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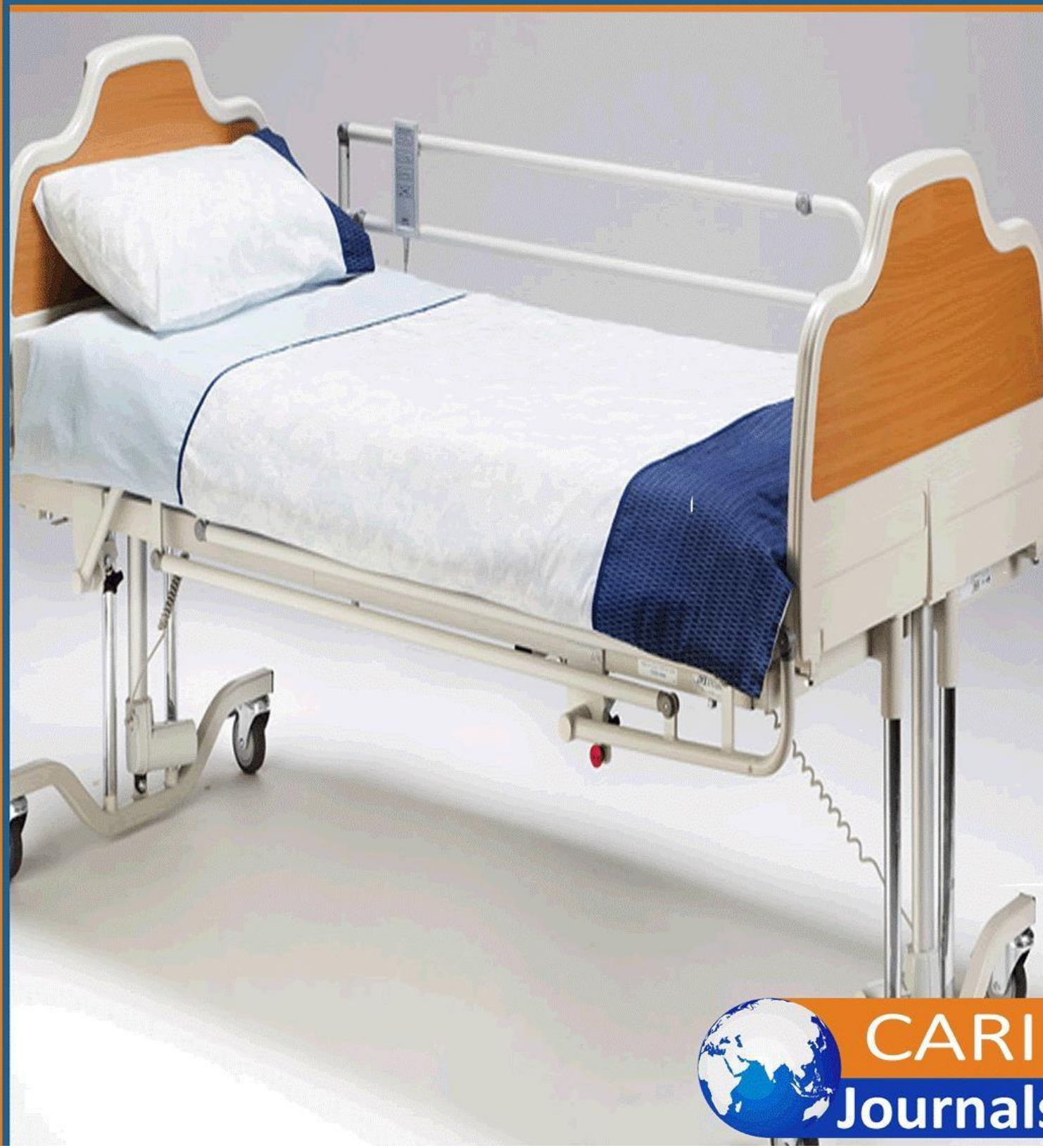

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Bacteriological and Biochemical Study of Natural Honey Available on the Mbujimayi Market

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Abstract

Purpose: to study the sterility and biochemical composition of natural honey available on the market in the town of Mbujimayi.

