Drug Leads Agents from Methanol Extract of Nigerian Bee (Apis mellifera) Propolis

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Drug leads agents from methanol extract of Nigerian bee (*Apis mellifera*) propolis

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ABSTRACT

Background: Propolis is a bee (*Apis mellifera)* product of plant origin with varied chemical composition depending on the ecology of the botanical origin. It has been reported in literature to possess various therapeutic effects both traditionally, clinical trial, and animal study. **Objectives:** In the present study bioactive principle in methanol extract of Nigerian bee (*A. mellifera)* propolis was determined by gas chromatography-mass spectrometry (GC/MS) study. **Materials and Methods:** The methanol extract of Nigerian bee (*A. mellifera*) propolis was characterized for its chemical composition by preliminary phytochemicals screening and GC/MS analysis using standard procedures and methods. **Results:** Phytochemical screening revealed the presence of flavonoids, saponins, alkaloids, tannins, cardiac glycosides, anthraquinones phlobatannins, and steroids while GC/MS chromatogram revealed nineteen peaks representing 60 different chemical compounds. The first compounds identified with less retention time (RT) (13.33s) were methyl tetradecanoate, tridecanoic acid, methyl ester, decanoic acid, methyl ester while squalene, all-trans-squalene, 2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-, (E,E)- and farnesol isomer a took longest RT (23.647s) to identify. Methyl 14-methylpentadecanoate, hexadecanoic acid methyl ester, methyl isoheptadecanoate, and methyl tridecanoate were the most concentrated constituent as revealed by there peak height (26.01%) while eicosanoic acid was the least concentrated (peak height 0.81%) constituent of Nigerian bee propolis. **Conclusion:** The presence of these chemical principles is an indication that methanol extract of Nigeria bee propolis, if properly screened could yield a drug of pharmaceutical importance.

KEY WORDS: Bee, gas chromatography-mass spectrometry, methanol, propolis, phytochemicals

INTRODUCTION

Over the last decades toxins and secretions from poisonous and venomous animals have been used as drugs and drug leads for treatment of numerous untreatable human ailments [1]. Leech salivary secretion exert antimicrobial agents and has been reported to be used in treatments of back pain, Snake Venom serve as anticancer, anti-diabetics and anti-hypertensive agents, secretion from cone snail *Conus magus* used in treatment of chronic pain [1], while hemolymph from African land snail has been reported for their hepatocurative effect against CCl_4 intoxicated rats [2].

Honey bees are members of genus *Apis*, and perennial insect species that can utilize nearly all habitats of the world. They have been in existence for more than 1000 decades. There are about seven species of the honey bees with a total of 44 subspecies [3]. Honeybees produce high-quality foods in the form of honey, building materials in the form of propolis, and chemical defenses inform of bee venom and propolis [4].

Propolis is a sticky honeybee resinous product produced by the honeybees to shut the cracks, and act as moisture and thermo stabilizer in the hive [5]. Bee propolis has been documented for it bacteriostatic, immunomodulatory, anti-inflammatory, anti-tumoral, anti-oxidative, hypotensive [6], hepatoprotective

and pancreato protective [7], and antibacterial properties as well as for the treatments of atherosclerosis among many other uses [8]. Recently, we also reported the hepatocurative [5], hematopoietic [9], and its toxicological effects on serum, and tissues of rats following chronic exposure [10,11].

It is worth noted that despite the high activity, propolis is very stable compounds, retaining their potency up to several years. These stable organic compounds would confer different chemical properties and could be implicated in the biological and toxicological effects of the propolis [10]. However, the chemical composition of bee propolis has been reported to be varied qualitatively and quantitatively, depending on the environmental plant ecology [12]. Since, propolis is a bee product of plant origin, thus at different geographic locations the source plants might vary with respect to the local flora [4]. Literatures have documented the chemical composition of bee propolis from a different region of the word [13-20], however, none of this study was on propolis of Nigerian origin. With the aimed of bridging the gap in knowledge, and the present study sort to evaluate Nigerian bee propolis for its chemical composition.

