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

*Sylvester N. Ugariogu[1,*], Stephanie A. Ezirim[1], Ijeoma A. Duru[1], Samya S. Alenezi[2], John A. Anyam[3] & John O. Igoli[3]*

[1] Chemistry Department Federal University of Technology Owerri, Nigeria. [2]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, UK. [3]Department of Chemistry, Joseph Sarwuan Tarka University Makurdi, Nigeria

*Corresponding author: mastersylvester@yahoo.com

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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

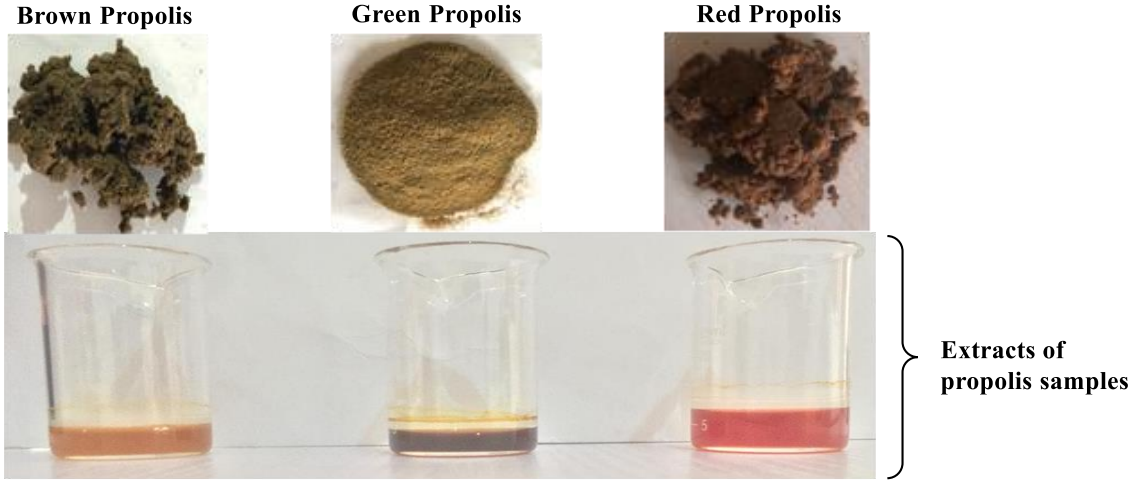
Propolis is a complex, resinous, gummy or sticky substance produced by honeybees from plants with the aid of bee enzymes. Propolis is naturally produced by bees from exudates, nectar, pollens and saps of trees. It has been reported to have numerous pharmacological activities and applications due to its 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**

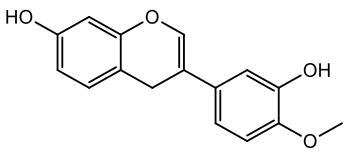
Prior to molecular docking analysis, proteins were pre-processed using Discovery Studio 2020. This step involvedthe removal of any hetero-groups, other chains and water molecules. The active site of the protein was identified using Discovery studio. Furthermore, the preparation of ligands and receptors 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**

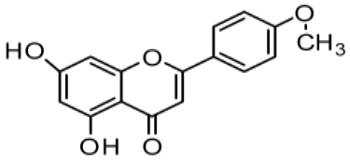
The statistical method used in the dereplication analysis was simple percentage, the number of the screened compounds from the extract were compared with the 31 compounds that were detected.

**Results and Discussion**

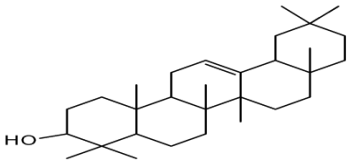
The results of the LC-MS for the extracts are reported in the appropriate tables and figures. The physicochemical parameters for the compounds using Mest ReNova software was also reported, the molecular docking analysis of the most prominent compounds were also reported by appropriate tables and figures 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.