Methods: This is a descriptive cross-sectional study, carried out from January 1st to April 30th 2023, a period of 4 months. The study was carried out in two laboratories in the Democratic Republic of Congo: the bacteriological laboratory of the Clinique MIBA located in the commune of Kanshi, city of Mbujimayi, province of Kasaï Oriental for the bacteriological study, and the Centre de Recherche Agro-alimentaire (CRAA) located in the commune of Lubumbashi, city of Lubumbashi, province of Haut-Katanga for the biochemical study. The study sample consisted of natural honey from three local origins: Kabinda (Lomami), Sankuru (Lusambo) and Kanyama (Haut Lomami), chosen on the basis of the availability of their honeys on the market in the town of Mbujimayi. The biochemical study consisted in measuring: water content, PH, sugars, proteins, protein identification, and mineral elements by atomic emission spectrophotometry. Germ testing included yeast, staphylococcus aureus, enterobacteria and salmonella.

Results: Bacteriologically, all three samples were sterile. Biochemically, the three honey samples contained four biochemical substances: carbohydrates, proteins, vitamins (B and C) and minerals (copper, iron, manganese, calcium, magnesium, zinc, sodium, potassium, nickel).

Unique contribution to theory, practice and policy: Local honey from Mbujimayi is sterile whatever its origin (all three origins). The biochemical composition was almost identical, with good antioxidant, antibacterial, nutritional and healing properties.

Key words: *Bacteriological and Biochemical Study, Natural Honey, Mbujimayi*

INTRODUCTION

Honey is the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants, or from secretions originating from living parts of plants, or from excretions left on them by sap-sucking insects. Bees collect these substances, transform them by combining them with specific internal substances, deposit them, dehydrate them, store them, and allow them to mature in the honeycomb. Except for filtered honey, no pollen or other components intrinsic to honey should be removed, unless necessary to eliminate foreign organic or inorganic matter [1, 2].

The physicochemical quality of honey is closely linked to its water content and storage temperature. Indeed, the higher the water percentage, the more fluid the honey becomes, with its viscosity decreasing significantly. For example, at 25 °C, honey containing 14% water can reach a viscosity of around 400 poises, compared to only 20 poises for honey with 20% water. [3].

This sensitivity to humidity and heat justifies the recommendations to store honey at temperatures below 25 °C, away from light and moisture, in order to preserve its properties over time. Moreover, honey's composition, rich in volatile organic compounds (VOCs), also contributes to its texture, aroma, and overall quality elements that can also be affected by improper storage [3].

Due to its low moisture and sugar profile, honey can be stored at room temperature in airtight jars for years. Various containers are accepted, although glass and food-grade plastic jars are most common [4].

Two main categories of components are identified in natural honey:

- **Major components**: These include the predominant carbohydrates (79.5%), mainly fructose (39%) and glucose (32%). Maltose, sucrose, and other polysaccharides are also present in smaller amounts. Honey contains inverted sugar resulting from the enzymatic action of invertase on sucrose and water [5, 6].
- **Minor components**: These include proteins, peptides, and various amino acids such as glutamic acid, alanine, arginine, cystine, and glycine; trace minerals (less than 1%); proteins like methylglyoxal (MGO), defensin-1, and flavonoids [7]; vitamins A, B, C, D, and K [8]; and organic acids (in very small quantities) including gluconic, acetic, palmitic, oleic, linoleic, citric, lactic, succinic, and formic acids [9, 10]. The presence of organic acids contributes to honey's pH, which typically ranges between 3.5 and 6, with an average of 3.9 [11, 12].

These biochemical substances are scientifically known to support wound healing and even bone consolidation [9, 10].

Many studies have highlighted honey's therapeutic properties, including antioxidant, nutritional, antibacterial, and wound-healing effects [13, 14, 15, 16].

Rationale for the Study

In our context, infected open wounds represent a significant proportion of surgical admissions.

Healing these wounds is often slow and challenging, requiring extended medical follow-up (several weeks or months). Conventional antiseptics often fail to yield rapid improvement. We aim to explore honey, which has demonstrated efficacy in the treatment of chronic wounds worldwide [17].

