GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Phytochemical analysis and antioxidant activity of honeys from wild colony and bee culture from five regions of the ivory coast

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GSC Biological and Pharmaceutical Sciences, 2024, 28(03), 140–148

Publication history: Received on 10 February 2024; revised on 15 July 2024; accepted on 18 July 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.28.3.0072

Abstract

Honeys have a very complex biological activity due to their composition of phenolic compounds which are very useful for the treatment of certain conditions. The objective of this study is to analyze the phenolic compounds and antioxidant activity of honeys from wild colonies and beekeeping on the Ivory Coast. This study was carried out in five regions of Côte d'Ivoire (Poro, Tchologo, Hambol, Bélier and N'zi). Twenty (20) samples from honey from wild colonies and beekeeping were collected and analyzed. The polyphenol content was quantified by the Folin-Ciocalteu colorimetric method. The aluminum trichloride (AlCl3) method was used to quantify the flavonoids in the extracts. The quantitative evaluation of the antioxidant potential of honeys was carried out by the DPPH test. The tests revealed the presence of polyphenols, tannins and flavonoids. High polyphenol contents were obtained with MSP (4.32 ± 0.02 mgEAG/g), MSH (3.7 ± 0.01 mg EAG/g), MAIPH (3.16 ± 0.02 mgEAG/g) honeys. g), MST (2.95 ± 0.01 mgEAG/g) and MSB (2.74± 0.02 mgEAG/g). MSH honey samples (1.48 ±0.05 mg QE/100 g); MSP (1.36 ± 0.03 mg QE/100 g) recorded the highest flavonoid values. The MAPIH sample (2.56 ± 0.04 mg EQ/g) recorded the highest tannin value. Significant antioxidant capacities were observed in honeys from wild Poro MSP colonies (82.384% at 80 mg/ml) IC_{50}= (44.86 mg/ml).

Keywords: Polyphénols; Flavonoids; Tannins; Antioxydants

1. Introduction

In Ivory Coast, honey previously collected by bee hunters for its organic activity has today become a product generating substantial income for the populations. Honey production has evolved from honey hunting to traditional beekeeping with traditional hives and then to modern beekeeping which now uses modern hives [15]. Moreover, the annual production of Ivorian honey is estimated at 20 tonnes [32]. This national honey production remains quite low and therefore fails to cover the needs of the populations despite the existing Ivorian honey-producing potential. Which makes Ivorian honey a very expensive product for socially modest people. Despite this, the production of honey by Ivorian beekeepers is compromised by the incidence of bee diseases and the lack of control of beekeeping practices. Also, it should be noted that the honeys thus produced and sold on the Ivorian markets are often of poor quality and have a limited shelf life due to the post-harvest activities applied. Some authors like [17] have worked on the collection, production and marketing of honey in the Department of Katiola (Centre-North). As for the work of [10], it focused on the analysis of pollen and antioxidant activities of honey from modern beekeeping and the identification of honey plants and physicochemical properties of honeys from the Worodougou region. Furthermore, very recent studies have been carried out by several authors to improve the productivity and quality of Ivorian honey [9]. Concerning the work carried out by [9], they focused on the characterization of pollen foraging activity by honey bees in central-eastern Côte d'Ivoire. These authors showed that controlling the foraging activity of plants by bees is necessary to better guide beekeepers with a view to better beekeeping productivity. Added to this is the work of [16] who respectively worked on the characterization of the physicochemical properties of honey harvested from the Sub-Prefecture of Cechi (Department

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of Agboville) and on the physicochemical, chemical, nutritional and biological study of honeys from ten (11) regions of Ivory Coast. It would therefore be interesting to address in this work all the analyzes relating to the quality of honey and thus be able to constitute a database to improve their quality. Therefore, this research work draws its originality and interest from the lack of substantial data on honeys from bee farms and wild bee colonies. It is with this in mind that the present study, which focuses on the contribution of the valorization of better quality honey produced in five regions of Côte d'Ivoire, through its phytochemical analysis and antioxidant activity, was initiated.

2. Materials and methods

2.1. Biological material

This study focuses on 20 honey samples collected in five production regions in Côte d'Ivoire (Poro, Tchologo, Hambol, Bélier and N'zi). The analyzes were carried out at the central laboratory of Nangui Abrogoua University in Abidjan.

