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Physicochemical Assessment and Drug Potential of Some Phenylpropanoid and Flavonoid Compounds of Ethyl Acetate Eluate from Umudike Propolis

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ABSTRACT

Propolis is a natural product produced from bees which have been acclaimed a medicinal product. In this study, greenish propolis samples collected from a private apiary in Umudike of Abia State, Nigeria were subjected to column chromatography using ethyl acetate as solvent. The eluates that were soluble in ethyl acetate only was subjected to liquid chromatography–mass spectrometry (LC-MS) and the result was analyzed using MestReNova (Mnova) software to identify and match the chemical compositions present in the eluate from some already isolated compounds from Propolis in Africa. The following compounds; 2-methyl-2-butenyl (E)-caffeate, Prenyl caffeate or 3-methyl-2-butenyl caffeate, 3-methyl-3-butenyl-(E)-caffeate, isosativan, calycosin, liquiritigenin, naringenin, eupatolitin, acacetin, quercetin, galangin and medicarpin were identified to be present in the sample and they were matched with their structure, matching score, percentage purity and retention time. Drug-like properties of the matched compounds were determined using the computed physicochemical properties from the Mnova software. The result showed the presence of some known compounds which have been used in medicinal chemistry and drug synthesis.

Keywords: Propolis, Physicochemical, Drug-like properties, Phytochemicals, LC-MS, Mnova.

Introduction

Propolis have been described as a complex resinous product due to its chemical composition which varies from place to place based on plant source available to the bees at collection site as they are gathered by honeybees.[1] It have been reported to plays an essential role in the hive as bees use it as a building material to seal holes, to repair and strengthen the thin borders of the comb, and for making the entrance of the hive tight which make it easier to defend against intruder and regulate weather. Moreover, it has been reported to possess defensive substances against microorganisms.[2] Propolis has biological and pharmacological properties which include antibacterial, anti-fungal, antiviral, anti-inflammatory, hepatoprotective, anti-oxidant and antitumor.[3] It chemical composition is diversified due to its botanical origin, climate condition and geography around the hive where it is collected from as the type of trees, plant and vegetation affects the chemical composition.[4] Propolis has been used anciently as a natural remedy for varieties of conditions, and recent interests have been renewed in reinvestigating the potentials of propolis for drug development with some significant advancement in the understanding of its chemistry and biological activity.[5–9]

Most propolis have been reported to compose of wax, fatty acids, resins, essential oils, pollens, enzymes, sugar, minerals, and microelements.[9] It is reported that over 500 phytochemicals have been identified collectively all over the world from propolis.[8]

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Phytochemical composition of propolis varies from place to place, season to season.[10] Propolis can be classified base on their botanical origin. Propolis samples from temperate regions have been reported to possess mostly phenolic phytochemicals that are seen in poplar bud, due to the trees around the region where the bees source their food are mostly poplar trees. It has been observed that this type of propolis is rich in flavonoids, cinnamic acids, esters, phenolic acids and other aromatic acids. [11] But those collected from tropical regions have been reported to have wider array of plant sources at their disposal, propolis from tropical regions has been characterized by the presence of other types of phytochemicals such as terpenoids, lignans, stilbenes, benzophenones, and phenoliclipids.[12–16] Researchers have used some standard hyphenated techniques like High performance liquid chromatography- diode array detector (HPLC-DAD), Gas chromatography-mass spectrometry (GC-MS), Liquid chromatography-mass spectrometry (LC-MS), and Liquid chromatography- tandem mass spectrometry (LC-MS-MS) to chemically profile propolis samples.[17–19] A recent study of the African propolis identified several phenylpropanoids and flavonoids such as coumaric acid, 2-methyl-2-butenyl (E)-caffeate, prenyl caffeate or 3-methyl-2-butenyl caffeate, 3-methyl-3-butenyl-(E)-caffeate, chicoric acid, cinnamic acid, isoferulic acid, β-amyrin, isosativan, calycosin, liquiritigenin, isoliquiritigenin, pinocembrin, naringenin, macarangin, eupatolitin, quercetin, acecetin and medicarpin. The extracts were also observed to possess significant antimicrobial activities.[20] However, the true identity of specific phytochemicals could not be authentically confirmed using the aforementioned techniques alone.[21] For this reason, we decided to focus these work on the use of Mnova software to search for de-replication of already isolated Phenylpropanoids and flavonoids from African propolis which were obtained using various preparative chromatography techniques and characterized unambiguously by means of mass spectrometry and nuclear magnetic resonance spectrometry (NMR) analysis. The physicochemical assessment and drug potential was based on the druglikeness properties of the compounds found in the eluate which is a qualitative concept used in drug design to show how druglike a substance can be

