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Dereplication Study and Pharmacological Potentials of Phytochemical Constituents of Nigerian Propolis from Umudike

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ABSTRACT

Liquid chromatography-mass spectroscopy (LC-MS) was used to detect the phytochemicals present in the extracts of propolis from Umudike. The dereplication study of the hexane and ethyl acetate extracts of green, brown and red propolis samples from a hive in Umudike Umuahia Nigeria were studied using MestReNova software. About 31 compounds previously reported from Africa propolis were detected. The results revealed the presence of 23compounds were found in the ethyl acetate extract of the brown propolis, while 20 were detected in both the ethyl acetate extract of the green and red propolis. The hexane extract of the brown propolis showed 20 while the hexane extracts of green and red propolis showed 16of thecompounds. The druglikeness screening of the compounds were carried out using MestReNova software by determining the physicochemical properties of the phytochemicals which showed positive potential based on the Lipinski rule of five for druglike compounds. Molecular docking using the compounds and some standard cancer drugs on Cyclin dependent kinase protein (PDB ID: 6GUE) to determine the inhibition ofcancer cells suggested that some of the compounds have potentials as anticancer drugs.

Keyword: Propolis, Dereplication, Druglike, Phytochemicals, anticancer activity.

INTRODUCTION

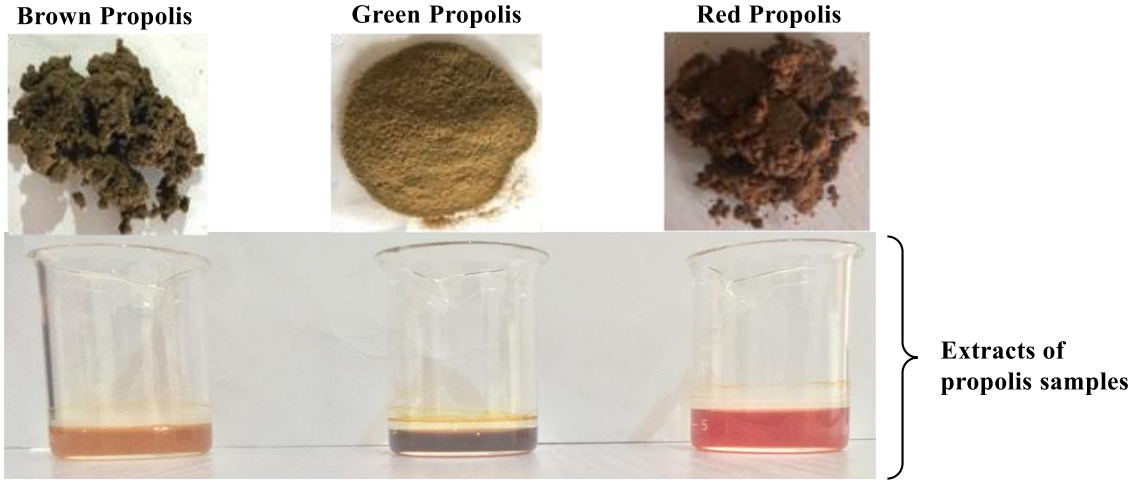
Propolis is a complex, resinous, gummy or sticky substance produced by honeybees from plants with the aid of bee enzymes. Propolis is naturally produced by bees from exudates, nectar, pollens and saps of trees. It has been reported to have numerous pharmacological activities and applications due to its complex phytochemical composition. Their constituents have been reported to vary with location, season, region and possibly bee type. Bees use propolis for a number of functions such as sealing of holes, strengthening the borders of the comb, protection of the hive entrance from intruders and maintenance of temperature in the hive. [1] Propolis has been reported to have hepatoprotective, hypotensive, immunomodulatory, antibacterial, antifungal, antioxidant, anti-inflammatory, antitumor and hematopoieticactivities. [2] The chemical composition of propolis has been reported to vary from hive to hive and depends on the local flora inthe site of collection,although all propolis samples have been found to have activity against microorganisms no matter the locationor weather and climatic conditions. [3-4] The therapeutic properties of propolis has long been recognized as it was used in homemade remedies by ancient Greeks, Romans and Egyptians. It is also used as a food additive and in cosmetics [4]·The major components of propolis are resins, wax, essential oils and phytocompounds. Elements such asmagnesium, nickel and iron have been reported to be present in small quantities, and the phytochemicals are mainlyterpenoids, flavonoids, fatty acids, phenolic acids and esters [4-5]· In south Africa, samples were collected from different locations and were found to contain β-pinene, α-pinene, dihydrosabinene, limonene, styrene, octanal and 1,8-cineole which was a makerfor western cape province and λ-terpinene, propanoic acid, furfural, 2-methoxy benzyl alcohol, hexanoic acid methylester [6] Nigerian propolis have also been investigated to be made-up of terpenoids and fats in central Nigeria while calycosin, liquiritigenin,

pinocembrin, medicarpin, 6-prenylnaringenin, 8-prenylnaringenin, propolin D**,** macarangin, xanthones, dihydrobenzofuran and riverinol were found in propolis from southern Nigeria. [7-8] Astrapterocarpan, 3,8-dihydroxy-9-methoxy-pterocarpan, vesticarpan, medicarpin, vestitol, broussonin B and 8-prenylnaringenin were also obtained from Nigerian propolis [9]·Propolis from different locations have shown vast biological activities with varying chemical composition and this could provide valuable leads to active components. Therefore, the study of propolis from new regions or locations is very important as it may uncover new biologically active compounds with significant pharmacological effects. Dereplication of propolis components helps to avoid the re-isolation and identification of known propolis constituents [4] Over 500 compounds have been identified from different propolis samples from around the World [5]·Cyclin-dependent kinases (CDKs) are serine/threonine kinases whose catalytic activities are regulated by interactions with cyclins and CDK inhibitors (CKIs). CDKs are key regulatory enzymes involved in cell proliferation through regulationof cell-cycle checkpoints and transcriptional events in response to extracellular and intracellular signals. Not surprisingly, the dysregulation of CDKs is a hallmark of cancers, and inhibition of a specific member is considered an attractive target in cancer therapy [10] In this study, LCMS was used to dereplicateknown compounds from the hexane and ethyl acetate extracts of three propolis samples from Umudike Umuahia, Abia State Nigeria while molecular docking was used to confirm the anticancer activity of the compounds.

Materials and methods

Samples

Propolis samples (green, red and brown) were obtained from hives in a private apiary in Umudike Umuahia, Abia State, Nigeria in September 2019. The samples were sliced into small pieces and ground to powder.



Extraction

150 g of each sample was transferred into a beaker, and extracted successively with 500 ml of n-hexane and ethyl acetate by maceration for 72 hours. The extracts were filtered to give the hexane and ethyl acetate extracts.

LC-MS analysis

Liquid chromatography-high resolution mass spectrometry (LC-HRMS) analysis was performed on an Accela 600 High Performance Liquid Chromatography (HPLC) system with an ACE C-18 column (150 × 3 mm, 3 µm particle size) (HiChrom, Reading UK) coupled to an Exactive (Orbitrap) mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). About 2 mg of the ethyl acetate and hexane extracts were dissolved in 1 mL of methanol and filtered and 10 µL of the filtrate was used for the analysis. The mobile phase was water with 0.1% formic acid as mobile phase A and acetonitrile with 0.1% formic acid as mobile phase B at a flow rate of 0.3 mL/minute. The gradient elution was programmed as follows: 0 –15 minutes linear gradient from 30% to 50% of B, 15 –25

minutes at 50% of B, 25 –40 minutes linear gradient from 50% to 80% of B, 40 –50 minutes at 80% of B, 50 –51 minutes increasing to 100% of B, 51 –59 minutes at 100% of B (with the flow rate increased to 0.5 mL/min) and at 61 minutes the solvent system was returned to 30% of B and held until the 70th minute. The samples were run in duplicates, the MS detection range was from m/z 100 –1500 and scanning was performed under ESI polarity switching mode. The needle voltages were −4.0 kV (negative) and 4.5 kV (positive) while the sheath and auxiliary gases were set at 50 and 17 arbitrary units respectively. The data obtained were split into positive and negative ions and the 'negative' dataset was processed using MZMine 2.14, with the masses selected between m/z 100–1200. Data were processed using Xcalibur 2.2 software from Thermo Fisher Scientific.24" [13]

Dereplication and Drugability studies

The study was carried outusing thesteps described by Chen Peng [14]

Correctly drawn structures of the compounds were uploaded onto the MestReNova software as. sdf files. Similarly, the structures were uploaded onto the LCMS result file and the match molecule function was used to obtain any molecular match. The physiochemical parameters of the compounds were also obtained using the Physchemfunction of the MestReNova software [14]