Figure 1 Sample of honey from the five regions of the Ivory Coast

2.2. Methods

2.2.1. Determination of total phenols

The total polyphenol contents were determined using the Folin-Ciocalteu colorimetric method [31]. To 1 ml of each aqueous extract of honey, diluted 1/10 with distilled water, are added 1.5 ml of Na2CO3 (17%, m/v) and 0.5 ml of Folin-Ciocalteu reagent (0.5N). The whole is incubated at 37 °C for 30 minutes. The absorbance is read at 720 nm against a blank without extract taken as a reference. The quantification of total polyphenols is done according to a linear calibration line produced by a gallic acid standard extract at different concentrations (0 to 1000 mg/ml) prepared under the same conditions as the sample.

Results are expressed as milligrams of gallic acid equivalent per gram of honey (mg EAG/g of honey). The total polyphenol content (Q) is calculated according to the formula of the following equation:

$$Q = (V \times C \times d) / m \text{ (in mg EAG/g of honey)}$$

With V: final volume of the extract in ml;
C: concentration of the extract in mg/ml;
d: dilution factor; m: mass of honey in g.

2.2.2. Assay of total flavonoids

The determination of total flavonoids was carried out using the aluminum trichloride method [20]

A volume of each honey extract (2 ml) was diluted 1/10 and mixed with 100 µl of $AlCl_3$. The absorbance is read at 404 nm and compared to that of quercetol taken as a standard (0.05 mg/ml), diluted under the same conditions and treated with the same quantity of reagent. The content of total flavonoids (F) expressed in milligrams of quercetol equivalent per gram of honey (mg EQ/g of honey) was calculated using the formula below:

F = (0.05 x Aext / Aq) x 100 x d / Cext (in mg EQ/g of honey)

With Axt: absorption of the extract
Aq: absorption of quercetol
Cq: concentration of the extract (mg/ml)
d: dilution

2.2.3. Determination of tannin content

The determination of condensed tannins in the different extracts was carried out according to the method described by [3]. For 400 µL of each sample or standard, 3 ml of a 4% methanolic vanillin solution and 1.5 ml of concentrated hydrochloric acid are added. The mixture is incubated for 15 min and the absorbance is read at 500 nm. The concentrations of condensed tannins are deduced from the calibration ranges established with catechin (0-300µg/ml), and are expressed in mg of catechin equivalent per g of sample (mgEC/g E)..

$$Q\ (mgEC/g\ E) = C_f * d / C_i$$

Cf: concentration of the extract in catechin equivalent (mgEC/ml)
d: dilution factor
Ci: concentration of the extract analyzed (g/ml)

2.2.4. Measurement of antioxidant power by spectrophotometry

The evaluation of the anti-radical potential of selective honey extracts was carried out following the methods described by [4]. The DPPH radical was solubilized in absolute ethanol, to obtain a solution with a concentration of 0.3 mg/ml. Different concentration ranges (80 mg/ml, 40 mg/ml, 20 mg/ml, 10 mg/ml, 5 mg/ml of each honey extract were prepared in absolute ethanol.

1 ml of honey and 2 ml of ethanolic solution of DPPH (1,1-diphenyl-2-picryl-hydrazyl) were respectively introduced into dry and sterile tubes. After shaking, the tubes were placed away from light for 30 minutes.

The absorbance of the mixture was then measured at 517 nm against a blank consisting of 1 ml of pure ethanol and 2 ml of DPPH solution. The positive reference control used is ascorbic acid (vitamin C) prepared under the same conditions as the samples.

The percentage of DPPH inhibition is calculated according to the formula:

$$\boldsymbol{PR}(\%) = (\mathbf{1} - \frac{\boldsymbol{A_e}}{\boldsymbol{A_b}}) \times \mathbf{100}$$

With
PR: percentage reduction
Ab: absorbance of white
Ae: absorbance of the sample.

2.2.5. Determination of inhibitory concentrations 50 (IC_{50})

The effectiveness of honey extracts was evaluated according to their median concentration (IC_{50}), defined as the concentration necessary to reduce 50% of the initial concentration of the DPPH radical. It was determined using Excel 2010 software.

2.3. Statistical analysis

Quantitative data are expressed as average plus or minus standard deviation and qualitative data as percentages. The data from the survey were collected for their use then transferred to the Microsoft Excel 2016 software which was used for the graphical representations and the results were presented in the form of tables and graphs.