MATERIALS AND METHODS

Collection of Bee Propolis

Propolis material was collected from an apiary in Akure, Ondo State, Nigeria. The identity of the Propolis was authenticated by an Entomologist in the Biological Sciences Department, Federal University of Technology, Minna, Nigeria. The Propolis material was chopped into small pieces and air dried in the Shade at room temperature for 2 weeks.

Preparation of Propolis Extract

200 g of Propolis pellets were percolated in 1600 mL of absolute methanol and subsequently allowed to stand in the shade for 48 h before filtration, using filter paper (Whatman No. 1). The extract concentrate was stored in air-tight vials in the refrigerator at 4°C, until needed for bioassay.

Phytochemical Analysis

Methanol extract from Nigeria bee propolis was screened preliminary for its phytochemical contents including flavonoids, saponins, alkaloids, tannins, cardiac glycosides, anthraquinones phlobatannins, and steroids according to the methods of Sofowora [21] as described by Lawal [22].

Gas Chromatography Mass Spectrometry (GC/MS) Analysis

The GC/MS analysis of methanol extract from Nigerian bee propolis was perform using GC-MS clarus 500 per kin Elmer system comprising an AOC-20i auto sampler. "The instrument is equipped with a VF 5 ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 μm film thickness." The temperatures employed were; column oven temperature 80°C, injection Temp 250°C at a pressure of 108.0 kPa, with total flow and column flow of 6.20 ml/min and 1.58 ml/min, respectively. The linear velocity was 46.3 cm/s and a purge flow of 3.0 ml/min. The GC program ion source and interface temperature were 200.00°C and 250.00°C, respectively, with solvent cut time of 2.50 min. The MS program starting time was 3.00 min which ended at 30.00 min with event time of 0.50 s, scan speed of 1666 μl/s, scan range 40-800 u, and an injection volume of 1 μl of the propolis extract (split ratio 10:1). The total running time of GC-MS was 30 min. The relative percentage of the extract was expressed as percentage with peak area normalization as previously reported by Lawal *et al.* [23].

Identification of the Components

Interpretation on the mass spectrum was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The fragmentation pattern spectra of the unknown components were compared with those of known components stored in the NIST library. The relative percentage amount of each bio-component was calculated by comparing its average peak area to the total area. The name, molecular weight, and structure of the components of the test materials were ascertained.

RESULTS

Table 1 shows the phytochemical composition of methanol extract from Nigeria bee propolis. The result revealed the presence of flavonoids, saponins, alkaloids, tannins, cardiac

Table 1: Phytochemical compositions of methanol extract of Nigerian bee propolis

Phytochemicals	Inference
Alkaloids	+
Flavonoids	+
Saponins	+
Alkaloids	+
Steroids	+
Anthraquinone	+
Tannins	+
Glycosides	+
Phlobatannins	+

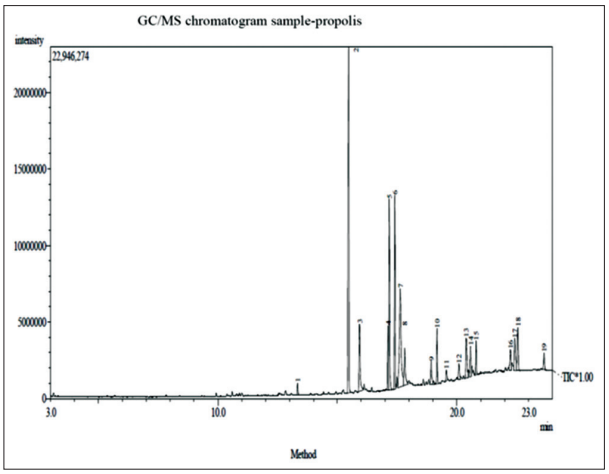

Figure 1: Gas chromatography mass spectrometry chromatogram of methanol extract of Nigerian bee (*Apis mellifera*) propolis

Table 2: Bio-active components identified in methanol extract of Nigerian bee (*Apis mellifera*) propolis using GCMS

