarpum (Cox),* an aromatic plant.
Place and Duration of Study: Department of Food Science and Technology (UFR-STA), University Nangui Abrogoua, between April 2016 and March 2017.
Methodology: The spice was obtained by drying and crushing the fruit pulp of *Cox*. The powder obtained was used to evaluate the sensory (colour, aroma, taste and smell) characteristics and antioxidants (phenolic compounds and vitamin A) using standard methods of analysis. The free radicals scavenging activities of the aqueous extract of the spice from *Cox* were determined and compared with that of a reference antioxidant (Ascorbic acid) prepared under the same conditions.
Results: The 50 % Inhibition Concentration (IC50) value of the aqueous extract of the spice (13.64 µg/mL) for DPPH radical was approximately equal to twice the value of Ascorbic acid (6.94 µg/mL). The concentration (813.69 µg / mL) of the aqueous extract of the spice led to 50 % inhibition of hydrogen peroxide radical. The iron reducing power of aqueous extract of spice was about 33 %

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that of Ascorbic acid at the same concentration. The spice also showed high phenolic content (2287.42 mg GAE/100 g DW). Tannins (314.95 mg tannic acid/100 g DW) were higher than flavonoids (211.57 mg quercetin /100 g DW). Catechin (494.95 µg /100g DW), epicatechin (3.21 µg /100g DW) and quercetin (6.22 µg/100g DW) were also detected. Furthermore, sensory tests showed a score for overall acceptability corresponding to pleasant levels.
Conclusion: The spice from *Cox* fruit presented interesting sensory characteristics. It contains also an appreciable amount of phenolic compounds which helped to highlight the potential biological activities of the spice. This spice could be considered as an important source of natural antioxidants.

Keywords: Coelocaryon oxycarpum; sensory properties; antioxidant activity; polyphenols; spice; aromatic plants.

ABBREVIATIONS

Cox : Coelocaryon Oxycarpum
DPPH : 2, 2-diphenyl-1-picryllhydrazyl
FRAP : Ferric reducing antioxidant power
TAC : Total phenolic acid content

1. INTRODUCTION

Aromatic plants are set of plants used in cooking and phytotherapy for the aromas they release and the active ingredients they contain. These plants are often grown as needed for their leaves, stems, bulbs, roots, fruits, seeds, flowers, and bark. Aromatic plants consist of spice and aromatic herbs. The term "spices" indicates chlorophyll-free aromatic products, which mainly originate from tropical countries, while "aromatic herbs" is used for plants whose fresh or dried herbaceous part is used [1]. Aromatic herbs and spices have been long used as preservative agents to extend the shelf-life of food and also to enhance or improve the flavour and organoleptic properties of different types of food due to their sensory properties [2,3]. They are an essential component of human nutrition and occupy a prominent place in the cultures of many people throughout the world. In Africa, Latin America, Asia and the Mediterranean, spices and herbs of various kinds are ubiquitous in the diet of the indigenous populations [4]. Singularly, spices have been reported as abundant sources of polyphenol compounds with strong antioxidant capacities [5] that could replace the synthetic antioxidants in food systems and offer additional health benefits. Therefore, this has resulted in a demand for antioxidants derived from natural sources as spices [6]. It has been reported that, global demand for aromatic and medicinal plants would grow rapidly with an estimated annual growth rate of between 10 and 20 % due to their organoleptic qualities and their therapeutic properties [7,8]. In this context, the characterisation of wild aromatic plants with a view to proposing new sources of antioxidant to meet nutritional and therapeutic needs seems to be a relevant approach. Therefore, this study focused on the fruit of *Coelocaryon oxycarpum* (*Cox*) which is an endemic aromatic plant that grows spontaneously in the form of a tree with a height of more than 40 m. This fruit used as a spice by the populations of the north-east of Côte d'Ivoire [9], has been recently reported as an excellent source of nutrients and very flagrant and pleasant once dried [10]. Thus, this work aimed to identify the sensory and antioxidant properties of this traditional spice.

2. MATERIALS AND METHODS

2.1 Sample Preparation

The spice is obtained by drying the fruit pulp of *Coelocaryon oxycarpum*. Thus, 2 kg of fresh pulps were pitted, washed with distilled water, drained, grated and dried in an oven at 45°C for 72 h. After drying, the fruit pulps were crushed using a Moulinex-type mixer. The powder obtained was sieved (250 µm) to obtain the fruit pulp powder (spice powder). The powder was put into boxes and stored in a cool dry place.

