South Asian Research Journal of Natural Products

Volume 6, Issue 2, Page 101-109, 2023; Article no.SARJNP.99246

GC-MS and Proximate Analysis of the Hydromethanol Extract of *Craterispermum schweinfurthi* Leaves

F. Saronee [a*], F. S. Amah-Tariah [a], B. C. Chinko [a] and D. V. Dapper [a]

[a] Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port-Harcourt, PMB-5323, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/99246

Original Research Article

Received: 05/03/2023
Accepted: 09/05/2023
Published: 19/05/2023

ABSTRACT

Introduction: On account of their accessibility, affordability and reduced side effects, approximately half of the world's population depends on herbal products for their primary healthcare needs. Although the possible potency and efficacy of some of these products have been documented, more studies are needed to accurately screen, identify and possibly characterize their numerous bioactive components.
Aim: The present study therefore, presents a preliminary report of proximate analysis and Gas Chromatography and Mass Spectrometry (GC-MS) assessment of the possible bioactive constituents of hydromethanol extract of C*raterispermum schweinfurthi* leaves.
Methodology: The air-dried leaves were powdered and subjected to selective sequential extraction using solvents of increasing polarity through percolation, namely, water and methanol to obtain the extract. GC–MS investigation of bioactive components of the extract was carried out using Agilent Technologies.

**Corresponding author: Email: saroneefridayt@yahoo.com;*

Results: Result of the proximate composition of hydro-methanol extract of C*raterispermum schweinfurthi* shows that residual moisture was found to be 28.2%; Ash 11%; Fibre 26.2%; Protein 30.43%; Lipid; 11.42% and Carbohydrate 32.61%. The extract was further subjected to Gas Chromatography-Mass Spectrometry. Obtained results suggests that the extracts of C*raterispermum schweinfurthi* leaves possess several bioactive compounds amongst which include: neophytadiene (most abundant), phytosterol and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol amongst others. The possible pharmacologic, biological effects and industrial application of the identified compounds in the extract are reviewed.
Conclusion: Our findings validate the basis for the use of the leaves of the plant in folk medicine for the treatment of a number of medical conditions in our locality; This is a new idea or perspective that adds to the existing body of knowledge in the area of phytochemistry and lethal toxicity study.

Keywords: Craterispermum schweinfurthi; GC-MS; herbal products; bioactive; biological effects.

1. INTRODUCTION

"On account of their accessibility, affordability and reduced side effects, approximately half of the world's population depend on herbal products for their primary healthcare needs" [1]. "The possible potency and efficacy of some of these products have been documented; however, more studies are required to accurately screen, identify and characterize their numerous bioactive ingredients. Attention should also focus on their modes of action, toxicity and possible interactions with orthodox medications" [2-5]. Reports show that plants are an invaluable primary source of successful drugs, hence the need for continuous screening to identify new compounds [6]. "An important aspect in the investigation of products of plant origin is the identification of the inherent biologically active compounds leading to further pharmacological, biological and scientific investigations" [7-9].

"Craterispermum schweinfurthi is distributed in tropical Africa, Madagascar and the Seychelles" [10-11]. *Craterispermum schweinfurthi* species are shrubs or small trees with axillary or supra-axillary inflorescences, paired at the nodes and often condensed. The anecdotal applications of C*raterispermum schweinfurthi* in traditional medicine are numerous. For instance, in traditional folklore medicine the seed, leaves, and inner bark have been described to have beneficial reproductive efficacies and relief in cases of stomach afflictions, ulcer, diabetes and fever [12]. Although scientific studies on the possible beneficial effects or otherwise of the leaves of *Craterispermum schweinfurthii* are relatively scanty in our environment, the identification of the bioactive compounds in the leaves have become necessary to enable further studies.

