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COMPARATIVE PHYTOCHEMICAL AND ANTINUTRITIONAL CONSTITUENTS OF NIGERIA SWEET AND BITTER HONEY VARIETIES

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ABSTRACT

This study elucidated the phytochemical and antinutritional composition of Nigeria sweet and bitter honey variety. Antinutritional composition, qualitative and quantitative phytochemicals of Nigerian sweet and bitter honey was carried out using standard methods. Qualitative Phytochemical analysis of the sweet and bitter honey revealed the presence of alkaloids, tannins, flavonoids, saponins glycoside with absence of anthraquinones and phlobatannins, while steroids was detected only in sweet honey. Subsequent quantification revealed no significant difference ($p>0.05$) in the Saponins and flavonoids contents of the two honey variety. However, the tannins content of bitter honey (0.02 ± 0.10) was significantly higher ($p<0.05$) than that of sweet honey (0.0006 ± 0.00). Antinutritional analysis showed that both sweet and bitter honey has high contents of oxalate 59.4 ± 2.31 mg/ml and 59.5 ± 2.03 mg/ml, Cyanide 19.13 ± 2.01mg/ml and 18.99 ± 2.04 mg/ml, and low contents of phytate 1.98 ± 0.00 mg/ml and 2.01 ± 0.10 mg/ml for sweet and bitter honey respectively. However, no statistically significant difference ($p>0.05$) was observed in the phytate, oxalate and cyanide content of the two honey verities. This study suggests that irrespective of the taste of honey sample, they are rich in nutrients and phytochemicals of medicinal significance.

KEYWORDS: Antinutrients, Sweet honey, Bitter honey and Phytochemicals.

INTRODUCTION

Honey is a gift of nature to mankind. Since ancient time, it has being in use in the household preparation. Honey is the sweet, viscous fluid produced by honeybees (*Apismelliflera*) using the nectar of flowers or secretions from living parts of plants (Codex Alimentarius, 2001).

Bees produce honey to act as a food store for the colony for periods when there are no flowers, or when the climate is adverse. For human, honey is a useful source of high carbohydrate food; it consists of approximately 70-80% sugar, mainly from fructose and glucose (White, 1975). In addition to water (usually 17-20 percent), honey also contains small amounts of other substances, including minerals, vitamins, proteins and amino acids adding nutritional variety to human diets. The 'ash' content of honey is mainly mineral trace elements. Minerals present are calcium, copper, iron, magnesium, manganese, potassium, sodium, and chlorides, phosphates, silicates and sulphates. These trace amounts of minerals may be important for human nutrition. Honey is widely used as a source of sugars for making honey wines and beers, and in the manufacture of many secondary products: breakfast cereals, bakery goods, and a multitude of other value-added products. Honey also contains a blend of flavonoids and phenolic acids which are antioxidants that eliminate potentially destructive free radicals in the human body (Sampath *et al*., 2010)

The major constituents of honey are nearly the same in all honey samples, however, the biochemical composition and physical properties of natural honeys varies greatly according to the plant species on which the bees forage (Cantarelli *et al*., 2008; Ebenezer & Olubenga, 2010; James *et al*., 2013; Adeniyi *et al*., 2014).

In many countries, honey is regarded more as a medicine or special tonic, rather than as an every-day food. Honey does have medicinal properties that are acknowledged increasingly by modern medicine. Ancient Egyptians, Assyrians, Chinese, Romans and Greeks have traditionally used honey as a medicinal remedy, for the management of wound healing, skin ailments and various gastrointestinal diseases (White *et al*., 2005). Modern research has shown that honey may possess anti-inflammatory activity and stimulate immune responses within a wound. The therapeutic importance of certain types of honey has been attributed to its secondary metabolites of antibacterial potency (Gheldof *et al*. 2002)

Phytochemicals are chemical compounds that are naturally found in plant. They are responsible for the colour and organoleptic properties of the plant (Lu *et al*., 2004). It is also referred to as those chemicals that may have biological significance but are not established as essential nutrients in plant (Brow and Arthur, 2001). Phytochemicals could be available as a dietary supplement, but the potential health benefits of phytochemicals are derived from consumption of the whole plant (Rao and Rao, 2007). Honey contains a variety of phytochemicals (as well as other substances such as organic acids, vitamins and enzymes) that may serve as sources of dietary antioxidants (Gheldof and Engeseth 2002; Gheldof *et al*., 2002).