Fig. 1. Photographs of fresh fruits and dried fruit pulp of *Coelocaryon oxocarpum*; A: fresh fruits, B: dried fruit pulp, C: dried fruit pulp powder.

2.2 Methods

2.2.1 Organoleptic Evaluation

Organoleptic analysis was carried out according to Dadzie and Orchard [11] to obtain descriptive data on the spice powder. A 10-point descriptive scale was used. A panel of 15 subjects trained consisting of male and female of University Nangui Abrogoua (Abidjan, Côte d'Ivoire) allowed to have information about the organoleptic properties. For the following attributes: brown colour, astringency, smell, ‘‘1” indicated that the attribute was low while ‘‘10” indicated that the intensity of the attribute was highest. Then, the sensation of taste ("1" represented “bland” and "10" represented “pungency”), appearance of taste ("1" represented “instantaneous” and "10" represented “progressive”) and persistence of taste ("1" represented “passenger or short” and "10" represented “permanent”) were determined. This test was performed in triplicate.

2.2.2 Hedonic test

A sensory panel consisting of 58 subjects untrained was also recruited in the same University. Each subject was first briefed with the important sensory evaluation conceptual knowledge. A hedonic scale of 9-points was used to rate the spice powder for the colour, taste, smell and aroma. A score of 1 represented "dislike extremely" and 9 represented "like extremely" [12]. The sensory analysis was performed in one room equipped with individual booths.

2.2.3 Determination of polyphenols

Total Polyphenols were extracted and determined using Folin Ciocalteu reagent [13]. One (1 g) of spice powder was soaked in 10 mL of 70 % methanol (v/v) and centrifuged at 1000 rpm for 10 min. An aliquot (1 mL) of supernatant was oxidised with 1 mL of Folin-Ciocalteu reagent and neutralised by 1 mL of 20 % (w/v) sodium carbonate. The reaction mixture was incubated for 30 min at ambient temperature and absorbance was measured at 725 nm by using a Spectrophotometer (Spectrophotometer invisible model MS-A 5100). The polyphenols content was obtained using a calibration curve of gallic acid (1 mg/mL) as standard.

2.2.4 Determination of flavonoids

The total flavonoids were evaluated using the method reported by Meda et al., [14]. Briefly, 0.5 mL of the methanolic extract was mixed with 0.5 mL methanol, 0.5 mL of AlCl3 (10 %, w/v), 0.5 mL of potassium acetate (1 M) and 2 mL of distilled water. The mixture was allowed to incubate at ambient temperature for 30 min. Thereafter, the absorbance was measured at 415 nm by using a spectrophotometer (spectrophotometer invisible model MS-A 5100). The total flavonoids were determined using a calibration curve of quercetin (0.1 mg/mL) as standard.

2.2.5 Determination of tannins

Tannins were quantified according to Bainbridge et al. [15]. One (1) mL of methanolic extract was mixed with 5 mL of vanillin reagent and the mixture was allowed to incubate at ambient temperature for 30 min. Absorbance was read at 500 nm by using a Spectrophotometer (Spectrophotometer invisible Model MS-A 5100). This method used a calibration curve of tannic acid (2 mg/mL) as standard.

2.2.6 Identification and quantification of phenolic compounds by HPLC analysis

The individual phenolic compounds were analysed by the method described by Ho et al., [16]. The phenolic extract previously prepared (50 mL) was diluted into 100 mL of distilled water and 20 µL were analysed using an Analytical HPLC unit (HPLC (Shimadzu Corporation, Japan) equiped with the binary pump (LC-6A) coupled to UV-VIS Detector (SPD-6A). Phenolic compounds were separated on a column ICSep ICE ORH-801(Length 25cm) at 30°C. The mobile phase consisted of 50 mM NaH4H2PO4 to pH 2.6 (Eluent A), a solution of acetonitrile/ NaH4H2PO4 (80:20, v/v) (Eluent B) and 200 mM acid o-phosphoric pH 1.5 (Eluent C). The operating time was 70 min with a flow rate of 1 mL/min. Phenolic compounds in methanolic extract of spice sample were identified through comparison of their retention times and UV-visible spectra with those obtained by injection of the standard solution under the same conditions. Peak area was used for quantification purposes, using external calibration with standards.