"Gas chromatography (GC) is a technique used for identification of minute volatile molecules including hormones and steroids" [13]. "It is widely used to analyse chemicals and screen drugs. Mass spectrometry (MS) quantifies the mass-to-charge ratio of ionic analysts" [14,15]. "Gas chromatography-Mass spectrometry (GC-MS) combined can thus separate complex mixtures and quantify analysts" [16,17]. "Relying on the National Institute of Standards and Technology Chemistry Web Book database [18] for many compounds, scholars can therefore identify and quantify inherent compounds and constituents".

As a response to the many reported anecdotal potentials and beneficial effects of C*raterispermum schweinfurthi* leaves, the present study attempts a preliminary determination of the lethal dose, proximate analysis, phytochemistry and GC-MS scan of possible active ingredients in the hydromethanol extracts of the leaves of the plant. This is with the intention that a phytochemical characterization of the extract could provide rationale for the use of the leaves of the plant in the treatment of common medical conditions in our environment.

2. MATERIALS AND METHODS

2.1 Collection, Identification and Extraction of Plant Materials

Fresh leaves of *Craterispermum schweinfurthii* were obtained from the University of Port Harcourt botanical garden. Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology, University of Port-Harcourt, Nigeria identified and authenticated the specimen and assigned a reference code; UPH/V/296. Voucher specimen was

subsequently deposited in the University herbarium. The plant leaves were gathered and all extraneous materials carefully removed. The leaves were air dried at room temperature for a minimum of 7 days after which it was pulverized into powder and the weighed quantity of 670.6g dissolved using Soxhlet device in 390ml of water-methanol mixture (25:75% v/v BDH) for three days in a jar. It was filtered and concentrated using a rotary evaporator at 40°C and the yield was 73%. Obtained extract was preserved in air tight containers and stocked at room temperature prior administration.

2.2 Acute Toxicity

The acute toxicity of the hydromethanol extract of C*raterispermum schweinfurthi* leaves was determined using Karber's method as modified by Aliu and Nwude, 1982 [19]. Lethal dose (LD_{50}) of the extract was found to be 3968mg/kg body weight.

2.3 GC-MS Analysis

GC–MS investigation of bioactive components of the hydromethanol extract of C*raterispermum schweinfurthi* leaves was carried out using Agilent Technologies at the Central Laboratory, University of Lagos, Nigeria. GC machine with model number GC7990A/MC-5975C, Santa Clara, USA assembled with HP5MS column was used. Spectroscopic determination by GC–MS entails electron ionization network which used high energy electrons of 70 eV, pure helium gas of 99.995% as carrier gas was used with a flow rate of approximately 1 mL/min. The temperature was initially set at 50 to 150 °C but was subsequently increased to 300 °C at about 10 °C/min. One microliter of the already prepared extract diluted with the respective solvents (water and methanol) was injected. Relative amount of the inherent chemical compounds in the extract of C*raterispermum schweinfurthi* was formulated in percentage on account of produced chromatogram peak area. Compounds identification was based on visible molecular structure and mass. Mass spectrum GC-MS interpretation was done using the NIST (National Institute Standard and Technology) database having over 62,000 patterns and Wiley library. Identified compound name, molecular weight, structure and peak value of the compounds of the material tested were determined by correlating with the library source. The relative percentage composition of component compounds was determined by correlating the total mass to the average peak area [20,21].

3. RESULTS AND DISCUSSION

The extract caused some neurological, behavioural, physical and other abnormalities at a dose of 3000mg/kg and above. As stated earlier, the lethal dose (LD_{50}) was found to be 3968mg/kg body weight while lethal concentration was observed to be between 3000mg/kg, 4000mg/kg body weight. Safe doses of administration were found to be between 100mg/kg and 2000mg/kg body weight implying that the extract is relatively and comparatively safe. According to Clarke and Clarke, 1997 [22], any compound with an oral LD_{50} above 1000 mg/kg bw may be considered to be of low toxicity and therefore relatively safe for consumption.

