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Polyphenolic profile, and antioxidant and antifungal activities of honey products in Benin

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The aim of this study was to characterize polyphenolic compounds, antifungal and antioxidant activities of polyfloral honey collected from three different phytogeographical zones in Benin duing dry and rainy seasons. Spectrophotometrically at 517 nm, DPPH scavenging activity of tested samples was measured, while antifungal activity, total polyphenols, flavonoids and condensed tannins were evaluted by Dohou, Singleton and Zhishen methods, and a slightly modified method of Sun, respectively. Results revealed that only season has significant influence (P <0.05) on the flavonoids content and total phenols. The highest total phenols (781 ± 46 µgGAE/gs) and flavonoids (528 ± 31 µgCE/g) contents were obtained respectively from honey samples of the rainy and dry seasons. The samples collected in the rainy season in the three zones, have better antifungal activity than those collected in the dry season. The lowest IC_{50} recorded was 27.58 ± 4.44 µg.µl^{-1} which showed that the highest antioxidant activity can be found in honey samples of the rainy season collected from the Sudanian zone. Overall, this study confirms that all investigated honey samples were good sources of polyphenolic compounds, and exhibit antifungal and antioxidant actvities.

Keywords: Antifungal, antiioxidant, honey, Benin.

INTRODUCTION

Honey is the natural sweet substance produced by *Apis mellifera* from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which bees collect,

transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature (European Union, 2002). It plays an important part in our nutrition and it is well-known for its positive effects on health. Honey has been reported to contain approximately 200 substances (sugars, minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids, enzymes and other phytochemical compound) and is considered to be an important part of traditional medicine (Ferreira et al., 2009). This natural product has been used since antiquity by the first humans for the treatment of burns, gastrointestinal disorders, asthma, infections and chronic injury, skin ulcers, cataracts and other eye diseases (Ghashm et al., 2010; Nasir et al., 2010; Wen et al., 2012; Saba et al., 2013).

Recently, in the light of oxidative stress phenomenon, the bankruptcy of a good number of conventional therapeutic agents, honey has been "rediscovered". Therapeutic potential such as antimicrobial and antiviral activity (Koc et al., 2009; Hamouda and Abouwarda 2011), antioxidant capacity (Erujewa et al., 2010; Hussein et al., 2011; Khalil et al., 2012) and anti-inflammatory effect (Mueller et al., 2010; Vallianou et al., 2014) were reported. In addition, honey as a source of antioxidant and/or an antifungal has been widely proven (Beretta et al., 2005; Küçük et al., 2007; Ferreira et al., 2009). Antioxidant activity of honey may be due to enzymatic and non-enzymatic antioxidants including glucose oxidase, catalase, peroxidase, ascorbic acid, derivative, carotenoids, organic acids and amino acids and proteins, the products of Maillard reaction and more than 150 polyphenolic compounds including flavonoids and phenolic acids. Pure honey and its dilutions have been reported to have inhibitory effects on fungi, and also inhibited their toxins production (Al-Waili and Haq, 2004). It has been reported to be effective against candidiasis, caused by *Candida albicans*, skin fungal infections such as the moth and athletes foot (Bansal et al., 2005). An antifungal action was observed on yeasts mainly, *Aspergillus* and Penicillium, as well as the totality of dermatophytes (Sampath et al., 2010). In addition, some studies have reported that the topical application of honey is effective in the treatment of seborrheic dermatitis and dandruff (Al-Waili, 2005). The quantity of these different compounds varies greatly depending on the floral and geographical origin of the honey. Additionally, the composition of honey is influenced by processing, handling and storage time (Gheldof et al., 2002; Bertoncelj et al., 2007).

Benin is a country with a diverse range of climates characterized by the relative weakness of the annual precipitation which vary from 900 to 1300 mm per year (Sinsin et al., 2004). More than hundred melliferous plant have been counted in Benin such as: *Acanthacees, Amaranthacees*, *Anacardiacees*, *Annonacees*, *Apocynacees*, *Asclépiadacees*, *Bignoniacees*, *Bombacacees*, *Borragninacees*, *Capparidacees*, *Césalpiniacees*, Commelinacees, Composed, *Cucurbitacees*, *Ebénacees*, *Euphorbiacees*, *Ficoidacees*, *Gramineous* (*Poacees*), *Lythracees*, *Méliacées*, Mimosacées, *Moracées*, *Musacées*, *Myrtacées*, *Rubiacées*, *Rutacées*, *Sapindacées*, *Sapotacees*, *Scrophuliacees*, Simaroubacees, *Sterculiacees*, *Verbénacees* and *Zygophyllacees* (Mensah et al., 2003; Yédomonhan et al., 2009). Honey has been used traditionally over the years by the people of Benin as food and as a traditional medicine in the treatment of several diseases