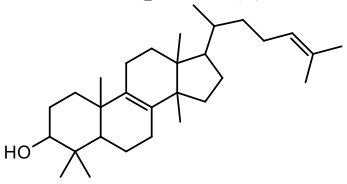
**Compound** (**1**)**:** Calycosin



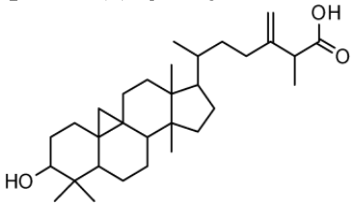
**Compound** (**2**)**:** Acacetin



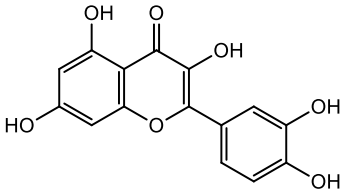
**Compound** (**3**)**: β-**amyrin



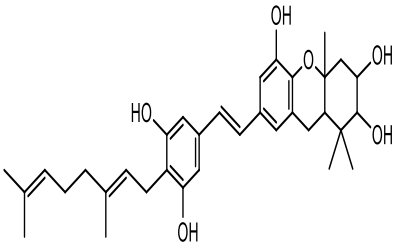
**Compound** (**4**)**:** Lanosterin



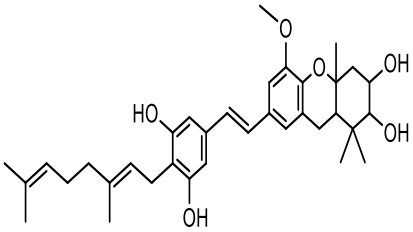
**Compound (5) :** Ambolic



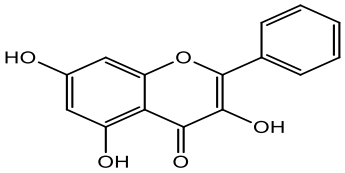
**Compound (6) :** Quercetin



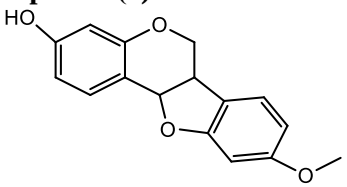
**Compound (7):** Schweinfurthin A



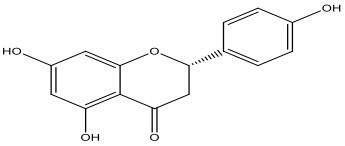
**Compound (8):** Schweinfurthin B



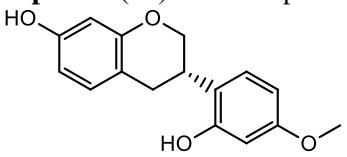
**Compound** (**9**)**:** Galagin



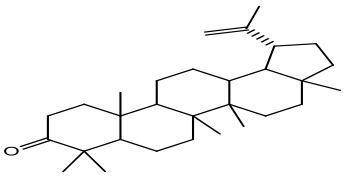
**Compound** (**10**)**:** Medicarpin



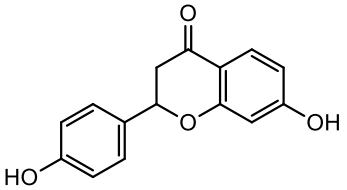
**Compound** (**11**)**:** Naringenin



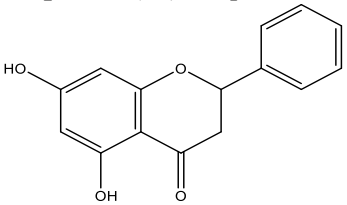
**Compound** (**12**)**:** Vestitol



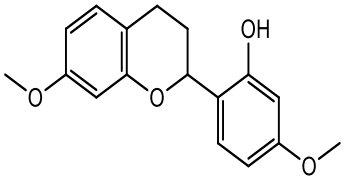
**Compound** (**13**)**:** Lupenone



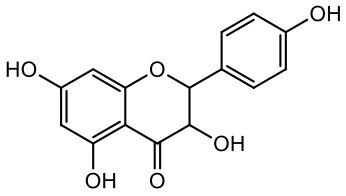
**Compound** (**14**)**:** Liquiritigenin



