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Gas Chromatography-Mass Spectroscopy Analysis and Chemical Composition of Ngaoundere, Cameroon Honey

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Abstract: The investigation was carried out to determine the possible chemical components and quantity of the component present in honey using GC-MS analysis. Traditionally the natural honey are used in the treatment of ulcers, wound healing, swells, asthma, cough, hyperacidity, leprosy, diuretic, antimicrobial, jaundice, diuretic activity, hypolipidemic effect, hepatoprotective activity and fever. In the present study, the honey has been subjected to GC-MS analysis. Fourteen chemical constituents have been identified, the major chemical constituents are 2, 4-Dimethyl-1-pentanol (9.23%), 3, 5-Dihydroxy-6-methyl-2, 3-dihydro-4H-pyran-4-one (8.91%), 2-Furancarboxaldehyde, 5-hydroxymethyl (36.02%), 2-Butoxyethyl acetate (11.11%). It could be concluded that the Ngaoundere, Cameroon honey contains various bioactive compounds.

Keywords: GC-MS Analysis, Ulcer, Chemical Component, Honey

1. Introduction

Honey is a natural product obtained from bee and it is widely sought due to its unique nutritional and medicinal properties, which are attributed to the influence of the different groups of substances it contains. Codex Alimentarius Commission defined honey as the natural sweet substance produced by honey bees, Apismellifera, from the nectar of plants or from the secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honey bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature [1, 2]. The bees are said to produce honey in order to serve as their source of food in times of scarcity or during harsh weather conditions [3].

Basically, natural honey is a sticky and viscous solution which contents carbohydrate (mainly glucose and fructose), water, protein, ash and minor quantities of amino acids, enzymes and vitamins as well as other substances like phenolic antioxidants [3-7]. Each of these minor constituents is known to have distinctive nutritional or medicinal properties and different applications of natural honeys [3]. Although the major constituents of honey are nearly the same in all honey samples, the precise chemical composition and physical properties of natural honeys differ according to the plant species on which the bees forage [3, 8-11]. Furthermore, differences in climatic conditions and vegetation are important factors that can affect the various properties of honey.

Honey is used for nutritional, medicinal and industrial purposes and it is an important commodity in the international market; serving as foreign exchange earner for many countries. Beekeeping is an age old tradition in Cameroon as in Nigeria but it is not considered as a profit making venture in most parts of the country [12, 13], honey production has largely been at a subsistence level [14]. However, honey is found in beehives in large quantities in Nigeria [15] and it has been recognized that honey production (beekeeping) has the potential to develop as a

prime agro-horticultural and forest-based industry which can well become a major foreign exchange earner if international standards are met. For instance, it was shown that in Adamawa State, a beekeeper with an average number of 27 beehives made an average of $1,119.29 per annum from the sales of honey and beeswax [16, 17]. Similarly, it was reported that in Ngaoundere (Ngaounda) in northern part of Cameroon, beekeepers with an average of 20 beehives made average revenue from sales of honey, bees wax and propolis amounting to about $2,148.42 per annum and $1,027.29 per annum for langstroth and top bar hives users, respectively [18, 19] had reported that in Adamawa State, only a small percentage (5.62%) of the farming population who were already in the practice of beekeeping actually perceived apiculture as a profitable enterprise and know of its profitability; majority (56.25%) of the rural farming community and about 36.25% of the urban farming community considered apiculture only as a sideline economic activity. The ecology of Cameroon like Nigeria varies from tropical forest in the south to dry savanna in the far north, yielding a diverse mix of plant and animal life. Available literature on the properties and qualities of Nigerian honey have largely focused on samples obtained in the southern parts of the country [19, 20, 21]; with very scarce information on samples obtained in the northern parts, especially the northeast sub-region where commercial beekeeping practice has been documented [17, 19]. These practices are similar to what is obtained in the northwest of Cameroon and the Adamawa region of Cameroon is a neighbor to the Adamawa State of Nigeria. Several Health Benefits has been attributed to honey viz: Honey contains flavonoids, antioxidants which help reduce the risk of cancers and heart disease, Recent research shows that honey treatment may help disorders such as ulcers, it has antibacterial, because the bees add an enzyme that makes hydrogen peroxide," said Peter Molan, director of the Honey Research Unit at the University of Waikato in New Zealand., it also Increase athletic performance, Honey helps with coughs particularly buckwheat honey. In a study of 110 children, a single dose of buckwheat honey was just as effective as a single dose of dextromethorphan in relieving nocturnal cough and allowing proper sleep.