The amount and type of these secondary metabolite and chemical compounds depends largely upon the floral source/variety of the honey bee species, geographic area, season, mode of storage, and even harvest technology and conditions (Gheldof *et al*., 2002). Furthermore, honey sweetness depends on high fructose content and acidity with pH of ranges from 3.2 to 4.5 (Amy and Carlos, 1996). The predominant acid found in honey is gluconic acid. Its presence in all honey originates largely from the activity of glucose oxidase which the bees add at ripening and to a lesser extent from the bacterial action which occurs (Ruiz-Argueso and Rodriguez-Navarro, 1973). A few plants give bitter honey: *Agave* sp. (sisal), *Datura* sp., *Euphorbia* sp., *Senecio* sp. – in some societies (for example, in East Africa), these honeys are very popular. In the present report, attempt was made to compare the qualitative and quantitative phytochemicals component of sweet and bitter varieties of Nigeria honey.

MATERIALS AND METHODS

Source of Materials

Nigerian sweet and bitter varieties of honey used for this study were obtained from Adekam Apiary Ala community, Akure South Akure, Ondo State, Nigeria. It was collected in an aseptic container and transfer to the Department of Biological science Federal University of Technology, Minna, where it was kept at room temperature for further analysis. All chemicals used were of analytical grade and were products of Sigma Chemical Co., USA. The distilled water used for all the washing, cleaning and preparation of solutions, was obtained from the Laboratory, Department of Biochemistry, Federal University of Technology, Minna.

Qualitative Phytochemicals

Steroids

A 0.5 g of each Nigerian sweet and bitter honey was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids (Sofowora, 1993).

Flavonoids

A portion of each Nigerian sweet and bitter honey was heated with 10 ml of ethyl acetate in a test tube over a steam bath for 3 minutes. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Yellow coloration was observed that indicated the presence of Flavonoids (Harborne, 1973; Sofowora, 1993).

Tannins

Five hundred milligram (0.5g) each of Nigerian sweet and bitter honey was boiled in 20 ml of distilled water in a test tube and filtered. Ferric chloride (0.1%) solution was added to the filtrate. The appearance of brownish green or a blue-black coloration indicates the presence of tannins in the test samples (Harborne, 1973).

Saponins

A 2.0 g of each Nigerian sweet and bitter honey was boiled in 20 ml of distilled water in a test tube in boiling water bath and filtered. A 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously to form a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion characteristic of saponins (Obadoni *et al*., 2001).

Alkaloids

A 0.5g of each Nigerian sweet and bitter honey was stirred with $5cm^3$ of 1% aqueous HCl on a steam bath. Few drops of picric acid solution were added to $2cm^3$ of the extract. The formation of a reddish brown precipitate was taken as a preliminary evidence for the presence of alkaloids (Trease and Evans 1989; Harborne, 1976).

Quantitative Phytochemicals and Antinutritional Analysis

Determination of Tannins

A 0.2 g portion of each Nigerian sweet and bitter honey was measured into a 50 ml beaker. 20 ml of 50 % methanol was added and covered with para film and placed in a water bath at

77-80^{o}C for 1hour. It was shaken thoroughly to ensure a uniform mixture. The extract was quantitatively filtered using a double layered Whatman No. 41 filter paper into a 100 ml volumetric flask, 20 ml of water was added, 2.5 ml Folin-Denis reagent and 10 ml of 17 % Na_2CO_3 were added and mixed properly. The mixture was made up to the marked level with distilled water mixed well and left undisturbed for 20minutes for the development of a bluish-green colour. The absorbances of the tannic acid standard solutions as well as the samples were read after colour development on a UV-Vis spectrophotometer model 752 at a wavelength of 760 nm (AOAC, 1984).