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with respect to factors like bioavailability.[22] Druglike properties include: Solubility which is the ability of the drug to dissolve in both water and fat, which can affects the orally administered drugs which needs to pass through the intestinal lining after it is consumed and the ability is known as LogP.[22] Potency at biological target, Ligand efficiency and lipophilic efficiency, Molecular weight can affect the druglikeness of a substance, any substance that has the potency to hit the biological target and have efficiency in binding with ligands is druglike, the lipinski's rule implies that the smaller the molecular weight of compound for drug the better and therefore suggested that drug candidate should have molecule weight less the 500.[23] The study gear towards contributing more specific reports on the current body of knowledge on the phytochemicals (phenylpropanoid and flavonoid), physicochemical parameter relating to drug lead agents in Umudike propolis.

Materials and Methods

Nigerian propolis sample was obtained from Umudike Umuahia, Abia State, Nigeria in September 2019. Solvents used were commercially obtained and re-distilled before use. TLC was performed using pre-coated TLC grade silica gel on Aluminum sheets (Pre-coated Silica gel PF254, Merck, Germany). 150 g of green grounded dried propolis sample was extracted with ethyl acetate 600 mL in a clean container for 72 hours via maceration, the extracts were filtered and the solvents were evaporated. The dried ethyl acetate extracts were subjected to column chromatography using ethyl acetate throughout over silica gel (230-400 mesh ASTM).[24]

LC-MS Analysis

Liquid chromatogram-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). 2 mg of the ethyl acetate extracts were dissolved in 1 mL of methanol and filtered. 10 µL of the filtrate was used for the analysis. The mobile phase used 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 minuutes 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 minutes. The samples were run in duplicate, 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 mass spectrometry software from Thermo Fisher Scientific.24

Mnova software analysis.

The prediction and the matching of the compound with already isolated compounds from literatures were done using MestreNova which is spectral data analyzing software, which can be run on Windows, Mac OS and whole range of Linux distributions. Mnova NMR processes data (1H, 13C or any other 1D NMR as well as any 2D correlations, such as Heteronuclear single quantum coherence spectroscopy (HSQC), Heteronuclear multiple band correlation (HMBC), nuclear overhauser effect spectroscopy (NOESY), correlated spectroscopy (COSY), total correlated spectroscopy (TOCSY), etc.) fully automatically, whilst preserving the raw data in the background to allow more detailed processing for the expert user, with a wealth of advanced functions. The analysis capabilities of the software are unmatched. Very advanced algorithmia enables best in class analysis of spectra (peak picking, integration, multiplet analysis, etc.) without user intervention, complemented by the ability to optimize results interactively. [25]

Results and Discussion

The LC-MS analysis of the eluate from the ethyl acetate extract of the propolis was done and analyzed with Mnova software for de-replication study. The results are shown in Figures 1 and 2 below.

The matching of phenylpropanoid compounds was done for the eluate to check the compounds present in the eluate and it was reported in Table 1. The matching was done for Caffeic acid, prenyl caffeate, methyl caffeate, isopentyl, 2-methyl-2-butenyl-(E)-caffeate, 3-methyl-3-butenyl-(E)-caffeate, Coumaric acid, Cinnamic acid, Isoferulic acid, caftaric acid and chicoric acid. But the matching showed positive for Prenyl caffeate or 3-methyl-2-butenyl caffeate, 2-methyl-2-butenyl-(E)-Caffeate, 3-methyl-3-butenyl-(E)-caffeate while it showed negative for others like caffeic acid, coumaric acid, cinnamic acids, caftaric acid, isoferulic acid and chicoric acid. These shows that out of the 11 phenylpropanoid compounds analyzed for in the eluate, only 3 of them were present in the eluate sample.

