

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

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Abstract

The fruit pulp of *Coelocaryon oxycarpum* (*Cox*) is consumed as spice in dried form by populations of North-Eastern part of Côte d'Ivoire. In this study, the sensory characteristics (color, aroma, taste and smell) as well as phenolic compounds and vitamin A contents of the spice were investigated. With regard to antioxidant activities, the extract of the spice powder was evaluated for scavenging ability on 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide radicals and reducing power by iron reduction technique method. The score for overall acceptability of the spice on a 9-point scale ranged from 6.22 ± 0.98 to 7.19 ± 0.93 , corresponding to the pleasant level. Total phenolics (2287.42 ± 106.89 mg GAE /100g DW) were largely high. Tannins (314.95 ± 1.28 mg tannic acid / 100 g DW) were higher than flavonoids (211.57 ± 3.88 mg quercetin / 100 g DW). Individual phenolic compounds such as catechin (494.95 ± 8.03 µg/100 g DW), epicatechin (3.21 ± 0.94 µg/100g DW), and quercetin (6.22 ± 1.53 µg/100g DW) were detected. The spice extract showed trapping activity on DPPH (IC50: 13.64 µg / mL) which was approximately twice the value of Ascorbic acid used as reference (IC50: 6.94 µg / mL). The spice extract exhibited also antioxidant activities by peroxide radicals and iron reducing methods. Therefore, the spice of *Cox* fruit could be considered as an important source of natural antioxidants. It could be used as ingredient for manufacturing of foods products due to its pleasant fragrance.

Keywords: *Coelocaryon Oxycarpum.*, sensory properties, antioxidant activity and polyphenols

1. Heading 1

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 (Richard, 1992). 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 flavor and organoleptic properties of different types of food due to their sensory properties (Kivilompolo et al., 2007; Park, 2011). They are an essential component of human nutrition and occupy a prominent place in the cultures of many people through 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 (Borquaye et al., 2017). Singularly, spices have been reported as abundant sources of polyphenol compounds with strong antioxidant capacities (Shan et al., 2005) that could replace the synthetic antioxidants in food systems and offer additional health benefits. Therefore, this has resulted in demand for antioxidants derived from natural sources as spices (Hossain et al., 2010). 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 (Helle et al., 2007; Elzbieta et al., 2008). In this context, the characterization 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 spice by the populations of the north-east of Côte d'Ivoire (Ouattara et al., 2016), has been recently reported as excellent source of nutrients and very fragrant and pleasant once dried (Sinh et al., 2017). Thus, this work aimed to identify the sensory and antioxidant properties of this traditional spice.

2. Material and Methods

Sample Preparation:

The spice is obtained by drying the fruit pulp of *Coelocaryon oxycarpum*. Thus, two (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.

Methods

Organoleptic evaluation:

Organoleptic analysis was carried out according to Dadzie and Orchard (1997) 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 our University (University Nangui Abrogoua, Abidjan, Côte d'Ivoire) allowed to have information about the organoleptic properties. For the following attributes: brown color, 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 "pungency" and "10" represented "burning"), appearance of taste ("1" represented "instantaneous" and "10" represented "progressive") and persistence of taste ("1" represented "passenger or short" and "10" represented "permanent") have been determined. This test was performed in triplicate.

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 color, taste, smell and aroma. A score of 1 represented "dislike extremely" and 9 represented "like extremely" (Lawless and Heymann, 2010). The sensory analysis was performed in one room equipped with individual booths.

Determination of Polyphenols:

Total Polyphenols were extracted and determined using Folin Ciocalteu reagent (Singleton et al., 1999). 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 oxidized with 1 mL of Folin-Ciocalteu reagent and neutralized 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.

Determination of Flavonoids:

The total flavonoids were evaluated using the method reported by (Meda et al., 2005). 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.

Determination of Tannins:

Tannins were quantified according to Bainbridge et al (1996). 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.

