Vol-4, Issue-1 PP. 8-15 ISSN: 2394-5788

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

SECONDARY METABOLITES AND ANTIOXIDANT PROPERTIES OFSOME HERBS AND SPICES (THYMUS VULGARIS, CAPSICUM ANNUM AND ALLIUM SATIVUM)

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ABSTRACT

This study focused on the determination of the secondary metabolites and the antioxidant properties of spices and herbs. Three different plants were used. It is Chilli pepper (*Capsicum annum*), Thyme (*Thymus vulgaris*) and Garlic (*Allium sativum*).Thus, a phytochemical screening and quantitative measurements were performed on extracts of these different plants (thyme, pepper and garlic).

The results obtained showed the presence of terpenoid compounds, flavonoids, tannins, phenols, saponins and alkaloids in these spices and herbs. These results revealed significant and variable rates of polyphenols and flavonoids. As for values, the rates recorded of polyphenols are 3.83 mg GAE / g for pepper (*Capsicum annuum), 3.33 mg GAE / g for thyme (Thymus vulgaris) and 0.58 mg GAE / g for garlic (Allium sativum*).As for the flavonoids, the contents were 28.43 mg EQC / g for thyme (Thymus vulgaris); 7.67 mg EQC / g for the pepper (Capsicum annuum) and 2.25 mg EQC / g for garlic (Allium sativum).

Through this study, an optimization of the use of these herbs and spices could be envisaged

Keywords: spices, secondary metabolites, antioxidants, flavonoids, polyphenols.

1. 1-INTRODUCTION

For millennia man draws in its environment, the elements necessary for its survival and well-being. Spices are used as a source of seasoning or traditional medicine cure (Majinda*et al.*, 2001). Recent surveys have shown that approximately 90% of the population of developing countries use herbal medicines for their treatment (Olsen, 2005; Jiofack*et al.*, 2010; Dibong*et al.*, 2011; Mpondo*et al.*, 2014). These plants which are garlic, thyme and chili, have within them, constituents known for their Phytotherapeutic and physiological activities. They are used in traditional medicine and phyto-therapeutic treatments through their various active ingredients (WHO, 2002; Biyiti*et al.*, 2004; Dibong*et al.*, 2011;Mpondo*et al.*, 2012; Yinyang*et al.*, 2014).

With the recurrent emergence of public health problems associated with the use of antibiotics as growth promotants in animal areas, the crude extracts of the plants began to have great interest as a potential source of natural bioactive molecules.They are used as an alternative for the treatment of infectious diseases and for the protection of foods against oxidation.Also, these plants would have positive effects on growth performance of poultry (Brenes and Roura 2010). These herbs and spices containing many active

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ingredients or secondary metabolites. They are extensively used in therapeutic environment as preventive agents' antioxidants, anti-microbial, anti-inflammatory, antiseptic and anti-diuretic (Kholkhal et al., 2013).

The present study on the garlic, thyme and chili is in the context of the search and recovery of bioactive natural substances and equipped of antioxidant activity. It is to identify the secondary metabolites to determine the phytochemicals components and antioxidant properties of these herbs and spices.

2. 2-MATERIAL ET METHODS

2.1 Spices and herbs

Three different plants were used in this study. It is the pepper (*Capsicum annum*), the thyme (*Thymus vulgaris*) and the garlic (*Allium sativum*). The pepper (*Capsicum annuum*) dried and milled was bought at the Forum market located in the town of Adjamé. Garlic powder (*Allium sativum*) and thyme (*Thymus vulgaris*) were purchased directly in a hyper-market (Sococé). All these products were stored separately in a dry place away from light at 25^{o} C.

2.2 Phytochemical treatments

The major classes of secondary metabolites such as alkaloids, flavonoids, quinones, phenols, tannins, saponins, sterols and polyterpenes have been screened according to the phytochemical methods described by Michel et al. (2011).Préparations de l'extrait brut

One gram of each spice is placed in an Erlenmeyer flask containing 20 ml of hot water. They are left to macerate for 24 hours. Then they are filtered. The filtrate obtained is used for the different tests to be performed.

2.3 Phytochemical screening

Different groups of compounds (sterol and polyterpenes, volatile compounds, alkaloids, tannins, polyphenols, flavonoids, saponins and quinones) contained in the extracts were demonstrated according to the methods described by Bekro et al. (2007). The detection of these chemicals is based on the solubility of the constituents, the reactions of precipitation and turbidity.