3. Results

3.1. Total polyphenols

The determination of total polyphenols gives us an overall estimate of the content of different classes of phenolic compounds contained in the samples analyzed [25]. (Figure 2).

The proportions of total polyphenols varied between 2.04 ± 0.01 mgEAG/g of honey and 4.32 ± 0.02 mgEAG/g of dry matter. High levels were obtained with MSP (4.32 ± 0.02 mgEAG/g), MSH (3.7 ± 0.01 mgEAG/g), MAIPH (3.16 ± 0.02 mgEAG/g) honeys. . Honeys from wild colonies MSP, MST, MSH, MSB obtained significantly high polyphenol values, except that of MSN which is lower at approximately 2.04 ± 0.01 mgEAG/g DM. As for beekeeping honeys, they recorded values ranging from 2.07 ± 0.01 mgEAG/g to 3.16 ± 0.01 mgEAG/g.

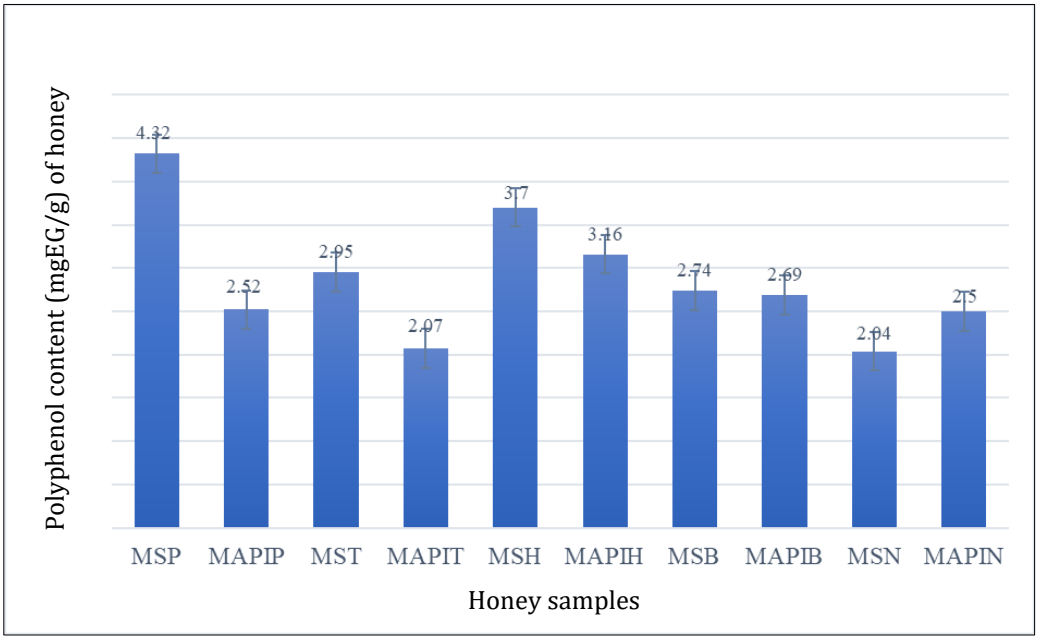

Figure 2 Total polyphenol contents of honey samples from the five regions of Côte d'Ivoire

3.2. Total flavonoids

The total flavonoid contents of honey from the five regions ranged from 0.88 ± 0.02 mg QE/100 g to 1.48 ± 0.05 mg QE/100g. (figure2).

MSH honey samples (1.48 ±0.05 mg QE/100 g); MSP (1.36 ± 0.03 mg QE/100 g); MAPIH (1.18±0.05 mg QE/100 g) recorded the highest values of flavonoids. While MAPIP honey samples (0.88 ± 0.02 mg QE/100 g); MAPIT (0.9 ± 0.01 mg QE/100 g) and MAPIN (0.94 ±0.02 mg QE/100 g) obtained the lowest values.