Identification and quantification of Phenolic Compounds by HPLC Analysis:

The individual phenolic compounds were analyzed by the method described by Ho et al. (1999). The phenolic extract previously prepared (50 mL) was diluted into 100 mL of distilled water and 20 µL were analyzed using an Analytical HPLC unit (HPLC (Shimadzu Corporation, Japan) equipped 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 NaH₂H₂PO₄ to pH 2.6 (Eluent A), a solution of acetonitrile/ NaH₂H₂PO₄ (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.

Determination of Vitamin A:

Vitamin A was determined according to AOAC (1990). 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), tetrahydrofuran (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.

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. (2002). Antioxidants reacted with the stable free radical DPPH[•] (purple color) and converted to 1, 1 diphenyl-2-picrylhydrazine with discoloration (yellow color). 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[•] solution by 50 %) was determined graphically.

$$\% \text{ inhibition} = (\text{Absorbance of control} - \text{Absorbance of sample}) \times 100 / \text{Absorbance of control}$$

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 (1986). Reaction mixtures were prepared by adding 2.5 mL of phosphate buffer (0.2 M, pH 6.6) 2.5 mL 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.

Hydrogen peroxide scavenging assay:

Hydrogen peroxide scavenging strength of sample was determined as described by Ruch et al. (1989). A solution of H₂O₂ (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 using wavelength at 230 nm.

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

Sensory properties of the spice powder:

The descriptive profile of the spice powder is shown in **Figure 1**. As a new product, the color of this spice obtained a descriptive score of 8.25 ± 1.49 . This score is characteristic of the brown color. The subjects identified other pronounced smells (8.64 ± 1.62), similar to that of pepper (7.44 ± 1.49). This spice has also an astringent savor (8.24 ± 1.54) in terms of taste. Furthermore, the sensation of taste was low (1.44 ± 0.53) and characteristic of pungency. 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 color, 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.

Polyphenols and Vitamin A:

Spices and aromatic herbs used, in foods for their flavor and in medicinal mixtures for their biological effects often contain high concentrations of phenolic compounds (Tsai et al., 2016). Recent interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activities of these phenolic compounds (Shashank and Abhay, 2013). 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) (Wojdyło et al., 2007), caraway (3.99 ± 0.44 mg GAE/ g DW) and nutmeg (0.63 ± 0.09 mg GAE/ g DW) (Vallverdú-Queralt et al., 2015). However, it was found lower than that recorded by (Djeddi et al., 2015) 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 (Dent et al., 2013), and drying process used for the plant material (Lemus-Mondaca et al., 2016). 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 (Peterson et al., 2006; Abeysinghe et al., 2007). Being phytochemicals, flavonoids cannot be synthesized by humans and animals (Koes et al., 2005), hence the spice could represent an interesting source of dietary flavonoids. Tannins form a group of phenolic compounds resulting partly from polymerization 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) (Abdou et al., 2010).

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 (Blokhina et al., 2003). Vitamin A was also reported to be involved in bone formation (Ekeanyanwu et al., 2010). Moreover, the lack of Vitamin A causes poor night vision and drying of cornea (Xerophthalma) (Hurt et al., 1980; Ameh et al., 2016). Its content (48.80 ± 5.63 mg / 100 g DW) for the spice was higher than that of other spices like *Myristica fragrans* ($14.57 \mu\text{g} / \text{g}$); *Piper guineense* ($7.08 \mu\text{g} / \text{g}$) and *Rosmarinus officinalis* ($14.87 \mu\text{g} / \text{g}$) (Okonkwo and Ogu 2014) J. Thus, the regular consumption of the spice could solve vitamin A deficiency.

Individual phenolic compounds:

With regard to individual phenolic compounds depicted in **Table 3**, data showed that catechin content was relatively high. Its average was $494.95 \pm 8.03 \mu\text{g} / 100$ 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 found in caraway ($9.78 \pm 0.39 \mu\text{g} / 100$ g DW) and ($5.62 \pm 0.42 \mu\text{g} / 100$ g DW), respectively (Vallverdú-Queralt et al., 2015) being under the rate in the spice. However, Baâtour et al. (2012) found values of $7.2\text{-}177 \mu\text{g} / \text{g}$ DW and $51\text{-}78 \mu\text{g} / \text{g}$ DW in marjoram, for catechin and epicatechin, respectively.

