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GC-MS Analysis of the Volatile Constituents and Antioxidant Activity of the Crude Honey Residue from Takum Local Government Area of Taraba State, Nigeria

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ABSTRACT: The GC-MS analysis of the volatile constituents of crude honey residue (VCHR) and its *in vitro* antioxidant study of the crude honey residue (CHR) has been carried out. Hydrodistillation technique was employed in the extraction of the VCHR. The various candidates present in the extract of crude honey residue (ECHR) were identified using the gas chromatography-mass spectrometry (GC-MS) instrumental method of analysis. The ECHR was investigated for *in vitro* antioxidant study by exploring the free radical scavenging potential via the 2,2- diphenyl-1- picrylhydrazyl, DPPH technique. A total of twenty-one (21) compounds were identified from the GC-MS analysis of ECHR with humulene (10.09%), α–methyl mannofuranoside (4.77%), 4,6-di-O-methyl-α-D-galactoside (13.25%), n-hexadecanoic acid (7.33%) and 9-octadecenoic acid (5.79%) as major constituents. The antioxidant study of ECHR witnessed an increasing activity with a free radical scavenging potential range of 4.16±0.01 to 16.47±0.12 as the concentration increased. The present study has shown the GC-MS analysis of the volatile constituents and its *in vitro* antioxidant activity of crude honey residue from Takum Local Government Area of Taraba State, Nigeria.

Keywords: GC-MS, crude honey residue, volatile constituents, antioxidant activity.

Introduction

Emerging threats of strains of infectious diseases and their rapid evolving adaptive nature have necessitated constant research and development in complementary medicines. This seeks to alienate the community challenge being faced by modern or scientific therapeutic protocols. The need for alternate medicines is aimed at exploring nature's depot of medicinal substances for a better wellbeing.

Crude honey residue, CHR, is the residual component of a strained crude honey. Medicinal values of crude honey and its residue have known phytochemical composition such as: alkaloids, flavonoids, phenol, terpenoids, steroids, saponins and essential oils (Hamilton -Amachree and Odokwo, 2022; Odokwo and Salawu, 2021; Alvarez-Suarez *et al*., 2010; Jose *et al*., 2010; Turkmen *et al*., 2006; Johnson *et al*., 2005; Tonks *et al*., 2003; Al-Mamary *et al*., 2002) which vary from one wild to another due to vegetative factors (Odokwo and Salawu, 2021; Abel and Banjo, 2012; Bankova *et al*., 2000).

Therapeutically, crude honey has been reported to be potent in the treatments of burns and bruises (Medhi *et al*., 2008), gastrointestinal (Ezz El-Arab *et al*., 2006), cardiac and hepatic (Rakha *et al*., 2008; Eteraf-Oskouei and Najafi, 2013) and oral hygiene (Eteraf-Oskouei and Najafi, 2013; *Medhi et al*., 2008). *In vitro* studies have shown the antioxidant and anti-infective studies of both the crude honey and its residues (Odokwo and Salawu, 2021; Eteraf-Oskouei and Najafi, 2013; Küç ük *et al*., 2007).

The present study is designed to investigate the volatile constituents and *in vitro* antioxidant study of crude honey residue. This is intended with the view of establishing volatile phytoconstitution profile of the crude honey residue and its free radical scavenging spree as basis for its nutraceutical potential.

Materials and methods

Sample collection: The crude honey was collected from the wild in Takum Local Government Area of Taraba State, Nigeria. The crude honey was extracted from its honeycomb into a sterile glass container and stored at 4 °C in the dark prior to analysis.
Reagents: The chemicals and reagents: N-hexane, anhydrous sodium sulphate, 2,2-diphenyl-1-picrylhydrazyl, methanol, ascorbic acid were all of analytical grade.
Extraction of volatile constituents: Crude honey residue (CHR) was obtained by straining off the brownish honey using sieving material. The standard method of hydrodistillation was employed in the extraction of the volatile constituents of crude honey residue (VCHR). The extraction lasted for two (2) hours. The extract of crude honey residue (ECHR) was collected over hexane, a non-polar organic solvent, dried over anhydrous sodium sulphate, Na_2SO_4 and stored in a sterile sealed vial below room temperature (Odokwo and Onifade, 2020).
The percentage yield, %PY of the oil was calculated using the equation:

$$\%PY = \frac{W_E}{W_{CHR}} \times 100$$

where: W_E – the weight of extract in grams, W_{CHR} – the weight of crude honeybee residue in grams
Identification of volatile constituents in ECHR: The various candidates present in ECHR were isolated and characterized using a Gas Chromatography, GC system 7890A hyphenated unto a Mass Spectrometry, MSD 5975C (GC-MS) system, Agilent Technologies equipment, 7683B series, with specifications described in literature (Anifalaje and Ibok, 2020; Odokwo and Onifade, 2020).
Antioxidant activity: ECHR was probed for its *in vitro* antioxidant activity by exploring its free radical scavenging potential via the 2, 2- diphenyl-1- picrylhydrazyl, DPPH technique. A serial solution of 1 ml of ECHR was prepared using a solution of DPPH in methanol and read at 517 nm. Ascorbic acid was used as standard. The free radical scavenging potential %FP was calculated using the equation:

$$\%FP = \frac{Abs_{sd} - Abs_E}{Abs_{sd}} \times 100$$

where: %FP is free radical scavenging potential, Abs_{sd} is absorbance of standard, Abs_E is absorbance of extract.

Results and Discussion

Volatile constituents: ECHR is characterized with a unique odour, colourless at room temperature with a calculated percentage yield (w/w) of 1.89.
The GC-MS chromatogram Figure 1 revealed the presence of some volatile compounds and are presented in Table 1. Twenty-one (21) volatile compounds were isolated and identified by the GC-MS instrumental method of analysis. The major constituents include: humulene, α –methyl mannofuranoside, 4,6-di-O-methyl-α-D-galactoside, n-hexadecanoic acid and 9-octadecenoic acid. The MS spectra of some selected compounds are presented in Fig. 2-6.
The volatile compounds are phytochemicals that can be grouped into the terpenes, sugars, fatty acids, fatty acids esters and other organic groups with unique homologous series. The terpenes constituents identified are hydrocarbons with the C-15 (three (3) C-5 isoprene) unit. They are sesquiterpenes by structural composition. Humulene and its isomeric counterpart, caryophyllene belong to this group. Humulene is an established sesquiterpene with spectrum of activities ranging from antibacterial **(**Jang *et al*., 2020), anti-inflammatory (Chaves *et al*., 2008), analgesic (Fidyt *et al*., 2016) to anticancer agent (Ambroẑ *et al*., 2019; Fidyt *et al*., 2016; Legault *et al*., 2003). The sugar derivative constituents include α –methyl mannofuranoside and 4, 6-di-O-methyl-α-D-galactoside are methylated products of the six (6) membered monosaccharides, mannose and galactose. They have been implicated as antifreezing agents (Wang *et al*., 2014) and in anti-tuberculosis therapy (Maretti *et al*., 2017). The fatty acids: n-hexadecanoic acid and 9-octadecenoic acid and the fatty acid esters: hexadecanoic acid methyl ester, butynedioioc acid di-2-propenyl ester and (Z,Z)-9,12-octadecadienoic acid methyl ester have been reported to exhibit antimicrobial activities (Shaaban *et al*., 2021; Davoodbasha *et al*., 2018; Krishnaveni *et al*., 2014; Chandrasekaran *et al*., 2008).