2.4 Quantitative determination of polyphenols

The assay of total polyphenols was carried out with the Folin-Ciocalteu colorimetric reagent according to the method cited by Wong et al. (2007).It is collected 100 µl of solution, added 1 ml of distilled water, 0.5 ml of Folin-Ciocalteau (1/10 dilution) and 1.5 ml Na_2CO_3 at 2% (w / v). All is well mixed. The mixture was incubated in the dark room at ambient temperature for 15 minutes. The absorbance of the blue solution of all samples was measured at 765nm using a gene As 1300 UV-Vis spectrophotometers. The values are expressed in milligrams of cafeic acid per gram of dry weight of plant powders. The results are expressed in milligrams gallic acid equivalent per gram of dry extract (EAG mg / g S).These results are obtained using linear regression curve shown in Figure 1.

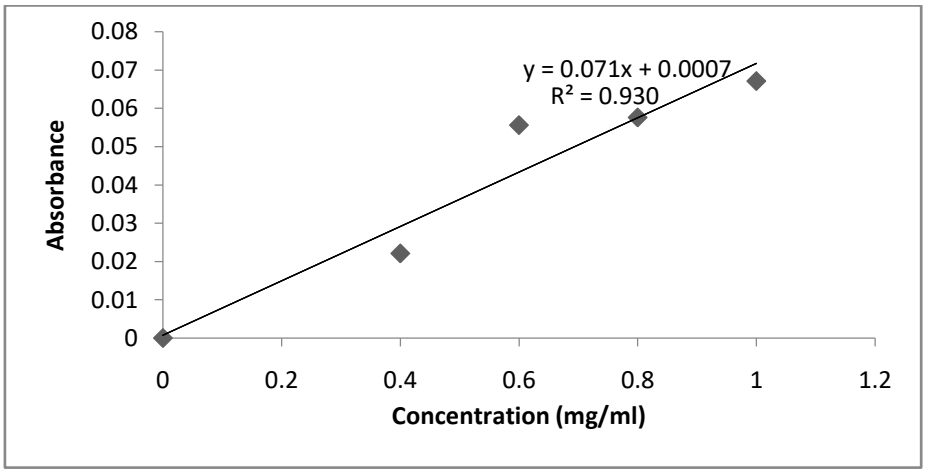

Figure 1: Standard curve of phenolic compounds

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2.5 Quantitative determination of flavonoids

The method used to quantify the flavonoids present in the extracts of spices and herbs is that of Marinolova et al. (2005) and Djeridane et al. (2006). In fact a volume of 2.5 ml of each plant extract is removed and placed in an Erlenmeyer flask.To this volume is added 750 µl of NaNO2. Then another volume of 750 µl of AlCl3 was added after 6 minutes in the dark. To this mixture, 5 ml of NaOH is added and made up with distilled water to the mark.The resulting mixture is thoroughly shaken and the absorbance read at 510 nm using a visible UV spectrophotometer (Jasco V-530), after ten minutes of incubation.

The dosage of flavonoids was performed by the colorimetric method. Quercetin, considered as positive control, has achieved a calibration curve. From this curve, it is deducted flavonoid content of various spices. It is expressed in mg quercetin equivalent (CE) per gram of material solids (Figure 2).