Although, honey is widely consumed by locals, very few data are available to support the medicinal claims for different types of honey samples from Benin. Honey is widely consumed in Benin, few data are available on the quality of commonly consumed honey. Investigations of honey samples collected from different geographic locations are necessary to provide local data. Furthermore, the data available for honey reported from other countries are not applicable to Benin because it varies in antioxidant capacity, antifungal activity and polyphenolic compounds. In this study, the authors aimed to investigate different honey samples collected from different regions in Benin and to value total phenolics, total flavonoids, condensed tanins, anfifungal and antioxidant activities of some polyfloral honey samples from Benin. To the best of the authors' knowledge, this is the first study to extensively investigate the different polyphenolic compunds, antifungal and antioxidant activities in various types of honey samples from Benin.

MATERIALS AND METHODS

Study area

Samples of honey were collected from three climatic zones (Sudanian, Sudano-guinean and Guinean) in Benin (Figure 1). Benin is located between the parallel 6° 15' and 12° 25' and extends on an area of 112 622 km^2. It is limited to the north by Niger and Burkina Faso, to the south by the Atlantic Ocean, to the west by Togo and on the east by Nigeria. Benin presents a diverse range of climates characterized by the relative weakness of the annual precipitation which vary from 900 to 1300 mm per year (Sinsin et al., 2004). In the Guinean zone from the coast (6°25 N) to the latitude of 7°30 N, there are four seasons (two rainy and two dry). It has an annual rainfall average of 1 200 mm with an average of 250 days of rain. The Sudano-guinean located between 7°30' N and 9°45' N with a uni modal (May-October) rainfall regime and the average annual rainfall varies from 900 to 1110 mm, distributed approximately over 113 days on average. The Sudanian zone is located between 9°45 N and 12°25 N with a 900 to 1100 mm as

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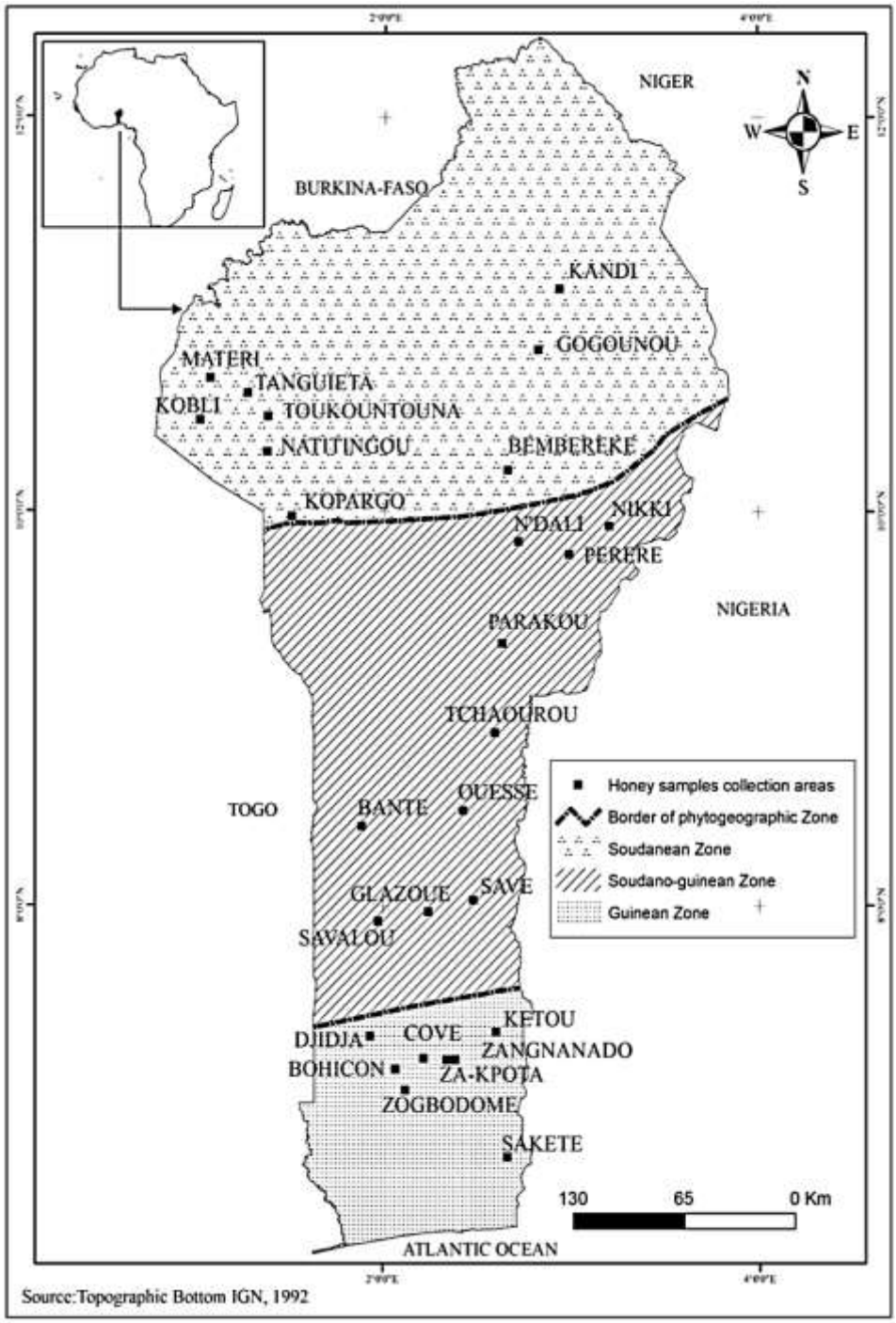