Scientific literature supports the use of honey in wound care, especially for infected wounds and diabetic foot ulcers of venous origin [18, 19, 20]. Given that honey is affordable and readily available locally, and considering the lack of data on the bacteriological and biochemical properties of natural honey in our region, we found it relevant to conduct this research to validate a cost-effective and accessible natural wound dressing for use in our setting.

General Objective: To assess the sterility and biochemical composition of natural honey available on the market in the city of Mbujimayi.

II. METHODS

This was a descriptive cross-sectional study conducted from January 1st to April 30th, 2023, over a 4-month period. This study was conducted in two laboratories: the bacteriological analysis was carried out at the Bacteriology Laboratory of the MIBA Clinic located in the Kanshi commune, Mbujimayi city, Kasai Oriental province; and the biochemical analysis was conducted at the Agro-Food Research Center (CRAA) located in the Lubumbashi commune, Lubumbashi city, Haut-Katanga province, Democratic Republic of Congo.

The study sample consisted of natural honey from three local sources: Kabinda (Lomami), Sankuru (Lusambo), and Kanyama (Haut-Lomami), chosen based on the availability of their honey on the Mbujimayi market. The honey was freshly harvested by producers and stored in hermetically sealed plastic jars at room temperature.

Upon collection, the three samples were stored under recommended conditions [4]. The primary containers used were plastic jars pre-sterilized in an autoclave. The storage environment consisted of cabinets and wall-mounted display cases in the operating room of Saint Jean BONZOLA General Referral Hospital, maintained at a constant temperature of 16°C (confirmed by a digital thermometer).

Honey Analysis

The following analyses were systematically performed in the selected laboratories: Biochemical Analysis, pH Measurement, Sugar Content Measurement, Protein Quantification, Vitamin Identification, Mineral Content Determination by Atomic Emission Spectrophotometry

I. Bacteriological Analysis

The microbial investigation focused on the detection of yeasts, *Staphylococcus aureus*, enterobacteria, and *Salmonella* species. One milliliter of each diluted honey sample was

inoculated and homogenized in Petri dishes. The cultures of honey and bacteria were grown on nutrient agar media [2].

RESULTS

III.1. Bacteriological Study

The samples analyzed in the bacteriological laboratory were found to be sterile.

III.2. Biochemical Study: Honey Constituents

In the three samples analyzed at the biochemistry laboratory, at least four biochemical substances were identified within normal ranges: carbohydrates, proteins, vitamins, and minerals (Table 1).

The detected vitamins were B and C (Table 2).

Several trace metals and micronutrients were identified, including copper, iron, manganese, calcium, magnesium, zinc, sodium, potassium, and nickel. Cobalt and chromium were not detected (Table 3).

Constituents	Usual values	Values found (by origin)		
		KABINDA	SUNKURU	KANYAMA
Carbohydrates (sugars)	78-80 %	81,7	82,4	82,8
Proteins (Defensin -1)	0,26 %	0 ,56	0,43	0,55
Humidity	15 – 30%	19,17	16,29	17,04
Ph	3,2 à 4,5	4,1	4,2	4,3
Vitamins (B, C)	Traces	++	+++	+++
Minerals (Mg, Ca+, K+, P, Zinc, etc.)	Less than 1% of sales	++	++	++

Table 1. Physicochemical components of honey according to its origin

Table 2. Vitamin content by honey origin

Samples	Vitamin A	Vitamin B	Vitamin C	Vitamin D	Vitamin E
KABINDA	Absent	(++)	(+++)	Absent	Absent
SANKURU	Absent	(++)	(+++)	Absent	Absent
KANYAMA	Absent	(++)	(+++)	Absent	Absent

Table 3. Trace element content by honey origin

Samples (Provenance)	Minerals (in mg)								
	Cu	Fe	Mr	Ca	Mg	Zn	Na	K	Ni
KABINDA	0,024	0,053	0,002	3,184	2,981	0,041	4,886	16,373	0,001
SANKURU	0,018	0,056	0,004	3,407	2,118	0,091	4,696	19,213	0,001
KANYAMA	0,016	0,054	0,005	5,369	3,775	0,022	4,249	19,701	0,001

DISCUSSION

IV.1. Bacteriological Study

Samples from the three origins (Sankuru/Lusambo, Kanyama/Haut-Lomami, and Kabinda/Lomami) tested in the bacteriological laboratory were found to be sterile. Our findings are consistent with those reported by Baudel, Achouri, and collaborators, who also confirmed the sterility of honey in their respective studies [2].