Result of the proximate analysis of the composition of hydromethanol extract of C*raterispermum schweinfurthi* shows the residual moisture to be 28.2%; Ash 11%; Fibre 26.2%; Protein 30.43%; Lipid; 11.42% and Carbohydrate 32.61%. Result of quantitative phytochemical screening of leaf extract of C*raterispermum schweinfurthi* leaves shows the percentage abundance of identified phytochemicals are as follows: polyphenols 2.20%, flavonoids 4.10%, tannins 6.00%, alkaloids 2.00%, and glycosides 1.80%.

Fig. 1 shows the GC-MS spectra of the hydromethanol extract of the leaves of C*raterispermum schweinfurthi*. It indicates the retention time and intensity counts of the various components of the extract. A minimum of 23 bioactive constituents were identified following GCMS analysis. The names of the identified compounds, retention times (RT), peak areas (percentage), chemical formular and molecular weights are presented in Table 1. As shown in Table 1, the predominant components in the hydromethanol extract of the leaves of C*ratererispermum schweinfurthi* include neophytadiene 35.70%, phytosterol 34.66% and 3,7,11,15-tetramethyl-2-hehadecen-1-ol 19.38%. Available literature showed that neophytadiene exhibits some antioxidant, anti-inflammatory and cardioprotective potentials [23]. According to Bouic, 2001: Awad et al. 2008: Cabral and Klein, 2017: Lesma et al. 2018 [24-27], phytosterol has a wide range of biological and pharmacological effects including: anticarcinogenic, immunomo-dulatory, aphrodisiac, anti-inflammatory, anti-diabetic, antioxidant and anticholesterol

properties. The compound 3,7,11,15-Tetramethyl-2-hexadecen-1-ol is a raw material for the production of vitamin K, and vitamin E, which are used as colour retention agents, additives, food stabilizers, colorants amongst others [28].

The possible pharmacologic and biological effects and known industrial application of the identified compounds in the hydromethanol extract of the leaves of *Craterispermum schweinfurthi* are as indicated in Table 2.

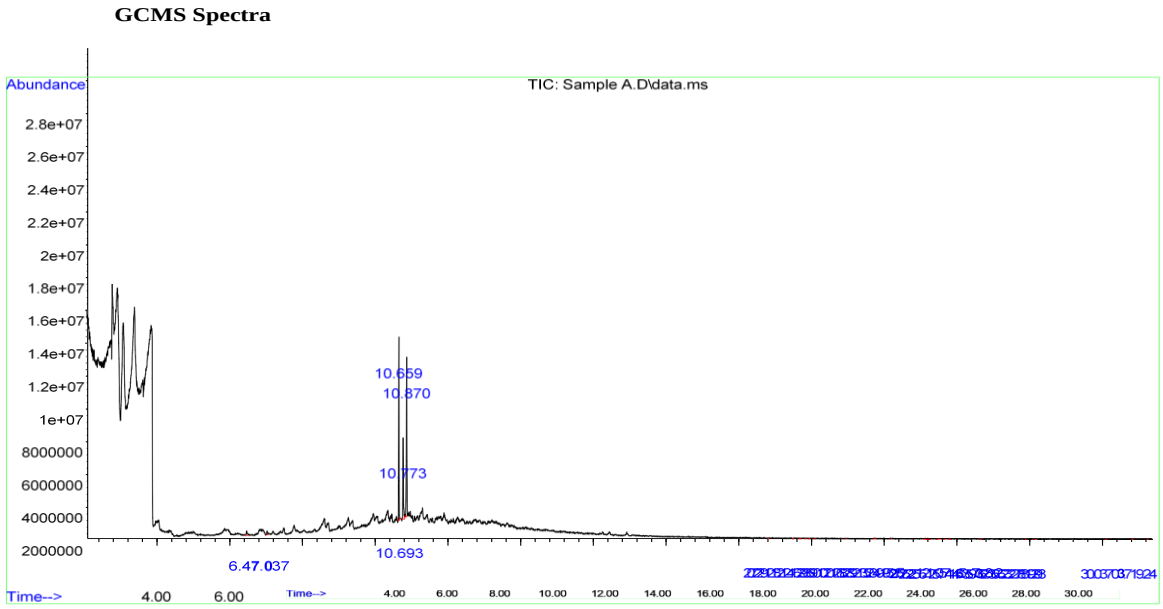

Fig. 1. GC-MS Total Ion Chromatogram of the hyromethanol extract of the leaves of *Craterispermum schweinfurthi*