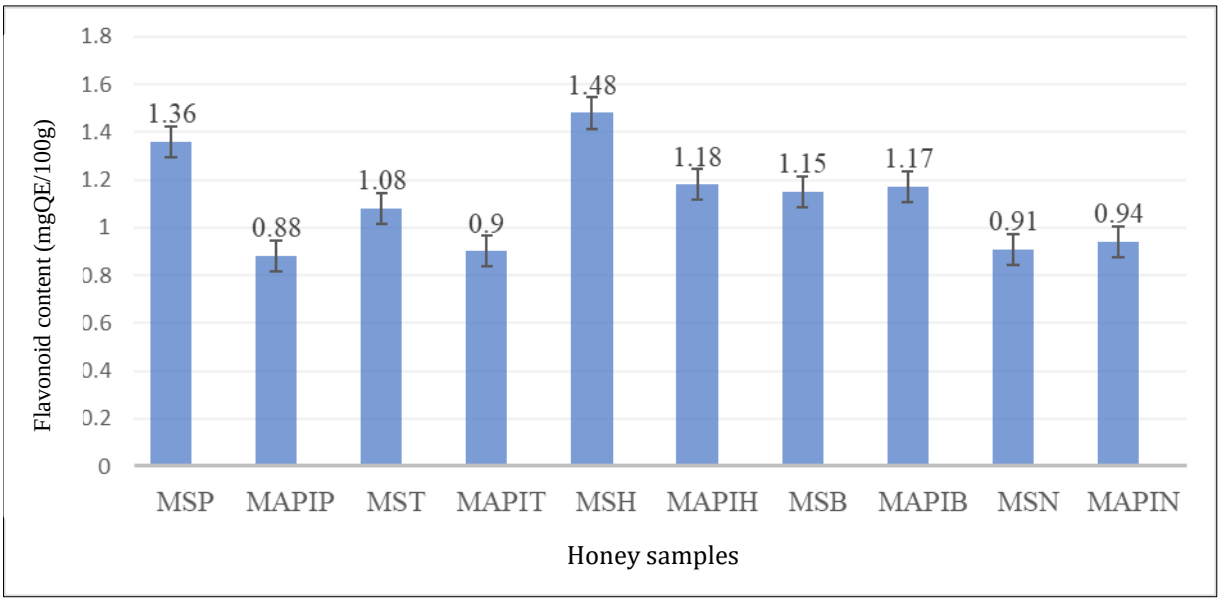

Figure 3 Total Flavonoid contents of honey samples from the five regions of Côte d'Ivoire

3.3. Condensed tannins

The contents of condensed tannins in honey samples vary between 1.1 ± 0.01 and 2.56 ± 0.04 mg EQ/g of honey (Figure 4). The sample from Hambol (MAPIH) with a rate of 2.56 ± 0.04 mg EQ/g of honey was the highest in flavonoids, followed by those from Poro (MSP) (2.53 ± 0.04 mg EQ/g of honey), Aries (MAPIB) (2.46 ± 0.03 mg EQ/g of honey), (MSB) (2.04±0.01). While the sample from the N'zi region (MSN) (1.1 ± 0.01), MST (1.45 ± 0.04), MAPIT (1.22 ± 0.04) obtained rates the lowest.

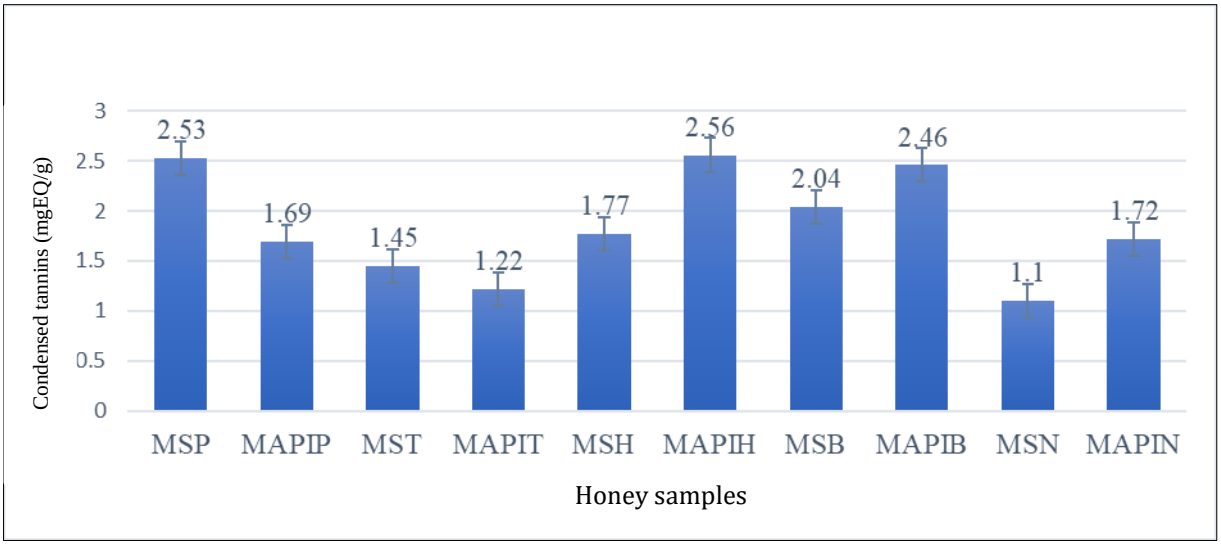

Figure 4 Condensed tannin contents of honey samples from the five regions of Côte d'Ivoire