Peak no	RT	Compound	MF	MW (g/mol)	Peak area (%)	Peak height (%)
1	13.333	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	0.63	0.89
1	13.333	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	228	0.63	0.89
1	13.333	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	186	0.63	0.89
2	15.468	Methyl 14-methylpentadecanoate	$C_{17}H_{34}O_2$	270	22.39	26.01
2	15.468	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	22.39	26.01
2	15.468	Methyl isoheptadecanoate	$C_{18}H_{36}O_2$	284	22.39	26.01
2	15.468	Methyl tridecanoate	$C_{14}H_{28}O_2$	228	22.39	26.01
3	15.930	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.97	5.04
3	15.930	Octadecanoic acid	$C_{18}H_{36}O_2$	284	5.97	5.04
3	15.930	Pentadecanoic acid	$C_{15}H_{30}O_4$	242	5.97	5.04
3	15.930	Heptadecanoic acid	$C_{17}H_{34}O_2$	270	5.97	5.04
4	17.118	9,12-Octadecadienoic acid, methyl ester, (E, E)	$C_{19}H_{34}O_2$	294	3.79	4.80
4	17.118	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322	3.79	4.80
4	17.118	7,10-Hexadecadienoic acid, methyl ester	$C_{17}H_{30}O_2$	266	3.79	4.80
4	17.118	9,12-Hexadecadienoic acid, methyl ester	$C_{17}H_{30}O_2$	266	3.79	4.80
5	17.174	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	11.53	14.33
5	17.174	13,16-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	11.53	14.33
5	17.174	6-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	11.53	14.33
5	17.174	7-Hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	268	11.53	14.33
5	17.174	9,12-Octadecadienoyl chloride, (Z, Z)-	$C_{18}H_{31}ClO$	298	11.53	14.33
6	17.398	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	11.18	14.61
6	17.398	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284	11.18	14.61
7	17.630	Oleic Acid	$C_{18}H_{34}O_2$	282	14.70	7.38
7	17.630	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	14.70	7.38
7	17.630	Erucic acid	$C_{22}H_{42}O_2$	338	14.70	7.38
7	17.630	Z-10-Pentadecen-1-ol	$C_{14}H_{26}O_2$	226	14.70	7.38
7	17.630	E-9-Tetradecenoic acid	$C_{14}H_{26}O_2$	226	14.70	7.38
8	17.817	Aqua Cera	$C_{22}H_{44}O_4$	372	3.68	2.81
9	18.925	2-Methylnonadecane	$C_{20}H_{42}$	282	2.09	1.72
9	18.925	n-Cetane	$C_{16}H_{34}$	226	2.09	1.72
9	18.925	2-Methyleicosane	$C_{21}H_{44}$	296	2.09	1.72
9	18.925	2,6,10,14-Tetramethylheptadecane	$C_{21}H_{44}$	296	2.09	1.72
9	18.925	7-n-Hexyleicosane	$C_{26}H_{54}$	366	2.09	1.72
10	19.168	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326	3.40	4.17
10	19.168	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	340	3.40	4.17
10	19.168	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354	3.40	4.17
10	19.168	Methyl 15-methylhexadecanoate	$C_{18}H_{36}O_2$	284	3.40	4.17
11	19.558	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.81	0.81
12	20.085	Chalcone, 2′,6′-dihydroxy-4′-methoxy	$C_{16}H_{14}O_4$	270	1.40	1.17
13	20.386	13-Tetradece-11-yn-1-ol	$C_{14}H_{24}O$	208	3.84	3.02
13	20.386	2-Octylcyclopropene-1-heptanol	$C_{18}H_{34}O$	226	3.84	3.02
13	20.386	(6Z,9Z)-6,9-Pentadecadien-1-ol	$C_{15}H_{28}O$	224	3.84	3.02
13	20.386	2-(7-Octenyl)oxirane	$C_{10}H_{18}O$	154	3.84	3.02
14	20.575	Eicosane	$C_{20}H_{42}$	282	2.10	2.27
14	20.575	Tetracosane	$C_{24}H_{50}$	338	2.10	2.27
14	20.575	Pentadecane	$C_{15}H_{32}$	212	2.10	2.27
15	20.804	Heptacosanoic acid, methyl ester	$C_{28}H_{56}O_2$	424	2.12	2.54
16	22.245	2,6,10-Trimethyltetradecane	$C_{17}H_{36}$	240	1.87	1.55
16	22.245	Octacosane	$C_{28}H_{58}$	394	1.87	1.55
16	22.245	Heptacosane	$C_{27}H_{56}$	380	1.87	1.55
16	22.245	Tetratetracontane	$C_{44}H_{90}$	618	1.87	1.55
16	22.245	11-Butyldocosane	$C_{26}H_{54}$	366	1.87	1.55
17	22.421	Phenol, 3-pentadecyl	$C_{21}H_{36}O$	304	3.76	2.41
17	22.421	Benzene, 1-methyl-4-(2-pentenyloxy)	$C_{12}H_{16}O$	176	3.76	2.41
17	22.421	Oxalic acid, dodecyl 2-methylphenyl ester	$C_{21}H_{30}O_4$	348	3.76	2.41
18	22.547	Triacontanoic acid, methyl ester	$C_{31}H_{62}O_2$	466	3.35	3.25
19	23.647	Squalene	$C_{30}H_{50}$	410	1.36	1.23
19	23.647	All-trans-Squalene	$C_{30}H_{50}$	410	1.36	1.23
19	23.647	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (E, E)-	$C_{15}H_{26}O$	222	1.36	1.23
19	23.647	Farnesol isomers	$C_{15}H_{26}O$	222	1.36	1.23