Table 1. Chemical formular, molecular weight and percentage composition of Identified Compounds in hydromethanol extract of leaves of *Craterispermum schweinfurthii* using GC-MS

S/No	Retention time in minutes	Name of compound	Chemical formula	Molecular weight (g/mol)	Percentage content (%)
1	10.659	Neophytadiene	$C_{20}H_{38}$	278	35.70
2	10.870	Phytosterol	$C_{29}H_{50}O$	414.7	34.66
3	10.773	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	19,38
4	6.470	1,3-Butadiene-carboxylic acid	$C_5H_6O_2$	98	2.09
5	7.037	Hexasiloxane,1,1,3,5,5,7,7,9,9,11, 11-dodecamethyl-	$C_{12}H_{38}O_5Si_6$	430	1.36
6	25.175	1,2-Dihydroanthra [1,2-d] thiazole-2,6,11-trione	$C_{15}H_7NO_3S$	281	0.64
7	25.106	Cyclodecasiloxane eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	740	0.58
8	23.699	Carbamic acid, (3-ethylphenyl)-, ethyl ester	$C_{11}H_{15}NO_2$	193	0.42
9	21.908	1H-Indo,5-methyl-2-phenyl-	$C_{15}H_{13}N$	207	0.32
10	21.662	Imidazo [1,2-a] pyridine-2(3H)	$C_7H_6F_3N_2O$	134	0.31
11	25.433	Chromium tricarbonyl [(1,10,11,12,13,14-η)-tricyclo [8.2.2.2(4,7)] hexadeca-4,6,10,12,13,15-hexaene]-	$C_{19}H_{16}CrO_3$	344	0.28
12	31.212	6,7-Benzo-phenothiazine-5,5-	$C_{16}H_{11}NO_2S$	281	0.27

		dioxide			
13	20.79	2,6-Dihydroxybenzoic acid, 3TMS derivative	$C_{16}H_{30}O_4Si_3$	370	0.27
14	25.782	Cholestan-3α-ol acetate	$C_{29}H_{50}O_2$	430	0.25
15	25.633	3-Amino-2-phenazinol ditms	$C_{18}H_{25}N_3OSi_2$	355	0.24
16	24.151	Phenol,6-methyl-2-[(4morpholinyl) methyl]-	$C_{12}H_{17}NO_2$	207	0.24
17	22.915	Phosphinic acid, diethyl-, (3-trifluoromethylphenyl) amide	$C_{11}H_{15}F_3NO$	265	0.24
18	28.128	2-naphthalenesulfonamide, 6-hydroxy-N-methyl-5- [2-phenyldiazenyl]-	$C_{17}H_{15}N_3O_3S$	341	0.23
19	25.496	9-Desoxo-9-x-acetoxy-3-desoxy-7,8,12-tri-O-acetylingol-3-one	$C_{28}H_{38}O_{10}$	534	0.22
20	26.623	Pyridine-3-carboxylic acid, 1,2,3,4-tetrahydro-5-cyano-4-(2-ethoxyphenyl)-6-mercapto-2-oxo-, methyl ester	$C_{16}H_{16}N_2O_4S$	332	0.22
21	27.899	3H-Pyrazol-3-one, 2,4-dihydro-2,4,4,5-tetramethyl-	$C_7H_{12}N_2O$	140	0. 21
22	30.794	Thiazole, 4-(4-iodophenyl)-2-(3-tolylamino)-	$C_{16}H_{13}IN_2S$	392	0. 21
23	22.068	Pseudosarsasapogenin	$C_{27}H_{44}O_3$	416	0.21