The honey samples presenting PR ≥ 50% are those of Poro (MSP) (82.384% at 80 mg/ml and 50.1355% at 40 mg/ml), N'zi (MAPIN) (71.56% at 80 mg/ml and 46.73% at 40 mg/ml) and Tchologo MAPIT (67.23% at 80 mg/ml and 41.03% at 40 mg/ml). The samples of Poro (MSP), N'zi (MAPIN), Bélier (MSB), Tchologo (MAPIT) obtained the highest percentages of reduction at 80 mg/ml respectively (82.4%%), (71.56%), (68.4%), (67.23%), (67.7506%) The lowest reduction potentials were observed in honey (MSN) (51%) in Djamalabo in. the N'zi region. (figure 5)

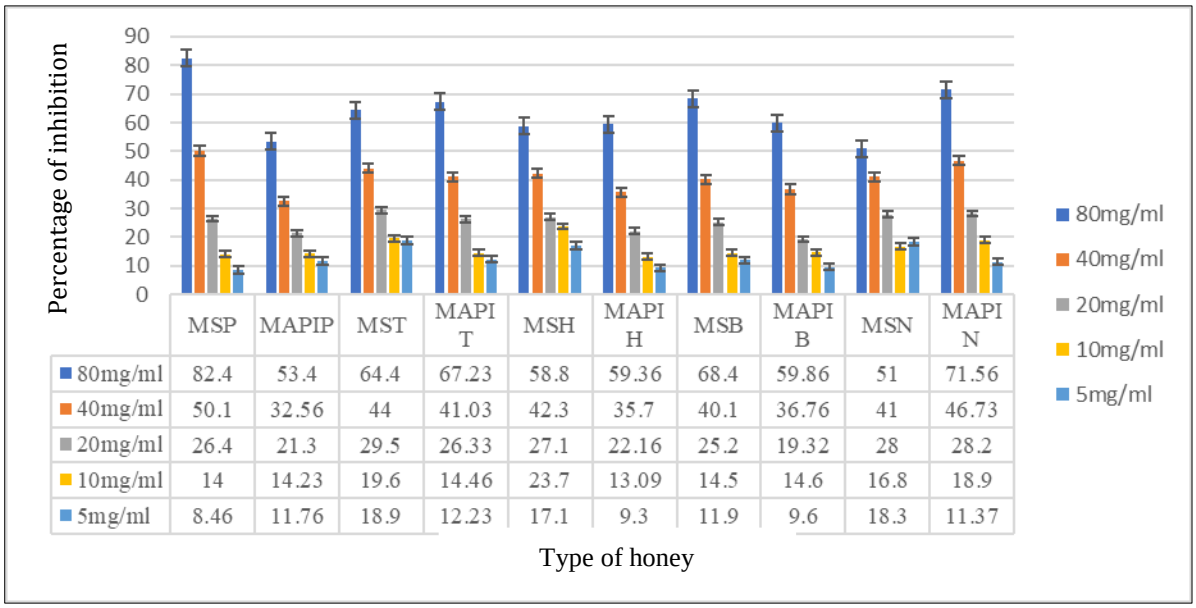

Figure 5 Percentage of inhibition (PI) of honeys from the five regions

The IC_{50} of honeys from wild colonies vary from 44.862 mg/ml (MSP) to 71.717 mg/ml (MSN). The lowest IC_{50}value comes from honey from wild colonies in the Poro region (MSP)(44.86 mg/ml) precisely in N'Bengué and the highest value comes from wild honey from the N' region. zi (MSN) (71.717 mg/ml) (Dimbokro). As for those of beekeeping honey, they vary from 53.025 mg/ml to 73.14 mg/ml. The lowest value comes from the N'zi region (MAPIN) (53.025 mg/ml) of Dimbokro. The highest value is obtained with honey from the Poro region (MAPIP) (73.14 mg/ml). All wild honeys obtained the lowest IC_{50}s except wild honey (MSN) from the N'zi region (Dimbokro). (figure 6)