Table 1. The volatile constituents of crude honeybee residue

Retention Time (min.)	Peak Area (%)	Identified Compounds	Molecular Formula	Molecular Weight
4.55	1.20	Octahydro-3,6-methanonaphth [2,3-b] oxirene-2,7-dione	$C_{11}H_{14}O_3$	194
7.196	1.22	Resorcinol	$C_6H_4(OH)_2$	110
9.069	2.17	Caryophyllene	$C_{15}H_{24}$	204
9.500	10.09	Humulene	$C_{15}H_{24}$	204
9.552	3.56	Decyl oxirane	$C_{12}H_{24}O$	184
10.143	0.95	2,5-bis(1,1-dimethylethyl) phenol	$C_{14}H_{22}O$	206
11.103	1.05	1-Octen-3-yne	C_8H_{12}	108
12.027	1.39	5-oxo-1-cyclopentenacetic acid	$C_6H_8O_3$	128
12.120	1.47	3-phenoxylpropionic acid	$C_9H_{10}O_3$	166
12.234	4.77	α –methyl mannofuranoside	$C_7H_{14}O_6$	194
12.276	2.77	2,2-diethoxy tetrahydrofuran	$C_8H_{16}O_3$	160
12.317	2.81	Pivaloin	$C_{10}H_{20}O_2$	172
12.567	13.25	4,6-di-O-methyl-α-D-galactoside	$C_8H_{16}O_6$	208
13.428	1.13	2-hydroxy-5-methylisophthaldehyde acetic acid	$C_{11}H_{10}O_4$	206
14.652	2.02	hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270
15.005	7.33	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256
15.726	1.49	Butynedioioc acid di-2-propenyl ester	$C_{10}H_{10}O_4$	194
15.934	2.06	(Z)-8-dodecen-1-ol	$C_{12}H_{24}O$	184
16.406	1.98	3-methylbicyclo [4.1.0] heptane	C_8H_{14}	110
16.614	2.89	(Z, Z)-9,12-octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294
16.666	5.79	9-octadecenoic acid	$C_{18}H_{34}O_2$	282

