Preliminary Investigation on the Phytochemical Constituents of Honey Samples from Ado-Ekiti, Ekiti State, Nigeria.

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Abstract
Demand for honey consumption nowadays is continuously increasing worldwide due to its multiple importance from food to medicine. The medicinal value of honey lies in the bioactive phytochemical constituents that produce health benefits to man. Investigation of the phytochemical constituents of the two honey samples procured from a beekeeper and a roadside seller in Ado Ekiti, Ekiti State, Nigeria was carried out to establish their phytochemical richness. The honey samples were analyzed and found to contain Tannins, Flavonoids, Alkaloids, Cardiac Glycosides, Phenols, Steroids and Reducing Sugar. Saponins, Terpenoids and Phlobatanins were absent in the two honey samples. Quantitative phytochemical analyses revealed the concentration of Tannins (19.33±0.17% and 13.88±0.33%), Flavonoids (25.2±10.32% and 23.49±0.23%)), Alkaloids (14.44±0.30% and 11.93±0.28%), Cardiac Glycosides (19.00±0.33% and 15.38±0.19%), Phenols (23.56±0.13% and 19.70±0.09%) and Reducing Sugar (28.15±0.85 and 26.08±0.30%) in the two honey samples respectively. The results showed that the two honey samples were rich in health promoting phytochemicals and this might justify honey as a potential source of phytomedicine.

Keywords: Beekeeper, Honey, Phytochemical, Ado Ekiti, Nigeria.

Enquête préliminaire sur les constituants phytochimiques des échantillons de miel d'Ado-Ekiti, Ekiti State, Nigeria

Résumé
La demande de consommation de miel de nos jours augmente constamment dans le monde entier en raison de son importance multiple, de la nourriture à la médecine. La valeur médicinale du miel réside dans les constituants phytochimiques bioactifs qui produisent des avantages pour la santé de l'homme. L'étude des constituants phytochimiques des deux échantillons de miel obtenus auprès d'un apiculteur et d'un vendeur ambulant à Ado Ekiti, l'État d'Ekiti, au Nigéria a été menée pour établir leur richesse phytochimique. Les échantillons de miel ont été analysés et se sont révélés contenir des tanins, des flavonoïdes, des alcaloïdes, des glycosides cardiaques, des phénols, des stéroïdes et du sucre réducteur. Les saponines, les terpénoïdes et les phlobatanines étaient absentes dans les deux

échantillons de miel. L'analyse phytochimique quantitative a révélé la concentration de tanins (19,33 ±0,17% et 13,88 ±0,33%), les flavonoïdes (25,2 ± 10,32% et 23,49 ±0,23%)), les alcaloïdes (14,44 ±0,30% et 11,93 ±0,28%), les glycosides cardiaques (19,00 ±0,33% et 15,38 ±0,19%), Phénols (23,56 ± 0,13% et 19,70 ± 0,09%) et Réduction du sucre (28,15 ±0,85 et 26,08 ± 0,30%) dans les deux échantillons de miel respectivement. Nos résultats ont montré que les deux échantillons de miel étaient riches en produits phytochimiques favorisant la santé et cela pourrait justifier le miel comme source potentielle de phytomédecine.

Mots-clés: Apiculteur, Miel, Phytochimique, Ado Ekiti, Nigéria

Introduction

The use of traditional medicine to treat infections has been practiced since the origin of mankind, and honey is one of the oldest traditional medicines considered to be very useful in the treatment of various human ailments. Honey is a sweet food made by honey bees (the genus *Apis*) collected by beekeepers and consumed by man. Honeybees collect nectar from flowers of plants as well as secretion from other living parts of plants to form dense and stable energy food called honey by a process of regurgitation and stored in wax honeycombs inside the beehive (CAC, 2001).