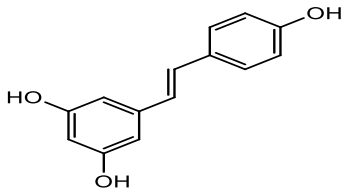
**Compound** (**15**)**:** Pinocemberin



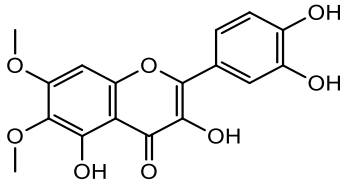
**Compound** (**16**)**:** Isosativan



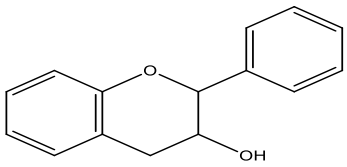
**Compound** (**17**)**:** Kaempferol



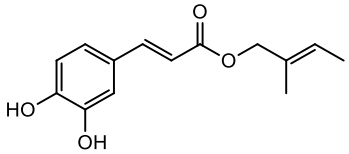
**Compound** (**18**)**:** Resveratrol



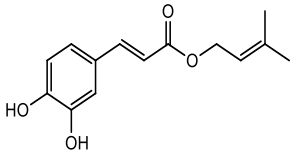
**Compound** (**19**)**:** Euptolin



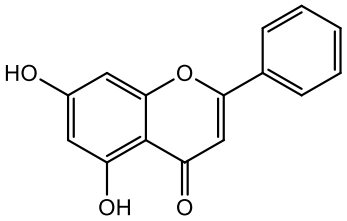
**Compound (20**)**:** Flavan-3-ol



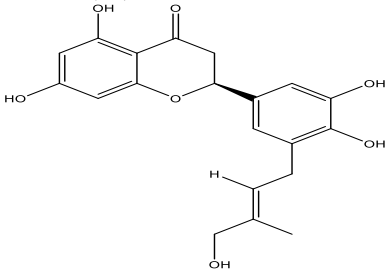
**Compound** (**21**)**:** 2-methyl-2-butenyl-(E)Caffeate



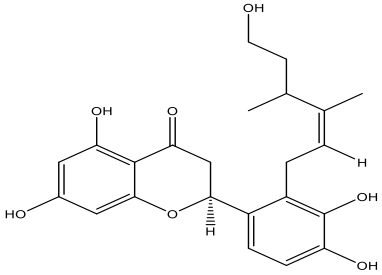
**Compound** (**22**)**:** 3-methyl-2-butenyl caffeate



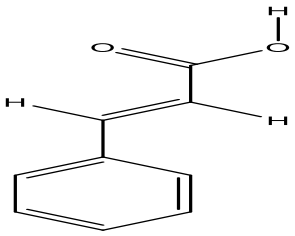
**Compound** (**23**)**:** Chrysin



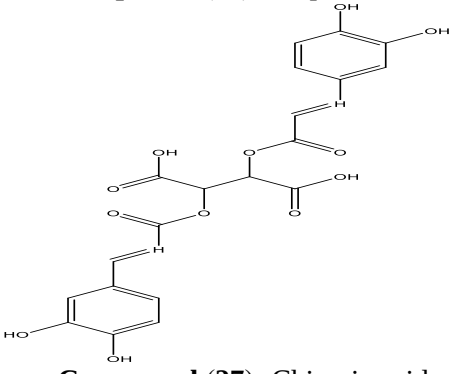
**Compound** (**24**)**:** Propolin B



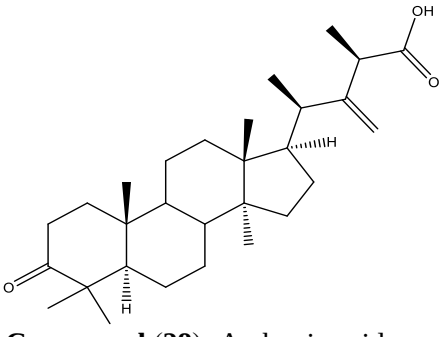
**Compound** (**25**)**:** Propolin A



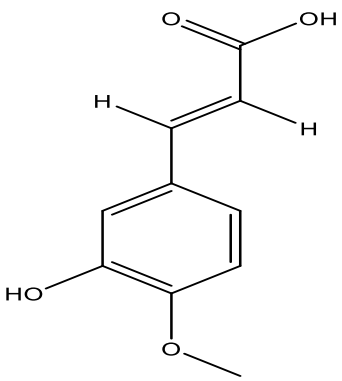
**Compound (26**)**:** Cinnamic acid



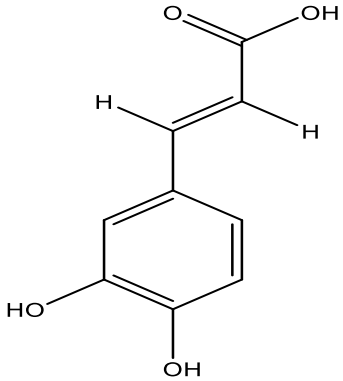
**Compound** (**27**)**:** Chicoric acid