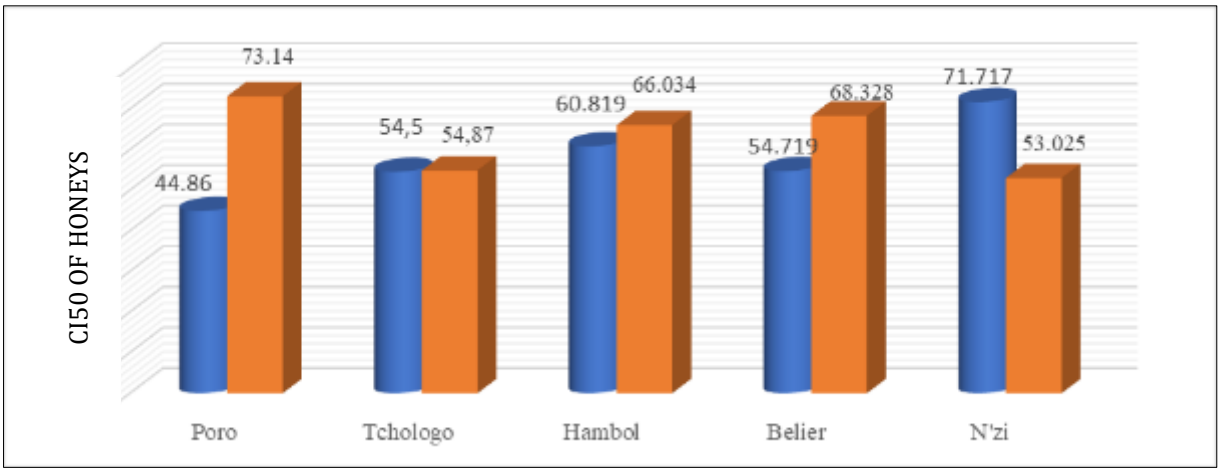

Figure 6 The **IC_{50}**of honeys from the five regions (in blue: wild honey; in brown: beekeeping honey)

4. Discussion

The determination of total polyphenols gives us an overall estimate of the content of different classes of phenolic compounds contained in the samples analyzed [25]. Figure 1 shows that the proportions of total polyphenols vary between 2.04 ±0.01 mgEAG/g of honey and 4.32±0.02 mgEAG/g of dry matter. The high levels obtained were those of MSP (4.32±0.02 mgEAG/g), MSH (3.7±0.01 mgEAG/g), MAIPH (3.16±0.02 mgEAG/g) honeys. . Honeys from wild colonies MSP, MST, MSH, MSB obtained significantly high polyphenol values which are respectively (4.32±0.02 mgEAG/g), (2.95±0.01mgEAG/g), (3 .7±0.01mgEAG/g), (2.74±0.02mgEAG/g). Except that of the MSN sample which was the lowest at approximately 2.04±0.01 mgEAG/g dry matter. As for beekeeping honeys, they recorded values ranging from 2.07±0.01 mgEAG/g to 3.16±0.01 mgEAG/g. Our results are lower than those of [5] who reported very high values (20-1810 mg GAE/Kg). The high content of total polyphenols recorded in these honey samples would be due to the

conservation of color after post-harvest activities and the low polyphenol content in these MSN honey samples from the N'zi region would be due to the high temperature in this region which had a negative impact on the chemical nature of the honeys. These results are lower than those obtained by [28] who show that the value of total phenolic content of multifloral honey varies between 141.14 and 247.81 mg GAE/ Kg. [12] also found that the average concentration of polyphenols for 4 samples of Algerian honey was established at 459.83±1.92 mg GAE/kg.

The total flavonoid content of honey from the five regions ranges from 0.88 ± 0.02 mg QE/100g to 1.48± 0.05 mg QE/100g. (figure II). Honey samples from wild colonies of Poro (MSP) (1.36± 0.03), Tchologo (MST) (1.08±0.04); Hambol (MSH) (1.48± 0.05), and Aries (MSB) (1.15±0.05) mg QE/100 g obtain the highest values of flavonoids. These different values obtained in these regions would be due to the harsh post-harvest treatment, the shelf life and the temperature in these regions. Most honeys from wild colonies obtained higher values than those from beekeeping honeys except wild honey (MSN) (0.91± 0.01) mg QE/100 g. These results are lower than those reported by [26] (290 mg REE/kg). According to [6], the variation in the flavonoid content of honey depends on the floral source, the region, the season, the environment of the hive and the honey species. [12] also show that the total flavonoid content is 233.99 ± 2.81 mg QE/kg and 227.57 ± 2.91 mg QE/Kg. These results corroborate with those [14]. According to them, flavonoids are low molecular weight phenolic compounds which are essential elements for the aroma and its antioxidant properties of honey.