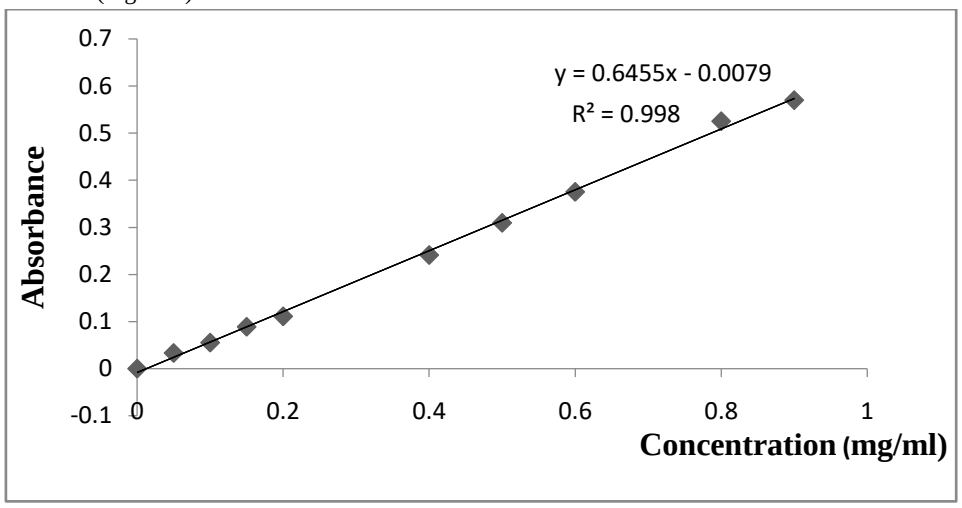

Figure 2: Flavonoids standard curve

2.6 Determination of Antioxidant Activity (AAO)

The anti-radical activity of plant extracts was determined using the stable free radical 2-2-diphenyl-1-picrylhydrazyl, DPPH (C18H12N5O6) which is one of the leading employee attempts to explore the use of plant extracts as antioxidant. This determination was made according to the method of Markowitz et al. (2007) and Michel et al. (2012).In four tubes, it is introduced 1 ml of each sample extract and 2.5 ml of Methanoic DPPH solution (0.3 mM). After stirring with a vortex, the tubes are placed in the dark room at ambient temperature for 30 minutes. The reading was performed by measuring the absorbance at 415 nm. The percentages of inhibition are determined using the following formula:

$$AAO\ (\%) = \frac{[DOc-(DO_t-DO_b)\ x100]}{DO_c}$$

AAO :Activité anti-oxydante

DO_c: absorbance of control tube (1mL of DPPH 2.5 mL of methanol)

DO_t: absorbance of the test tube (1 mL of DPPH + 2.5 ml of methanol)

DO_b: absorbance of the blank or sample tube (1 mL of DPPH + 2.5 ml of methanol extract)

The percentages of inhibition of DPPH depending on the concentrations of methanol extracts, have resulted in the concentration of antioxidant necessary to decrease the amount of initial DPPH to 50%. This value is called the EC50 effective concentration (mg / ml).

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2.7 Statistical analysis

Statistical analysis was performed using STATISTICA software StatSoft, version 7.0 (2009). The average values were the subject of an analysis of variance (ANOVA) with one factor and matched according to the multiple range test of Duncan 5% level. It has also permit to establish the average for each sample.

3. RESULTATS

3.1 Secondary metabolites detected

The results of phytochemical tests are shown in Table 1. Phytochemical tests have highlighted the presence of secondary metabolites in the chilli, garlic and thyme.From this test, it is shown that the intensity of secondary metabolites varies from one sample to another. Thus, the capsicum extract (*Capsicum annuum*) and that of thyme (*Thymus vulgaris*) contain sterol, volatile compounds, flavonoids. As for *Allium sativum*, it contains catechol tannins, alkaloids, saponins and quinones.

Table 1: Secondary metabolites detected in different extracts

Compounds	chilli pepper (*Capsicum annuum*)	Garlic (*Allium sativum L.*)	Thyme (*Thymus vulgaris L.*)
Sterol and polyterpenes	+++	-	++
Volatile compounds	++	-	++++
flavonoids	+++	+	++
gallic tannins	-	-	-
catechin tannins	-	++++	-
alkaloids	-	++	-
saponins	-	++++	++
Quinones	-	++++	++

(+) = low presence
(++) = medium presence
(+++) = abundant presence
(++++) = very abundant presence
(-) = Not detected or absence

3.2 Contents of polyphenols

Figure 3 shows the polyphenol content of the studied herbs. It reveals that *Thymus vulgaris* is the spice containing more polyphenols with a content of 3.88 ± 0.13 mg EAG / g MS followed by *Capsicum annuum*with 3.33 ± 0.2913 mg EAG / g MS. *Allium sativum is a plant that has the least polyphenols (0.58 ± 0.1413 mg EAG / g MS).*