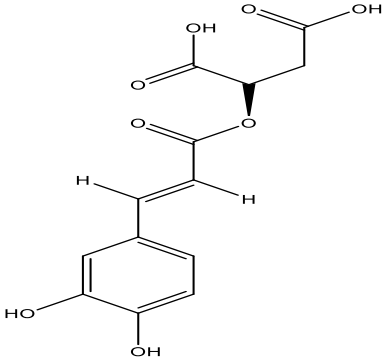
**Compound** (**28**)**:** Ambonic acid



**Compound (29):** Isoferulic Acid



**Compound (30):** Caffeic acid



**Compound (31):** Cafteric acid

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

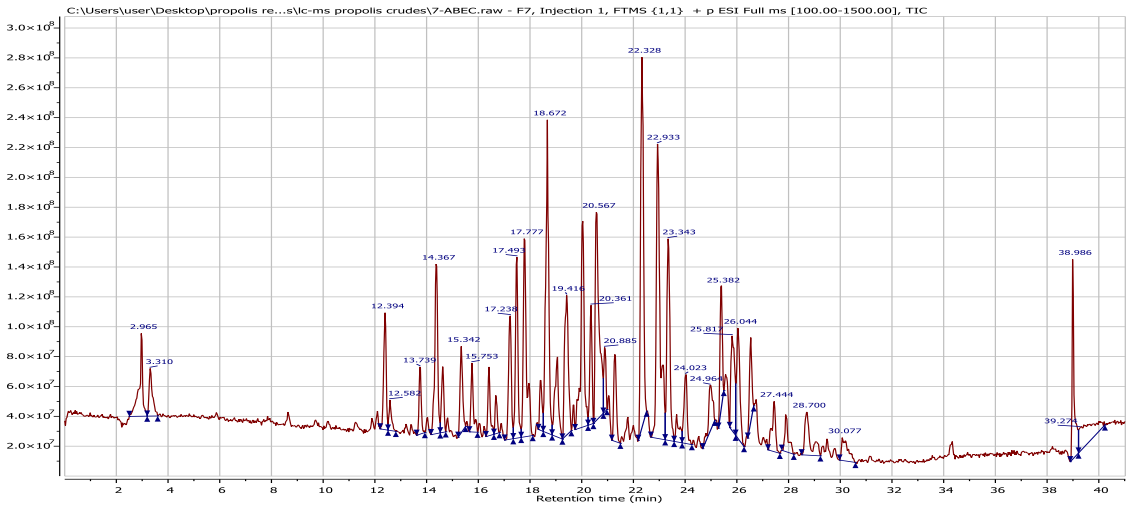



**Figure (2):** Result of LC-MS of the ethyl acetate extract of the brown propolis.

**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.

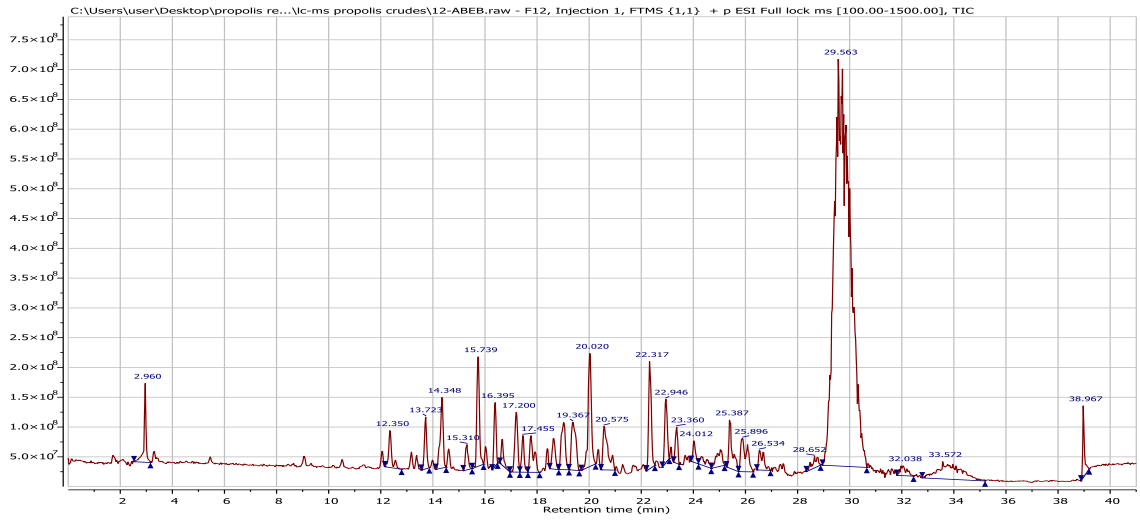