Protein receptors ligand retrieval and preparations

The three-dimensional (3D) structures of some anticancer and anti-inflammation drugs, with the compounds from the sample were retrieved from PubChem website in simple document format. They were optimized using Open babel in Python Prescription (version 0.8) which converted the ligands energetically to the most stable structures using Merk Molecular Force Field 94 (MMFF94). Similarly, the 3D X-ray crystallographic structure of the CDK2/CyclinA in complex with AZD5438 (Cyclin dependent inhibitors) was retrieved from the RCSB protein data bank (PDB) (https://www.rcsb.org/) with ID 6GUE having 1.99 A resolution with no mutation. The protein was then prepared for docking and minimized using the relevant tools in Discovery studio

Molecular Docking

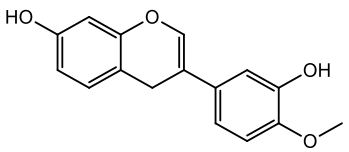
Prior to molecular docking analysis, proteins were pre-processed using Discovery Studio 2020. This step involvedthe removal of any hetero-groups, other chains and water molecules. The active site of the protein was identified using Discovery studio. Furthermore, the preparation of ligands and receptors in the PDBQT file format were carried out in the AutoDock tool. The molecular docking was carried out using AutoDock Vina to understand the interaction between receptors and ligands. A rigid-flexible docking was performed after setting a grid box surrounding the binding sites of the receptors at exhaustiveness = 8, center_x = -7.5617226552, center_y = -21.8191182329, center_z = 18.6456113747, size_x = 23.7075432599, size_y = 22.2959662644, size_z = 21.6130191782.

Dereplication Statistics

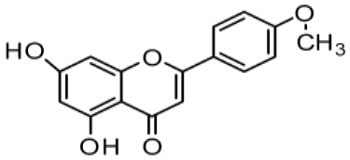
The statistical method used in the dereplication analysis was simple percentage, the number of the screened compounds from the extract were compared with the 31 compounds that were detected.

Results and Discussion

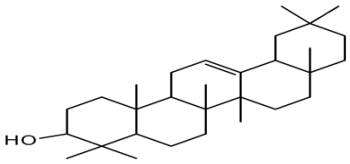
The results of the LC-MS for the extracts are reported in the appropriate tables and figures. The physicochemical parameters for the compounds using Mest ReNova software was also reported, the molecular docking analysis of the most prominent compounds were also reported by appropriate tables and figures below. Figure 1 and table 1 showed the LC-MS result of ethyl acetate extract of brown propolis and its dereplication, other results follow suit.



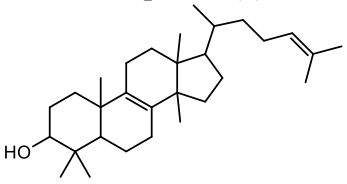
Compound (**1**)**:** Calycosin



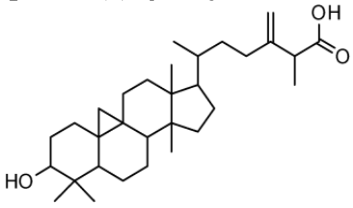
Compound (**2**)**:** Acacetin



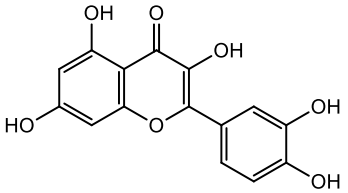
Compound (**3**)**: β-**amyrin



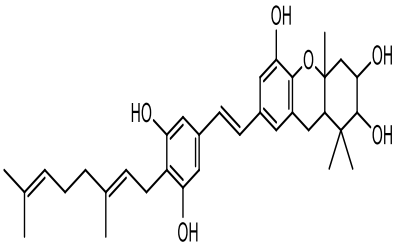
Compound (**4**)**:** Lanosterin



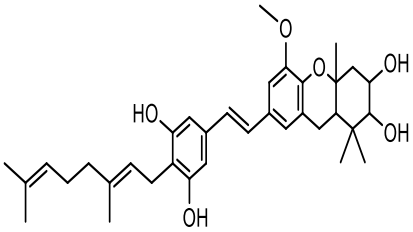
Compound (5) : Ambolic



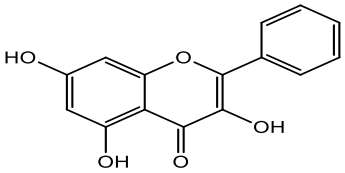
Compound (6) : Quercetin



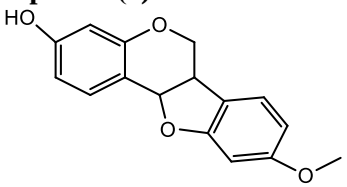
Compound (7): Schweinfurthin A



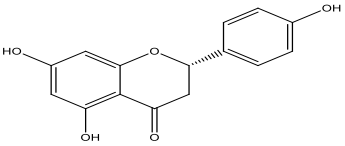
Compound (8): Schweinfurthin B



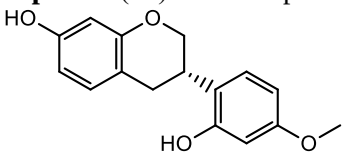
Compound (**9**)**:** Galagin



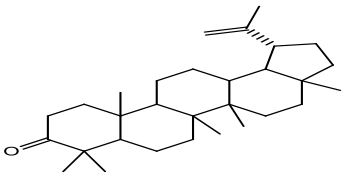
Compound (**10**)**:** Medicarpin



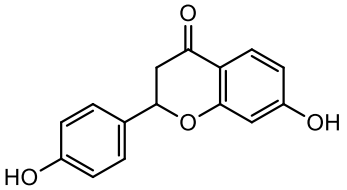
Compound (**11**)**:** Naringenin



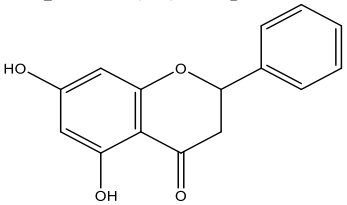
Compound (**12**)**:** Vestitol



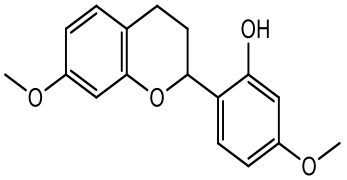
Compound (**13**)**:** Lupenone



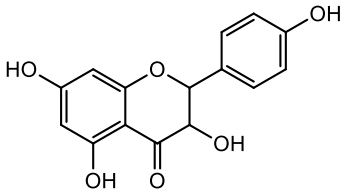
Compound (**14**)**:** Liquiritigenin



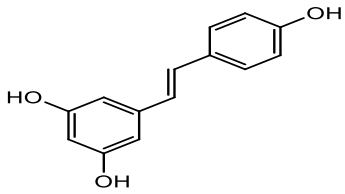
Compound (**15**)**:** Pinocemberin



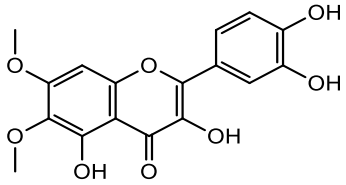
Compound (**16**)**:** Isosativan



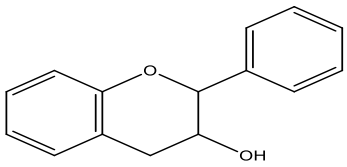
Compound (**17**)**:** Kaempferol



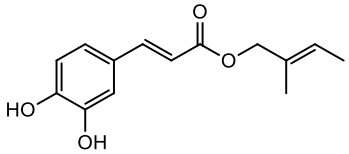
Compound (**18**)**:** Resveratrol



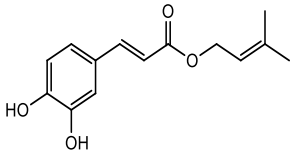
Compound (**19**)**:** Euptolin



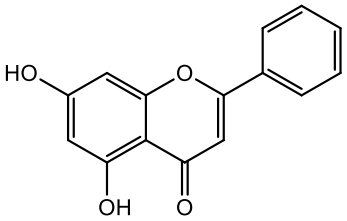
Compound (20)**:** Flavan-3-ol



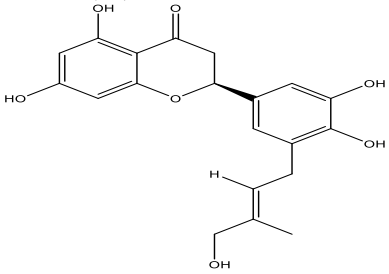
Compound (**21**)**:** 2-methyl-2-butenyl-(E)Caffeate