The matching of flavonoid compounds was done for the eluate to check the compounds present in the eluate and it was reported in table 2. The matching was done for Calycosin, eupatolitin, acacetin, quercetin, galangin, naringenin, medicarpin, isosativan, macarangin and liquirtigenin. But the matching showed positive for the entire compounds analyzed for except for macarangin which showed negative in the matching. The result showed the matching score, MS purity and retention time for the LC-MS. The matching score above 0.5 showed a good match but those below 0.5 indicate low match. The result showed that the compounds in the eluate are mostly flavonoids and few phenylpropanoids.

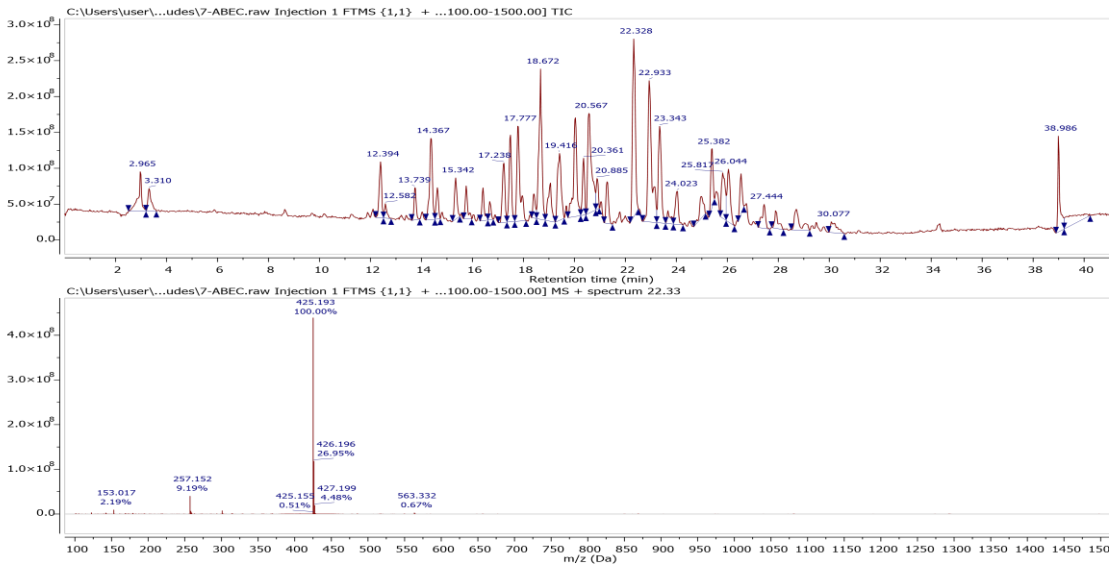

Figure 1: LC Chromatogram of the ethyl acetate extract of propolis.