Figure 1. Sources of honey.

annual rainfall average, distributed over 145 days.

Sampling

A total of sixty polyfloral honey (n = 60) samples were collected from thirty different locations in three different phytogeographical zones (Sudanian, Sudano-guinean and Guinean) in Benin (Figure 1) during two seasons, namely dry and rainy. Indeed, thirty honey samples were colleted by season and same beekeepers were visited each season. The details of the honey, including the honey's location and zone, are described in Table 1. All honey collections were performed between January and December, 2015. The samples were refrigerated (4 to 5°C) in airtight plastic containers until further analysis. All analyses were conducted in triplicate. The samples collection periods vary according to the zones. In the

Table 1. Source of the investigated benese polyfloral honey.

Total honey samples by zone	Honey samples of the dry season by zone (n=10)	Honey samples of the rainy season (n=10)	Localities (n=10 by zone)
Zone Sudanean (n =20)	SoD_1	SoR_1	Bembèrèkè
	SoD_2	SoR_2	Cobly
	SoD_3	SoR_3	Copargo
	SoD_4	SoR_4	Gogounou/Bagou
	SoD_5	SoR_5	Kandi
	SoD_6	SoR_6	Matéri
	SoD_7	SoR_7	Natitingou Korimbéré
	SoD_8	SoR_8	Natitingou centre
	SoD_9	SoR_9	Tanguiéta
	SoD_{10}	SoR_{10}	Toucountouna
Sudano-Guinean zone (n=20)	SgD_1	SgR_1	Bantè
	SgD_2	SgR_2	Glazoué/Kpakpaza
	SgD_3	SgR_3	N'dali/Sinisson
	SgD_4	SgR_4	Nikki Biro
	SgD_5	SgR_5	Ouessè/Laminou
	SgD_6	SgR_6	Parakou/Monastère
	SgD_7	SgR_7	Pérèrè
	SgD_8	SgR_8	Savalou/Ouéssè
	SgD_9	SgR_9	Savè/Yaoui
	SgD_{10}	SgR_{10}	Tchaourou/Kinikpanhoun
Guinean Zone (n=20)	GuD_1	GuR_1	Bohicon 1
	GuD_2	GuR_2	Bohicon2
	GuD_3	GuR_3	Covè
	GuD_4	GuR_4	Djidja
	GuD_5	GuR_5	Ketou
	GuD_6	GuR_6	Saketé
	GuD_7	GuR_7	Zakpota
	GuD_8	GuR_8	Zangnanado
	GuD_9	GuR_9	Zogbodomey/Dovogon
	GuD_{10}	GuR_{10}	Zogbodomey/Mongon

D: Dry season; R : rainy season; So: samples of the Sudanian zone, Sg: samples of the Sudano-guinean zone; Gu: samples of the Guinean zone.

Sudanian and Sudano-guinean zones, all samples were collected in 2015 from November to April (dry season) and from June to September (rainy season), whereas, in the Guinea area, samples were collected from November to March and July to September (for dry season) and from April to July and September to October for the rainy season in the same year.

Reagents

Gallic acid, catechin, quercetin, 2,2-diphenyl- 1-picrylhydrazyl (DPPH) and Folin-Ciocalteu's reagent, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium carbonate (Na_2CO_3), aluminum chloride ($AlCl_3$), sodium nitrite ($NaNO_2$), chlorhydric acid (HCl), sodium hydroxide (NaOH) and BIOMATE 3S UV-Visible Spectrophotometer used were purchased from Merck (Darmstadt, Germany).

Fungal strains used

Four reference fungal strains namely, *Aspergillus parasiticus* (CMBB 20), *Aspergillus ochraceus* (CMBB 91), *Aspergillus fumigatus* (CMBB 89) and *Aspergillus clavatus* (NCPT 97) were used in this study. These strains were obtained from the Laboratory of Quality Control of Medicines of the Health Ministry and have been stored in the Laboratory of Biology and Molecular Typing (University of Abomey-Calavi, Benin).