Determination of Saponins

A 0.5 g of each Nigerian sweet and bitter honey was added to 20 ml of 1NHCl and was boiled for 4 hours. After cooling it was filtered and 50 ml of petroleum ether was added to the filtrate for ether layer and evaporated to dryness. 5 ml of acetone ethanol was added to the residue. 0.4 ml of each was placed in 3 different test tubes. 6 ml of ferrous sulphate reagent was added followed by 2 ml of concentrated H_2SO_4. It was thoroughly mixed after 10 minutes and the absorbance was read at 490 nm. Standard saponin was used to plot the calibration curve (Oloyed, 2005).

Determination of Flavonoids

Total flavonoid was determined using aluminium chloride colorimetric method (Chang *et al*., 2002). Quercetin was used as standard to generate the calibration curve. Exactly 0.5 ml of the diluted sample was added into test tube containing 1.5 ml of methanol. 0.1 ml of 10% $AlCl_3$ solution and 0.1 ml sodium acetate ($NaCH_3COO^-$) were added, followed by 2.8 ml of distilled water. After incubation at room temperature for 30minutes, the absorbance of the reaction mixture was read at 415 nm. The amount of 10% $AlCl_3$ was substituted by the same amount of distilled water in blank.

Determination of phytate

The phytic acid content was determined using a modified indirect colorimetric method of Wheeler and Ferrel (1971). The method depends on an iron phosphorus ratio of 4:6 and is based on the ability of standard ferric chloride to precipitate phytate in dilute HCI extract of the sample. 5g of the sample was extracted with 20ml of 3% trichloroacetic acid and filtered. 5ml of the filtrate was used for the analysis; the phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding 5ml of IM NaOH. The precipitate was dissolved with hot 3.2M HNO_3 and the absorbance and immediately at

480nm. Preparation of standard curve for phytic acid was done as follows: standard curve of different $Fe(NO_3)_3$ concentrations was plotted against the corresponding absorbance of spectrophotometer to calculate the ferric iron concentration. The phytate phosphorus was calculated from the concentration of ferric iron assuming 4:6 Iron: phosphorus molar ratio.

Determination of oxalate

The titrimetric method of Day and Underwood (1986) was used in the determination of oxalate in of each of the sample. 150 ml of 15N H_2SO_4 was added to 5 g of the pulverized sample and the solution was carefully stirred intermittently with a magnetic stirrer for 30 minutes and filtered using Whatman No 1 filter paper, after which 25 ml of the filtrate was collected and titrated against 0.05M standardized $KMnO_4$ solution until a faint pink color appeared that persisted for 30 seconds.

Determination of Cyanide

Cyanide content was determined by alkaline picrate method as described by Onwuka, (2005). A 5g of each Nigerian sweet and bitter honey was dissolved in 50ml of distilled water in a cooked conical flask and the extraction was allowed to stand over-night, filtered. 1ml of sample filtered was mixed with 4ml alkaline picrate in a corked test tube and incubated in a water bath for 5mins. After colour development (reddish brown colour) the absorbance was read at 490nm, the absorbance of the blank containing 1ml distilled water and 4ml alkaline picrate solution was also recorded. The cyanide content was extrapolated from cyanide standard curve prepared from different concentration of KCN solution containing 5-50µg cyanide in a 5001 conical flask followed by addition of 25ml of INHCI.

Statistical analysis

Values were analyzed using statistical package for social science (SPSS) version 16 and presented as means ± SE of the mean. Comparisons were carried out by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The level of significance was set at $P < 0.05$ (Adamu and Johnson, 1997).