This paper reports on the biochemical properties of honey from the northwest of Cameroon Africa.

2. Materials and Method

2.1. Collection and Preparation of the Honey Samples

The honey was harvested from Ngaoundere in the Adamawa region of Cameroon and used for the study. The sample was collected freshly in sterile containers (labeled with numbers, place and date of collection) and stored at ambient temperature until analyzed. Unwanted material such as wax sticks, dead bees and particles of combs were removed by straining the samples through cheesecloth before analysis.

2.2. GC-MS Analysis

2.2.1. Instruments and Chromatographic Conditions

For the identification of phytoconstituents in honey was carried out by subjecting the sample to GC-MS analysis. The sample (2 µl) was injected into a RTX-5 column (60 m X 0.25 mm i.d., film thickness 0.25 µm) of GC-MS (model GC-MS-QP-2010 plus, Shimadzu Make). Helium was used as a carrier gas at a constant column flow 1.2 ml/min at 173 kpa inlet pressures. Temperature programming was maintained from 10 0°C to 200 °C with constant rise of 5 °C/min and then held isothermal at 200 °C for 6 min; further the temperature was increased by 10 °C/min up to 290 °C and again held isothermal at 290 °C for 10 min. The injector and ion source temperatures were 270 °C and 250 °C, respectively. The wax was dissolve in methanol (HPLC grade) injected with a split ratio of 1:10. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 950 Dalton.

2.2.2. Identification of Components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

3. Result and Discussion

GC-MS analysis

GC-MS chromatogram of honey in Figure 1 indicates the presences of fourteen phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library 2005, the fourteen phytoconstituents were characterized and identified (Table 1). The various phytochemicals which contribute to the medicinal activity of honey are natural in nature. Traditionally, the natural honey are used in the treatment of ulcers, wound healing, swells, asthma, cough, hyperacidity, leprosy, diuretic, antimicrobial, jaundice, diuretic activity, hypolipidemic effect, hepatoprotective activity and fever. The GC-MS analysis identified the following major chemical constituents namely 2, 4-Dimethyl-1-pentanol, 3, 5-Dihydroxy-6- methyl-2, 3-dihydro-4H-pyran-4-one, 2-Furancarboxaldehyde 5-hydrox methyl, 2-Butoxyethyl acetate. These compounds are known to have some biological activity such as antibacterial, antifungal, antioxidant and wound healing power activity as previously reported. Also a study showed that 100% inhibition was observed on MH agar with honey in *Pseudomonas aeruginosa*, eoagulase-positive *Staphylococcus*, *Proteus mirabilis*, and *Citrobacter diversus*, *Klebsiella pneumonia* at 25% concentration (v/v) honey concentration. The activity found in the previous works could be attributed to the presence of this secondary metabolite identified from honey and bee wax [16].

Although 2-Furancarboxaldehyde, 5-hydroxymethyl (FCHM) the most abundant chemical constituent is not yet

been ascertained as a harmful substance however, its content in honey are imposed in many countries. This is to guard against distribution of honey of low quality. FCHM is an indication of overheating, storage at elevated temperature or an aged sample or adulterated with invert sugars (20, 21). The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners.

Furthermore, the geographical origin of honey is a relevant factor that can influence its different composition. The accumulation of phytochemicals such as (carbohydrates, phenolic compounds and volatile compounds) is dependent on climatic conditions (sunlight, moisture), soil characteristics and other factors; therefore, it is reasonable to believe that differences between honeys obtained from different countries are bound to be different due to the compositions of pollen or nectar, which have the greatest influence on the chemical composition [22, 23].