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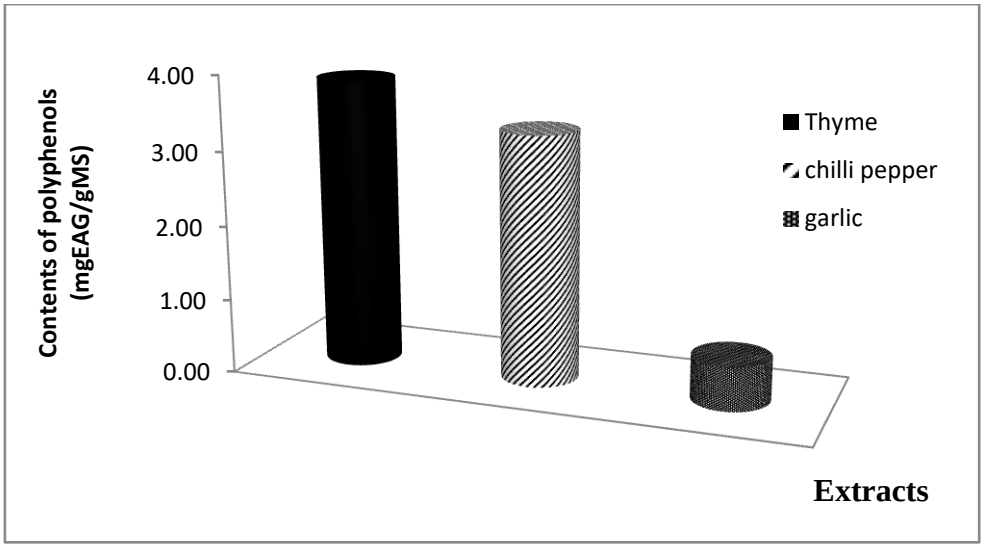

Figure 3: polyphenol contents of different extracts

3.3 Contents of flavonoids

The analysis of figure 4 shows that thyme (Thymus vulgaris) is very rich in flavonoids with a content of 28.43 ± 0.81 mg EQ / g ES. It is followed by thechilli pepper (Capsicum annuum) with 7.67 ± 0.14 mg EQ / g ES. Garlic (*Allium sativum)*is low in flavonoids with a content of 2.25 mg EQ / g ES.

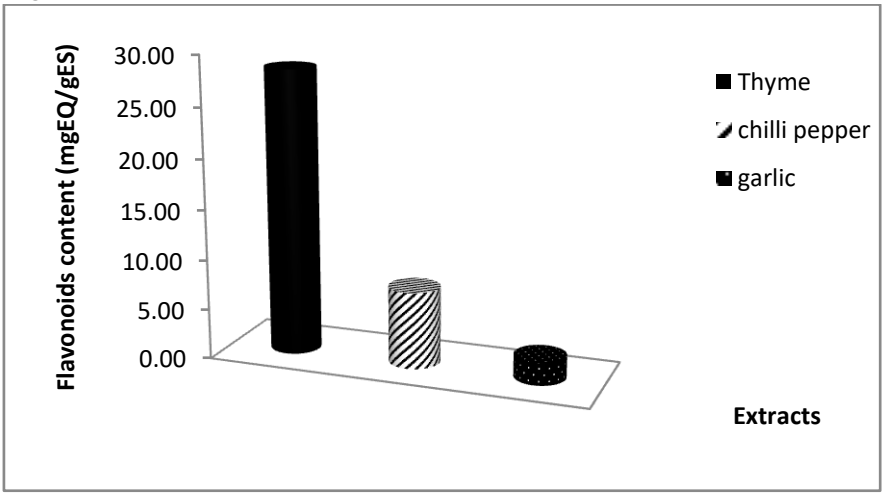

Figure 4: flavonoid contents of different extracts

3.4 Antioxidant activity

Figure 5 shows the antioxidant activity of different spices and herbs. The analysis revealed that garlic (*Allium sativum*) is the spice that has anti-oxidant highest activity (57.57% ± 0.61). It is followed by *Capsicum annuum* (47.30% ± 0.56). Thyme (*Thymus vulgaris*) is the spice that got the lowest antioxidant activity (42.84% ± 0.55).