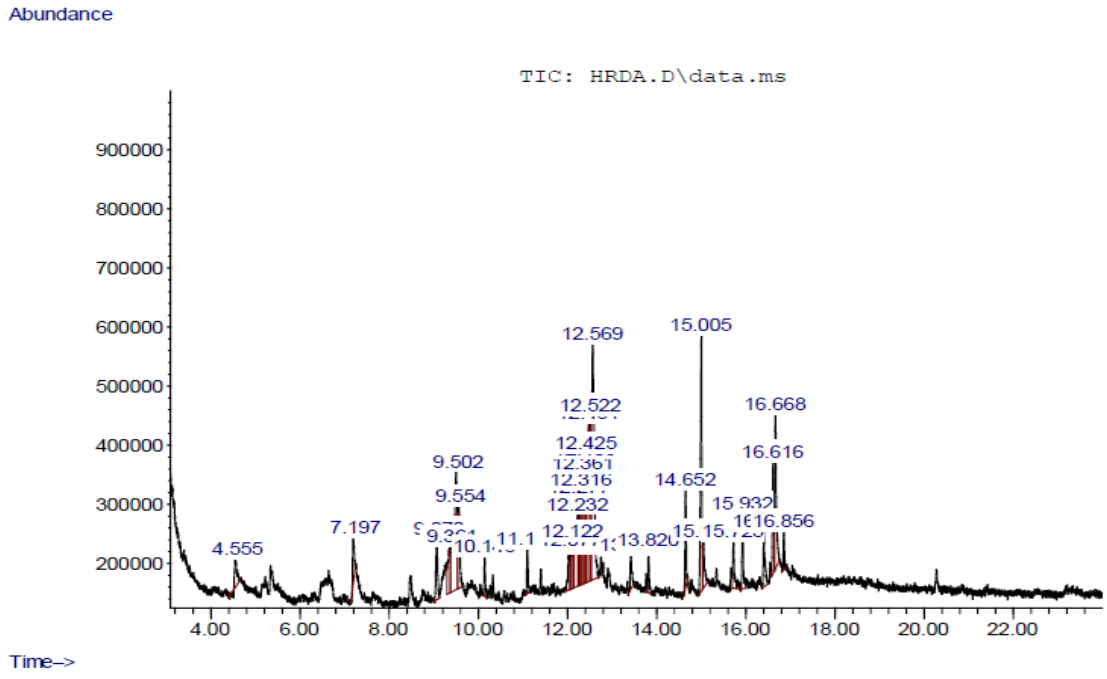

Figure 1: GCMS chromatogram of the volatile components of crude honeybee residue

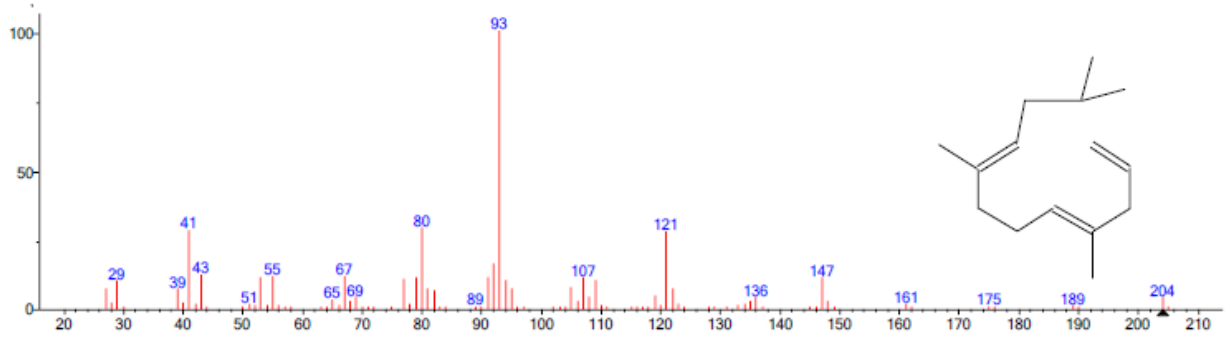

Figure 2: MS spectrum of humulene

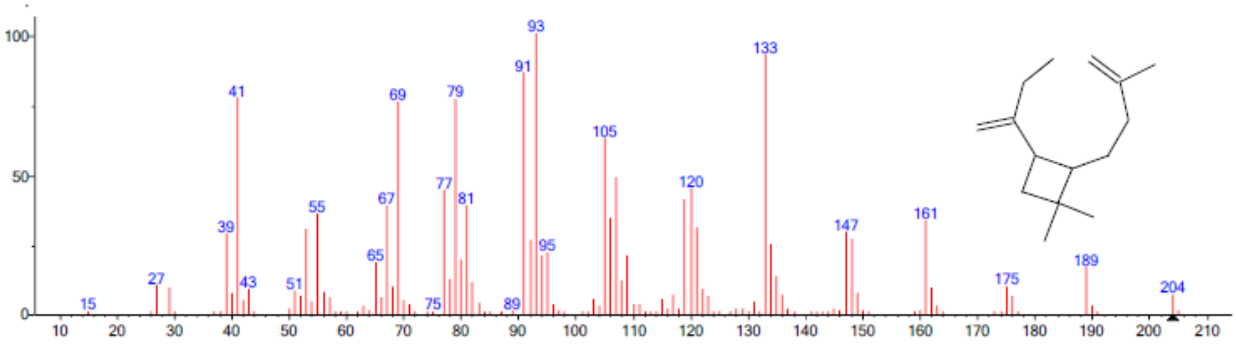

Figure 3: MS spectrum of caryophyllene

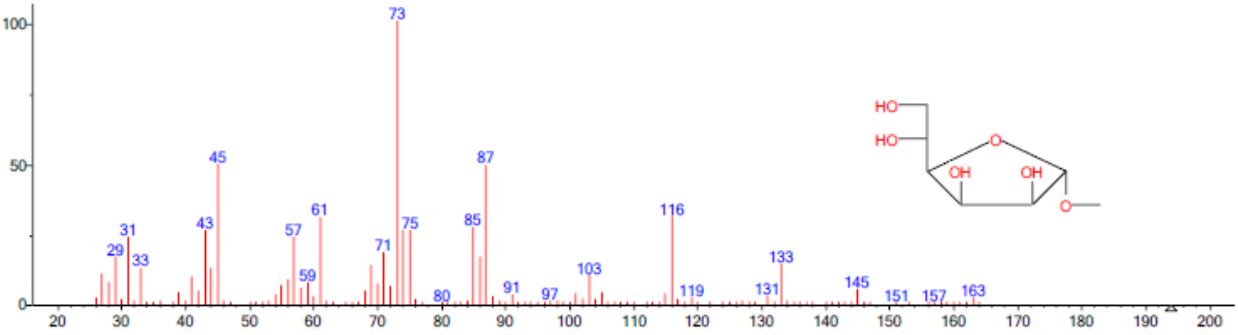

Figure 4: MS spectrum of α –methyl mannofuranoside

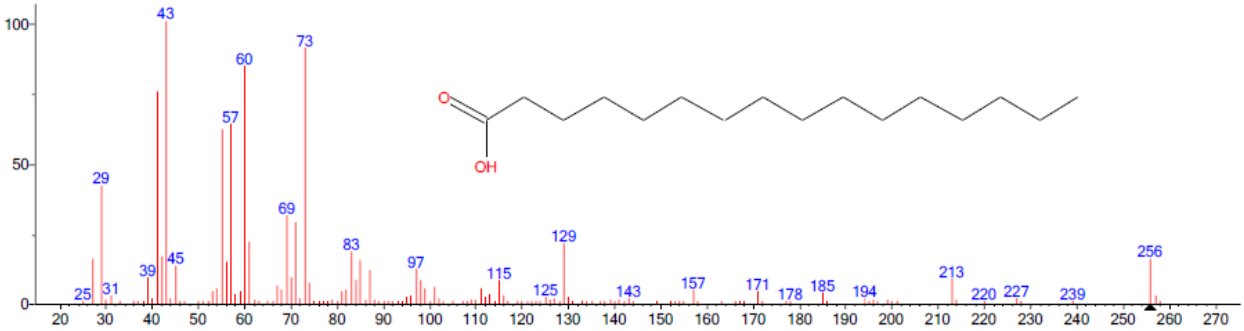

Figure 5: MS spectrum of n-hexadecanoic acid

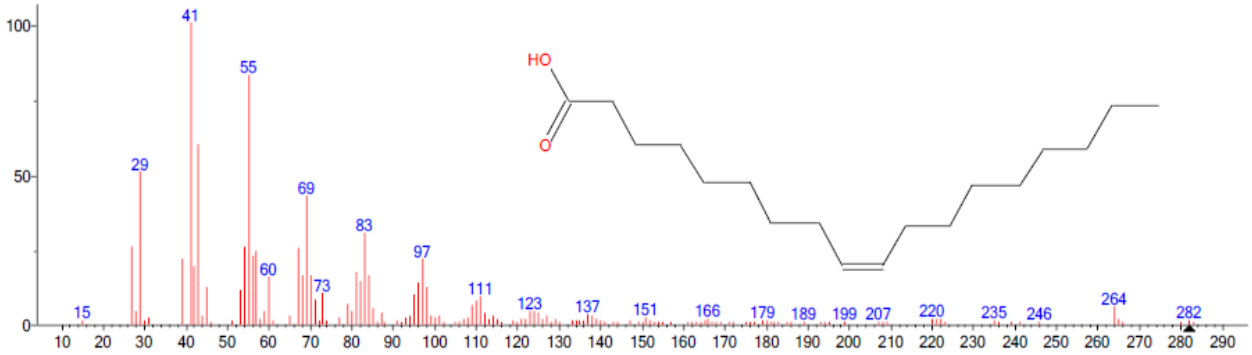

Figure 6. MS spectrum of 9-octadecenoic acid

Antioxidant Studies of the extract of crude honey residue (ECHR): The result of the *in vitro* free radical scavenging potential of ECHR was established using the DPPH techniques. The result is presented in Table 2. The free radical scavenging potential of ECHR increases as concentration increases. The free radical potential was highest at 2000 µg/ml and least at 125 µg/ml. The free radical potential was however, lower when compared to that of the organic standard being used. The lower free radical potential could be credited to the synergistic masking consequence of the non-polar volatile constituents.