Honey is a complex mixture of carbohydrate (mainly fructose and glucose) and other minor substances such as organic acids, amino acids, proteins, minerals, pollen grains, vitamins, waxes and lipids (Sanz *et al.*, 2004; Saxena *et al.,* 2010). In most ancient cultures, honey has been used for both nutritional and medicinal purposes. Apitherapy, an alternative medicine branch has been developed in recent year, which offers treatments using honey and other bee products against many diseases. Honey is considered as one of the most useful non-wood forest products which has significant livelihood value and also plays an important role in strategy for sustainable and conservation of forest (Kuster *et al.,* 2006). Further more, honey is noted as a promising plant product, which could contribute to increase intake of some essential nutrients and health-promoting phytochemicals.

Phytochemicals are found in plant-based foods and are known to have protective or disease preventive properties acting as antioxidants, antimicrobial, anti-inflammatory, antitumor and anticancer effects amongst others (Harborne, 1973). The medicinal properties of honey lie in some chemical substances (phytochemicals) that produce a definite physiological action on human body. The knowledge of the chemical constituents of honey would be valuable in discovering the actual value of folklore remedies. Honey has a wide range of phytochemicals including polyphenols which act as antioxidant. These phytochemicals have been reported to vary according to the geographical and climatic conditions (Khali *et al.,* 2010). Raw honey is rich in antioxidants. Honey contains, in addition to flavonoids, other antioxidants such as catalase and ascorbic acid. Ferreire *et al.* (1992) reported that there have been 33 flavonoids identified in honey with concentration ranging from 0.005 to 0.01%. Recently, there has been increasing interest in determination of antioxidant activity of honey. Several reports have shown that antioxidant activity of honey varied widely

depending on the floral source, geographical as well as climatic condition of the area where the honey is sourced. Also, it was reported that the honey processing, handling and storage affect honey antioxidant activities to a less degree (Aljadi and Kamaruddin, 2004).

Phytochemical screening is one of the techniques to identify new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenols, steroids, tannins, etc. Studying the phytochemical nature of bioactive components of the bee products makes room for synthetic modifications for better pharmacokinetic profile (Rajinder Kaur *et al.,* 2013). Similarly, analysis of potent health protective phytochemical of honey locally available is of vital important as it provides information on the nutritional status of the honey. Such phytochemical screenings in various honey samples were conducted in various parts of the world. Khaliqur *et al.* (2013) reported phytochemical analysis and chemical compositions of different branded and unbranded honey samples in Pakistan. Bhuvaneswari *et al.* (2014) made available the report on the quality and phytochemical constituents of honey from Chennai, India. Phytochemical screening and biological activity of raw honey from Zimbabwe was reported by Dzomba *et al.* (2012). Other researchers such as Nwankwo *et al.* (2014) carried out phytochemical screening and antimicrobial activities of apiary honey produced by honey bee *(Apis mellifera)* on clinical strains of *Staphylococcus aureus, Eschericha coli* and *Candida albicans* in Nsukka, Nigeria. However, little or no information is available regarding the phytochemical constituents of honey consumed in Ekiti State, Nigeria. Hence, the present study was carried out to determine the phytochemical constituents of honey samples from Ekiti State, Nigeria.

Materials and Methods

Collection of honey samples

An unbranded honey sample was procured from a bee farmer in Ado Ekiti and the other honey sample was procured from a roadside honey seller at Ilokun, Ado Ekiti in February, 2016. The honey samples were stored in airtight plastic containers at room temperature for three weeks. The samples were taken to Biochemistry Laboratory, Department of Biochemistry, University of Lagos, for phytochemical analysis.

Phytochemical analysis

Phytochemical analysis was carried out according to Tease and Evan (1989); Harbornes (1991); Tease and Evans (2002) and Edeoga *et al.* (2005). The honey samples were used directly rather than extracting using a solvent.

Qualitative phytochemical analysis

Test for tannins: Two millilitres of honey sample each was dissolved in 10 ml of water. 1ml of this solution was put in a test tube and a few drops of 0.1% Ferric Chloride were added. A brownish green colouration indicated a positive test for tannins (Trease and Evans, 1989).

Test for saponins: Ten millilitres of distilled water was added to 5 ml of honey sample and boiled. The mixture was shaken vigorously to obtain a stable persistent froth. Frothing that persisted for 30 minutes showed the presence of saponins (Odebiyi and Sofowora, 1978).