Polyphenolic analysis

Total phenols

The total polyphenol content was analyzed by spectrometry at 765 nm using Folin-Ciocalteu reagent (Singleton et al., 1999). In this method, 0.5 ml of honey (0.1 g/ml) was mixed with 2.5 ml of the

Folin-Ciocalteu's reagent diluted 1/10 with water. After 5 min, 2 ml of sodium carbonate (0.2 g/ml) was added to the mixture. Absorbance at 765 nm was recorded after 10 min of reaction at 37°C, against a blank with water. The polyphenol concentration was estimated with a calibration curve using a solution of 0.1 g/l of gallic acid as standard (0, 0.25, 0.05 and 0.1 g/l). The results were reported as mean ± standard deviation and expressed as microgram of gallic acid equivalent per gram of honey (µgGAE/g).

Flavonoids

Flavonoid content in each honey sample was measured using the colorimetric assay developed by Zhishen et al. (1999). Honey extract (1 ml) was mixed with 4 ml of distilled water. At the baseline, 0.3 ml of $NaNO_2$ (5%, w/v) was added. After 5 min, 0.3 ml of $AlCl_3$ (10% w/v) was added, followed by the addition of 2 ml of NaOH (1 M) 6 min later. The volume was then increased to 10 mL by the addition of 2.4 ml distilled water. The mixture was vigorously shaken to ensure adequate mixing and the absorbance was read at 510 nm. A calibration curve was created using a standard solution of quercetin (0.5 g/l). The results were expressed as microgram of catechin equivalent per gram of honey (µgCE/g).

Condensed tannins

Content in condensed tannins was determined by a slightly modified method of Sun et al. (1998). To 50 µl of honey (0.1 g/ml) was added 3 ml of vanillin (4%) and 1.5 ml of HCl. The mixture was then incubated at room temperature (25 ± 2°C) for 15 min. The mixture was vigorously shaken to ensure adequate mixing and the absorbance was read at 500 nm. Concentrations of condensed tannins are deducted from a calibration range established with quercetin (1g/l) and are expressed as microgram of catechin equivalent per gram of honey (µgQE/g).

Antifungal activity

The *in vitro* antifungal activity of the honey samples was evaluted according to the method previously described by Dohou et al. (2004). The assay was performed on the Potato-Dextrose Agar medium. Briefly, 2.5 ml of honey sample were mixed with 12.5 ml of the sterilized potato-destrose agar medium before it was transferred to sterile Petri plates for solidification. After the medium solidification, a sterile 6 mm disc pretreated with fungal strain was placed in each Petri plate. Plates were incubated at 25 ± 1°C for five days. Each treatment was replicated twice. Fungal radial growth was mesured by averaging the two diameters taken from each colony. Percentage growth inhibition of the fungal colonies was calculated using the formula:

$$\text{Inhibition percentage (\%)} = \frac{\text{Control's growth} - \text{Treatment's growth}}{\text{Control's growth}} \times 100$$

Antioxidant activity

The DPPH methode was conducted using an adapted method of Scherer and Godoy (20009). Practically, equal volumes (2 ml) of DPPH (200 µg/ml) and honey extracts (100 µg/ml) were mixed in screw tube serial and allowed to stand in darkness for 20 to 30 min at room temperature. Then, the absorbance was read at 517 nm and the blank was a mixture of methanol and DPPH (v:v). The inhibitory percentage of DPPH radiical indicating the antioxydant activity of honey extract was obtain using the formula ed by Schmeda-Hirschman et al. (2003).

The concentration providing 50% inhibition (IC_{50}) was determined graphically using a calibration curve in the linear range by plotting the honey extract concentration and the corresponding scavening effet. Antiioxydant activity index (AAI) was calculated according to the formula used by Scherer and Godoy (2009).

Statistical analysis

Microsoft Excel 2010 has been used for the seizure and the treatment of the data. Data of the polyphenolic compounds (total phenols, flavonoids and condensed tannins), inhibition percentage of the growth of the fungal strains and the IC_{50} obtained for the antioxydant activity were subjected to analysis of variance (ANOVA), respectively, for two factors, namely collection season and phytogeographical zone at probability level of 0.05 and the Student Newman-Keuls (SNK) tests using the Statistical Analysis System (SAS, Version 9.2) software in order to determine the influence of the seasons and zones on the content of polyphenolic compounds, antifungal and antioxidant activity of the different honey samples. Pearson correlation test was conducted with the Minitab 14 (Mtb v. 14) software to establish links between the polyphenolic compound, antifungal and antioxydant activity of the different honey samples.