**Figure (3):** Result of LC-MS ethyl acetate extract of green propolis.

**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.

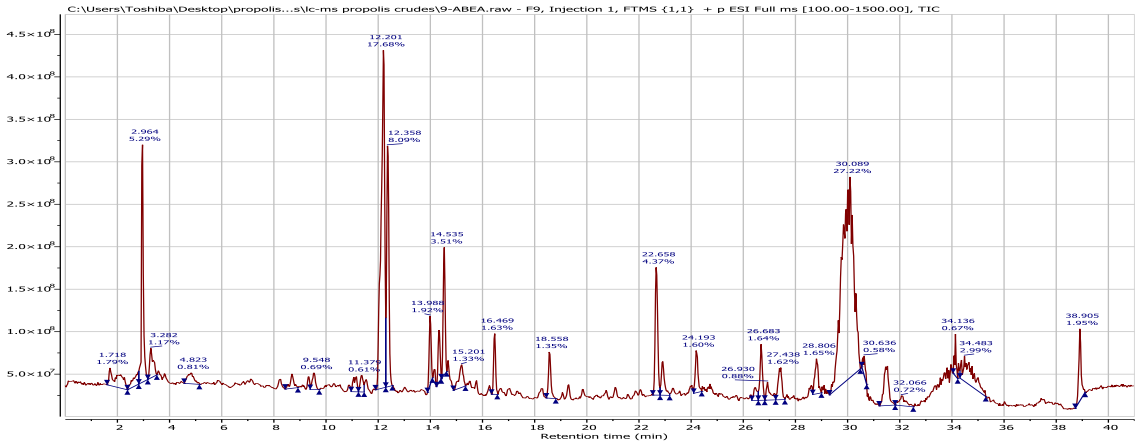



**Figure (4):** Result of LC-MS Ethyl Acetate Crude extract of Red Propolis.

**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]

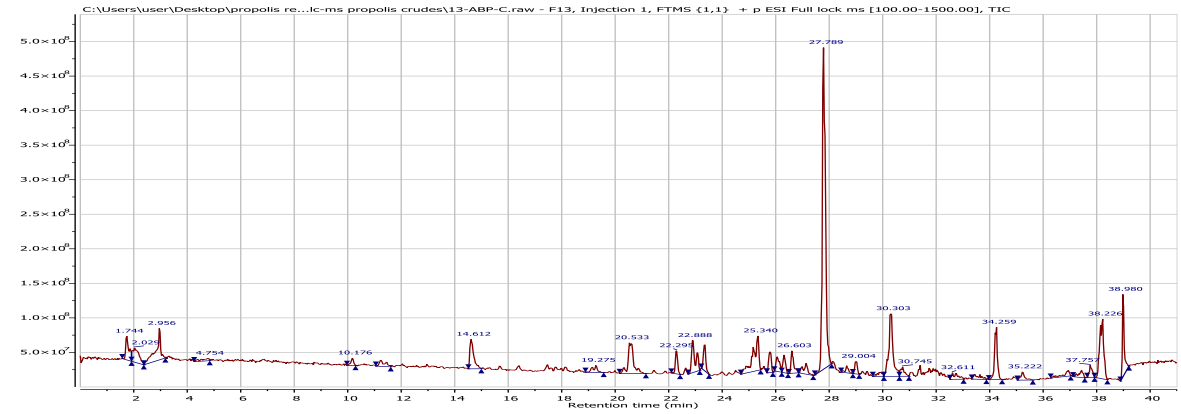



**Figure (5):** Result of LC-MS Hexane crude extract of Brown Propolis.

**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.

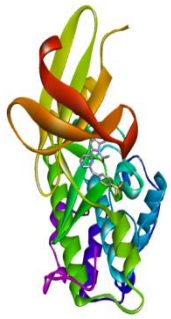
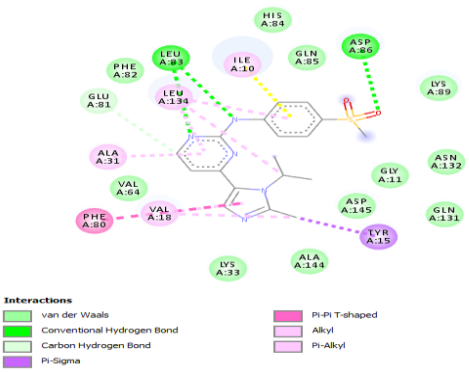
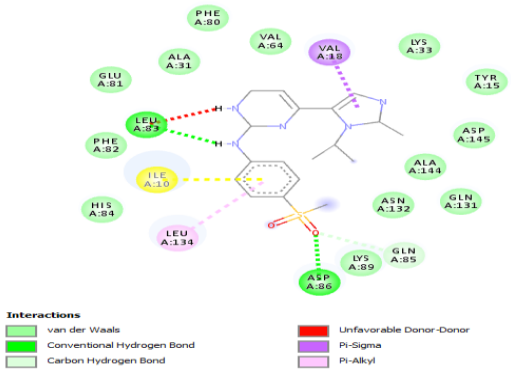


