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Comparative Chemical and Quantitative Analysis of Flavonoid Contents In Propolis Samples From South East (Abia) and South West (Ibadan) Of Nigeria

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ABSTRACT

Propolis has been used as medicinal agent for centuries. Among the many compounds identified in propolis, flavonoids are considered to be responsible for its main biological activities. Therefore, determination of flavonoids in propolis is a key parameter for evaluation of propolis quality. To continue our research on chemical composition of propolis samples, spectrometric and chromatographic analyses of flavonoid contents collected from two different regions in Nigeria (South East, Abia) and South West, Ibadan) were investigated. Gas chromatography-mass spectrometry technique was used for qualitative analysis. Quantitative flavonoid contents were determined as flavones, flavonols and flavanones expressed as rutin, quercetine and naringenin Equivalent, respectively, using HPLC and UV spectroscopy. Phytochemical screening results of Abia propolis hexane extract (ABPHE), Abia propolis ethanol extract (ABPEE), Ibadan propolis hexane extract (IBPHE), and Ibadan propolis ethanol extract (IBPEE) indicated the presence of flavonoids, alkaloids, steroidal nucleus, saponins, reducing sugars and phenols, while tannins were only detected in ABPEE and IBPEE. The GC-MS analysis identified zingiberene, alpha-farnesene, beta-bisabolene, beta-sesquiphellandrene, phytol and trans-geranylgeraniol in ABPHE and beta-caryophellene, humulene, trans-sesquisabinene hydrate, eremophilene. lauric acid, methyl ester, beta-sesquiphellandrene, lauric acid, ethyl ester, spathulenol, methoxyeugenol, asarone and junipar camphor in IBPHE.
The UV/VIS results gave Total Flavonoid Contents (TFCs) for ABPHE (5.59 %) and ABPEE (3.12 %) as 8.70 % whereas TFCs for IBPHE (1.81 %) and IBPEE (12.65 %) as 14.46%. The HPLC results gave TFCs for ABPHE (2.82 %) and ABPEE (3.27 %) as 6.09 % whereas IBPHE (0.37 %) and IBPEE (3.76 %) as 4.13 %. There was no statistically significant difference in the TFCs of the South-east propolis and that of the South-west propolis ($p > 0.10$) from the two methods.Our findings indicated that despite variability in the concentrations of flavones, flavonols and flavanones contents in the two samples collected from two regions in Nigeria, their total flavonoid contents were comparable to propolis samples collected from China and Rome, Italy. The two propolis samples from South-east and South-west are endowed with flavonoids and can be exploited for pharmaceutical uses.

Keywords: propolis, essential oils, flavonoids, GC-MS, HPLC, Nigeria.

INTRODUCTION

The chemical composition of propolis, a natural resinous honey bee product is very complex. It is interesting to know that people identify with honey more than propolis produced by same insect, bee and as such pay less attention to such a pharmacologically endowed product. A significant number of papers dealing with propolis chemistry have been published and this led to the understanding that chemical composition of propolis is highly variable and depends on the local flora at the site of collection (Marcucci, 1995 and Bankova *et al*., 2000). The distinct chemistry of propolis from different origins leads to the expectation that the biological properties of different propolis samples will be dissimilar. However, it is a fact that propolis serves as a functional defense measure of bees against microorganisms, and has antibacterial and antifungal activity (Bankova, 2005). Kujumgiev *et al*. (1999) compared the antimicrobial (antibacterial, antifungal and antiviral) activity and chemical composition of propolis from diverse geographic origins. It was demonstrated that in spite of the great differences in the chemical composition of propolis from different geographic locations, all samples exhibited significant antibacterial and antifungal activity.

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This study and similar studies led to the believe that, although different propolis samples from different geographic and climatic zones may show different chemical composition, the biological activity of propolis, especially its activity against microorganisms is expected to be always present in samples from different geographic and climatic zones.