Arts et al. (2000) 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 ($6.22 \pm 1.53 \mu\text{g} / 100$ g 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 (Lakhanpal and Rai., 2007).

Ultimately, the richness of this spice in polyphenols, flavonoids, tannins and individual phenolic compounds could provide it with very interesting antioxidant potential likely to be evaluated by other methods.

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 **Figure 2**. The IC_{50} value of the spice extract (IC_{50} : $13.64 \mu\text{g} / \text{mL}$) calculated from the logarithmic regression curve was approximately equal to twice the value of Ascorbic acid (IC_{50} : $6.94 \mu\text{g} / \text{mL}$). Note that less is the value of IC_{50} , powerful is 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. (2007) polyphenols appear to be effective hydrogen donors to the DPPH radical due to their ideal chemical structure. Particularly, this may account for flavonoids and their components as catechin, and tannins which were identified in this spice. Flavonoids are known as the most active antioxidants of the food plants (Kholkhal, 2014). In general, antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups (Bors et al., 1990). 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 (Shukla et al., 2009). As a health benefit, the consumption of the spice would allow better protection of lipids against the action of free radicals responsible for metabolic diseases such as cancer, cardiovascular diseases and cellular aging (Scalbert, et al., 2005).

Reducing power activity:

Results obtained during the study of the antioxidant activity by the iron reduction technique are depicted in the **Figure 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.

Hydrogen peroxide scavenging assay:

Figure 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 $\mu g / mL$), near to the value (63.09 %; 750 mg/L) of infusion of *Thymus Numidicus* leaf (Djeddi et al., 2015). Thus, the spice 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 (Kholkhal, 2014). 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 (Sikorski and Kolakowski., 2003; Chun et al., 2005).

4. Conclusion

The spice of Cox fruit exhibited interesting sensory characteristics that may account for its valorization. It contains appreciable amount of phenolic compounds. The related trapping activity of free radicals and iron reducing activity highlighted on the potential biological activities of the spice. Therefore, the spice of Cox fruit could be considered as an important source of natural antioxidants likely to fight against body oxidative stress by its regular consumption. It could also be used as ingredient for manufacturing of food products due to its pleasant fragrance. Taken together, these properties should encourage its marketing.

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Conflict of Interest The authors declare that they have no conflicts of interest

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Notes

Note 1. This is an example.

Note 2. This is an example for note 2

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

| Sensory attribute | Color | 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).



Figure 1. Plot of scoring averages for descriptive analyses (out of 10) for the spice powder of of Cox.

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).

Table 3. Phenolic compounds ($\mu\text{g}/100\text{g DW}$) 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 \pm SD (n = 3).

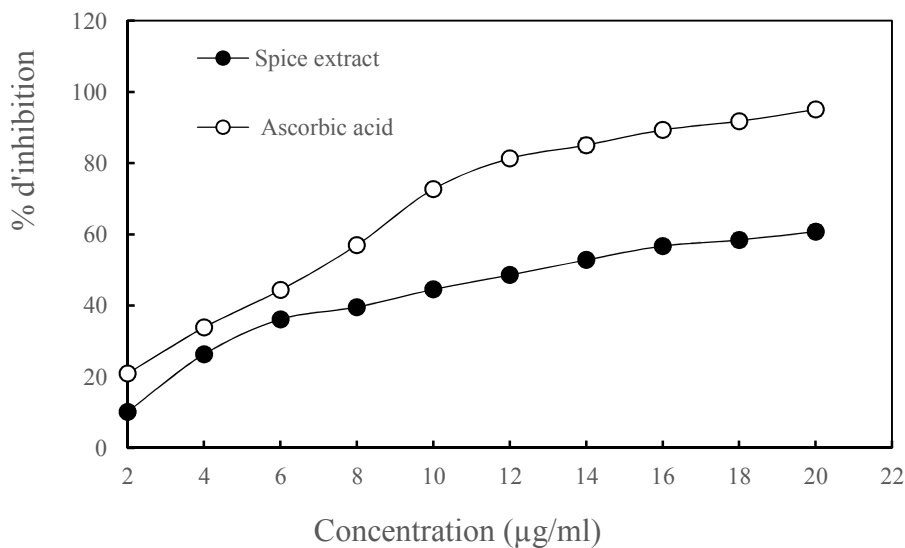


Figure 2. The DPPH radical trapping activity of the extract of the spice from Cox fruit and Ascorbic acid