Table 2: Free radical reducing potentials crude honey residue

Conc. µg/ml	Volatile constituents	Standard
2000.00	16.47±0.12	96.18±0.00
1000.00	13.96±0.00	95.88±0.09
500.00	11.28±0.00	95.42±0.15
250.00	8.06±0.11	94.96±0.09
125.00	4.16±0.01	94.35±0.15

The data are presented as mean of triplicates±standard deviation

Conclusion

The major phytochemicals as revealed by the GC-MS analysis, include: humulene (10.09%), α –methyl mannofuranoside (4.77%), 4,6-di-O-methyl-α-D-galactoside (13.25%), n-hexadecanoic acid (7.33%) and 9-octadecenoic acid (5.79%) constitute part of the volatile phytoconstitution of the crude honey residue. The volatile constituents, VCHR can be classified into terpenes, sugars, fatty acids, fatty acids esters and other organic groups with unique homologous series. The antioxidant activity as established by free radical scavenging potential witnessed an increasing effect as concentration increased. The presence of phytochemicals and the observed antioxidant activity are justifications for its nutraceutical potential. This could be explored in the search for lead drug substances in the face of emerging threat of global diseases.

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References

Abel AA, Banjo, AD: Apitherapy in Southern Nigeria: An assessment of therapeutic potentials of some honeybee products. J Pharm Biomed Sci, 2(2): 9-15.2012.

Al-Mamary Tk, Jose MS, Vassya B: Propolis: Is there a potential for development of new drugs. J Ethnopharmacol, 133: 253-260. 2010.

Alvarez-Suarez JM, Tulipani S, Romandini S, Bertoli E, Battino M: Contribution of honey in nutrition and human health: a review. Med J Nutr Metab, 3: 15-23. 2010.

Ambroẑ M., Śmatová M., Śadibolová M., Pospiŝilová E., Hadravská V, Kaŝparova M, SkarkaváVH., Králová V, Skálová L: Sesquiterpenes α-humulene and β-caryophyllene oxide enhance the efficacy of 5-flurorouracil and oxaliplatin in colon cancer cells. Acta Pharm, 69(1): 121-128. 2019.

Anifalaje EO, Ibok MG: Chemical compositions and antioxidant activities of *Albizia lebbeck* L. essential oils. J Essent Oil-Bear Plants, 23(4): 810-820.2020.

Bankova V, Castro SL, Marcucci MC: Propolis: Recent advances in chemistry and plant origin. Apidologie, 31: 3-15. 2000.

Chandrasekaran M, Kannathasan K, Venkatesalu V: Antimicrobial activity of fatty acid methyl ester of some selected members of chenopodiaceae. Z Naturforsch C J Biosci, 63 (5-6): 331-336. 2008.

Chaves JS, Leal PC, Pianowisky L, Calixto JB: Pharmacokinetics and tissue distribution of the sesquiterpene alpha humulene in mice. Planta Med, 74(14): 1678-1683. 2008.

Davoodbasha M, Edachery B, Nooruddin T, Lee S, Kim J: An evidence of C16 fatty acid esters extracted from microalga for effective antimicrobial and antioxidant property. Microb Pathog, 115: 233-238. 2018.

Eteraf-Oskouei T, Najafi M: Traditional and modern uses of natural honey in human diseases: A review. Iran. J Basic Med Sci, 16(6):731-742. 2013.

Ezz El-Arab AM, Girgis SM, Hegazy ME, Abd El-khalek AB: Effect of dietary honey on intestinal microflora and toxicity of mycotoxins in mice. BMC Complement Altern Med, 6:1-13. 2006.