Due to phyto-geographic dependence of chemical composition of propolis, it has been a rich source of wide variety of secondary metabolites. More than 200 compounds have been identified in propolis from different sources (Ibrahim, 2001). Research has shown that the major biologically active constituents of propolis from European and North American sources are flavones, flavanones and flavanols (Garcia-Viguera *et al*., 1993). Besides these flavonoids, alcohols, aldehydes, aliphatic and aromatic acids, chalcones, terpenoids, steroids, sugars and amino acids have also been identified in propolis (Greenaway *et al*., 1991). These compounds are believed to be responsible for the biological activities of propolis. Among these many compounds identified in propolis, flavonoids are considered to be responsible for the main biological activities. Flavonoids are water soluble polyphenolic molecules containing at least 15 carbon atoms. They are widely distributed in the leaves, seeds, bark and flowers of plants. They are found in most plant materials. The flavonoids consist of 6 major subgroups: chalcones, flavones, flavonols, flavanones, anthocyanins and isoflavonoids. Rutin, quercetin and naringenin are three important flavonoids of documented pharmaceutical importance. They belong to the subgroups flavones, flavonols and flavanones respectively. Flavonoids are increasingly becoming very popular because they have many health promoting effects attributed strongly to their antioxidant and chelating abilities. Therefore, determination of flavonoids in propolis is regarded as an important quality parameter. The objectives of this study were to identify, isolate, characterize and compare the bioactive compounds of the propolis samples as well as to quantitate and compare their flavonoid contents.

MATERIALS AND METHODS

Propolis collection

South Eastern Nigeria propolis was collected from a Honeybee cultivator in Isi-Ala Ahaba, Ikwuano Local Government Area of Abia State while South Western Nigeria propolis was collected from Forest Research Institute of Nigeria (FRIN), Jericho Hill, Ibadan, Oyo State. The propolis were cut into smaller sizes and stored at room temperature for use.

Reagents and Equipment

Rutin, quercetin, naringenin, aluminium chloride, and potassium acetate were obtained from Sigma–Aldrich. n-hexane, ethanol, methanol, hydrochloric acid, and orthophosphoric acid were of analytical grade. Water bath (Jubalo TW20), analytical weighing balance (Mettler Toledo AL- 204), Rotary evaporator (Heidolph laborate 4010 digital), Agilent Technologies GC-MS (model 6890), T90+ UV/VIS Spectrometer (PG Instruments Ltd), and Agilent High-performance liquid chromatograph.

Extraction of Sample material

Propolis samples were sequentially extracted with non-polar and polar solvents to obtain extracts with different compositions. South-eastern Nigeria propolis (50 g) was extracted with 500 ml of n-hexane at room temperature for 9 days while periodically agitating the content. The extracts were filtered using Whatman No. 1 filter paper, the filtrates were bulk collected and concentrated *in vacuo* on a rotary evaporator to yield a dark brown waxy extract (17.55 g) referred to as Abia propolis hexane extract (ABPHE). The propolis residues were collected and further extracted with 500 ml of 99.8 % ethanol as done with n-hexane. The ethanol extracts were filtered, bulk collected and concentrated to yield a light brown gummy extract (12.33 g) referred to as Abia propolis ethanol extract (ABPEE). Same was done to South-west Nigeria propolis to obtained a yellowish-brown gummy extract (10.68 g) referred to as Ibadan propolis hexane extract (IBPHE) and a brown waxy extract (7.80 g) referred to as Ibadan propolis ethanol extract (IBPEE).

Phytochemical screening

Phytochemical screening was carried out on the ABPHE, ABPEE, IBPHE and IBPEE. Portions of the four extracts were reconstituted and subjected to phytochemical screening for flavonoids, alkaloids, steroidal nucleus, saponins, phenols, reducing sugars and tannins using standard methods as described by Evans (1996) and Silva *et al*. (1998).

Gas chromatography-Mass spectroscopy (GC-MS) analysis

GC-MS analysis was carried out on aliquot of ABPHE and IBPHE using a Gas chromatograph hyphenated with mass spectrometer. The analysis was carried out using total ion monitoring mode. Ions were obtained by electron ionization mode. Helium was used as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. Acquisition time for ABPHE and IBPHE were 18 mins and 15 mins respectively and their molecular ions (mass range: 40–500 m z^{-1}) were

monitored for identification. The relative percentage of the constituents was expressed as percentage by peak area normalization. Identification of components was based on their retention indices, relative to a series of recorded compounds on the capillary column under the same operating conditions and computer matching with the GC–MS spectra from the installed NIST Mass Spectral Library.