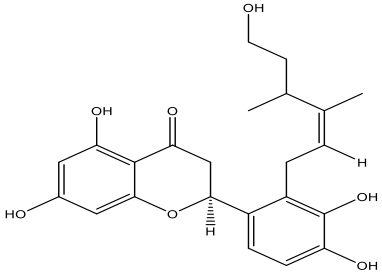
Compound (**22**)**:** 3-methyl-2-butenyl caffeate



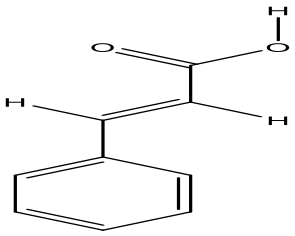
Compound (**23**)**:** Chrysin



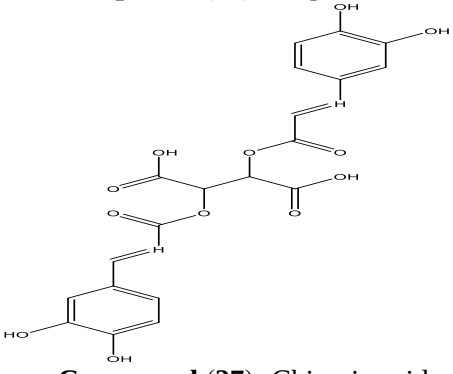
Compound (**24**)**:** Propolin B



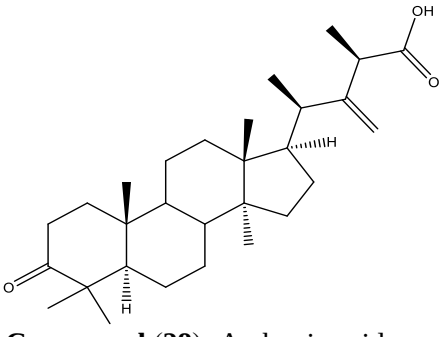
Compound (**25**)**:** Propolin A



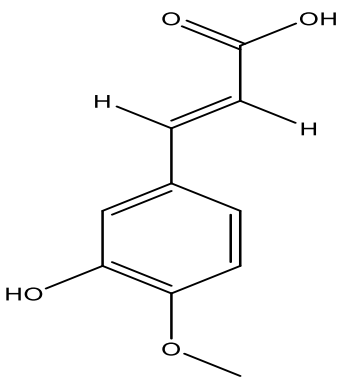
Compound (26)**:** Cinnamic acid



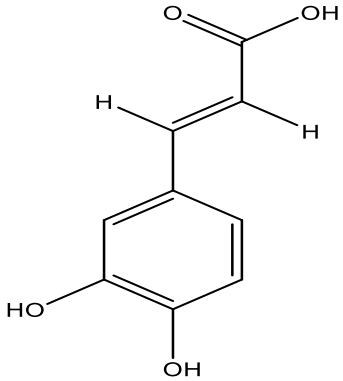
Compound (**27**)**:** Chicoric acid



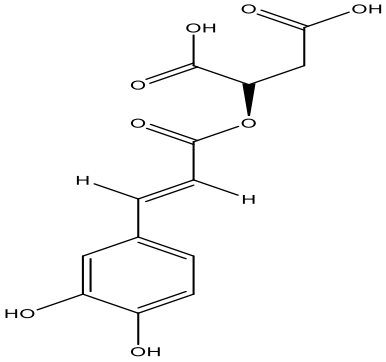
Compound (**28**)**:** Ambonic acid



Compound (29): Isoferulic Acid



Compound (30): Caffeic acid



Compound (31): Cafteric acid

Figure (1): Structures of compounds, their Names and Numbers.

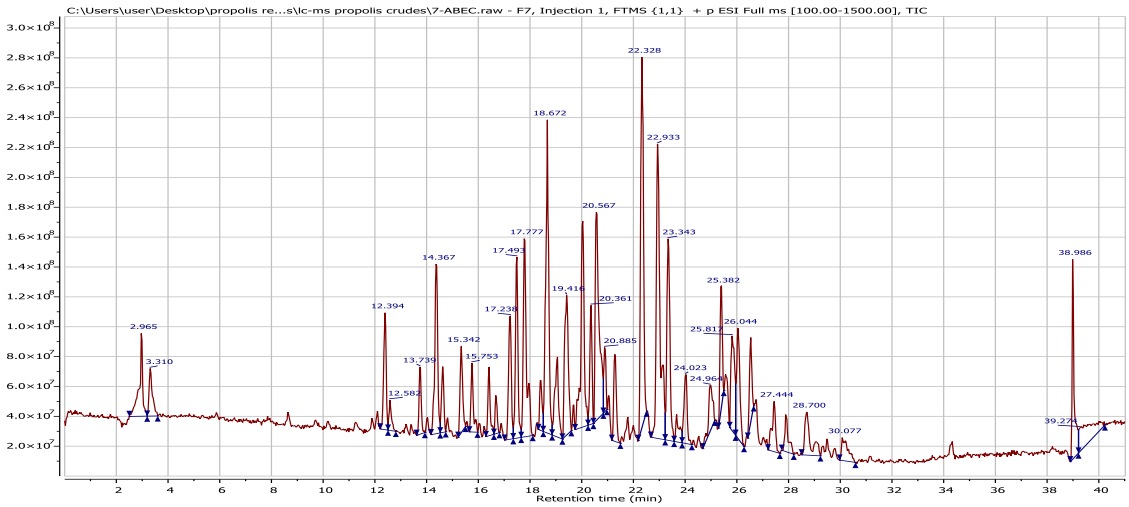


Figure (2): Result of LC-MS of the ethyl acetate extract of the brown propolis.

Table (1): Result of the dereplication analysis of the LC-MS of ethyl acetate extract of the brown propolis.

Compounds	Molecular formula	Molecular mass	Match score	% purity	Retention Time
Calycosin	$C_{16}H_{12}O_5$	284.068	1.000	0.143	20.54
Acacetin	$C_{16}H_{12}O_5$	284.068	1.000	0.143	20.54
β-Amyrin	$C_{30}H_{50}O$	426.386	1.000	0.811	25.82
Lanosterin	$C_{30}H_{50}O$	426.386	1.000	0.811	25.82
Ambolic acid	$C_{31}H_{50}O_3$	470.376	1.000	0.795	25.38

Compounds	Molecular formula	Molecular mass	Match score	% purity	Retention Time
Quercetin	$C_{15}H_{10}O_7$	302.043	0.999	0.071	9.76
Schweinfurthin A	$C_{34}H_{44}O_6$	548.314	0.999	0.269	22.00
Schweinfurthin B	$C_{35}H_{46}O_6$	562.329	0.999	0.303	23.72
Galangin	$C_{15}H_{10}O_5$	270.053	0.999	0.071	9.76
Medicarpin	$C_{16}H_{14}O_4$	270.089	0.998	0.071	9.76
Naringenin	$C_{15}H_{12}O_5$	272.068	0.996	0.147	11.88
Vestitol	$C_{16}H_{16}O_4$	272.105	0.995	0.147	11.88
Lupenone	$C_{30}H_{48}O$	424.371	0.994	0.713	22.33
Liquiritigenin	$C_{15}H_{12}O_4$	256.074	0.992	0.084	9.68
Pinocemberin	$C_{15}H_{12}O_4$	256.074	0.992	0.084	9.68
Isosativan	$C_{17}H_{18}O_4$	286.121	0.990	0.065	20.04
Kaempferol	$C_{15}H_{10}O_6$	286.048	0.988	0.065	20.04
Resveratrol	$C_{14}H_{12}O_3$	228.079	0.988	0.140	2.99
Eupatolin	$C_{17}H_{14}O_8$	346.069	0.983	0.225	18.58
Flavan-3-ol	$C_{15}H_{14}O_2$	226.099	0.965	0.137	2.97
2-methyl-2-butenyl-(E)Caffeate	$C_{14}H_{16}O_4$	248.105	0.944	0.081	12.12
3-methyl-2-butenyl caffeate	$C_{14}H_{16}O_4$	248.105	0.944	0.081	12.12
Chrysin	$C_{15}H_{10}O_4$	254.058	0.944	0.080	12.12

The dereplication analysis ofthe LC-MS of the ethyl acetate extract of the brown propolis showed 23 compounds. The compounds that were present have been isolated from Africa propolis. [13,15] Cinnamic acid, isoferulic acid, caftaric acid, propolin A, propolin B, chicoric acid, ambonic acid and epicatechin were all absent in the extract.