RESULTS

Phytochemicals

Table 1 shows the result of qualitative phytochemical analysis of Nigerian sweet and bitter honey. The results revealed the present of alkaloids, tannis, flavonoids, saponins, terpenes and absence of resin in both honey sample while steroids was detected only in sweet honey.

Quantification of the phytochemicals revealed no statistical significant difference in saponin and flavonoids content of Nigerian sweet and bitter honey. However, tannin contents (0.02g/ml) of the bitter honey were significantly higher than that of sweet honey (0.000615g/ml) (Table 2).

Antinutritional

Antinutritional analysis of the samples shows no statistical significant difference ($p>0.05$) in the phytate, oxalate and cyanide content of Nigerian sweet and bitter honey (Table 3).

Table 1: Qualitative phytochemical composition of Nigerian sweet and bitter honey

Phytochemicals	Sweet honey	Bitter honey
Tannins	+	+ +
Resin	-	-
Saponin	+++	+++
Flavonoid	+	+
Steroid	+	_
Terpenes	+	++
Alkaloid	+	+
Balsam	++	+
Carbohydrate	+	+

Key: ++ = relatively presence, + =presence, - = Absence

Table 2: Quantitative phytochemical and antinutritional composition of Nigerian sweet and bitter honey.

Samples	Saponins (g/ml)	Flavonoids (ug/ml)	Tannins (mg/ml)
Sweet honey	3.15 ± 0.01^{a}	20.81 ± 0.01^{a}	0.0006 ± 0.00^{b}
Bitter honey	3.05 ± 0.20^{a}	18.92 ± 0.01^{a}	0.02 ± 0.10^{a}

*Values with different superscript alphabet on the same column are significantly different at $p<0.05$ level of significant.

Data are Mean ± SEM of triplicate determination.

Table 3:Antinutritional composition of Nigerian sweet and bitter honey

Sample	Cyanide(mg/ml)	Phytate(mg/ml)	Oxalate(mg/ml)
Sweet honey	$19.13\pm2.01^{a*}$	1.98 ± 0.00^{a}	59.4 ± 2.31^{a}
Bitter honey	18.99 ± 2.04^{a}	2.01 ± 0.10^{a}	59.5 ± 2.03^{a}

*Values with the same superscript alphabet on the same column are not significantly different at $p>0.05$ level of significant

Data are Mean ± SEM of triplicate determination.

DISCUSSION

Phytochemical

This study revealed that Nigerian sweet and bitter honey are rich in various medical important phytochemicals that possess physiological activity (Sofowora, 1993). Flavonoids are most commonly known for their antioxidant activity. They are transformers which modify the body‘s reactions to carcinogens, viruses, and allergens. They show anticancer, anti-inflammatory, antimicrobial and anti-allergic activity (Balch and Balchi, 2000; Ekam and Ebong, 2007), and may be useful in therapeutic roles (Jisika*et al*, 1992). The flavonoids contents of sweet and bitter honey 20.81±0.01 and18.92±0.01(µg/ml) respectively are higher than the flavoinoids contents of other medicinal plants. The presence of alkaloids in both honey tested is an indication of their medicinal potentials.

Alkaloids are the most efficient therapeutically significant plant substance. Pure isolated alkaloids and their synthetic derivatives are use as basic medicinal agent for their analgesis, antispasmodic and antibacterial effect (Stray, 1988), Alkaloids has been found to have microbiocidal effect and the major anti-diarrheal effect is probably due to their effects on small intestine and antihypertensive antifungal, antiinflammatory, antifibrogenic effect (Ghosal*et al*., 1996). Some alkaloids are useful against HIV infection as well as intestinal infection associated with AIDS (McDevith*et al.*, 1996).

Saponins are used in veterinary vaccines as adjuvant (e.g. foot-and-mouth disease vaccines) helping to enhance immune response. They are also mild detergents and can be used commercially as well as for research (Belch *et al.*, 2000). They can also be used in intracellular histo-chemistry staining to allow antibody access to intracellular proteins (Belch *et al.*, 2000).