Quantitative Analysis

Ultraviolet/visible (UV/VIS) analysis

Preparation of propolis solutions

Propolis solutions of ABPHE, ABPEE, IBPHE and IBPEE were prepared as follows. 0.5 g of concentrated propolis extracts were reconstituted with 25 ml of 80% ethanol (v/v) for 24 h at room temperature and filtered. The filtrates were subjected to the determination as follows;

Determination of flavones and flavonols

Flavones and flavonols in propolis were expressed as rutin and quercetin equivalent. Rutin and quercetin were used to make the calibration plots (standard solutions of 10, 20, 30, 40 and 50 μg/ml of rutin and quercetin in 80% ethanol (V/V)). To the standard solutions of rutin, quercetin and extracts (0.5 ml) were added 1.5 ml 95% ethanol (V/V), 0.1 ml 10% aluminium chloride (m/V), 0.1 ml of 1 mol/L potassium acetate and 2.8 ml water. The volume of 10 % (m/V) aluminium chloride was substituted by the same volume of distilled water in the blank (Ivan *et al*., 2004). After incubation at room temperature for 30 mins, the instrument was calibrated with the blank, the standard solutions were scanned and the absorbances were measured at 272 nm and 446 nm for rutin and quercetin respectively on UV/VIS spectrophotometer. The absorbances of the extracts were slotted into the regression equations obtained from the calibration plots to evaluate the concentrations of flavones and flavonols.

HPLC analysis

Instrumentation and Chromatographic conditions

The HPLC analysis was done on an Agilent® HPLC apparatus, equipped with model pump (Agilent series 1100), a degasser, an injection valve with a 20 µL loop and an ultraviolet detector. Separation was attained on C 8 column(Hypersil)(250 X 4.6 mm, 5 µm) and using mobile phase consisting of 0.3 g/L solution of orthophosphoric acid adjusted to pH 3.0 as solvent A and methanol as solvent B. The elution was carried out with flow rate of 1.5 mL/min in a gradient manner; A: B; 60 : 40 (0 – 20 mins); 45:55 (20-21 mins) and 0: 100 (21-25 mins). All chromatographic experiments were performed at room temperature (25 ^{o}C ± 2 ^{o}C) and detector set to 370 nm.

Preparation of standard solutions for calibration plot

Standard stock solutions of rutin, quercetin and naringenin were prepared by accurately weighing 10 mg of each standard into 50 ml volumetric flask and dissolved in a mixture of 20 ml of methanol; 15 ml dilute hydrochloric acid and 5 ml of water and the content made up to 50 ml with same mixed solvents. Serial dilutions of the stock solutions were made to obtain working solutions of concentration in the range of 10-100 µg/ml which were used to obtain the calibration plots.

Preparation of propolis solution

Exactly 200 mg of ABPHE, ABPEE, IBPHE and IBPEE were dissolved in 20 ml of methanol separately and the resulting solutions were filtered using filter paper (Whatman No.1). Subsequently, 15 ml dilute hydrochloric acid and 5 ml of water were added, and the solutions were made up to 50 ml with methanol. 10 ml of these solutions were transferred into vials. The vials were heated on a water-bath for 25 mins (European Pharmacopoeia, 2012) and allowed to cool at room temperature. Each sample (20 µl) was injected into the HPLC system.

Determination of flavones, flavonols and flavanones

Flavones, flavonols and flavanones in propolis were expressed as rutin, quercetin and naringenin equivalent. The peak areas of the extracts were slotted into the regression equations obtained from the calibration plots to evaluate the concentrations of flavones, flavonols and flavanones.

Statistical Analysis

Statistical analysis was evaluated using Student's t-test at 95 % confidence level. P-value of <0.05 was considered to be statistically significant.

RESULTS

Results of phytochemical screening

Results of phytochemical screening of ABPHE, ABPEE, IBPHE and IBPEE showed the presence of alkaloids, Flavonoids , saponins, reducing sugars, cardiac glycosides, tannins and phenols. No tannin was confirmed present in ABPHE and IBPEE