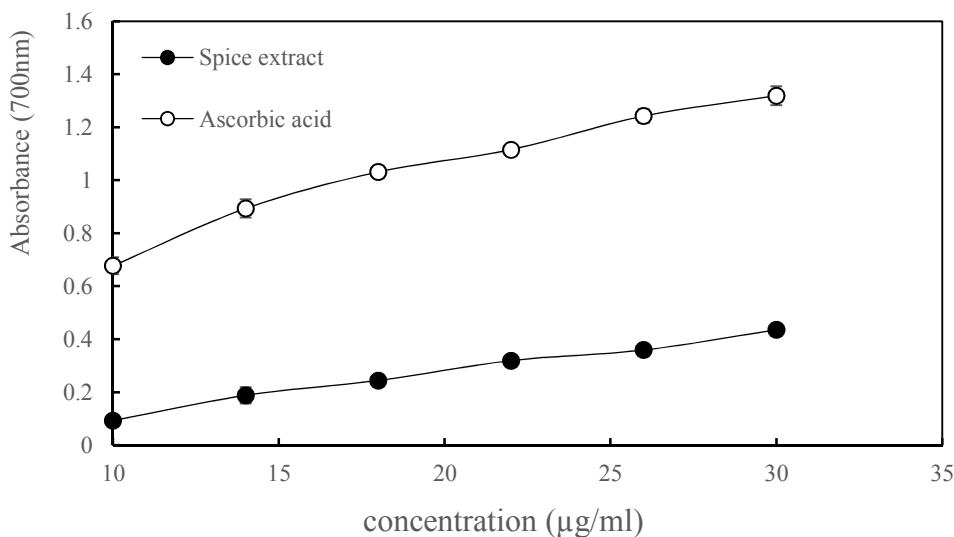


Figure 3. Reducing power of the extract of spice from Cox fruit and Ascorbic acid.

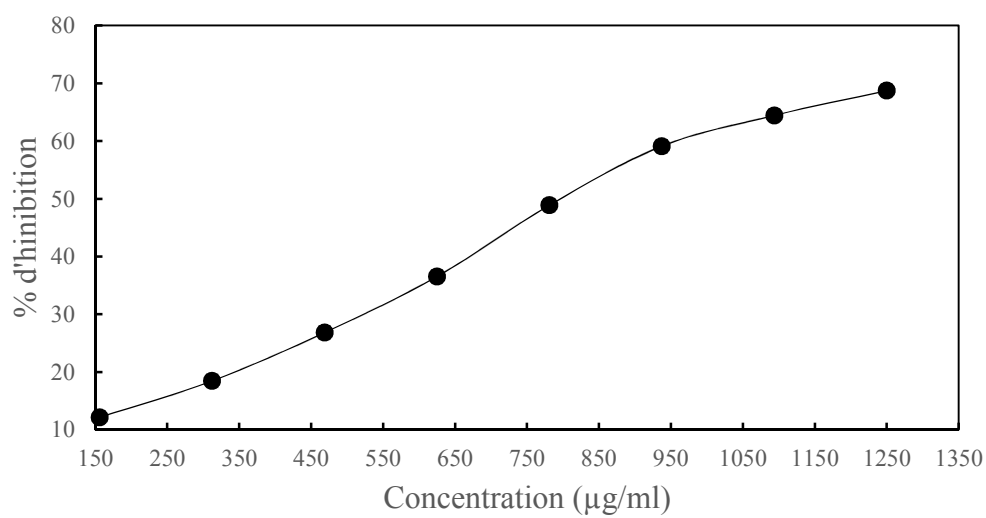


Figure 4. Hydrogen peroxide (H_2O_2) radical trapping activity of the extract of the spice from Cox fruit