RESULTS AND DISCUSSION

Total polyphenols, flavonoids and condensed tannins

The results of the polyphenolic compounds of different honey samples are shown in Table 2. The analysis of variance shows that there is no significant difference ($p > 0.05$) in condensed tannins, but there is a significant difference ($p < 0.05$) in total polyphenols and highly significant difference ($p < 0.001$) in total flavonoids content between seasons. However, following the collection area, no significant difference ($p>0.05$) was observed in these three (total polyphenols, flavonoids and condensed tannins) compounds (Table 3). The standard curve conducted with the gallic acid (R^2 = 0.9913) helped to determine the total polyphenols in honey samples. The phenolic concentration of honey in this study ranged from 672 ± 35 to 861 ± 113 µgGAE/g with an average of 781 ± 46 µgGAE/g in samples of the rainy season and 631 ± 38 to 691 ± 43 µgGAE/g in samples of the dry season with an average value of 668 ± 26 µgGAE/g. It was found that the high average content in totals polyphenols (781 ± 46 µgGAE/g) was obtained in the rainy season, while the lowest (668 ± 26 µgGAE/g) was obtained in dry season samples. The calibration curve of cathechin (R^2 = 0.9952) was used to assess the total flavonoids in honey samples. The same variation trend was observed with total flavonoids in terms of harvest area and season, with the only difference where the higher content of flavonoids (528 ± 31 µgCE/g) was obtained in the dry season, whereas the lowest (280 ± 31 µgCE/g) was recorded in the rainy season. Total flavonoids content shows that this group of compound is in high proportion in the dry season among the total polyphenol obtained in honey samples. Considering the condensed tannins, they varied from 776

Table 2. Phenolic compounds of honey samples (means ± standard error) according to the seasons and phytogeographical areas.

Seasons	Zones	**Total polyphenols** (µgGAE/g)	**Total flavonoids** (µgCE/g)	**Condensed tannins** (µgQE/g)
Rainy	Sudanian	861±113^{a}	223±42^{a}	776±70^{b}
	Sudano-guinean	809±67^{a}	347±74^{a}	834±49^{b}
	Guinean	672±35^{a}	270±36^{a}	1055±61^{a}
	Mean	781±46^{A}	280±31^{B}	888±40^{A}
Dry	Sudanian	631±38^{a}	519±55^{a}	992±114^{a}
	Sudano-guinean	682±56^{a}	512±71^{a}	1063±99^{a}
	Guinean	691±43^{a}	552±38^{a}	765±70^{a}
	Mean	668±26^{B}	528±31^{A}	940±58^{A}

GAE: Gallic acid equivalent, QE: quercetin equivalent, CE: catechin equivalent. Means followed by the same letter of the same character and for the same factor are not significantly different ($p > 0.05$).

Table 3. Analysis of variance (p-value) for seasons and phytogeographical areas.

Sources of variation	**DF**	**Fisher values**		
		Total polyphenols (µgGAE/g)	**flavonoids (µgCE/g)**	**condensed Tannins (µgQE/g)**
Seasons	1	4.51* (0.04)	30.09 *** (<0.0001)	0.61 ns (0.44)
Zone	2	0.65ns (0.52)	0.59 ns (0.56)	0.32 ns (0.73)
Season x zone	2	1.85ns (0.17)	0.85 ns (0.43)	6.73 ** (0.002)

DF: Degree of freedom; ns: $P > 0.05$ (no-significant); *: $p < 0.05$ (significant); **: $p < 0.01$ (highly significant); ***: $p < 0.001$ (very highly significant).

± 70 to 1055 ± 61 µgQE/g with an average of 888 ± 40 µgQE/g in rainy season honey samples. However, with an average of 940 ± 58 µgQE/g, condensed tannins vary from 765 ± 70 to 1063.66 ± 99 µgQE/g in honey samples of the dry season.

Antifungal activity

The antifungal activity of the honey samples shows a reduction of the rapid multiplication by fragmentation or by budding filaments (mycelial development) of the four fungal strains used. The percentages of inhibition vary according to the strains, the area and samples harvest period (Table 4). The samples collected in the rainy season in Sudanian zone have the best percentages of mycelial development inhibition (59.0 ± 12.1 to 68.4 ± 2.2%) on the four species of *Aspergilus*. The most remarkable antagonisms effect was observed on *Aspergilus parasiticus* (36.2%) and *Aspergilus ochraceus* (54.9%) for the honey samples collected in the dry season. In addition, the highest resistance was observed for *A. fumigatus* (43.7 %) and *A. clavatus* (49.1 %) using the samples collected in the dry season in the Guinean zone. Of the strains, *A. parasiticus* is the most sensitive (68.4 ± 2.2%), while *A. ochraceus* is more resistant. The analysis of variance of the mycelial growth inhibition rate revealed that neither the season nor harvest area did not influence ($p > 0.05$) the percentage of inhibition of *A. parasiticus* (Table 4). However, both zone and season highly affected ($p < 0.01$ to $p < 0.001$) the inhibition proportion of *A. clavatus*. Only the collection area has a significant effect ($p < 0.01$) on the rate of inhibition of *A. fumigatus*. In addition, the inhibition percentage of *A. ochraceus* was influenced ($p < 0.05$) by the season and the interaction between season and area (Table 5).