Results of GC-MS analysis

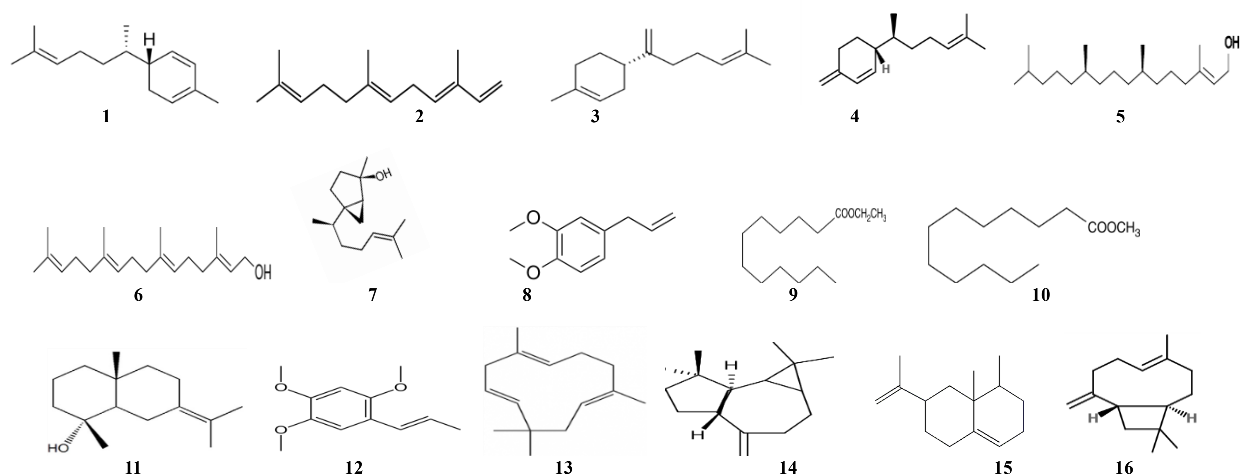
Table 1: Some compounds in ABPHE

Peak#	R. Time (mins)	Area %	Compound
1	8.56	30.78	Zingiberene
2	8.64	7.96	Alpha-farnesene
3	8.72	16.94	Beta-bisabolene
4	8.92	16.56	Beta-sesquiphellandrene
5	16.29	9.59	Phytol
6	17.53	18.16	Trans-geranylgeraniol

Retention time (R.Time, mins)

Table 2: Some compounds in IBPHE

Peak#	R. Time (mins)	Area%	Name of compound
1	7.79	12.29	Beta-caryophellene
2	8.20	4.28	Humulene
3	8.59	17.22	Trans-sesquisabinene hydrate
4	8.71	13.19	Eremophilene
5	8.79	4.30	Lauric acid methyl ester
6	8.92	4.85	Beta-sesquiphellandrene
7	9.62	13.30	Lauric acid ethyl ester
8	9.70	2.31	Spathulenol
9	9.84	2.16	Methoxyeugenol
10	10.36	4.69	Asarone
11	10.59	21.42	Juniper camphor



Figures 1: Structures of identified compounds; (**1**) zingiberene, (**2**) alpha-farnesene, (**3**) beta-bisabolene, (**4**) beta-sesquiphellandrene, (**5**) phytol, (**6**) trans-geranylgeraniol, (**7**) trans-sesquisabinene hydrate, (**8**) methoxyeugenol, (**9**) lauric acid ethyl ester, (**10**) lauric acid methyl ester, (**11**) juniper camphor, (**12**) asarone, (**13**) humulene, (**14**) spathulenol, (**15**) eremophilene, and (**16**) beta-caryophellene.

Quantitative Analysis Results

Results of Ultraviolet/visible (UV/VIS) analysis

Table 3: Physical properties and UV/Vis flavonoid contents of the propolis extracts

Extract type	Appearance	Colour	Smell	% Flavones*	% Flavonols**
ABPHE	Waxy	Dark brown	Aromatic	2.13	3.46
ABPEE	Gummy	Light brown	Aromatic	1.93	1.18
IBPHE	Gummy	Yellowish-brown	Aromatic	0.93	0.88
IBPEE	Waxy	Brown	Aromatic	9.43	3.22

Expressed as *rutin equivalent, ** quercetin equivalent

Results of HPLC analysis

Results of HPLC analysis of ABPEE, ABPEE, IBPHE and IBPEE, expressed as rutin, quercetin and naringenin equivalent.