2.2.7 Determination of vitamin A

Vitamin A was determined according to AOAC [17]. Five (5) g of sample was extracted with 20 mL of methanol. The stock of standard (Sigma Aldrich Analytical grade reagent) was prepared by dissolving 0.01 g of the standard (retinol) in

methanol. A HPLC system (Shimadzu SPD 20A) equipped with UV detector (PAD) and C18 ODS column (250 x 4.6 Cluzeau France) was used in isocratic mode for analysis. Mobile phase consisting of acetonitrile (55 mL), tetrahydrofurane (37 mL) and water (8 mL) at 1.5 mL/minute flow rate and 10 µl of each sample/standard were injected and monitored at 325 nm wavelength.

2.2.8 Antioxidant assay by DPPH

The scavenging activity of the spice was compared to that of a reference antioxidant (Ascorbic acid) prepared under the same conditions. Free radical scavenging activities of different concentrations of samples were assayed using a stable DPPH (2, 2-diphenyl-1-picryl-hydrazyl radical) according to Choi et al., [18]. Antioxidants reacted with the stable free radical $DPPH^{\cdot}$ (purple colour) and converted to 1, 1 diphenyl-2-picrylhydrazine with discolouration (yellow colour). Various concentrations (2 mL) of each sample (spice extract and Ascorbic acid) were added to 1 mL of a DPPH radical solution in methanol. The absorbance was measured at 517 nm against blank by using a Spectrophotometer (Spectrophotometer invisible model MS-A 5100). Percentage of free radical scavenging activity was expressed as inhibition percentage from the given formula and 50 % Inhibition Concentration (IC50: Concentration of compound decreasing the absorbance of a $DPPH^{\cdot}$ solution by 50 %) was determined graphically.

% inhibition = (Absorbance of control – Absorbance of sample) x 100 / Absorbance of control

2.2.9 Reducing power

The reducing power of the spice was compared to that of a reference antioxidant (Ascorbic acid) prepared under the same conditions. Aqueous solutions of samples (spice extract and Ascorbic acid) were prepared to determine their reducing power by modifying the method of Oyaizu [19]. Reaction mixtures were prepared by adding 2.5 mL of phosphate buffer (0.2 M, pH 6.6), 2.5 mL of Potassium Ferricyanide (1 %) and varying concentrations of samples (10-30 µg/mL). After the reaction mixtures were incubated for 30 min, allowed to cool at room temperature (28°C) and 2.5 mL of 10 % TCA (Trichloroacetic acid) were added to each reaction mixture, and then centrifuged at 4000 rpm for 5 min. The supernatant (2.5 mL) was separated in the test tube and added with 2.5 mL of distilled water and 0.5 mL FeCl3 (1 %) and allowed to react for 10 min at room temperature and the absorbance was measured at 700 nm.

2.2.10 Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging strength of sample was determined as described by Ruch et al. [20]. A solution of H_2O_2 (4 mM) was prepared in phosphate buffer (pH 7.4). Reaction mixtures contained different concentration of test samples and absorbance values were measured after 30 min at 230 nm wavelength.

2.2.11 Statistical analysis

All experiments were carried out in triplicate and data were expressed as mean ±standard deviation (SD) or standard error of mean (SEM).

3. RESULTS AND DISCUSSION

3.1 Sensory Properties of the Spice Powder

The descriptive profile of the spice powder is shown in Fig. 1. As a new product, the colour of this spice obtained a descriptive score of 8.25 ± 1.49. This score is characteristic of the brown colour. The subjects identified other pronounced smells (8.64 ± 1.62), similar to that of pepper (7.44 ± 1.49). In terms of taste, this spice has also an astringent savour (8.24 ± 1.54). Furthermore, the sensation of taste was low (1.44 ± 0.53) and characteristic of bland. The appearance of taste was progressive, and the persistence of taste was passenger or short (2.41 ± 0.90).

In addition to these descriptive results, the spice was accepted by the subjects according to the hedonic test. Indeed, the mean scores given by the sensorial panel for the colour, smells, taste and aroma acceptability of the spice powder are presented in Table 1. Mean score for overall acceptability on a 9-point scale ranged from 6.22 ± 0.98 to 7.19 ± 0.93, corresponding to pleasant level. Thus, the spice could be accepted by consumers.