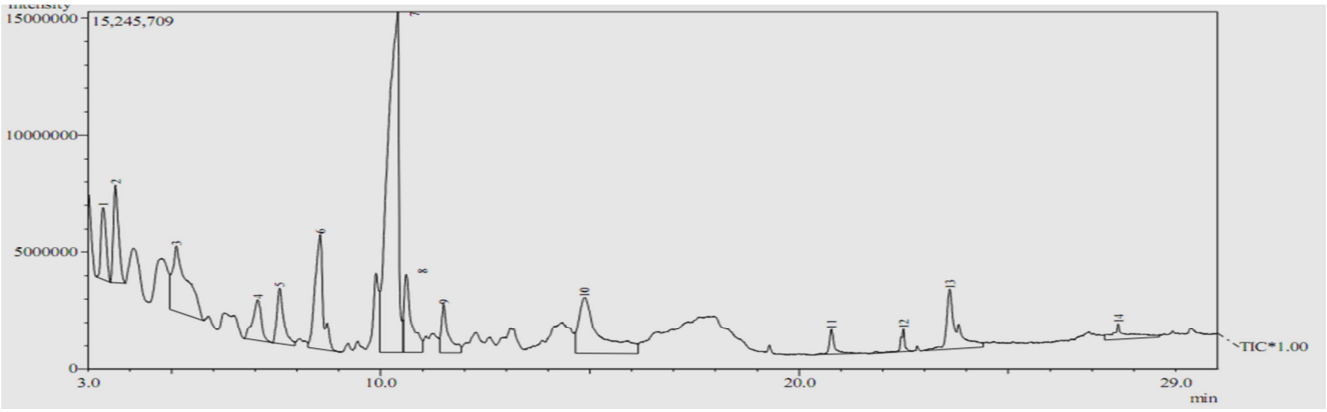

Figure 1. *GC-MS Chromatogram of honey wax.*

Table 1. *Phytocomponents identified in the honey by GC-MS.*

PN	RT	Name of the compound	Mol. Form	P.A. %	SI (%)	MW
1	3.36	2-Furancarboxaldehyde	$C_5H_4O_2$	3.56	94	96
2	3.66	alpha.-Furfuryl alcohol	$C_5H_6O_2$	4.87	90	98
3	5.12	2,4-Dimethyl-1-pentanol	$C_7H_{16}O$	9.23	77	116
4	7.06	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	C6H8O3	3.44	87	128
5	7.59	Methyl 1-methylcyclopropyl ketone	$C_6H_{10}O$	3.70	86	98
6	8.56	3,5-Dihydroxy-6-methyl-2,3dihydro-4H-pyran-4-one	$C_6H_8O_4$	8.91	91	144
7	10.40	2-Furancarboxaldehyde, 5-hydroxymethyl	$C_6H_6O_3$	36.02	82	126
8	10.62	2-Hexanone, 3-methyl-4-methylene	$C_8H_{14}O$	5.68	83	126
9	11.51	2H-Imidazol-2-one, 1,3-dihydro-4-methyl	$C_4H_6N_2O$	3.31	80	98
10	14.90	2-Butoxyethyl acetate	$C_8H_{16}O_3$	11.11	80	160
11	20.82	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	1.31	92	256
12	22.51	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	0.88	92	296
13	23.66	Oleic Acid	$C_{18}H_{34}O_2$	5.82	94	282
14	27.69	Pentafluoropropionic acid, 2-ethylhexyl ester	$C_{11}H_{17}F_5O_2$	2.15	78	276

+ PN= peak number, RT= retention Time in minutes, MW=Molecular weight in Dalton, SI= Similarity index in percentage to the mass peak in the NIST database.

4. Conclusion

In the present study fourteen chemical constituents have been identified from honey by Gas Chromatogram-Mass spectrometry (GC-MS) analyses and the Ngoundewre, Cameroon honey contains fourteen (14) chemical compounds with 2-Furancarboxaldehyde, 5-hydroxymethyl as the most abundant that could be bioactive.

Recommendation

Isolation of individual phytochemical constituents and subjecting them to biological activity may give fruitful results. However, due to the vast traditional used of honey; further studies will need to be undertaken to ascertain fully its bioactivity and it toxicity profile.

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