Table 4: HPLC percentage (%) flavonoid contents of the propolis extracts

Extract type	% Flavones*	% Flavonols**	% Flavanones***
ABPHE	0.86	0.31	1.65
ABPEE	Not detected	0.02	3.25
IBPHE	0.006	0.09	0.27
IBPEE	2.48	0.76	0.53

Expressed as *rutin equivalent, ** quercetin equivalent and *** naringenin equivalent

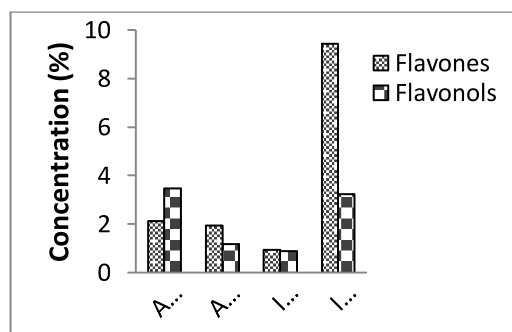


Figure 2: Percentage (%) Flavonoid contents of ABPHE, ABPEE, IBPHE, and IBPEE by UV/VIS spectrometry.

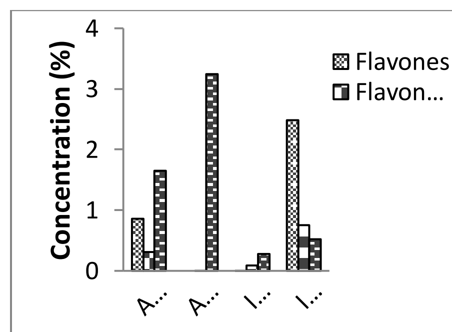


Figure 3: Percentage (%) Flavonoid contents of ABPHE, ABPEE, IBPHE and IBPEE by HPLC assay.

DISCUSSION

In spite of the phyto-geographic dependence of the chemical composition of propolis, flavonoids are important secondary metabolites ubiquitous in propolis hence their identification and quantitation are regarded as important parameters for assessing the quality of propolis samples. Two propolis samples collected from two different regions in Nigeria; South East, Abia and South West, Ibadan were investigated for their chemical compositions and flavonoid contents. To obtain extracts with different compositions, the propolis samples were extracted with n-hexane and subsequently with 96 % ethanol to obtain Abia propolis hexane extract (ABPHE), Abia propolis ethanol extract (ABPEE), Ibadan propolis hexane extract (IBPHE) and Ibadan propolis ethanol extract (IBPEE). It was observed that phyto-geographic differences have little influence on the physical properties of the extracts. All the extracts had aromatic smell, were waxy or gummy and of different shades in brown colour as shown in Table 3.

The phytochemical analysis indicated the presence of flavonoids, alkaloids, phenols, steroidal nucleus, saponins and reducing sugars in all the extracts; while tannins were detected only in ABPEE and IBPEE. The importance of these phytochemicals in phytomedicines has been documented. Flavonoids as a group and the secondary metabolite of interest in this research study have long been recognized to possess many biological activities, some of which are; anti- inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities (Tapas *et al*., 2008). Their free radical scavenging property particularly makes them useful in management of inflammatory diseases e.g. tumour and oxidative stress- related diseases. Also compounds with phenolic groups have been known to have antiseptic and antioxidant properties (Evans, 2002). Therefore, the identification of these phytochemicals in these propolis samples corroborates their uses for prevention of parasites as well as for inhibition of fungal and bacterial growth in the hives of these bees. These findings also substantiate the uses of propolis for treatment of ailments traditionally, as food supplements and in preparation of cosmetic products.

Propolis is reported to contain approximately 10 % essential oils (Bankova *et al*., 2000). The n-hexane extracts were subjected to GC-MS analysis to investigate their essential oils components. ABPHE was identified to contain zingiberene, alpha-farnesene, beta-bisabolene, beta-sesquiphellandrene, phytol and trans-geranylgeraniol as shown in Table 1; Figure 1. This is an indication of the flora in the area from which this propolis was collected. Zingiberene is a monocyclic sesquiterpene hydrocarbon and one of the major constituents of the oil of ginger (*Zingiber officinale*), from which it gets its name. It can contribute up to 30% of the essential oils in ginger rhizomes and it gives ginger its distinct aroma and flavoring. Zingiberene is a natural antioxidant and serves as antiviral and antifertility agent (Wang *et al*., 2012). Another compound identified is; α-farnesene, an acyclic sesquiterpene hydrocarbon and a member of a group of six closely related sesquiterpenes; farnesenes. It differs from its isomer; β-farnesene by the location of one double bond and also known to exist as four stereoisomers. Two of the α-farnesene stereoisomers are reported to occur in nature. (E, E)-α-Farnesene is the most common isomer, commonly found in the coating of apples and other fruits and it is responsible for the characteristic odour in green apple. (Z, E)-α-Farnesene has also been isolated from the oil of perilla. Both isomers are also insect semiochemicals and they act as alarm pheromones in termites (Šobotník *et al*., 2008) or food attractants for the apple tree pest, the codling moth (Hern & Dorn, 1999).