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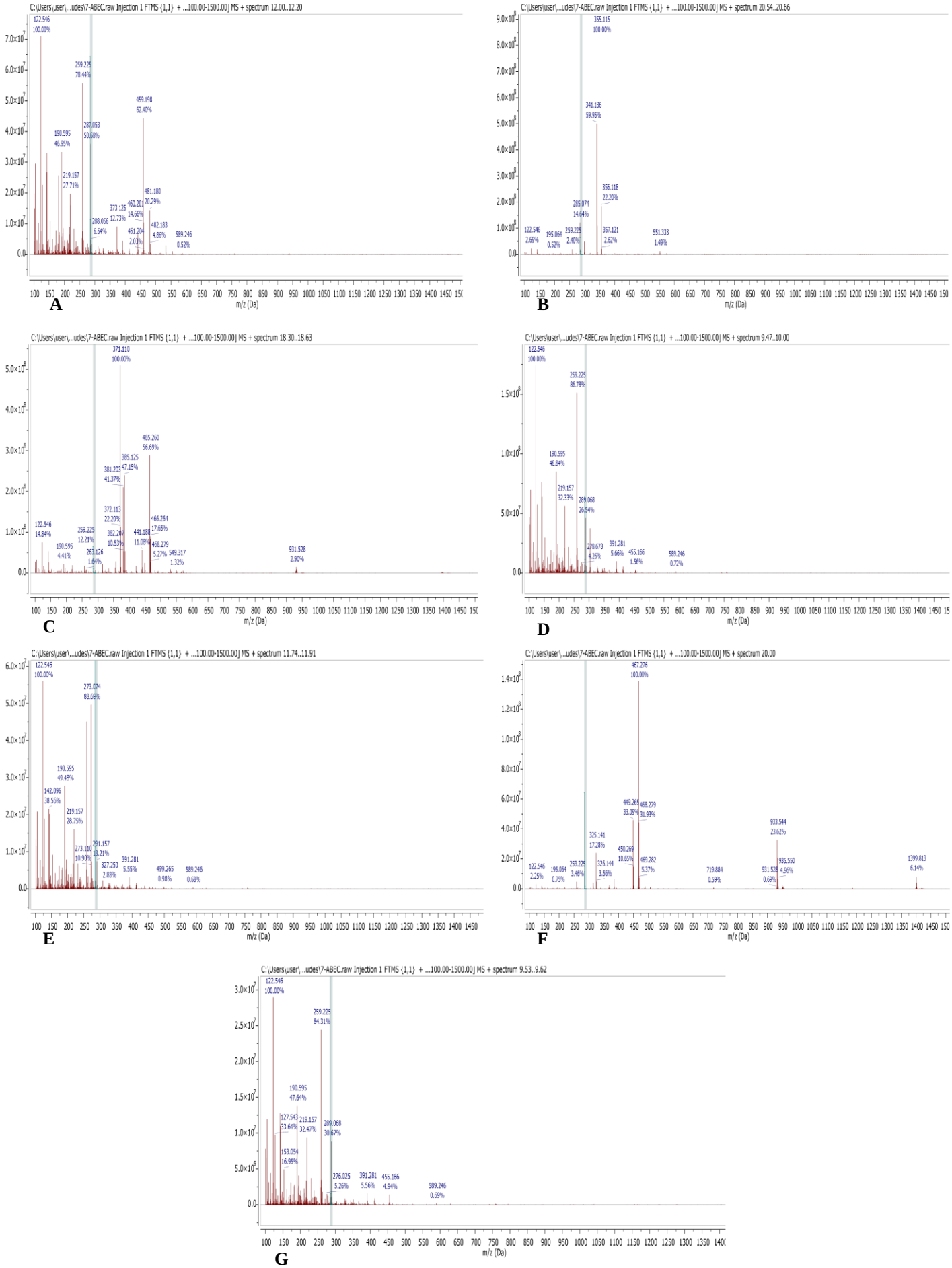

Figure 2: Mass Spectra of the compounds eluted at different retention times. **(A)** 12.12 min **(B)** 20.54 min **(C)** 18.58 min **(D)** 9.76 min **(E)** 11.88 min **(F)** 20.04 min **(G)** 9.689 min

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The physicochemical parameters that were checked for include; Octanol/water partition coefficient-(Log P), polar hydrogen bond donor-(HBD), polar hydrogen bond acceptor-(HBA), water solulibility of drug-(Log S), distribution coefficient-(Log D), blood-brain barrier-(Log BB). The result of the physicochemical parameters of the compounds isolated from the eluates of the propolis reported in ta ble 3 and 4 showed that their log P is between 1.873-3.731 Eupatolilin having the lowest Log P of 1.873 while Isosativan having the highest of 3.731. Log P have been reported to play an important role in helping scientists reduce the liabilities of new drug candidate by helping in predicting the actual transport of a compound around the body. It has been reported to affects drug formulation, dosing, clearance and toxicity. [26] Log P has been reported to provide information to know whether a substance can be absorbed by the body or living tissue or be easily disseminated or carried away by water, a negative value for log P has been indicated to represent that the compound has a higher affinity for the aqueous phase (it is more hydrophilic) while when log P = 0 it shows that the compound is equally partitioned between the lipid and aqueous phases. A positive value for log P has been reported to represent a higher concentration in the lipid phase (i.e. the compound is more lipophilic) and the entire log P for the tested compounds are positive. Log P has been reported to be an important parameter in the pharmaceutical industries in understanding the behavior of drug molecules in the body, the LogP of drugs used for central nervous system has been reported to be in the range of 1.35-1.8, while drugs intended for sub-lingual absorption are always less than 5 [26] and all the Log P for the tested compounds are less than 5 therefore suggesting the compounds as having drugs potency. The polar HBD (Hydrogen bond donor) for all the compounds present in the sample ranges from 8.407 - 39.499.Isosativan having the lowest HBD of 8.407 and quercertin highest value of 39.499. Hydrogen bond donor is a bond or molecule that supplies the hydrogen atom of a hydrogen bond. The
value of polar hydrogen bond acceptor ranges from 19.697 – 34.410 with medicapin having the lowest value of 19.697 and eupatolilin having the highest value of 34.410. HBA and HBD have been used in the quantitative estimate of druglikeness as HBD should be <5 and HBA < 10)[27] The value of Log S ranges from -4.352 to -2.607 with calycosin having the lowest value of -4.352 and narigenin having the highest value of -2.607. The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. Typically, a low solubility goes along with bad absorption and therefore the general aim is to avoid poorly soluble compounds. Estimated log S value is a unit stripped logarithm (base 10) of the solubility measured in mol/liter. More than 80% of the drugs in the market have a (estimated) log S value greater than -4.[28]