In general, the samples collected in the rainy season in the three climatic zones, have antifungal activities better than those collected in the dry season. The rain may play a beneficial role in the concentration of active principle antimicrobials.

Antioxidant activity

The antioxidant activity of each sample of honey was determined regarding the antiradical activity against the free radical-1-diphenyl-picryl hydrazyl (DPPH) using IC_{50}, representing the concentration necessary for the inhibition of 50% of the free radicals. Thus, a lower value of IC_{50} in honey indicates a greater ability to neutralize free radicals.The antioxidant profiles obtained reveal that all honey samples possess antioxidant activities dose-dependently. The IC_{50} values vary from a season to another and from a collection area to another (Figure 2). The IC_{50} values of the rainy season samples are weaker than those of the dry season in the three phytogeographical zones. Thus, the analysis of variance

Table 4. Percentage inhibition of the honey samples related to the zones and the harvest seasons.

Inhibition (%) (Means ± standard error, n = 10)					
Seasons	**Areas**	***A. parasiticus***	***A. ochraceus***	***A. fumigatus***	***A. clavatus***
Dry	Sudanian	63.8±3.6^{a}	45.1±3.2^{b}	63.4±2.8^{a}	56.7±2.0^{a}
	Sudano-guinean	66.8±2.4^{a}	53.0±3.3^{ab}	58.9±2.2^{a}	58.9±2.4^{a}
	Guinean	65.7±3.0^{a}	57.9±2.8^{a}	56.3±2.4^{a}	50.9±1.3^{b}
	Mean	65.4±1.7^{A}	52.0±2.0B	59.5±1.5^{A}	55.5±1.2^{B}
Rainy	Sudanian	68.4±2.2^{a}	59.1±2.2^{a}	64.3±2.2^{ab}	63.9±1.8^{a}
	Sudano-guinean	66.8±2.8^{a}	57.0±2.8^{a}	59.0±3.9^{a}	61.9±1.8^{a}
	Guinean	64.5±2.9^{a}	55.6±1.9^{a}	57.9±2.4^{b}	57.3±2.2^{a}
	Mean	66.6±1.5^{A}	57.2±1.7^{A}	63.1±1.5^{A}	61.0±1.2^{A}

n: Number of honey samples by zone and by season, means with different letters are significantly different with probability level of 5% according to Student Newman-Keuls test.

Table 5. Analysis of variance (p-value) for seasons and phytogeographical zone.

Sources of variation	**DF**	**Values**			
		A. parasiticus	***A. ochraceus***	***A. fumigatus***	***A. clavatus***
Season	1	0.24 (0.62) ns	4.41 (0.04) *	3.22 (0.07) ns	12.50 (0.0008) ***
Area	2	0.19 (0.83) ns	1.20 (0.31) ns	5.75 (0.005) **	7.11 (0.0018) **
Season *Area	2	0.60 (0.55) ns	3.61 (0.03) *	0.32 (0.73) ns	0.67 (0.5159) ns

DF: Degree of freedom; ns: p >0.05 (no-significant) *: p < 0.05 (significant): ** p < 0.01 (highly significant). ***: p < 0.001 (very highly significant).

showed significant variation ($p < 0.05$) of IC_{50} between seasons in the Guinean zone, very significant ($p < 0.001$) in the Sudanian zone area and non-significant ($p > 0.05$) in the Sudano-Guinean. The lowest IC_{50} (27.58 ± 4.44 $\mu g.\mu l^{-1}$) which shows the highest antioxidant activity was recorded for the samples of the rainy season collected in the Sudanian zone area. On the other hand, the largest IC_{50} (47.46 ± 2.62 $\mu g\ \mu l^{-1}$), was recorded in the samples collected in the dry season in Guinean zone. Similar to the IC_{50}, the index of antioxidant activity (IAA) also varied according to the seasons and collection areas. Indeed, for the rainy season, the IAA values recorded were 1.62 ± 0.91 (Guinean zone), 1.61 ± 0.84 (Sudano-guinean) and 2.03 ± 0.76 (Sudanian zone). For the dry season, the lowest index (1.06 ± 0.11) was obtained with the samples of the Guinean zone, followed by that of the Sudano-guinean zone (1.25 ± 0.41) and finally Sudanian zone (1.47 ± 0.72). These different profiles show the importance and the antioxidant power of pure honey. The samples of the rainy season are richer in antioxidant compounds.