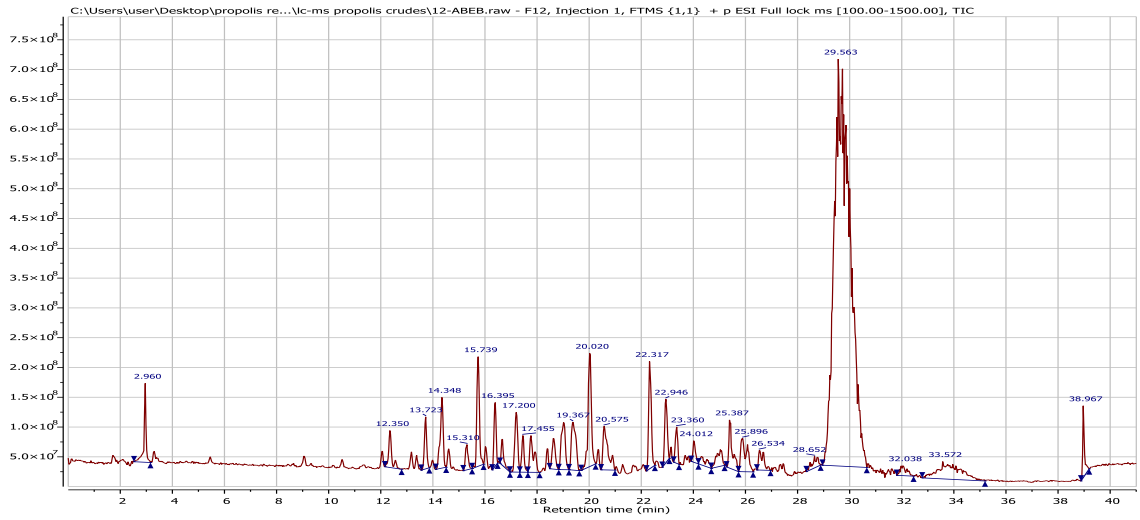


Figure (3): Result of LC-MS ethyl acetate extract of green propolis.

Table (2): Result of the dereplication analysis of the LC-MS result of ethyl acetate extract of the green propolis

Molecule	Molecular formula	M.weight	Match score	% purity	RT
β-Amyrin	$C_{30}H_{50}O$	426.386	1.000	0.754	25.90
Lanosterin	$C_{30}H_{50}O$	426.386	1.000	0.754	25.90

Molecule	Molecular formula	M.weight	Match score	% purity	RT
Ambolic Acid	$C_{31}H_{50}O_3$	470.376	1.000	0.741	25.39
Propolin B	$C_{25}H_{30}O_7$	442.199	0.999	0.490	15.74
Propolin A	$C_{25}H_{30}O_7$	442.199	0.999	0.490	15.74
Schweinfurthin B	$C_{35}H_{46}O_6$	562.329	0.999	0.305	23.78
Schweinfurthin A	$C_{34}H_{44}O_6$	548.314	0.999	0.211	16.76
Cinnamic acid	$C_9H_8O_2$	148.052	0.999	0.066	29.72
Quercetin	$C_{15}H_{10}O_7$	302.043	0.998	0.068	20.02
Liquiritigen	$C_{15}H_{12}O_4$	256.074	0.998	0.040	29.56
Pinocembrin	$C_{15}H_{12}O_4$	256.074	0.998	0.040	29.56
Chicoric acid	$C_{22}H_{18}O_{12}$	474.080	0.996	0.011	29.86
Lupenone	$C_{30}H_{48}O$	424.371	0.995	0.707	22.32
Ambonic acid	$C_{31}H_{48}O_3$	468.360	0.994	0.135	27.47
Calycosin	$C_{16}H_{12}O_5$	284.068	0.989	0.038	2.09
Acacetin	$C_{16}H_{12}O_5$	284.068	0.989	0.038	2.09
Flavan-3-ol	$C_{15}H_{14}O_2$	226.099	0.989	0.080	2.93
Isosativan	$C_{17}H_{18}O_4$	286.121	0.988	0.068	20.02
Kaempferol	$C_{15}H_{10}O_6$	286.048	0.987	0.068	20.02
Resveratrol	$C_{14}H_{12}O_3$	228.079	0.985	0.158	2.96

20 compounds were found to be present in the green propolis ethyl acetate extract while 11 were absent. The compounds present have been isolated from African propolis. [13, 15] The following compounds were not present in the extract, galangin, medicarpin, naringenin, vestitol, eupatolin, 2-methyl-2-butenyl(E) caffeate, 3-methyl-3-butenyl(E) caffeate, chrysin, isoferulic acid, cafteric acid and epicatechin.

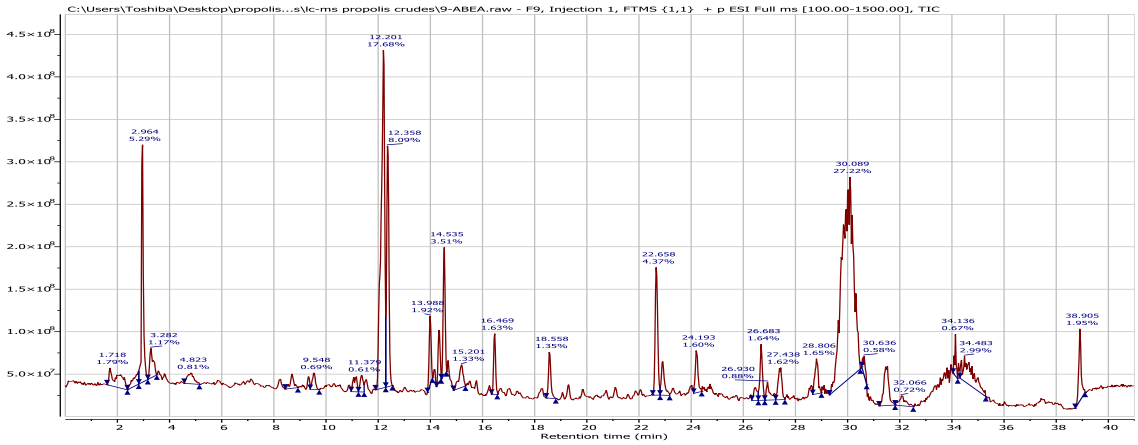


Figure (4): Result of LC-MS Ethyl Acetate Crude extract of Red Propolis.

Table (3): Result of the dereplication analysis of the LC-MS result of ethyl acetate extract of Red propolis.

Name	Molecular formula	Molecular weight	Match score	Ms purity	Retention time
Cinnamic acid	$C_9H_8O_2$	148.052	1.000	0.053	30.09
Methyl caffeate	$C_{10}H_{10}O_4$	194.058	0.999	0.021	12.20
Isoferulic acid	$C_{10}H_{10}O_4$	194.058	0.999	0.021	12.20
Caffeic acid	C_9H_8O	180.042	0.999	0.076	12.22

Name	Molecular formula	Molecular weight	Match score	Ms purity	Retention time
Caftaric acid	$C_{13}H_{12}O_9$	312.048	0.997	0.079	12.36
Vestitol	$C_{16}H_{16}O$	272.105	1.000	0.383	22.66
Pinocembrin	$C_{15}H_{12}O_4$	256.074	1.000	0.344	16.47
Chrysin	$C_{15}H_{10}O_4$	254.058	0.997	0.231	22.66
Acacetin	$C_{16}H_{12}O_5$	284.068	0.996	0.163	23.29
β-Amyrin	$C_{30}H_{50}O$	426.386	0.996	0.588	25.80
Lanosterin	$C_{30}H_{50}O$	426.386	0.996	0.588	25.80
Ambolic Acid	$C_{31}H_{50}O_3$	470.376	0.999	0.664	25.34
Schweinfurthin B	$C_{35}H_{46}O_6$	562.329	0.968	0.004	22.93
Schweinfurthin A	$C_{34}H_{44}O_6$	548.314	0.980	0.013	27.90
Liqiuritigen	$C_{15}H_{12}O_4$	256.074	0.998	0.180	22.93
Ambonic Acid	$C_{31}H_{50}O_3$	468.360	0.997	0.606	38.23
Isosativan	$C_{17}H_{18}O_4$	286.121	0.996	0.082	21.89
Kaempferol	$C_{15}H_{10}O_6$	286.048	0.995	0.059	20.53
Reservatrol	$C_{14}H_{12}O_3$	228.079	0.996	0.098	30.30
Galangin	$C_{16}H_{14}O_4$	270.089	0.997	0.094	25.16