Table 2. Possible pharmacologic and biologic effects & industrial uses of identified compounds in the hydromethanol extract of C*raterispermum schweinfurthi* leaves

Name of bioactive compound	Possible pharmacological, biological effects and industrial uses
Neophytadiene	Neophytadiene exhibits some antioxidant, anti-inflammatory and cardioprotective potentials [23].
Phytosterol	Phytosterol has a wide range of biological and pharmacological effects including: anticarcinogenic, immunomodulatory, aphrodisiac, anti-inflammatory, anti-diabetic, antioxidant and anticholesterol properties [24-27].
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	3,7,11,15-Tetramethyl-2-hexadecen-1-ol is a raw material for the production of vitamin K, and vitamin E, which are used as color retention agents, additives, food stabilizers, colorants amongst others [28].
1,3-Butadiene-carboxylic acid	Most butadiene is used to make synthetic rubbers for the manufacture of tyres, grommets and elastic bands [29]. Toxic to human beyond trace level [30]
Hexasiloxane, 1,1,3,5,5,7,7,9,9,11,11-dodecamethyl-	Antimicrobial, Antiseptic, Hair Conditioning agent, Skin-Conditioning Agent, emollient, Solvent [31]
1,2-Dihydroanthra[1,2-d] thiazole-2,6,11-trione	Found in naturally occurring peptides, and utilized in the development of peptidomimetics (i.e., molecules that mimic the function and structure of peptides) [32].
Cyclodecasiloxane, eicosamethyl-	Emollient, in personal care products, lubricant and de-foaming agent [33].
Carbamic acid, (3-ethylphenyl)-, ethyl ester	Some carbamate esters have use as muscle relaxants [34].
1H-Indo,5-methyl-2-phenyl-	Indoles and derivatives are promising against tuberculosis, malaria, diabetes, cancer, migraines, convulsions, hypertension, bacterial infections of methicillin-resistant *Staphylococcus aureus* (MRSA) and even viruses [35-39].

Name of bioactive compound	Possible pharmacological, biological effects and industrial uses
Imidazo [1,2-a] pyridine-2(3H)-one	Anti-ulcer and cardioprotective effects [40-41].
Chromium, tricarbonyl[(1,10,11,12,13,14-η)-tricyclo [8.2.2.2(4,7)] hexadeca-4,6,10,12,13,15-hexaene]-	Used as reagents for organic synthesis of aromatic compounds [42].
6,7-Benzo-phenothiazine-5,5-dioxide	Possess psycholeptic, sedative, hypnotic, anxiolytic, anticonvulsant, muscle relaxant, and amnesic actions [43,44].
2,6-Dihydroxybenzoic acid, 3TMS derivative	Occurs naturally in Phyllanthus acidus and in the aquatic fern Salvinia molesta. A human xenobiotic and plant metabolite [45].
Cholestan-3α-ol acetate	Main flavor component of mushrooms [46].
3-Amino-2-phenazinol ditms	Key intermediate for the preparation of several fluorescent dyes (e.g., rhodamine B). Useful as hair dye colorants and stabilizers for chlorine-containing thermoplastics [47].
Phenol,6-methyl-2-[(4morpholinyl) methyl]-	Phenol is widely used as an antiseptic to treat sore throat [48]. An active ingredient in some oral analgesics [49].
Phosphinic acid, diethyl-, (3-trifluoromethylphenyl) amide	Acts as a pH adjuster in cosmetics and skin-care products [50] and as a sanitizing agent in the dairy, food, and brewing industries [51].
2-naphthalenesulfonamide, 6-hydroxy-N-methyl-5- [2-phenyldiazenyl]-	Naturally occurring phenolic compound used as a flavoring agent; possesses some anticancer and anti-inflammatory potentials [52,53].
9-Desoxo-9-x-acetoxy-3-desoxy-7,8,12-tri-O-acetylingol-3-one	Improves vision and yeast production [54].
Pyridine-3-carboxylic acid, 1,2,3,4-tetrahydro-5-cyano-4-(2-ethoxyphenyl)-6-mercapto-2-oxo-, methyl ester	Nicotinic acid or niacin is used as a precursor of vitamin B 3 synthesis [55].
3H-Pyrazol-3-one, 2,4-dihydro-2,4,4,5-tetramethyl-	Used in people with familial adenomatous polyposis [56] and in the treatment of osteoarthritis [57].
Thiazole, 4-(4-iodophenyl)-2-(3-tolylamino)-	The thiazole ring is notable as a component of the vitamin thiamine (B1) [58].
Pseudosarsasapogenin	Lowered blood sugar and reversed diabetic weight gain in experimental mice [59]. Halted the decline in muscarinic acetylcholine receptors (mAChRs) in animal models of Alzheimer's disease [60].