MF: Molecular formula, MW: Molecular weight, GCMS: Gas chromatography mass spectrometry

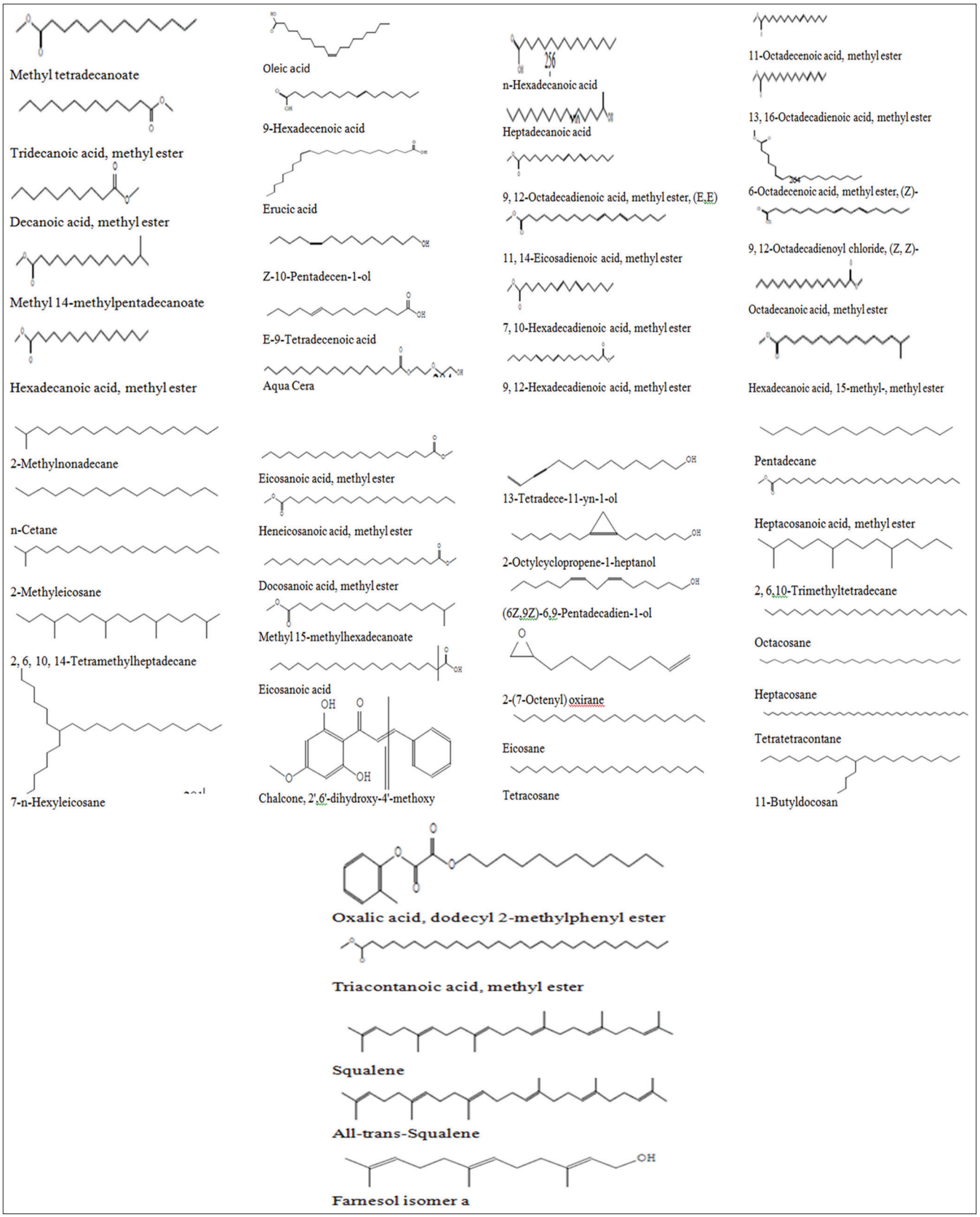

Figure 2: Structures of some chemical compounds identified from methanol extract of Nigerian bee propolis by gas chromatography mass spectrometry

glycosides, anthraquinones phlobatannins, and steroids in methanol extract of Nigeria bee propolis. The GC/MS chromatogram revealed nineteen peaks [Figure 1] representing 60 different chemical compounds. The chemical compounds with their molecular formula, molecular weight, retention time (RT), and % peak area are presented in Table 2 while the chemical structures as revealed by the GC/MS were shown in Figure 2.

DISCUSSIONS

The use of and search for, plant-derived drugs have accelerated in recent years. Biochemist, pharmacologists, botanists, microbiologists, and natural-products chemists globally are continuously in search of natural-products for bioactive phytoconstituents that could serve as a drug lead for treatment of various human ailments [24].

Phytochemicals elicit varied pharmacological and biochemical effects when administered by animals [25]. The present study revealed the present of various important phytochemicals in methanol extract from Nigerian bee propolis [Table 1]. Flavonoids are phenolic compounds with important roles in scavenging free radicals and thus play vital roles in preventing oxidative stress associated disorder. Alkaloids also possess a significant pharmacological property [26]. Tannin is non-toxic compounds that are known for there antidiarrheal, antifungal, antihemorrhoidal, and antioxidant agents [27]. Saponin has also been reported for there anti-inflammatory, cardiac depressant, and hypercholesterolemic [25].