3.2 Polyphenols and Vitamin A

Spices and aromatic herbs used in foods and medicinal mixtures for their flavor and biological effects often contain high concentrations of

phenolic compounds [21]. Recent interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activities of these phenolic compounds [22]. As revealed by the analysis Table 2, polyphenols content of the spice studied (2287.42 ± 106.89 mg GAE / 100 g DW) was largely high than that found for spices and aromatic herbs of various sources such as *Echinacea purpurea* (15.15 ± 0.13 mg GAE/100 g DW), *Carum Cavi* (0.07 ± 0.00 mg GAE/100 g DW) [23], caraway (3.99 ± 0.44 mg GAE/ g DW) and nutmeg (0.63 ± 0.09 mg GAE/ g DW) [24]. However, it was found lower than that recorded by Djeddi et al., [25] for methanol extract of *Thymus numidicus* (513.40 mg GAE/g DW). Note that the efficiency of phenolic extraction depends on the extraction method, solvent type [26], and drying process used for the plant material [27]. Among phenolic compounds, flavonoids which constitute one of the most important groups in plant products were revealed in the spice. Their content (211.57 ± 3.88 mg quercetin / 100 g DW) was higher than that of grapefruits (7.12 mg quercetin / 100 g DW) and strawberries (17.53 mg quercetin /100 g DW) (Haddadi, 2005), those fruits that are well known for their important antioxidant properties [28, 29]. Being phytochemicals, flavonoids cannot be synthesised by humans and animals [30], hence the spice could represent an interesting source of dietary flavonoids. Tannins form a group of phenolic compounds resulting partly from polymerisation of flavonoids units. Their level (314.95 ± 1.28 mg tannic acid / 100 g DW) was also higher in the spice studied than in *Piper guineense* (93.09 mg / 100 g DW) and *Capsicum frutescens* (99.03 mg / 100 g DW) [31]. Another important component revealed in the spice, was Vitamin A. Vitamin A, Vitamin C, and Vitamin E are dietary antioxidants, commonly present in vegetables and fruits which help to inhibit the free radicals [32]. Vitamin A was also reported to be involved in bone formation [33]. Moreover, the lack of Vitamin A causes poor night vision and drying of cornea (Xerophthalma) [34, 35]. Its content (48.80 ± 5.63 mg / 100 g DW) for the spice was higher than that of other spices like *Myristica frangrans* (14.57µg /g); *Piper guineense* (7.08 µg /g) and *Rosmarinus officinalis* (14.87µg /g) [36]. Thus, the regular consumption of the spice could solve vitamin A deficiency.

3.3 Individual Phenolic Compounds

With regard to individual phenolic compounds depicted in Table 3, data showed that catechin content was relatively high. Its average was 494.95 ± 8.03 µg/100 g DW. Note that, catechins are the most reduced form of the C3 unit in flavonoid compounds, extensively researched due to their biological activity. Catechin and epicatechin were determined in cumin with respective levels of (9.78 ± 0.39 µg / 100 g DW) and (5.62 ± 0.42 µg / 100 g DW) [24].These values were lower than those recorded in the spice. However, Baâtour et al. [37] found values of 7.2-177 µg/g DW and 51-78 µg/g DW in marjoram, for catechin and epicatechin, respectively. Arts et al. [38] reported that, although most fruits and some legumes contain catechins, the levels vary from 4.5 to 610 mg/kg. Compared to catechin, the level of quercetin in the spice was low (6.22 ± 1.53 µg/100g DW). Quercetin has been used as a nutritional supplement and may be beneficial against a variety of diseases. Some of the beneficial effects include cardiovascular protection, anticancer, antitumor, anti-ulcer, anti-allergy, anti-viral, anti-inflammatory activity, anti-diabetic, gastroprotective effects, antihypertensive, immunomodulatory, and anti-infective [39]. Ultimately, the richness of this spice in

Table 1. Average score out of 9 for sensory analysis of the spice powder of *Cox*

Sensory attribute	Colour	Smell	Aroma	Taste
Score	7.19 ± 0.93	6.89 ± 1.46	6.70 ± 1.02	6.22 ± 0.98

Values represent Mean ± SD (n = 3).