The result of the de-replication analysis done on the LC-MS result of the ethyl acetate extract of the Red propolis showed that out of the 31 compounds analyzed for 20 were present why 11 were absent. The compounds that were present have been isolated from Africa propolis. [13,15]

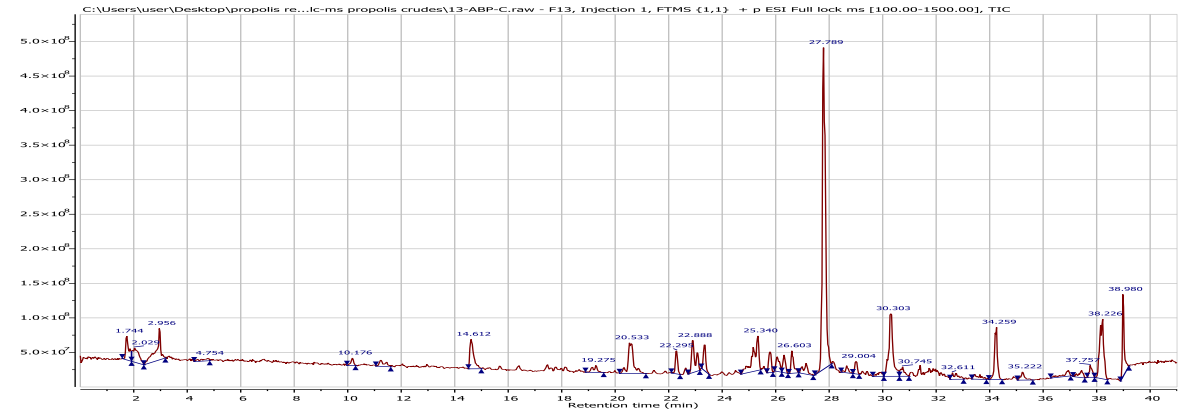


Figure (5): Result of LC-MS Hexane crude extract of Brown Propolis.

Table (4): Result of the dereplication analysis of the LC-MS result of hexane extract of brown propolis.

Molecule	MolecularFormula	Molecular Weight	Match Score	MS purity	Retention Time
Galangin	$C_{16}H_{14}O_4$	270.089	1.000	0.094	25.16
Ambonic acid	$C_{31}H_{50}O_3$	468.360	1.000	0.606	38.23
Ambolic acid	$C_{31}H_{50}O_3$	470.376	0.999	0.664	25.34
Medicarpin	$C_{15}H_{10}O_5$	270.053	0.999	0.094	25.16
Liquiritigenin	$C_{15}H_{12}O_4$	256.074	0.998	0.180	22.93
Cinnamic acid	$C_9H_8O_2$	148.052	0.998	0.032	27.79
Pinocembrin	$C_{15}H_{12}O_4$	256.074	0.998	0.180	22.93

Molecule	MolecularFormula	Molecular Weight	Match Score	MS purity	Retention Time
Eupatolin	$C_{17}H_{14}O_8$	346.069	0.998	0.232	24.49
Isosativan	$C_{17}H_{18}O_4$	286.121	0.998	0.082	21.89
Quercetin	$C_{15}H_{10}O_7$	302.043	0.998	0.094	25.16
β –Amyrin	$C_{30}H_{50}O$	426.386	0.996	0.588	25.80
Lanosterin	$C_{30}H_{50}O$	426.386	0.996	0.588	25.80
Resveratrol	$C_{14}H_{12}O_3$	228.079	0.996	0.098	30.30
Kaempferol	$C_{15}H_{10}O_6$	286.048	0.995	0.059	20.53
Lupenone	$C_{30}H_{48}O$	424.371	0.994	0.500	22.29
Schweinfurthin A	$C_{34}H_{44}O_6$	548.314	0.980	0.013	27.90
Schweinfurthin B	$C_{35}H_{46}O_6$	562.329	0.968	0.004	22.93
Calycosin	$C_{16}H_{12}O_5$	284.068	0.958	0.069	32.84
Acacetin	$C_{16}H_{12}O_5$	284.068	0.958	0.069	32.84
Epicatechin	$C_{15}H_{14}O_6$	290.079	0.875	0.268	26.32

The dereplication study of the 31 compounds assess showed that 20 compounds were present in the extract while 11 were absent. The compounds present have isolated from African propolis. [15] Naringenin, vestitol, flavan-3-ol, 2-methyl-2-butenyl (E) Caffeate, 3-methyl-3-butenyl (E) caffeate, chrysin, isoferulic acid, cafteric acid, propolin A, propolin B and chicoric acid were absent from the extract.

Table (5): Result of the dereplication analysis of the LC-MS result of hexane extract of Red propolis.

Name	Molecular formula	Molecular Weight	Match score	Ms purity	Retention time
Acacetin	$C_{16}H_{12}O_5$	284.07	1.00	0.172	21.93
Vestitol	$C_{16}H_{16}O_4$	272.11	1.00	0.050	22.69
Naringenin	$C_{15}H_{12}O_5$	272.07	1.00	0.344	22.69
Kaempferol	$C_{15}H_{10}O_6$	286.05	0.998	0.135	10.62
Chrysin	$C_{15}H_{10}O_4$	254.06	0.992	0.216	22.69
Liquiritigenin	$C_{15}H_{12}O_4$	265.07	1.000	0.344	30.73
Ambolic Acid	$C_{31}H_{50}O_3$	470.376	0.999	0.349	31.53
Ambonic	$C_{31}H_{50}O_3$	470.376	0.999	0.263	31.55
Lupenone	$C_{30}H_{48}O$	424.371	0.989	0.117	27.20
β -amyrin	$C_{30}H_{50}O$	426.386	0.959	0.075	27.70
Lanosterol	$C_{30}H_{50}O$	426.386	0.959	0.075	27.70
Caftaric acid	$C_{13}H_{12}O_9$	312.2	0.997	0.071	12.39
Caffeic acid	$C_9H_8O_4$	180.2	0.979	0.092	12.24
Cinnamic acid	$C_9H_8O_2$	148.05	1.00	0.053	30.09
Methyl caffeate	$C_{10}H_{10}O_4$	194.05	0.999	0.021	12.20
Isoferulic	$C_{10}H_{10}O_4$	194.05	0.999	0.021	12.20

The result of the de-replication analysis done on the LC-MS result of the Hexane extract of the Red propolis showed that out of the 31 compounds analyzed for 16 were present while 15 were absent. The compounds that were present have been isolated from Africa propolis. [13,15]

Table (6): Result of the dereplication analysis of the LC-MS result of hexane extract of Green propolis.

Molecule	Molecular formula	M.weight	Match score	% purity	RT
Ambolic Acid	$C_{31}H_{50}O_3$	470.376	1.000	0.741	25.39
chweinfurthin B	$C_{35}H_{46}O_6$	562.329	0.999	0.305	23.78
Schweinfurthin A	$C_{34}H_{44}O_6$	548.314	0.999	0.211	16.76
Cinnamic acid	$C_9H_8O_2$	148.052	0.999	0.066	29.72
Quercetin	$C_{15}H_{10}O_7$	302.043	0.998	0.068	20.02
Liquiritigen	$C_{15}H_{12}O_4$	256.074	0.998	0.040	29.56
Pinocembrin	$C_{15}H_{12}O_4$	256.074	0.998	0.040	29.56
Chicoric acid	$C_{22}H_{18}O_{12}$	474.080	0.996	0.011	29.86
Lupenone	$C_{30}H_{48}O$	424.371	0.995	0.707	22.32
Ambonic acid	$C_{31}H_{48}O_3$	468.360	0.994	0.135	27.47
Calycosin	$C_{16}H_{12}O_5$	284.068	0.989	0.038	2.09
Acacetin	$C_{16}H_{12}O_5$	284.068	0.989	0.038	2.09
Isosativan	$C_{17}H_{18}O_4$	286.121	0.988	0.068	20.02
Kaempferol	$C_{15}H_{10}O_6$	286.048	0.987	0.068	20.02
Resveratrol	$C_{14}H_{12}O_3$	228.079	0.985	0.158	2.96
Medicarpin	$C_{15}H_{10}O_5$	270.053	0.999	0.094	25.16

The result of the de-replication analysis done on the LC-MS result of the hexane extract of the green propolis showed that out of the 31 compounds analyzed for 16 were present while 15 were absent. The compounds present have been isolated from Africa propolis. [13,15] From the result calycosin, acacetin, β-amyrin, lanosterin, ambolic acid, quercetin, scheweinfurthin B and A, lupenone, liquiritigenin, pinocembrin, isosativan, kaempferol and resveratrol were present in all extract while isoferulic acid and caftaric acid were absent in all the extract. chrysin, 3-methyl-3-butenyl (E) caffeate, 2-methyl-2-butenyl (E) caffeate, naringenin and vestitol were present in only extract of ethyl acetate of brown propolis, while propolin A, B and chicoric acid were present in only ethyl acetate of green propolis extract. epicatechin was present in only hexane extract of brown propolis. Cinnamic acid and ambonic acid were present in the extracts of ethyl acetate of green propolis and hexane extract of brown propolis. galangin, medicarpin and eupatolin were present in the ethyl acetate of brown propolis and hexane extract of brown propolis. While flavan-3-ol was present in ethyl acetate of both green and brown propolis. The result confirmed the reports of previous studies that indicates that the chemical composition of propolis varies with the location, colour and method of extraction of propolis. [3-4, 7-8] The result suggested that Ethyl acetate extract of brown propolis have higher (23/31) composition of the 31 compounds screened for dereplication. The hexane extracts of green and red propolis have the lowest (16/31). Suggesting that the propolis from Umudike contained more of the mid-polar compounds than the non-polar, as ethyl acetate is a mid-polar solvent while Hexane is a non-polar solvent. The result also suggested that more of the compounds screened for de-replication were at the brown propolis than other coloured propolis.