Correlation between polyphenolic compounds, antifungal and antioxidant activities

Table 6 shows the correlations between the various polyphenolic compounds, antifungal and antioxidant activity of the honey samples. It appears from the analysis of this table that there was a positive and considerable correlation ($r = 0.941$, $p < 0.01$) between antifungal activity of *A. clavatus* and antioxidant activity expressed in IC_{50}. In other words, honey samples with high antifungal activity against *A. clavatus* were the richest in antioxidant compounds. A considerable negative correlation was observed between the antifungal activity of *A. ochraceus* and the total phenols content ($r = -0.943$, $p < 0.01$), in other words, the honey samples which have a high total phenol content had a low fungal activity against *A. ochraceus*.

DISCUSSION

The study on the biochemical content and biological activity carried out on homey samples collected in Benin showed variation of their composition in secondary metabolites. Considering the total polyphenols, there is no significant variation between the collection areas but their within the season of samples collection ($p < 0.05$). It is the same for the total flavonoids ($p < 0.001$). Thus, this data showed that the rain may have a beneficial effect on the formation and expression of this class of molecules. In addition, Campone et al. (2014) argued that the polyphenols (flavonoids) of honey can be distinguished according to their origin. Some of them come from the

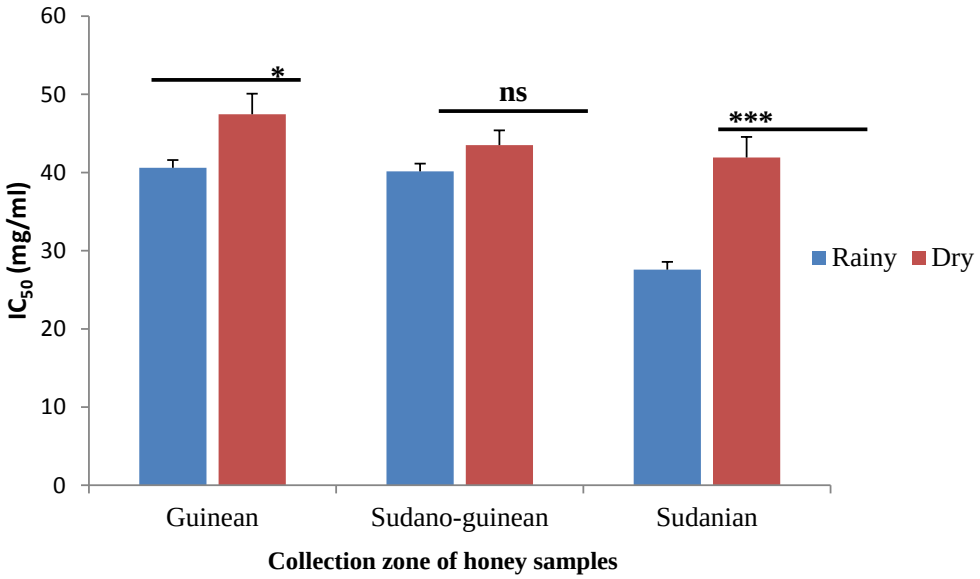

Figure 2. Variation of IC_{50} according to the seasons and samples collection zone. ns = P >0.05 (no-significant); * = p < 0.05 (significant); *** = p < 0.001 (very highly significant).

Table 6. Correlation coefficient of Pearson between different variables.

Variables	*A. parasiticus*	*A. ochraceus*	*A. fumigatus*	*A. clavatus*	IC_{50}	T. P.	F.	C.T.
A. parasiticus	1							
A. ochraceus	0.692	1						
A. fumigatus	0.203	-0.344	1					
A. clavatus	0.628	0.195	0.609	1				
IC_{50}	0.359	-0.018	0.679	0.941**	1			
T.P.	-0.562	-0.943**	0.194	-0.208	-0.054	1		
F.	0.445	0.63	0.008	0.662	0.643	-0.672	1	
C. T.	0.623	0.696	-0.727	0.080	-0.221	-0.671	0.139	1