Log BB value is used to predict the permeability of new druglike compound comparing it concentration in the brain and in the blood. It has been reported that three prediction zones have be defined:
- Ratio <20% Log BB<-0.7 – Red zone - No permeability
- Ratio >=20% and <50% Log BB>=-0.7 and <-0.30 – Yellow zone - Possible permeability
- Ration >= 50% - Green zone - High probability of permeability. [29]

It was observed that the optimal log BB cutoff is as follows: compounds having log BB values ≥ 0.3 can readily penetrate the BB, compounds with values between 0.3 <logBB< −1 can still pass the BBwhile compounds having log BB values < −1 are poorly diffused into the brain.[30]

LogD is used to help predict in-vivo permeability of active compounds in drug discovery. It can help us evaluate and predict the likely behavior of a compound prior to synthesis. LogD has been reported to be the appropriate descriptor for lipophilicity of ionizable compounds because it accounts for the pH dependence of a molecule in aqueous solution. The distribution coefficient, log D, has been defined as the ratio of the sum of the concentrations of all forms of the compound (ionized plus un-ionized) in each of the two phases, one essentially always aqueous; as such, it depends on the pH of the aqueous phase, and log D = log P for non-ionizable compounds at any pH[31] The log D for all the matched compounds range from 1.661-3.598 for quercetin and 2-methyl-2-butenyl (E)-Caffeate respectively. Drugs with lower melting point are more likely to be well absorbed than higher melting points drugs. 32 Most of the matched compounds have high melting points which can have reduce melting point after undergoing drug production processes.

Table 1: Result of molecular match in the eluate for Phenylpropanoids

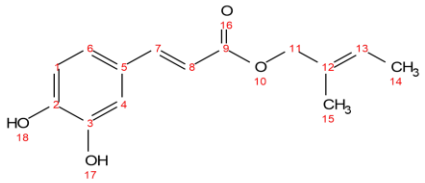
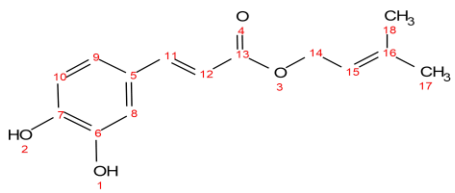
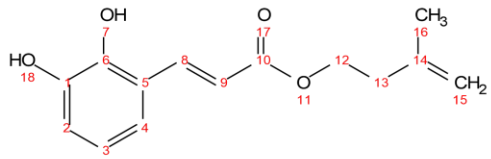
Molecule	Formular	Molecular Weight	Match Score	MS purity	Retention Time
 2-methyl-2-butenyl (E)-Caffeate	$C_{14}H_{16}O_4$	248.105	0.944 Match	0.081	12.12
 Prenyl Caffeate or 3-methyl-2-butenyl caffeate	$C_{14}H_{16}O_4$	248.105	0.944 Match	0.081	12.12
 3-methyl-3-butenyl-(E)-caffeate	$C_{14}H_{16}O_4$	248.105	0.944 Match	0.081	12.12