Test for flavonoids: Five milliliters of diluted ammonia solution was added to each honey sample in a test tube, followed by the addition of concentrated sulphuric acid. A yellow colouration was observed if flavonoid compounds are present (Trease and Evans, 1989).

Test for phlobatannins: One millilitre of honey sample was boiled with 2% Hydrochloric (HCL) acid solution. Red precipitate indicated the presence of phlobatannins.

Test for Alkaloids: To each of the honey sample, 1% HCL and 6 drops of Mayer's reagent and Dragendroff"s reagent was added to 5 ml of each honey sample. An organic precipitate showed the presence of alkaloids in the honey sample investigated.

Test for terpenes / terpenoids: The Salkowski test was adopted, 5 ml of each of the honey sample was mixed with 2 ml of Chloroform and 3 ml of concentrated $H_2SO_4$was carefully added to form a layer. An interface with a reddish brown colouration was formed to show positive result for the presence of terpenes/terpenoids.

Test for steroids: Five millilitres of honey sample was weighed and 10 ml of Chloroform was added. 2 ml acetic anhydride and few drops of concentrated H_2SO_4 were also added. Appearance of blue green ring indicated the presence of steroids.

Test for Cardiac glycosides: The Keller-Killani test was used. 5 ml of each honey sample was treated with 2 ml of glacial acetic acid containing one drop of Ferric chloride solution. This was under laid with 1 ml of concentrated H_2SO_4. Appearance of a brown ring indicated the presence of the cardiac glycosides.

Quantitative phytochemical analysis

The quantitative amounts of phytochemicals, which were found in the honey samples were determined using standard procedure as described by Obadoni and Ochuko (2001); Trease and Evans (2002) and Amakura *et al*. (2009).

Results

The results of the qualitative and quantitative investigations of the two honey samples are presented in Tables 1 and 2 respectively. The results revealed the presence of phytochemicals such as tannins, flavonoids, alkaloids, cardiac glycosides, phenols, steroids and reducing sugar. However, saponins, terpenoids and phlobatanins were not found in the honey samples analyzed. The quantitative phytochemical analysis of honey sample from the beekeeper (sample A) were found to be 19.33±0.17% tannins, 25.21±0.32% flavonoids, 14.44±0.30% alkaloids, 19.00±0.33% cardiac glycosides, 23.56±0.13% phenols and 28.15±0.84% reducing sugar while 13.88±0.33%, 23.49±0.23%, 11.93±0.28%, 15.38±0.19%, 19.70±0.09% and 26.08±0.30% were recorded for tannins, flavonoids, alkaloids, cardiac glycosides, phenols and reducing sugar respectively for honey obtained from the roadside seller (sample B).

Discussion

The determination of phytochemicals in this study could have been responsible for the generally acclaimed honey medicinal properties as well as honey physiological activities. The medicinal values of honey have been reported by several authors (Efem *et al.,* 1988; Dumfort *et al.,* 2000). Tannins have been reported to quicken the healing of wounds and inflamed mucos membranes (Farquar, 1996). Tannins have also shown potential antibacterial and antiviral effects (Akiyama, 2001). These findings are in agreement with the work of Khaliqur *et al.* (2013) whose study revealed the presence of tannins in seven different honey samples from Pakistan. The above results are however, contrary to the finding of Bhuvaneswari *et al.* (2014) who reported negative result for tannins in three honey samples procured from Channai, India. Appreciable amount of

alkaloids were determined in the two honey samples. Alkaloids are very important in medicine and constitute most of the valuable drugs. Pure isolated alkaloids are noted for analgesic, antispasmodic and bactericidal effects (Okwu and Okwu, 2004).

The presence of phenols further showed that the honey could be used as anti-inflammatory, anti-clotting and immune enhancers. Phenols have the ability to block specific enzyme that causes inflammation. Previous study of Khalil *et al.* (2010) supported the claim that phenolic antioxidant from processed honey are bioactive and can increase antioxidant activity of plasma. Schramn *et al.* (2003) submitted that honey could enhance antioxidant defense system in healthy adults when used in some foods instead of traditional sweetness. Rumbaoa *et al.* (2009) also reported that phenols have the ability to retard lipid in oils and fatty foods thereby reducing heart related diseases. Phenols can as well reduce the risk of cancer by interfering with all stages of the cancer process (Hollman, 2001).