Table 2. Composition in few polyphenols and Vitamins A of the spice from *Cox* fruit

Compound	Values
Polyphenols (mg GAE/100 g DW)	2287.42 ±106.89
Tannins (mg tannic acid/100 g DW)	314.95 ± 1.28
Flavonoids (mg quercetin/100 g DW)	211.57 ± 3.88
Vitamin A (mg/100 g DW)	48.80 ± 5.63

Values represent Mean ± SD (n = 3)

polyphenols, flavonoids, tannins and individual phenolic compounds could provide it with very interesting antioxidant potential likely to be evaluated by other methods.

3.4 Free Radical Scavenging Activity

DPPH radical trapping method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts. By this method, the extract of the spice exhibited effective free radical scavenging activity Fig. 2. The IC50 value of the spice extract (IC50: 13.64 µg/mL) calculated from the logarithmic regression curve was approximately equal to twice the value of Ascorbic acid (IC50: 6.94 µg/mL). Note that the IC50 represents the concentration causing 50% inhibition. The lower the IC50 is, powerful will be the antioxidant. Nevertheless, this unpurified extract of the spice was able to reach half the activity of vitamin C. Thus, bioactive substances contained in the spice would be powerful antioxidants. This antioxidant activity could be linked to its richness in polyphenols. According to Turkmen et al. [40] polyphenols appear to be effective hydrogen donors to the DPPH radical due to their ideal chemical structure. Particularly, this may be attributed to the high contents of flavonoids and tannins in the spice. Flavonoids are known as the most active antioxidants in plant foods [41]. In general, antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups [42]. Catechin, which is a class of flavonoids with a high polarity, has been reported for its ability to bind free radicals and prevent cellular aging [43]. The presence of these phenolic compounds and the antioxidant activity of this spice could constitute a better protection of lipids against the action of free radicals responsible for metabolic diseases such as cancer, cardiovascular diseases and cellular ageing [44].

Table 3. Phenolic compounds (µg/100g DW) of Cox fruits

Phenolic compound	Values
Arbutin	0.78 ± 0.10
Catechin	494.95 ± 8.03
Epicatechin	3.21 ± 0.94
Quercetin	6.22 ± 1.53

Values represent Mean ± SD (n = 3).

3.5 Reducing Power Activity

Results obtained during the study of the antioxidant activity by the iron reduction technique are depicted in the Fig. 3. These results indicate the presence of reducing agents in the extract of the spice. The reducing power is dose dependent, but largely lower than that of Ascorbic acid. It represented about 33 % that of Ascorbic acid at the same concentration, while the DPPH radical scavenging activity was about 54.68 % the capacity of Ascorbic acid. This observation suggests that compounds involved in the two types of reaction are not the same and only one method cannot well describe the antioxidant potential.

3.6 Hydrogen Peroxide Scavenging Assay

Fig. 4 shows the capacity of the spice extract to trap the peroxide radical (H_2O_2.). This method indicated that the extract achieved an inhibition of 50% at a concentration (813.69 µg / mL), near to the value (63.09 %; 750 mg/L) of infusion of *Thymus Numidicus* leaf [25]. Thus, the spice

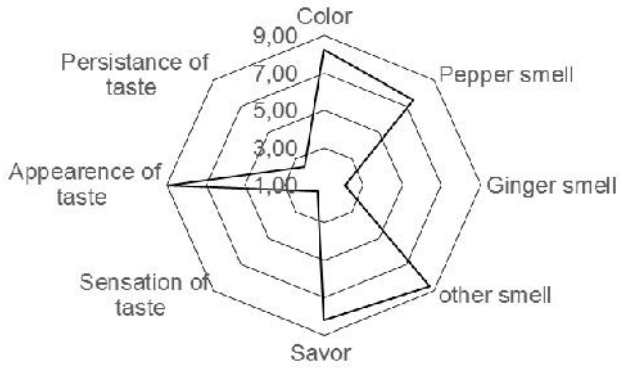

Fig. 2. Plot of scoring averages for descriptive analyses (out of 10) for the spice powder of *Cox*