Table (7): Result of the Physiochemical and druglikeness potential analysis of the compounds analyzed for dereplication in both LC-MS result of ethyl acetate and hexane extract of the brown, red and green propolis using Mestre-Nova.

Molecule	Log P	HBD	HBA	Log S	B.P.	M.P.	LogD	Log BB
Calycosin ($C_{16}H_{12}O_5$)	2.884	2	3	-4.352	735.453	510.858	3.024	-0.174
(Acacetin) ($C_{16}H_{12}O_5$)	2.997	2	3	-4,280	737.453	496.238	2.888	-0.369
Quercetin ($C_{15}H_{10}O_7$)	1.925	5	2	-3.371	837.939	619.463	1.661	-0.741
Galagin ($C_{15}H_{10}O_5$)	2.669	3	5	-3.882	746.289	514.477	2.515	-0.519
Medicarpin ($C_{16}H_{14}O_4$)	3.086	1	3	-3.300	563.706	427.464	2.760	0.318
Narigenin ($C_{15}H_{12}O_5$)	2.123	3	2	-2.607	673.869	500.452	1.665	-0.335
Isosativan ($C_{17}H_{18}O_4$)	3.731	1	3	-3.129	590.019	394.871	3.439	0.265
Kaempferol ($C_{15}H_{10}O_6$)	2.356	4	2	-3.591	795.946	569.416	2.117	-0.649
Eupatolin ($C_{17}H_{14}O_8$)	1.873	4	4	-3.964	829.137	589.071	1.744	-0.455
Flavan-3-ol ($C_{15}H_{14}O_2$)	3.016	1	1	-3.430	522.507	350.623	2.890	0.106
2-methyl-2-butenyl-(E)-caffeate ($C_{14}H_{16}O_4$)	3.278	2	2	-3.524	600.708	373.001	3.598	-0.023
3-methyl-2-butenyl caffeate ($C_{14}H_{16}O_4$)	3.129	2	2	-3.335	616.291	414.134	3.073	0.002
Epicatechin ($C_{15}H_{14}O_6$)	1.672	5	1	-1.995	707.692	567.897	1.299	-0.371
β-Amyrin ($C_{30}H_{50}O$)	7.788	1	0	-7.724	648.477	472.174	7.811	0.802
Liquiritigen ($C_{15}H_{12}O_4$)	2.325	2	2	-2.998	636.226	459.525	2.045	-0.127
Lanosterin ($C_{30}H_{50}O$)	8.295	1	0	-7.763	674.767	436.765	8.755	0.989
Chrysin ($C_{15}H_{10}O_4$)	3.062	2	2	-4.199	709.435	479.071	2.988	-0.409
Lupenone ($C_{30}H_{48}O$)	6.389	0	1	-7.437	654.047	478.149	6.792	0.332
Pinocembrin ($C_{15}H_{12}O_4$)	2.436	2	2	-2.897	624.211	445.513	2.064	-0.205
Schweinfurthin A ($C_{34}H_{44}O_6$)	7.751	5	1	-7.622	887.055	588.213	7.890	-0.660
Schweinfurthin B ($C_{35}H_{46}O_6$)	8.000	4	2	-7.993	865.415	550.440	8.188	-0.490
Vestitol ($C_{16}H_{16}O_4$)	3.670	2	2	-3.175	661.825	432.760	3.351	0.373
Cinnamic acid ($C_9H_8O_2$)	1.978	1	1	-2.059	554.205	403.689	-0.558	0.219
Isoferulic acid ($C_{10}H_{10}O_4$)	1.482	2	2	-1.920	624.265	470.902	-1.114	0.167
Caftaric acid ($C_{13}H_{12}O_9$)	-0.308	5	4	-1.898	773.148	592.443	-6.725	-0.174
Propolin B ($C_{25}H_{30}O_7$)	4.802	5	2	-4.603	799.739	569.866	4.598	-0.620
Propolin A ($C_{25}H_{30}O_7$)	4.835	5	2	-4.451	804.454	574.071	4.656	-0.609
Resveratrol ($C_{14}H_{12}O_3$)	3.145	3	0	-3.254	727.493	487.985	2.924	0.131

Molecule	Log P	HBD	HBA	Log S	B.P.	M.P.	LogD	Log BB
Chicoric acid ($C_{22}H_{18}O_{12}$)	1.292	6	6	4.157	974.582	729.895	-4.944	-0.201
Ambonic acid ($C_{31}H_{48}O_3$)	4.938	1	2	-6.763	759.945	573.946	2.669	-0.070
Ambolic acid ($C_{31}H_{50}O_3$)	5.413	2	1	-7.087	770.485	573.111	2.586	-0.360

The results showed that the compounds detected in the propolis samples have significant drugability (Druglikeness) considering the value of their log P (Partitioning), Log D (Distribution) Log BB (absorption), log S (Solubility and metabolism), HBA (Hydrongen bond acceptor), HBD (Hydrogen bond donor) and molecular weight (molecule size). Which are used in checking druglikeness and are the assessment of structural features and properties of a molecule to known whether it can serve as drug or non-drug. The Lipinski rule states that a molecule considered to be a drug must conform to the entire rule with only one violation.

– Not more than 5 hydrogen bond donor
– Not more than 10 hydrogen bond acceptors
– A molecular mass less than 500 dalton
– Log P not more than 5. [16]

Most of the compounds screened for adhere to Lipinski rule. Except for Schweinfurthin A and B, which have molecular weights of more than 500 daltons and LogP greater than 5. Other compounds have molecular mass of less than 500 and Log P of less than 5 except for Ambolic acid, lupenone, β-Amyrin and lanosterin which have Log P of 5.413, 6.389, 7.788 and 8.295 respectively. All compounds HBD and HBA adhered to the lipinski rule except for chicoric acid which have 6 HBD. This shows that most of the compounds can serve as drug lead. Log P shows whether a substance can be absorbed by the body. 1.35-1.8 is idea for compounds for central nervous system. less than 5 for sub-lingual. HBA and HBD is used in the quantitative estimate of druglikeness HBD should be less than 5 and HBA should be less than 10. Log S shows the solubility of the compound which affects absorption and it should be greater than -4. Log BB is use to predict the permeability of the compound. Log D predict in-invo permeability it also predicts the behaviour of a compound. Compound with lower melting points absorbed more than those with higher melting points. [17]

The table below shows the compounds present in the extracts and their reported medicinal uses, pharmacological properties and their references.

Table (8): Compounds and their pharmacological activities.