Another interesting compound identified is β-bisabolene, a sesquiterpenes identified and isolated in a wide variety of plants including lemon, cardamom, cubeb, cotton and oregano. They have excellent functions such as pheromones, flavor and fragrance. Its isomer, γ-Bisabolene, increases the levels of tumor suppressor p53, decreases the expression of metastasis promoter Snail1, inhibits the proliferation of tumor-initiating cells, suppresses epithelial-to-mesenchymal transition and the progression of metastasis, as well as supports tumor relapse-free survival in breast cancer patients (GBMD, 2015).

Phytol, also an acyclic diterpene alcohol has been isolated and can be used as a precursor for the manufacture of synthetic forms of vitamin E (Netscher, 2007) and vitamin K1 (Daines, 2003). Insects, such as the sumac flea beetle, are reported to use phytol and its metabolites (e.g. phytanic acid) from host plants as chemical deterrents against predation (Vencl *et al*., 1998). It has bactericidal activity against *S. aureus* (Inoue *et al*., 2005) and can also be used commercially in the fragrance and cosmetic industries (McGinty *et al*., 2010).

Last but not the least compound identified from ABPHE is Trans-geranylgeraniol, a Trans form of geranylgeraniol and a diterpene alcohol which plays a role in several important biological processes. It is an intermediate in the biosynthesis of other diterpenes, vitamins E, K and is also used in the post-translational modification known as geranylgeranylation. Geranylgeraniol is a pheromone for bumblebees and a variety of other insects and a potent inhibitor of *Mycobacterium tuberculosis in vitro* (Vik *et al*., 2007) and *S. aureus* (Inoue *et al*., 2005).

Furthermore, beta-sesquiphellandrene, trans-sesquisabinene hydrate, methoxyeugenol, lauric acid ethyl ester, lauric acid methyl ester, juniper camphor, asarone, humulene, spathulenol, eremophilene, and beta-caryophellene as shown in Table 2 were identified in IBPHE.

Beta-caryophyllene, a sesquiterpene widely distributed in plants have several excellent biological activities attributed to it such as anti-inflammatory, antibiotic, antioxidant, anticarcinogenic and as local anaesthetic. Previous studies showed that a non-cytotoxic concentration of beta-caryophyllene significantly increased the anticancer activity of alpha-humulene and isocaryophyllene on MCF-7 cells (Legault & Pichette, 2007). On the other hand, Humulene, also known as α-humulene or α-caryophyllene is an isomer of β-caryophyllene, and the two are often found together as a mixture in many

aromatic plants. It produces similar effects to dexamethasone, and was found to decrease the edema formation caused by histamine injections (Passosa & Fernandesa, 2007). It is also known to produce inhibitory effects on tumor necrosis factor-α (TNFα) and interleukin-1 β (IL1B) generation in carrageenan-injected rats (Fernandes *et al*., 2007) and has been found to have anticarcinogenic activity on MCF-7 cells. It also inhibited cell growth by about 50% alone and 75% with beta-caryophyllene (Legault & Pichette, 2007).

Spathulenol, another compound identified from IBPHE is a sesquiterpene alcohol and has been found to produce immunoinhibitory effect. It showed the capacity to inhibit proliferation in the lymphocytes and to induce apoptosis in these cells possibly through a caspase-3 independent pathway (Ziaei *et al*., 2010).