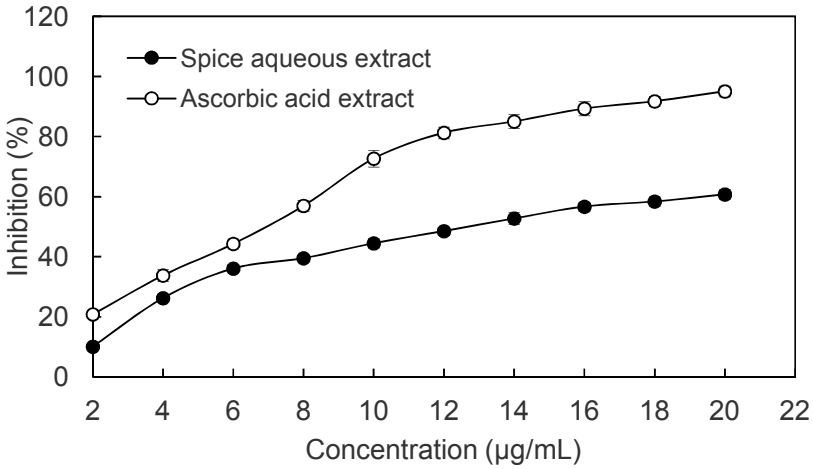

Fig. 3. The DPPH radical trapping activity of the extract of the spice from *Cox* fruit and Ascorbic acid

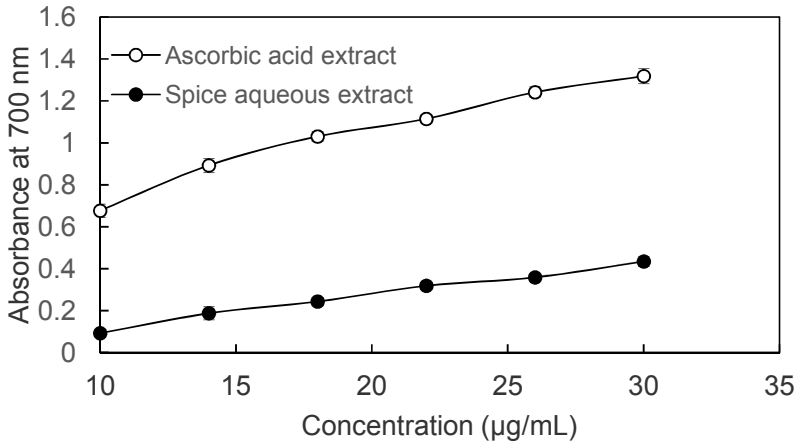

Fig. 4. Reducing power of the extract of spice from *Cox* fruit and Ascorbic acid.

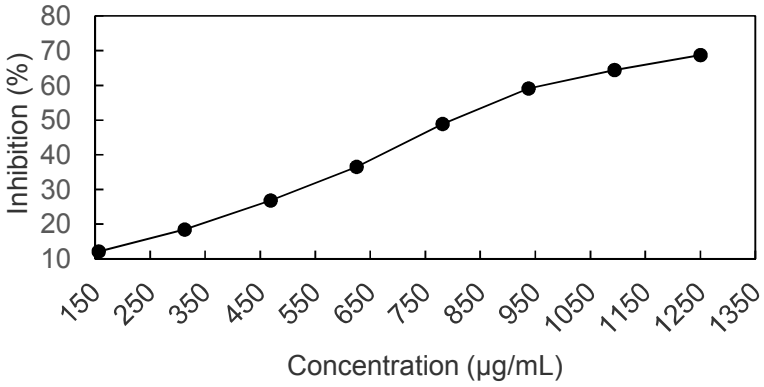

Fig. 5. Hydrogen peroxide (H_2O_2) radical trapping activity of the extract of the spice from *Cox* fruit

extract could be considered as a good electron donor, able to accelerate the conversion of hydrogen peroxide (H_2O_2) into water (H_2O) and consequently its elimination [41]. This reaction could also be attributed to polyphenols. Their role as a natural antioxidant in plants is very beneficial in the prevention and treatment of diseases related to oxidative stress and high blood pressure [45,46].

4. CONCLUSION

The spice of *Cox* fruit exhibited interesting sensory characteristics that may account for it valorisation. It contains appreciable amount of phenolic compounds. The free radical scavenging activities, as well as the reducing activity of iron, have made it possible to highlight the potential biological activities of this spice. Therefore, the spice of *Cox* fruit could be considered as an important source of natural antioxidants likely to fight against body oxidative stress when regularly consumed. It could also be used as ingredient for manufacturing of foods products due to its pleasant flagrance. Taken together, these properties should encourage it marketing.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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