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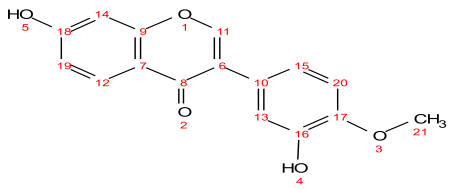
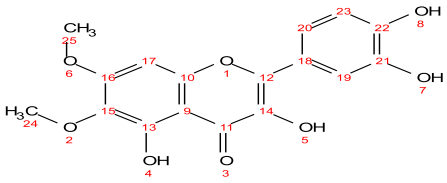
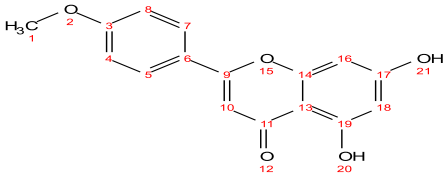
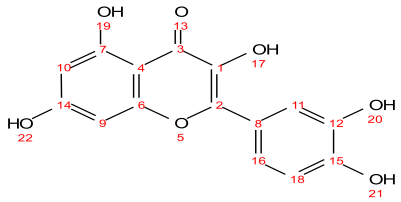
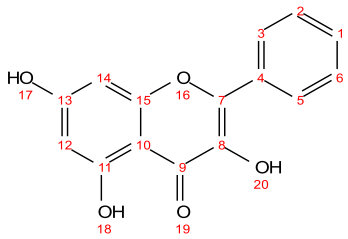
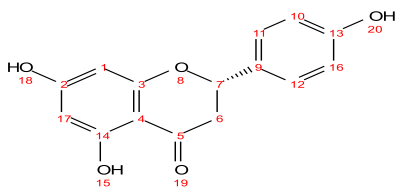
Table 2: Result of molecular match in the eluate for Flavonoids

Molecule	Formulae	Molecular Weight	Match score	MS purity	Retention Time
 Calycosin	$C_{16}H_{12}O_5$	284.068	1.000 Match	0.143	20.54
 Eupatolitin	$C_{17}H_{14}O_8$	346.069	0.983 Match	0.225	18.58
 Acacetin	$C_{16}H_{12}O_5$	284.068	1.000 Match	0.143	20.54
 Quercetin	$C_{15}H_{12}O_8$	302.043	0.999 Match	0.071	9.76
 Galangin	$C_{15}H_{10}O_5$	270.053	0.999 Match	0.071	9.76
 Naringenin	$C_{15}H_{12}O_5$	272.068	0.996 Match	0.147	11.88

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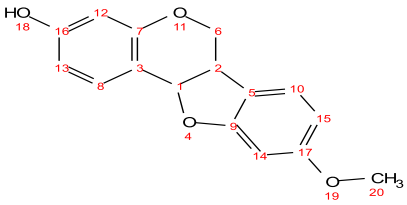
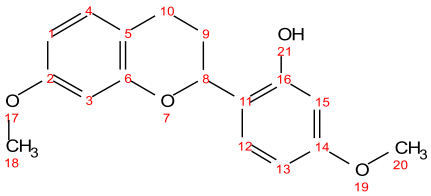
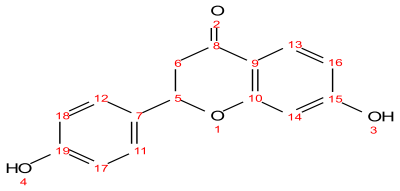
	$C_{16}H_{14}O_4$	270.089	0.998 Match	0.071	9.76
Medicarpin					
	$C_{17}H_{18}O_4$	256.074	0.992 Match	0.084	20.04
Isosativan					
	$C_{15}H_{12}O_4$	256.074	0.992 Match	0.084	9.68
Liquiritigenin					

Table 3: Result of the physicochemical parameter of the Phenylpropanoids compounds

Compound Name	LogP	Polar HBD	Polar HBA	LogS	Boiling point	Hydroxyl radical attack	Melting Point	LogD	LogBB
2-methyl-2-butenyl(E)-Caffeate	3.278	18.669	26.908	-3.524	600.708	-8.309	373.001	3.598	-0.023
Prenyl Caffeate or 3-methyl-2-butenyl caffeate	3.129	18.873	28.299	-3.335	616.291	-8.330	414.134	3.073	-0.002
3-methyl-3-butenyl-(E)-caffeate	2.999	17.221	25.649	-3.168	606.331	-8.558	357.151	2.923	0.056