Phytochemicals	Pharmacological activities and medicinal uses	Reference
Calycosin	Treatment of tumors, inflammation stroke and cardiovascular disease, interaction with ER receptors on the cell membrane, modulation of MAPK signaling pathway	18
Acacetin	Neuroprotective, cardioprotective, antidiabetic, anti-cancer, anti-inflammatory and antimicrobial activities.	19
Quercetin	Antioxidant, antiviral, anticancer, antimicrobial, antiinflammatory, neuroprotective and antitumor, heptoprotective cardiovascular protection, protective of the reproductive system and antiobesity agent.	20
Galangin	Antiviral, antimicrobial, antidiabetic and anticancer properties	21
Medicarpin	Antifungal, antibacterial, antiinflammation, antitumor, antiosteoporosis, antimalarial, antioxidant, inhibition of	22

Phytochemicals	Pharmacological activities and medicinal uses	Reference
	neuraminidase and melannin synthesis estrogenic and anti-estrogenic activity, anticlastogensis, immunosuppressive activity and inhibition of acetylcholinal	
Narigenin	Antioxidant, antitumor, antiviral, antibacterial, anti-inflammatory, antiadipogenic, antihepatitis c, anti-aging and cardioprotective effects.	23
Vestitol	Antimicrobial and anti-inflammatory activities	24
Liquiritigenin	It also has Anti-psychostimulant and antimonoamine oxidases which result in it use in therapy for disorder of the CNS. It also has anti-parkison disease memory enhancing, anti-Alzeheimer's neuroprotection against Glutamate-induced toxicity. neuroprotection against stroke, Neuroprotection against Brain Giloma, Activity against HIV-I-Associated neurocognitive disorder, anti-nociception activities, antibacteria, anti-inflammatory estrogen receptor signaling activities, anti-periodontitis, antiasthmatic effects, antidiabetic activities, anti-osteoporosis, hepatoprotective, anti-mutagenic and anti-cancer activities.	25
Pinocemberin	antimicrobial, anti-inflammatory, antioxidant and anticancer activites. It can also be used as neuroprotective against cerebral ischemic which can be antiexcitoxic and apopotic effect. It can reduce reactive oxygen species, regulate apoptosis, protect blood-brain barrier, modulate mitochondrial function it also has potential to treat ischemic stroke	26
Chrysin	Treatment of degenerative disorders with cytotoxic and anti-inflammatory functions.	27
Epicatechin	Antioxidant, antiviral, antimalarial and anticarcinogenetic, anti-hyperlipidaemic, anti-inflammatory, cytoprotective and antidiabetes	28
Eupatolin	Anticancer, anti-oxidant and anti-inflammatory	29
Flavan-3-ol	Anticancer, anticardiovascular disease, antineurodegenerative disease and diabetes, antiosteoporosis, anti-inflammatory and anti-parasites	30
Propolin A	Induces cytotoxicity effect in human melanonia A2058 cells induced apoptosis in A2058 cells. Have strong ability to scavenge free radicals. Strong antioxidants	31
Kaempferol	Usedful in reducing the risk of chronic diseases like cancer, inflammation, apoptosis, angiogenesis and metastatis. Strong antioxidant	32
β-amyrin	Antiinflammation and antitumor agents	33
Lupenone	Antiviral, anti-inflammatory, anticancer and anti-diabetes pharmacological activities including chemopreventive activity and can treat chagas disease without toxicity	34
Cinnamic acid	Antioxidants and antimicrobial (antifungal, antiviral and antibacterial).	35
Isoferulic acid	An Antioxidant. used in treatment of diabetes, coronary heart disease, anginapectoris, heart stroke and cardiovascular disease	36
Caftaric acid	It have antioxidant, anti-inflammatory, anti-mutagenic, chemopreventive, antidiabetic, hepatoprotective,	37

Phytochemicals	Pharmacological activities and medicinal uses	Reference
	anticarcinogenic, anti-hypertensive, anti-metabolic syndrome, anti-obesity and neuroprotective effects	
Chicoric acid	Have anticancer, anti-obesity, antiviral, anti-diabetic, anti-HIV, anti-oxidant, anti-microbial and neuroprotection effect	38
Methyl caffeate	anti-bacterial, antifungal, antitubercolsis, antioxidant, anti-inflammatory, anticancer, cytotoxic and apoptotic effects	39, 40
Scheweinfurthins	They have anti-proliferative activity against human cancer cells. They exhibit various biological activities which include anticancer, antimicrobial, cytotoxicity and radical scavenging effects	41
Resveratrol	Antioxidant potential, it exhibit anti-tumor activity, anticancer, anti-inflammatory, cardioprotective, phytoestrogenic and neuroprotective activities	42
Lanosterin	Sterol lipid	43

The table 9 below shows the result of the molecular docking done with propolis compounds and some anticancer drugs against a CDK protein (6GUE) a cancer protein this was used because most of the medicinal claims of the compounds from table 8 showed that most of the compounds are anti-inflammatory, antitumor and anticancer this will help to confirm the pharmacological claims of the compounds.

The compounds from propolis and some anti-cancer drugs like 5-fluorouracil, floxuridine, azacitidine, cladribine, capecitabine and vismodegib were docked on the protein from cancer cell that was cocrystalized with CDK2/CyclinA in complex with AZD5438 (Cyclin dependent inhibitor) and the results are reported below.

Table (9): Result of the molecular docking of propolis compounds on CDK protein with PDB ID 6gue

Compounds	Pubchem ID	Binding affinity
Narigenin	932	-8.9
5-Fluorouracil	3385	-5.1
Floxuridine	5790	-7
Azacitidine	9444	-6.7
Cladribine	20279	-7.5
Capecitabine	60953	-7.9
Pinocemberin	68071	-9
Epicatechin	72276	-8.3
β-amyrin	73145	-7.7
Lupenone	92158	-8.9
Vestitol	92503	-8.5
Liquiritigenin	114829	-8.8
Lanosterin	246983	-10
Medicarpin	336327	-8.3
Cinnamic acid	444539	-6.5
Resveratrol	445154	-8.3
Scheweinfurthin-A	643462	-9.1
Scheweinfurthin-B	643463	-9
6-Mercaptopurine	667490	-4.9

Compounds	Pubchem ID	Binding affinity
Methyl caffeate	689075	-6.8
Isoferulic acid	736186	-6.6
Flavan-3-ol	3707243	-8.4
Eupatolin	5273755	-8.4
Quercetin	5280343	-9
Acacetin	5280442	-8.5
Calycosin	5280448	-9.2
Kaempferol	5280863	-8.9
Chrysin	5281607	-8.9
Galangin	5281616	-8.6
Chicoric acid	5281764	-8.8
Caftaric acid	6440397	-7.7
Propolin A	10411087	-9
Cocrystalline Ligand	16747683	-9
Vismodegib	24776445	-9.5

From the result it was shown that lanosterin, vismodegib, calycosin and scheweinfurthin A have the best Binding affinity which were smaller than -9 showing a better binding compared to the other compounds and drugs. Therefore, they were chosen for modeling to check their bonds. The other compounds showed favorable binding affinities as they showed binding activities between -7 to -9, of all the compounds analysed they were all good and having binding affinity of less than -7 except for 6-Mercaptopurine, Isoferulic acid, Methyl caffeate, Cinnamic acid, Azacitidine and 5-Fluorouracil which are between -4 to -6.7 these show that most of the phytocompounds from propolis have better binding affinities than the control drugs and their amino acids-ligand interactions are better due to low binding affinity showing that the compounds binding easily with the amino acids at the binding site which suggest that the compounds can act as Cyclin dependent inhibitor which inhibits the growth and multiplication of cancer cells.

Figure (6): Raw protein.

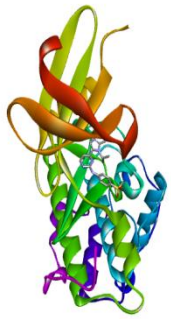
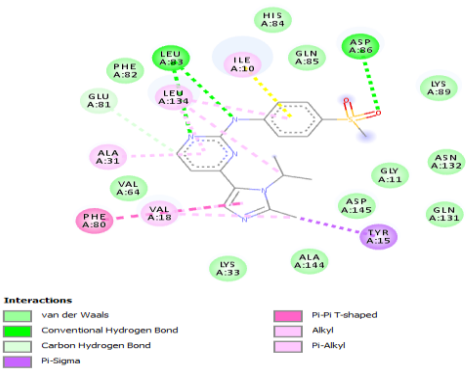
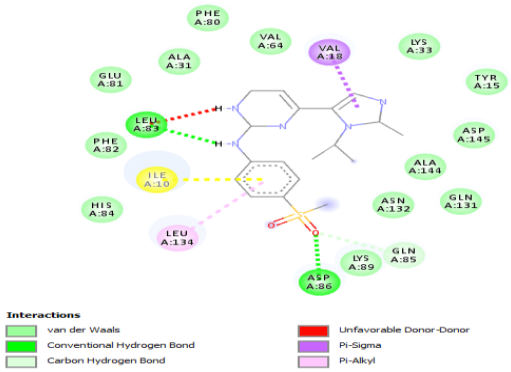


Figure (7): Prepared protein.