Flavonoids have been reported to have antioxidant and detoxification activities and many health-promoting effects (Akroun, 2011). Antioxidant in honey adds health benefits to honey. Although honey may not serve as a major source of dietary antioxidant, it shows that honey has the potential to play a role in providing antioxidant in a pleasing form. As a result of honey palatable taste, consumers may readily prefer honey to plant derived antioxidants. Honey can be a flavourful and supplementary source of antioxidants (Jaganathan and Mandal, 2009). Antioxidant properties of honey act as an anti depressant during high emotion, physical and intellectual stress (Jaganathan and Mandal, 2009). This supports the assertion that honey provides a tranquilizing sensation to the mind which leads to calmness where there is restlessness and agitation. It should be noted that the antioxidant activity of honey depends on its botanical sources and also to a great extent its geographical origin (Vela *et al.*, 2007).

Table1. Qualitative phytochemical screening of two honey samples in Ekiti State (Bee farmer and roadside seller)

Phytochemical	*Sample A*	*Sample B*
Tannins	+	+
Flavonoids	+	+
Saponins	-	-
Alkaloids	+	+
Cardiac glycosides	+	+
Terpenoids	-	-
Phenols	+	+
Phlobatanins	-	-
Steroids	+	+
Reducing sugar	+	+

"+" indicates presence "-" indicates absence

Table2. Quantitative phytochemical composition of the two honey samples obtained in Ekiti State (Bee farmer and roadside seller honey)

Phytochemical(%)	*Sample A*	*Sample B*
Tannins	19.33±0.17	13.88±0.33
Flavonoids	25.21±0.32	23.49±0.23
Alkaloids	14.44±0.30	11.93±0.28
Cardiac glycosides	19.00±0.33	15.38±0.19
Phenols	23.56±0.13	19.70±0.09
Reducing sugar	28.15±0.84	26.08±0.30

Values are the mean ± S.D

Cardiac glycosides are natural substances that act on the heart and its importance in treatment of cancer (breast cancer), colon cancer, bladder cancer and lung cancer have been reported by Frese *et al*. (2006). Cardiac glycosides act on the heart muscles and increase renal flow (Borokini and Omotayo, 2012). The presence of steroids in the two honey samples are of great importance because of their relationship with sex hormones. Steroids increase protein synthesis, help in bone and muscles growth as well as enable athletes to train more intensively for longer periods. Identification of steroids lends credence to the claim that honey produce dramatic effect upon mental perception during athletic performance. In addition, steroids are reported as controlling agent for tropical diseases such as eczema. In this finding, saponins and terpenoids were absent. This is contrary to the work of Aruna *et al.* (2014) who identified saponins and terpenoids in manuka honey investigated. They also reported that all the four honey samples screened lacked tannins. Interestingly, the concentrations of these phytochemicals obtained in the honey samples analyzed were relatively higher compared to what was reported for some notable Nigerian vegetables. Raimi *et al.* (2014) reported that *Manihot esculentus* and *Abelmoschus esculentus* contained 9.83% and 8.94% tannins, 5.45% and 4.71% flavonoids respectively. Similarly, Dike (2010) confirmed the presence of alkaloids, flavonoids and tannins in fruits and seeds of 15 plant species in Nigeria and reported that the concentrations of these phytochemicals ranged between (0.18%-1.46%) for alkaloids, (0.04%-0.65%) for flavonoids and (0.12%-0.36%) for tannins.

Conclusion

The findings from this work established that honey obtained from samples A and B were rich in bioactive compounds that can promote good health. Presence of these phytochemicals support the inestimable medicinal attributes of honey as well as its efficacies as antimicrobial, anti-inflammatory, antioxidant and anti-cancer. The results of this research showed that honey procured from the roadside vendor is medicinally better when compared to honey from the bee farmer. However, further analysis need to be carried out to investigate the nutritional status of these honey as well as their botanical sources.

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