**: p < 0.01 (highly significant); T.P. = total polyphenols; F. = flavonoids; C.T.= condenseds taninns.

nectar of plants visited by the bees, pollen, honeydew, or even the propolis. This statement confirms in part the hypothesis of departure for honeys of vegetable origin because the rain promotes a better growth of the plants organs and a good availability of their constituents. The total polyphenols would therefore be more available in plants in the rainy season than in dry season. The total polyphenols rates obtained independently in the season are three times greater than those reported (248.8 mgGAE/100 g) by Djossou et al. (2013) in Benin during their work on the physicochemical characteristics of city market honey samples collected in Cotonou. This difference may be due to the quality of the honey analyzed because some traders in quest to maximize their profits, proceed to the alteration of the quality of the honey by dilution. In addition, Beretta et al., (2005) showed some values on unifloral and multifloral honey samples of Africa (52.2 to 789.6 mg/kg) and Bertoncelj et al. (2007) on Slovakia honey samples (64 to 1304 µgGAE/g). Also, values raging between 226.2 and 727.8 mg/kg were reported in Portugal (Ferreira et al., 2009) and from 102.1 to 1085 mg/kg in the north-east of Brazil (Liberato et al., 2011). The main explanation for this difference could be linked to the unequal distribution of polyphenols both in quantity and quality across the different organs of a plant used by bees to produce honey.The amount of flavonoids in the samples collected in rainy (280 ± 31 µgCE/g) and dry seasons (528 ± 31 µgCE/g) displays a great variability within seasons (p < 0.05). The levels of flavonoids obtained in this study, are higher than the 170 - 286.5 µgGAE/100 g obtained in Burkina Faso (Méda et al., 2005) and Tualang honeys (Ibrahim et al., 2011). The flavonoids are phenolic low molecular weight compounds essential for aroma and

antioxidant properties of honey.

The recent interest in these substances has been stimulated by the potential benefits to the health arising from their antioxidant and anti-radicular activities against coronary heart diseases and cancer (Saba et al., 2011). For the condensed tannins, the interaction zone and season showed a significant difference ($p < 0.01$). As tannins are part of polyphenols, we can say that their expression may be influenced by edaphic and climatic factors. Indeed, Evans (1999) have shown that humidity, type of soil, location of plants (Eldridge and Kwolek, 1983), season during which the plant was used by bees (Salminen et al., 2004) and part of the plant used (Eldridge and Kwolek, 1983) may have an effect on the phytochemical constituents. A great variability in the content of polyphenolic compounds within the samples has been observed. This variability corroborate many scientific work carried out to identify potential chemical floral markers of honey. The studies carried out by various authors have shown that the phenolic compounds vary in function of the geographic origins and floral honey (Méda et al., 2005; Beretta et al., 2005; da Silva et al., 2013). The antifungal activity reflects difference between the responses of four fungal strains in presence of the honey samples. An interaction between the strains and the samples collection areas seems to define a particular pattern of effectiveness. Thus, the samples collected in the rainy season in Sudanian zone have the best percentages of inhibition. This observation may be related to good concentration of flavonoids and tannins in the honey samples of rainy season. The flavonoids are reported as phenolic compounds synthesized by plants in response to a microbial infection (Batawita et al., 2002). These are *in vitro* broad-spectrum antimicrobial whose antifungal properties have been reported (Babayi et al., 2004; Ulanowska et al., 2006). Their activity is probably due to their ability to complexed the soluble proteins and the extracellular proteins. Tannins also have very interesting antifungal properties (Okigbo et al., 2006) through various mechanisms such as the membranes destruction, enzymes inhibition, substrates deprivation and metal ions complexation. Tannins stick to proline rich proteins and then interfere with the proteins synthesis. Additional mechanisms are the alteration of biomembranes stability, proteins synthesis and important enzymes in the metabolism.

The antioxidant capacity of the honey samples, expressed in IC_{50} varies from 27.6 to 47.5 µg/µl. The values of the antioxidant activity obtained in the present study are similar to those obtained (7.2 to 53.8 mg/ml) in Slovakia (Bertoncelj et al., 2007). However, they are lower than those obtained by Ferreira et al. (2009) and Liberato et al. (2011) in honeys in Portugal (106.7 to 168.9 mg/ml) and the Northeast of Brazil (4.2 to 106.7 mg/ml). The antioxidant powers obtained with the honeys studied confirm the quality of these samples. The same trend of efficiency observed with the fungal strains was also observed for the antioxidant activity. This note shows that samples collected in the rainy season can be strongly used as antifungal and antioxidant agent. Considering the antioxidant activity index (AAI), according to Scherer and Godoy (2009), it can be said that, only the samples collected during the rainy season in Sudanian zone have a AAI>2.0. These samples have therefore a very strong antioxidant activity. However, all other samples have a strong antioxidant activity.

Conclusion

This study succesfully obtained scientific data on polyphenolic compounds, antifungal and antioxidant activities of honey samples produced in Benin. The polyphenolic compounds such as total polyphenols, flavonoids and condensed tannins have revealed the value of the honey products in each of the three phytogeographic zones of Benin. These secondary metabolites varied considerably depending on the season (dry and rainy) and the collection zone (Sudanian, Sudano-Guinean and Guinean). This study also revealed the antifungal power of the honey samples. The percentages of inhibition (antifungal drug) varied according to the strains, the area and the samples collection period. The samples collected in the rainy season in the three climatic zones, have antifungal activities better than those collected in the dry season. The antioxidant profiles obtained indicate that all samples possess dose-dependent antioxidant activities. The IC_{50} values vary from a season to another and according to the collection area. The IC_{50} values of the rainy season samples are weaker than those of the dry season independent of the zones.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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