Figure(8): Interaction of the Co-ligand before docking



Figure(9): Co-Ligand interaction after docking

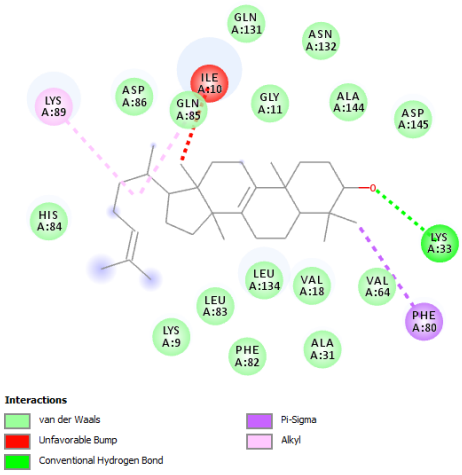


Figure (10): Interaction of Lanosterin.

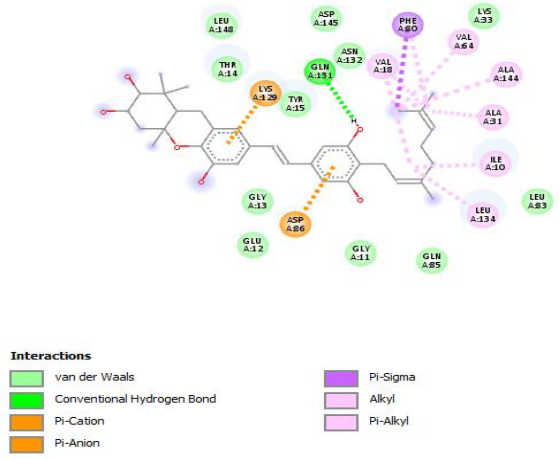


Figure (11): Interaction of Schweinfurthin A

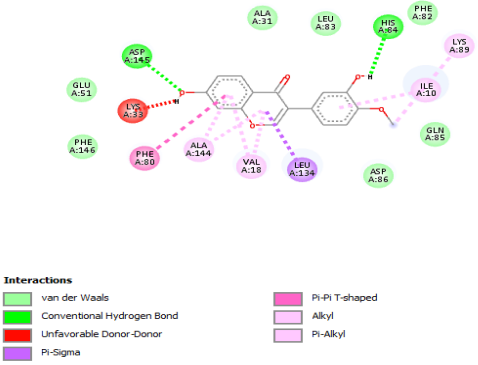


Figure (12): Interaction of Calycosin.

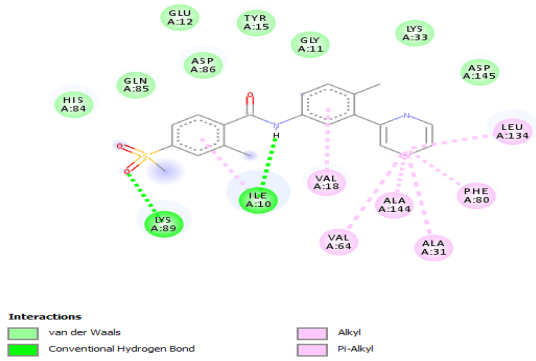


Figure (13): Interaction of Vismodegib.

Table 10 show the strong bonding interaction of the best binding compounds, standard drug and the cocrystalline ligand

Table (10): Protein-Ligand interaction of the better binding compounds.

Compound	Binding affinity	Type of interaction	Amino acids
Lanosterin	-10	Conventional hydrogen bond Pi-Sigma Alkyl	LYS 33 PHE 80 LYS 89
Schweinfurthin A	-9.1	Conventional hydrogen bond Pi-cation Pi-Anion Pi-sigma Alkyl and Pi-alkyl	GLN 131 LYS 129 ASP 86 PHE 80 VAL 18, VAL 64, ALA 144, ALA 31, ILE 10, LEU 134
Calycosin.	-9.2	Conventional hydrogen bond Pi-Sigma PI-Pi T shape Alkyl and Pi-Alkyl	ASP 145, HIS 84 LEU 134 PHE 80 LYS 89, ILE 10, ALA 144, VAL 18
Vismodegib	-9.5	Conventional hydrogen bond Alkyl and Pi-Alkyl	LYS 89, ILE 10 VAL 18, VAL 64, ALA 144, ALA 31, PHE 80, LEUU 134
Cocrystalline-Ligand	-9	Conventional hydrogen bond Carbon hydrogen bond Pi-Sigma Pi-Alkyl	ASP 86, LEU 83 GLN 85 VAL 18 LEU 134

Note: The van der waal bond was not included in the table as it is not a strong bond.

The Cocrystallized-ligand interactions with the protein of the CDK2/CyclinA before docking showed hydrogen bond on the following amino acids LEU 83, HIS 84, with Vander-waal bond at GLY 11, LYS 33, VAL 64, PHE 82, HIS 84, GLN 85, LYS 89, GLN 131, ASN 132. ALA 144 and ASP 145 with Carbon-Hydrogen bonds, Pi-Sigma bonds, Pi-Pi-T shaped, Alkyl, Pi-alkyl bonds with various amino acids. The interactions after docking showed that Lanosterin have hydrogen bond at LYS 33 but have van der waal bonds with the following amino acids HIS 84, ASP 86, GLN 85, GLN 131 etc this show that the docking was at the same site and the interaction was similar with the one from the Co crystalline ligand. Scheweinfurthin A has hydrogen bond interaction at GLN 131 and Van der waal interaction at LEU 10, GLY 11, TYR 15 LYS 33, LEU 83, GLN 85, ASN 132 these also show that the interaction was at the same site with the same amino acids. The same thing applies with Calycosin which have 2 hydrogen bonds at HIS 84 and ASP 145 with other bonds with different amino acids at the same site. The control drug Vismodegib also has 2 hydrogen bonds at ILE 10 and LYS 89 with other bonds on the amino acids at the active site. The Co-crystallized ligand was also dock on the protein and it interacted with the amino acids with hydrogen bonds at LUE 83 and ASP 86 with other bonds at various amino acids on the same site. These show that the interaction from the docking was at the active site hence the docking result reflect the interaction of the accurate site.

Vismodegib is a drug for the treatment of basal cell carcinoma. The drug also underwent clinical trials for metastatic colorectal cancer, small-cell lung cancer, advanced stomach cancer, medulloblastoma, chondrosarcoma and pancdrosarcoma around June 2011. [44]

The Protein 6GUE is a cancer protein having cyclin-dependent kinase 2 and cyclin-A2 as macromolecules in the 4 chains A-D and unique ligand 4-(2-methyl-3-propan-2-yl-imidazol-4-yl)-{N}-(4-methylsulfonylphenyl) pyrimidin-2-amine in chain A. The chain A and ligand A was chosen for the docking due to the appropriation of the ligand. Cyclin-dependent kinase (CDK) inhibitors are responsible for treatment of cancer as dysregulation of the cell cycle characterizes many cancer subtypes. Potent CDK2 inhibitors target certain cancers. [45]

Therefore the binding of the compounds to the CDK can inhibit the cell multiplication and growth of the cancer cell as it binds to the active site.The result from the docking showed that this propolis compounds have anticancer properties as the binding affinity were very competitive with the standard drugs used in the docking therefore validated the report of previous works by researchers like Forma which reported that propolis compounds can inhibit proliferation, angiogenesis, and metastasis of cancer cells and stimulate apoptosis. [46] The study also supported the report of Elumalai et al which reported that the presence of caffeicacid, phenethyl ester (CAPE), artepillin C, and chrysin and other propolis compounds are responsible for the anticancer potential of propolis. They also reported that propolis and its active compounds inhibit cancer progression by targeting multiple signaling pathwaysincluding phosphoinositide 3-kinases (PI3K)/Akt and mitogen-activated protein kinase (MAPK) signaling molecules, and inducecell cycle arrest. Induction of apoptosis by propolis is mediated through extrinsic and intrinsic apoptotic pathways. [47] from the above result it can be suggested that propolis compounds have great potentials from cancer treatment.

CONCLUSION

The dereplication analysis using LC-MS was useful in showing some of the isolated compounds therefore saving the time and cost of re-isolating already existing compounds. Most of the compounds present in the extracts were reported to be medicinal with high pharmacologically activities which scientifically validate the ethno-medical claim of propolis. The molecular docking showed their anticancer activities. The results also validate and confirm that the composition of propolis may depend on the colour and solvent of extraction.

Conflict of Interest:

The authors declare no conflict of interest

Author’s Declaration:

The authors hereby declare that the work is original and that any liability for claims relating to the content of this article will be borne by them.

Consent approval for publication

All the authors agreed that the work should be published.

Availability of data and materials

The authors declared that the data and materials for this study is available.

Author's contribution

Sylvester N. Ugariogu: conceptualization, writing-original draft, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, and writing review & editing. **Stephanie A. Ezirim**: conceptualization, writing-original draft, investigation, methodology, supervision, validation, visualization,

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