**Figure (7):** Prepared protein.




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




**Figure(9):**Co-Ligand interaction after docking

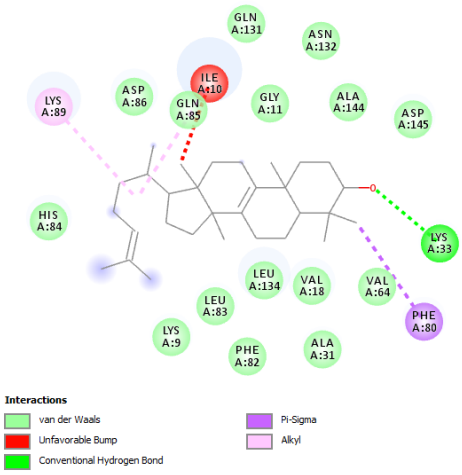



**Figure (10):** Interaction of Lanosterin.

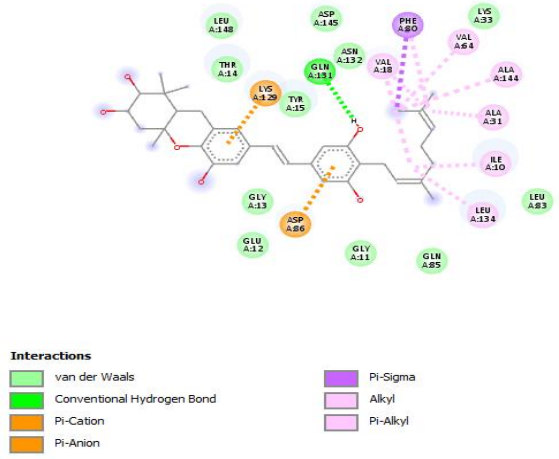



**Figure (11):**Interaction of Schweinfurthin A

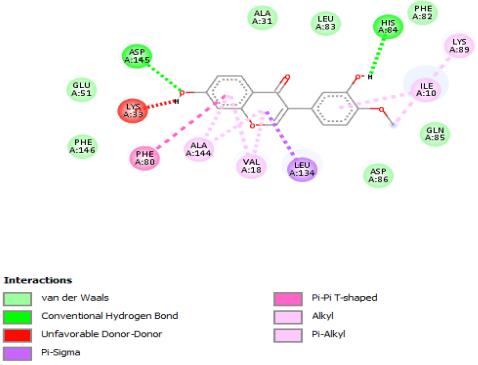



**Figure (12):** Interaction of Calycosin.

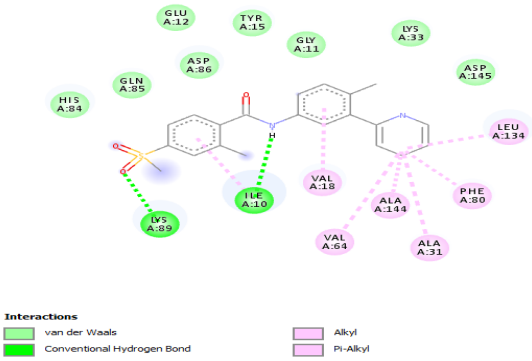



**Figure (13):** Interaction of Vismodegib.

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