The contents of condensed tannins in honey samples vary between 1.1 ± 0.01 and 2.56 ± 0.04 mg EQ/g of honey (Figure 3). The sample from Hambol (MAPIH) with a rate of 2.56 ± 0.04 mg EQ/g of honey was the highest in flavonoids, followed by those from Poro (MSP) (2.53 ± 0.04 mg EQ/g of honey), Aries (MAPIB) (2.46 ± 0.03 mg EQ/g of honey), (MSB) (2.04±0.01). While the sample from the N'zi region (MSN) (1.1±0.01), MST (1.45± 0.04), MAPIT (1.22± 0.04) are the least students. The tannin results show that our wild honeys come from tree trunks and leaves. The presence of tannins in the different honey samples shows that honeys have biological activities similar to tree bark such as that of Parkia biglobosa in the treatment of certain conditions (smallpox, chickenpox).

This is consistent with the results of [22] who demonstrated the presence of tannins and sterols in leaves and trunk bark.

The honey samples presenting PR ≥ 50% are those of Poro (MSP) (82.384% at 80 mg/ml and 50.1355% at 40 mg/ml) of Aries (MSB) (68.4% at 80 mg/ml). ml and 40.1% at 40mg/ml). The Poro (MAPIP) and Aries (MAPIB) samples obtained the lowest reduction percentages at 80 mg/ml respectively (53.4%) and (59.86%).

The strong antioxidant potential in polyphenolic compounds such as flavonoids and tannins could explain the anti-DPPH radical activity of the extracts. These phenolic compounds, through their antioxidant activity, prevent cardiovascular diseases.

It is reported in the literature that phytophenols constitute par excellence one of the main families with high antioxidant potential [29]. They are good scavengers of free radicals by donating hydrogen [10]. Studies have also demonstrated the role of free radicals in pathologies such as inflammatory diseases and liver conditions [18]. The majority of honeys have a total polyphenol content of less than 50 mg GAE/100g. Polyphenols or more broadly volatile secondary compounds give honey its flavor, aromas and color.

Indeed, according to the literature, the lower the IC_{50}, the more effective the extract [20]. On the other hand, the Poro samples have a notable high antioxidant potential (44.86 mg/ml) compared to Vitellaria honey from Burkina Faso, which presented IC_{50}s between 1.37 ± 0.03 and 2.43. ± 0.08 [19]. In short, the most active honey is that of wild honey from the Poro region (MSP) precisely in N'Bengue with IC_{50} (44.86mg/ml), then the sample (MST) (54.5mg/ml) from Lafokpokaha in the Tchologo region for honey from wild bee colonies. All honeys from wild colonies are more active than beekeeping honeys depending on their flavonoid and tannin content. The IC_{50} is the concentration necessary to reduce 50% of the initial concentration of the DPPH radical. Considering the results on phenolic compounds, the northern regions are full of a wide range of medicinal honey plants.

5. Conclusion

The phytochemical analyzes and antioxidant activities of honeys from wild colonies and beekeeping from five of Ivory Coast were determined. It appears from this study that samples of honey from wild colonies have very high phenolic compounds compared to honey from beekeeping. The presence of polyphenolic compounds such as tannins and flavonoids can be explained by the anti-radical DPPH activity of the extracts. The most active honeys are those from the Poro region (MSP) precisely in N'Bengue with a low IC_{50}, and that of the sample (MST) from Lafokpokaha in the Tchologo

region. In short, all honeys from wild colonies in the northern regions are more active than honeys from beekeeping depending on their flavonoid and tannin content. It would therefore be interesting to opt for honeys from the far north in the treatment of metabolic diseases and certain conditions such as tonsillitis, constipation, smallpox and chickenpox.

Consequently, it seems fundamental to us to consider:

To take essential precautions to ensure the standardization and rationalization of beekeeping techniques, hive manufacturing processes and storage processes to improve the quality of honey.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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