Fidyt K, Fiedorowicz A, Strzadala L, Szumny A: β-caryophyllene and β-caryophyllene oxide-natural compounds of anticancer and analgelsic properties. Cancer Med, 5(10): 3007-3017. 2016.

Hamilton-Amachree A, Odokwo EO: Phytoconstitution, anti-oxidant and iron chelating profiling of 30% acetone extract of crude honey residue in Obudu Local Government area of Cross Rivers State, Nigeria. World Sci News, 172: 165-178. 2022.

Jang H, Rhee K, Eom Y: Antibacterial and antibiofilm effects of α-humulene against *Bacteroides fragilis*. Can J Microbiol, 66(6): 389-399. 2020.

Johnson JE, Sepe HA, Miano CL, Brannan RG, Alderton AL: Honey inhibits lipid oxidation in ready-to-eat ground beef patties. Meat Sci, 70: 627-631. 2005.

Krishnaveni M, Dhanalakshimi R, Nandhini N: GC-MS analysis of phytochemicals, fatty acids profile, antimicrobial activity of *Gossypium* seeds. Int J Pharm Sci Rev Res, 27(1): 273-276. 2014.

Küçük M, Kolayi S, Koraoḡlu S, Ulusoy E, Baltaci C, Candan F: Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chem, 100:526-534. 2007.

Legault J, Dahl W, Debiton E, Madelmont J: Antitumor activity of balsam fir oil: production of reactive oxygen species induced by alpha-humulene as a possible action mechanism. Planta Med, 69(5): 402-407. 2003.

Maretti E, Costantino L, Rustichelli C, Leo E, Croce MA, Buttini F, Truzzi E, Lannuccelli V: Surface engineering of solid lipid nanoparticle assemblies by methyl α-mannopyranoside for active targeting to macrophages in anti-tuberculosis inhalation therapy. Int J Pharm, 528(1-2): 440-451. 2017.

Medhi B, Puri A, Upadhyay S, Kaman L: Topical application of honey in the treatment of wound healing: a meta analysis. JK Sci, 10:166-169. 2008.

Oche JI, Johnson TO, Akinsanmi AO, Jaryum KH, Francis T: *In vitro* antioxidant activity and inhibition of Fe^{2+} and SNP lipid peroxidation of African Mistletoes (*Tapinanthus globiferus*) from three selected host plants in Jos Plateau State Nigeria. J Appl Life Sci Int, 20(4): 1-10. 2019.

Odokwo EO, Onifade MS: Free radical scavenging effect of 2-(3[3Nitrophenylamino] propanoyloxy)-benzoic acid. Int J Chem Chem Process, 4(2): 9-13.2018.

Odokwo EO, Onifade MS: Volatile constituents of the leaves and stem of *Justicia secunda* Vahl. Commun Phys Sci, 6(2): 827-834. 2020.

Odokwo EO, Salawu RA: Chemical and biological profiling of n-hexane extract of crude honeybee residue. World Sci News, 152(2021): 69-81. 2021.

Rakha MK, Nabil ZI, Hussein AA: Cardioactive and vasoactive effect of natural wild honey against cardiac malperformance induced by hyperadrenergic activity. J Med Food, 11:91-98. 2008.

Shaaban MT, Ghaly MF, Fahmi SM: Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria. J Basic Microbiol, 61(6): 557-568. 2021.

Tonks AJ, Cooper RA, Jones KP, Blair S, Parton J, Tonks A: Honey stimulates inflammatory cytokine production from monocytes. Cytokine, 21: 242-247. 2003.

Turkmen N, Sari F, Poyrazoglu ES, Velioglu YS: Effects of prolonged heating on antioxidant activity and colour of honey food. Food Chem, 95: 653-657. 2006.

Wang S, Wen X, DeVries AL, Bagdagulyan Y, Morita A, Golen JA, Duman JG, Rheingold AL: Molecular recognition of methyl-α-d-mannopyranoside by antifreeze (glyco) protein. J Am Chem Soc, 136(25): 8973-8981. 2014.