Table 4: Result of the physicochemical parameter of the flavonoid compounds

Compound Name	LogP	Polar HBD	Polar HBA	LogS	Boiling point	Hydroxyl radical attack	Melting Point	LogD	LogBB
Calycosin	2.884	18.544	26.837	-4.352	735.453	-8.405	510.858	3.024	-0.174
Eupatolitin	1.873	31.290	34.410	-3.964	829.137	-7.899	589.071	1.744	-0.455
Acacetin	2.997	17.370	29.799	-4.280	737.453	-8.260	496.238	2.888	-0.369
Galangin	2.669	23.520	21.922	-3.882	746.789	-8.379	514.477	2.515	-0.519
Quercetin	1.925	39.499	31.214	-3.371	837.989	-7.423	619.463	1.661	-0.741
Isosativan	3.731	8.407	21.308	-3.129	590.019	-8.677	394.871	3.439	0.265
Liquiritigenin	2.325	18.973	25.687	-2.998	636.226	-8.842	459.525	2.045	-0.127
Naringenin	2.123	25.947	29.104	-2.607	673.869	-8.179	500.452	1.665	-0.335
Medicarpin	3.086	10.220	19.697	-3.300	563.706	9.041	427.464	2.760	0.318

Conclusion

The matching of the compounds in the eluate showed more positive results for flavonoids than phenylpropanoid indicating that the eluate composes more of flavonoids. The physicochemical parameters of most of the compounds present in the eluate showed great drug potentials except for the high HBA, HDB and melting point which can be reduced during drug production processes and purification, the Log P, Log D, Log S and Log BB which is the main parameters that is checked for drugability is within the range, this showed that the eluate have a great drug potential.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