Flavonoids contents were determined in the two propolis samples and four extracts (ABPHE, ABPEE, IBPHE and IBPEE) using two analytical techniques; Ultraviolet/visible (UV/VIS) Spectrometry and High-performance liquid chromatography (HPLC) assay. The UV/VIS flavonoids content assay was done using calibration plot method and the flavones and flavonols in the samples expressed as rutin and quercetin equivalents as shown in the results, figure 2. The determined flavonoid contents of ABPHE were 2.13 % of flavones and 3.46 % of flavonols while those for ABPEE were 1.93 % of flavones and 1.18 % of flavonols. Thus, the Total determined Flavonoid Contents (TFCs) were 5.59 % of ABPHE and 3.11 % of ABPEE making 8.70 % of the South-eastern propolis extracts. In addition, from IBPHE, flavones (0.93 %) and flavonols (0.88 %); IBPEE, flavones (9.43%) and flavonols (3.22 %) were also calculated. Thus, the TFCs were 1.81% of IBPHE and 12.65 % of IBPEE making 14.46% of the South-western propolis extracts.

The HPLC flavonoids determination was done using three standards, rutin for determination of flavones, quercetin for flavonols, and naringenin for flavanones. The results are shown on Table 4. In ABPHE, 0.86 % of flavones, 0.31 % of flavonols and 1.65 % of flavanones were calculated while in ABPEE, 0.02 % of flavonols and 3.25 % of flavanones were also calculated as shown in Figure 3. Flavones were not detected in ABPEE. Thus, the TFCs were 2.82 % of ABPHE and 3.27 % of ABPEE making 6.09 % of the South-Eastern propolis extracts. Furthermore, in the IBPHE, 0.006 % of flavones, 0.09 % of flavonols and 0.27 % of flavanones were calculated, while in the IBPEE, 2.48% of flavones, 0.76 % of flavonols and 0.53 % of flavanones were also calculated. Thus, the TFCs were 0.37 % of IBPHE and 3.76 % of IBPEE making 4.13% of the South-western propolis extracts.

There were no statistically significant differences in the TFCs of the South-east propolis and that of the South-west propolis ($p > 0.10$) from both methods, although the UV/VIS flavonoid yield is more than the HPLC method.

Despite the fact it is well known that, not all propolis samples are the same; as chemical composition of propolis is highly variable and depends on the local flora or the vegetation available to bees to create the propolis (Marcucci, 1995 and Bankova *et al*., 2000). However, the results of this showed that despite the variability in the concentrations of flavones, flavonols and flavanones in the propolis samples, the total flavonoid concentrations were comparable. This is an indication of comparable quality and biological activities. Furthermore the obtained TFCs show that these samples are fairly rich in flavonoids and corroborate that flavonoids are one of the main group of biologically active chemical constituents of propolis and may serve for estimation of propolis quality. These findings can also be compared to the flavonoid content of propolis Brazil of botanical origin *Baccharis sp*. which presents 0.77-2.69 % of flavone/flavonols (Woisky & Salatino, 1998) and 0.15-0.65 % of flavone/flavonols (Silva *et al*., 2006). Furthermore, findings from Nigerian propolis can be comparable to similar studies of Chang *et al*., 2002 who studied the composition of Chinese propolis (≈6% flavone/flavonols, ≈11% flavanones/dihydroflavonols), and Taiwanese propolis (≈3% flavone/flavonols, ≈20% flavanones/dihydroflavonols), Our investigations showed that the South-eastern Nigerian propolis flavonoids concentration (2.13 % flavones/3.46 % flavonols) can be compared to Romanian propolis with reported amount of ≈4 % flavone/flavonols, ≈6 % flavanones/dihydroflavonols and about 10 % total flavonoids (Mărghitas *et al*, 2007) while the South-west Nigerian propolis (14.46 %) is similar in flavonoids concentration to Chinese propolis (≈6 % flavone/flavonols, ≈11% flavanones/dihydroflavonols and about 17 % total flavonoids.

CONCLUSION

This study provides results of comparative qualitative and quantitative chemical studies carried out on a bee product, propolis collected from the South-east and South-western part of Nigerian. Four sesquiterpenes and two diterpene alcohols were identified in the South-eastern Nigerian propolis sample while five sesquiterpenes, two sesquiterpenoids, two esters, one benzenoid and ether were identified in the South-western Nigerian propolis sample in addition to other

phytochemicals which justifies their ethno medicinal uses. In addition, quantitative flavonoids content determination showed that in spite of the variability in the concentrations of the flavones, flavonols and flavanones, the samples had comparable total flavonoid concentrations (TFCs) which were similar and comparable to those of propolis from other parts of the world. This study suggests further studies in investigating the influence of the differences in the concentrations of flavones, flavonols and flavanones on the pharmacological activities of these propolis samples.

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