Steroids are also of importance and interest in pharmacy due to their relationship with sex hormones (Okwu, 2001) and promote immune function in the skin and also reduce inflammation (Bell, 2008). The presence of this phytochemical in is an indication that Nigerian sweet and bitter honey can be given to expectant ruminant animals and those that deliver without the release of their placenta.

Tannin is non toxic and can generate physiological responses in animals that consume them (Scalbert, 1991). Tannin can be toxic to filamentous fungi, yeast and bacterial. The higher content of tannins in bitter honey as compared to the sweet honey suggests that the bitter

honey will be more active as antifungal, antibacterial, antidiarrheal, antioxidant and antihemorrhoidal agent (Khaliqur *et al.*, 2013).Tannins also have astringent property, plant containing tannin has been reported to be used for healing of wounds, varicose ulcers, hemorrhoids, frost bile and burn in herbal medicine (Igboko, 1983) thus bitter honey will be more suitable for this purpose.

Anti-Nutrients

Antinutritive factors limit the use of many plants for food because they elicit deleterious effects in both man and animals (Kubmarawa *et al*., 2008). Oxalate is quantitatively the most abundant. Antinutritive component of Nigeria sweet and bitter honey, 59.40±2.31 and 59.50±2.03 respectively. Oxalates have been known to cause irreversible oxalate nephritis when ingested in large doses. It is an antinutrient and prevents the absorption of some vital nutrients in food, especially divalent metals (Ca^{2+}, Mg^{2+} etc) and fatty acids by forming salts. Oxalate intoxification (high ingestion of oxalate) causes malasorption syndromes leading to steatorrhoea, in which fatty acids are not absorbed, causing formation of insoluble calcium salt of fatty acid (Vasudevan and Sreekumari, 2000). Oxalates also cause gastrointestinal tract irritation, blockage of the renal tubules by calcium oxalate crystals, development of urinary calculi and hypocalcaemia (Blood and Radostits, 1989)

Next to oxalate, cyanide is the second most abundant antinutrient in sweet (19.13±2.01mg/ml) and bitter (18.99±2.04mg/ml) Nigeria honey. It has been established that excess cyanate in the body inhibits the cytochrome oxidase. This may stop ATP formation and the release of inorganic phosphate to body tissues. Consequently, the body suffers energy deprivation and subsequent death. High level of HCN has been implicated in cerebral damage and lethargy in man and animal.

The knowledge of the phytate level in foods is necessary because high concentration can cause adverse effects on the digestibility. Phytate diet of 1-6% over a long period of time decreases the bioavailability of mineral elements in mono gastric animals. Phytic acid is a strong chelator, forming protein and mineral-phytic acid complexes thereby decreasing protein and mineral bioavailability (Soetan and Oyewole, 2009, Fasusi *et al*., 2003; Erdman, 1979,). Phytate is associated with nutritional diseases such as rickets in children and osteomalacia in adult humans respectively. However, the phytate are quantitatively the least abundant antinutritive contents of Nigeria sweet and bitter honey. It is established that only high content of these antinutrient selicite deleterious effect in the body metabolism.

Reduction of antinutrients in foods may be necessary especially when their levels are higher than those generally regarded as safe for human consumption. This can be accomplished through different hydrothermal treatments, which also enhances the nutritional qualities: increase palatability and digestibility of foods (Adeniji *et al.*, 2007). The antinutrients presents in both honey samples, irrespective of their concentration are natural components of honey. This is indicating the importance and necessity in taking moderate amount of honey in our daily diet. The presence of the antinutrients especially oxalate in high proportion may be attributed to their flora source.

CONCLUSION

In conclusion, Nigeria sweet and bitter honey varieties are rich sources of important phytochemicals of pharmacological significance. They also contain moderate level of antinutrient especially the oxalate, which is part of their basic chemical constituents. Both the honey types can therefore be exploited in search for new drugs, with higher efficacy and delayed resistance potentials.

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