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

References

1. Falcao SI, Freire C, Vilas-Boas M. A Proposal for Physicochemical Standards and Antioxidant Activity of Portuguese Propolis. J Am oil Chem Soc. 2013; (1):1-15.
2. Bankova VS, De Castro SL, Marcucci MC. Propolis: recent advances in chemistry and plant origin. Apidologie 2000; 31(1):3-15.
3. Banskota AH, Tezuka Y, Kadota SH. Recent progress in pharmacological research of propolis. Phytother Res. 2001; 15(7):561-561.
4. Bankova V. Chemical diversity of Propolis and the problem of standardization. J Ethnopharmacol. 2005; 100(2005):114-117.
5. Burdock GA. Review of the biological properties and toxicity of bee propolis (propolis). Food Chem Toxicol. 1998; 36(4):347-363.
6. Castaldo S and Capasso F. Propolis, an old remedy used in modern medicine. Fitoterapia 2002; 73:(1):1-6.
7. Wagh VD. Propolis: a wonder bees product and its pharmacological potentials. Adv Pharmacol Sci. 2013; 308249.
8. Huang S, Zhang CP, Wang K, Li G, Hu FL. Recent advances in the chemical composition of propolis. Molecules 2014; 19(12):19610-19632.
9. Silva-Carvalho R, Baltazar F, Almeida-Aguiar C. Propolis: a complex natural product with a plethora of biological activities that can be explored for drug development. Evid-Based Compl Altern Med. 2015; 206439.
10. Bueno-Silva B, Marsola A, Ikegaki M, Alencar SM, Rosalen PL. The effect of seasonson Brazilian red propolis and its botanical source: chemical composition and antibacterial activity. Nat Prod Res. 2017; 31(11):1318-1324.
11. Bankova V. Recent trends and important developments in propolis research. Evid-Based Compl Altern Med. 2005; 2(1):29-32.
12. Petrova A, Popova M, Kuzmanova C, Tsvetkova I, Naydenski H, Muli E, Bankova V. New biologically active compounds from Kenyan propolis. Fitoterapia 2010; 81(6):509-514.
13. De Castro VF, Ishidaa G, Negri A, Salatino MF, Bandeira CL. A new type of Brazilianpropolis: prenylated benzophenones in propolis from Amazon and effects against cariogenicbacteria. Food Chem. 2011; 125:966-972.
14. Piccinelli AL, Mencherini T, Celano R, Mouhoubi Z, Tamendjari A, Aquino RP, Rastrelli L. Chemical composition and antioxidant activity of Algerian propolis. J Agric Food Chem. 2013; 61(21):5080-5088.
15. Kardar MN, Zhang T, Coxon GD, Watson DG, Fearnley J, Seidel V. Characterisation of triterpenes and new phenolic lipids in Cameroonian propolis. Phytochem. 2014; 106:156-163.
16. Papachroni D, Graikou K, Kosalec I, Damianakos H, Ingram V, Chinou I. Phytochemical analysis and biological evaluation of selected African propolis samples from Cameroonand Congo. Nat Prod Commun. 2015; 10(1):67-70.
17. Hernandez IM, Cuesta-Rubio O, Campo Fernandez M, Perez RA, Deoca Porto MR, Piccinelli AL, Rastrelli L. Studies on the constituents of yellow Cubanpropolis: GC-MS determination of triterpenoids andflavonoids. J Agric Food Chem. 2010; 58(8):4725-4730.
18. Sun YM, Wu HL, Wang JY, Liu Z,Zhai M, Yu RQ. Simultaneous determination ofeightflavonoids in propolis using chemometrics-assisted high performance liquid chromatography-diode array detection. J Chromatogr B. 2014; 962:59-67.
19. Noureddine H, Hage-Sleiman R, Wehbi B, Fayyad-Kazan H,Hayar S, Traboulssi M, Alyamani OA, Faour WH, El-Makhour Y. Chemical characterization and cytotoxicactivity evaluation of Lebanese propolis. Biomed Pharmacother. 2017; 95:298-307.
20. Blicharska N and Seidel V. Chemical Diversity and Biological Activity of African Propolis. Springer Nature Switzerland AG. Prog Chem Org Nat Prod. 2019; 109:415-450.
21. Siheri W, Zhang T, Ebiloma GU, Biddau M, Woods N, Hussain MY, Clements CJ, Fearnley J, Ebel RE, Paget T, Muller S, Carter KC, Ferro VA, De Koning HP, Watson DG: Chemical and antimicrobial profiling of propolis from different regions within Libya. PLoSOne 2016; 11(5):1-16.
22. Uetrecht J. Prediction of a new drug's potential to cause idiosyncratic reactions". Curr Opin Drug Discov Dev. 2001; 4(1):55-59.
23. Uetrecht J. Idiosyncratic drug reactions: past, present, and future". Chem Res Toxicol. 2008; 21(1):84-92.
24. Sami B, Okoro H, Igoli NP, Igoli JO. Isolation of Isosativan from Nigeria Red Propolis. Trop J Nat Prod Res. 2020; 4(3):77-79.
25. What is MestreNova? Software. Retrieved from https://guides.libraries.uc.edu/c.php g=222784&p=1473465 Software in Chem-Bio library [cited 2020 Jun 15]
26. Bhal SK. Log P- making sense of the value. Advanced chemistry Development, Inc.Toronto ON, Canada. [Online] [cited 2020 Jun 29]. Available from www.acdlabs.com.
27. Brito MA. Pharmacokinetic study with computational tools in the medicinal chemistry course. Braz J Pharm Sci. 2011; 47(4):797-805.
28. Log S Calculation www.Organic-chemistry.org/prog/logs.html Retrieved 30/06/2020
29. Chevalier R. Penetration assessment of diatarysuppliments and drugs through the blood brain barrier for potential treatment of parkinson's disease. [Online 2018] [cited 2020 June 12] Available from https://www.biorxiv.org/content/10.1101/362541v2
30. Sarun K, Chanin N, Lertyot T, Apapan S, Chartchalerm I, Virapong P. Influence of logBB cut-off on the prediction of blood-brain barrier permeability. Biomed Appl Technol J. 2013;1(1):16-34.
31. Bhal SK. Lipophilicity Descriptors: Understanding When to Use LogP & LogD Advanced Chemistry Development, Inc. Toronto, ON, Canada online [cited 2020 Jun 13]. Available from http://www.acdlabs.com
32. Chu KA and Yalkowsky SH. An interesting relationship between Drug absorption and melting point. Int J Pharm. 2009; 373(1-2):24-40.