GC/MS chromatogram of methanol extracts of Nigerian bee (*Apis mellifera)* Propolis shows nineteen peaks [Figure 1]. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of RT. The heights of the peak indicate the relative concentrations of the components present in the sample. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds are giving rise to the appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library [28].

When the mass spectra of the constituents from the propolis were compared with the NIST library, a total of 60 different chemical compounds were characterized and identified. The first compounds identified with less RT (13.33s) were methyl tetradecanoate, tridecanoic acid, methyl ester, decanoic acid, methyl ester while squalene, all-trans-squalene, 2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-, (E,E)- and Farnesol isomer a [Figure 2] took longest RT (23.647s) to identify.

Methyl 14-methylpentadecanoate, hexadecanoic acid methyl ester, methyl isoheptadecanoate, and methyl tridecanoate were the most concentrated constituent as revealed by there peak height (26.01%) while eicosanoic acid was the least concentrated (peak height 0.81%) constituent of Nigerian bee propolis. The presence of these chemical principles is an indication that in methanol extract of Nigeria bee propolis if properly screened could yield a drug of pharmaceutical importance.

CONCLUSIONS

In the present study 60 compounds have been identified. The presence of various bioactive principles in Nigerian Bee propolis extract is an indication that Nigerian propolis extract, if properly screened could yield a drug of pharmacological significance. However, the isolation of individual constituents and subjecting it to biological activity will be of medical significant.

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