IV.2. Biochemical Study

The biochemical analysis of our samples revealed more than four standard physicochemical components, including carbohydrates, proteins, vitamins (B-complex and C), mineral salts, and several trace elements: copper, iron, manganese, calcium, magnesium, zinc, sodium, potassium, and nickel. The pH and moisture levels were within normal limits (Tables 1, 2, and 3).

Carbohydrate levels were within the normal range: 81.7%, 82.5%, and 82.8% for Kabinda, Sankuru, and Kanyama, respectively. These findings align with values reported in the literature for similar honey types [3, 4]. Protein contents were also within standard ranges: 0.56%, 0.43%, and 0.55%. Comparable values were reported in an Algerian study by Boukhari Z in 2019 [40].

Moisture levels were acceptable, with values of 19.17%, 16.29%, and 17.04%. According to the Codex Alimentarius and European Honey Directive, the maximum moisture content should not exceed 21%. In high-quality honey, over 95% of samples tested have a moisture content below 18.5% [3]. Our results fall within or near this standard range.

The pH values reported in our study were acidic: 4.1, 4.2, and 4.3 depending on origin. Previous studies have found pH values ranging between 3.2 and 4.5, with an average of 3.9. All honeys are known to have an acidic pH [3, 4, 6].

Our results thus corroborate those of other authors who found a similar biochemical composition [21, 22]. This supports honey's well-documented properties, including antioxidant effects, nutritional benefits due to its ~80% sugar content (primarily glucose and fructose), odor reduction, and antibacterial activity attributed to its acidic pH (3.5–6), hydrogen peroxide content, and proteins [23, 24].

The therapeutic properties of honey (sterility, antibacterial and healing effects) have long been documented and confirmed more recently through various research studies leading to multiple publications [2]. Several works show honey's effectiveness in debriding infected wounds, such as abscesses, burns, ulcers of various etiologies, surgical wounds, and traumatic injuries [6–11, 25–26]. Honey's antibacterial action results from hydrogen peroxide produced by glucose oxidase, breaking down sugars into hydrogen peroxide and gluconic acid. The latter increases acidity, making honey a hostile environment for bacterial growth. Hydrogen peroxide is an effective antimicrobial when present in sufficiently high concentrations [2, 12, 13, 15–20]. Beyond hydrogen peroxide, honey's antimicrobial action is also attributed to its osmolarity, which promotes exudation and reduces edema in wounds, and its acidic pH. Comparative studies have shown honey pH to range between 6.2 and 4.2; the more acidic the honey, the more it inhibits bacterial proliferation [14, 27–28].

It should be noted that other researchers have found a richer biochemical profile in their samples, notably with the presence of methylglyoxal (MGO), which was not detected in our study. These variations are thought to be related to the botanical origin of the nectar, as the floral species influence the chemical composition of honey [12, 29–30].

Only B and C vitamins were identified in our samples, while others (A, D, E, K, etc.) were absent—contrary to Boukhari's study, which reported incidental presence of vitamins A, D, and K [3].

Unique Contribution to Theory, Practice and Policy

The sterility of the local honey studied has been confirmed for all three sources. Its biochemical composition is consistent with data from the literature, particularly regarding its carbohydrate, protein, vitamin, and trace element content, as well as its moisture level and pH.

Thus, the three samples have been proven to be sterile, antioxidant, antibacterial, and nutritional, and therefore possess wound-healing properties. There is consequently no objection to evaluating this substance as a treatment option for infected wounds, as supported by previous studies.

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Compliance with Ethical Standards

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Ethical Approval Statement

This study was approved by the Ethics Committee of the Official University of Mbuji-Mayi (UOM). It was conducted in strict accordance with the principles set forth in the Declaration of Helsinki.