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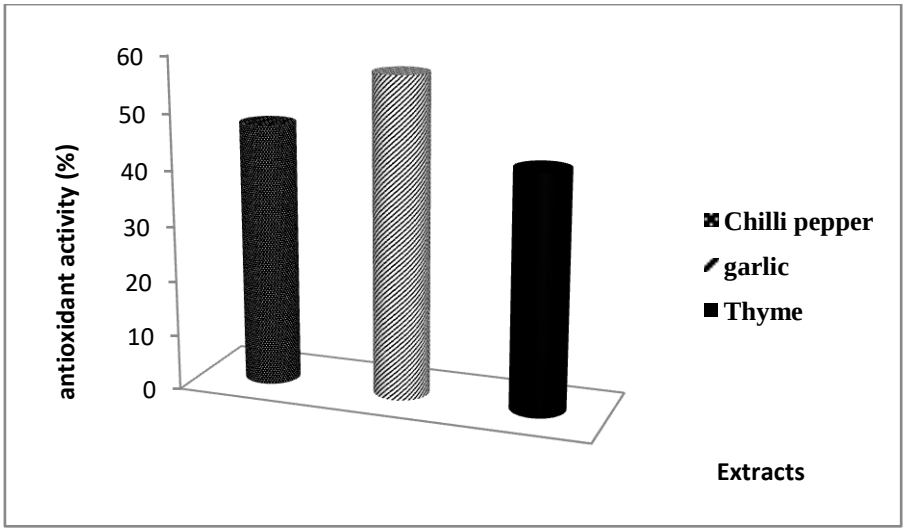

Figure 5: antioxidant activity of different extracts

4. DISCUSSION

Phytochemical screening showed that *Thymus vulgaris* and *Capsicum annum* are rich in phenolic compounds, flavonoids and terpenoid compounds.As for Allium sativum, it contains more alkaloids, tannins, saponins and terpene compounds. Considering their composition, they could have a role of protection against attacks by animals and insects. These components are known for their herbal and physiological activities (Kholkhal*et al*., 2013). The work of Kholkhal*et al*. (2013) also showed that Thymus is rich in flavonoids. These data are comparable with our results since the tests revealed the presence of flavonoids and phenolic compounds with significant amounts in the leaves of Thymus vulgaris.Quantitative phytochemical found considerable and variable contents of polyphenols and flavonoids. In crude extracts, the highest contents of polyphenols were found in the extract of *Capsicum annuum* (3.83mg GAE / g) subsequently in the leaves of *Thymus vulgaris* (3.33mgGAE / g) and finally in *Allium sativum* (0.58 mg GAE / g).Flavonoids are concentrated mainly in extracts of Thymus vulgaris sheet (28.43 mg EQC / g), subsequently in *Capsicum annuum* (7.67 mg EQC / g) and finally in Allium sativum (EQC 2.25 mg / g). The richness of these flavonoids in plants, explain their pharmacological properties. These compounds are known primarily for their role in reducing the permeability of blood capillaries and strengthening their resistance (Wichtl and Anton, 2003).Some anti-allergic properties, antispasmodic and their Hepatoprotectors are attributed. Flavonoids have antioxidant, anti-inflammatory and have a positive role in the treatment of cardiovascular diseases and neurodegenerative (Bruneton, 1999).In some cases, they are known for their anti-viral activities and anti-tumor. The determination of total phenols content is of great importance in plants. It intervenes in the growth process, protection of plants against insect pests. In addition, they contribute to give color and flavor specific to fruit and vegetables (Kever*set al*., 2007; Padilha*et al.*, 2015). Our results are consistent with Kever*set al*. (2007). These showed that chilli pepper is a plant which has a much higher concentration of phenolic compounds. Phenolic compounds are secondary metabolites normally synthesized by plants during development or in response to stress conditions (Morales-Soto *et al*, 2013; Padilha*et al*, 2015).The studied extracts also have a great antioxidant. Garlic is one that got the highest rate. The antioxidant activity of our plants is due to the main active ingredients and the presence in them of phenolic and flavonoid compounds.Thus, according Talbi*et al*. (2015), the antioxidant power of so-called medicinal plants used in African countries is mainly due to phenolic compounds.

5. CONCLUSION

In sum, *Capsicum annuum*, *Allium sativum* and *Thymus vulgaris* are important sources of phenolic compounds and flavonoids.They have a very large antioxidant capacity. The richness of these plants in different compounds that the plants could be used as antibiotics in the avian world, and as food supplements in preventing various diseases encountered in human and animal environment.

6. ACKNOWLEDGEMENTS

The authors sincerely thank Labograin Africa for the financial help in the realization of this study.

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