4. CONCLUSION

In conclusion, the present study has been able to determine the possible chemical compounds present in the hydromethanol leaves extract of C*raterispermum schweinfurthi* using the GC-MS technique. The potential pharmacological, biological effects and industrial applications of these identified compounds were highlighted. Our preliminary results suggest that the leaves of C*raterispermum schweinfurthi* is a valuable resource of potentially new compounds of health benefit and validates the reported anecdotal usefulness of the leaves.

ETHICAL APPROVAL

Ethical approval was sought and obtained from our Institutional Ethical Committee vide a communication referenced: UPH/CEREMAD/REC/MM82/024 and dated 23rd November, 2021. All experiments were conducted in accordance with the guidelines for the care and use of laboratory animals [61].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sujeong M, Siwoo L, Kihyun P, Sang-Jae L, Byung-Hee K, Younghwa B. Effect of traditional east asian medicinal herbal tea (HT002) on insomnia:a randomized controlled pilot study. Integr. Med. Res. 2019;8(1):15–20.
2. Yu-Ching L, Gar-Hwa L, Tsai-Yu L, Tien-Sheng T, Tsung-Hsun T, Wang-Chuan C, Cheng-Chung L, Keng-Chang T. Development of anti-aflatoxin B1 nanobodies from a novel mutagenesis-derived synthetic library for traditional Chinese medicine and foods safety testing. J. Biol. Eng. 2023;17(1):30-35.
3. Ajay K, Feroz K, Dharmendra S. Exploration of medicinal plants as sources of novel anticandidal drugs. Curr. Top. Med. Chem. 2019;19(28):2579-2592.
4. Falodun A. Herbal medicine in africa-distribution, standardization and prospects. Res. J. Phytochem. 2010;4(3):154-161.
5. Pelkonen O, Xu Q, Fan T. Why is research on herbal medicinal products important and how can we improve its quality. J. Trad. and Comple. Med. 2014;4(1):1-7.
6. Atanasov AG, Waltenberger B, Pferschy EM, Wenzig T, Linder C, Wawrosch P, Uhrin AL. Discovery and resupply of pharmacologically active plant-derived natural products:a review. Biotechnol Adv. 2015;3(8):1582-1614.
7. Guo F, Feng L, Huang C, Ding H, Zhang X, Wang Z. Phenylflavone derivatives from Broussonetia papyrifera in hibit the growth of breast cancer cells *In vitro* and *In vivo*. Phytochem Lett. 2013;6(3):331-336.
8. Momin MA, Bellah SF, Rahman SM, Rahman AA, Murshid GM, Emran TB. Phytopharmacological evaluation of ethanol extract of Sida cordifolia L. roots. Asian Pac. J. Trop. Biomed. 2014;4 (1):18-24.
9. Farid MM, Hussein SR, Ibrahim LF, Desouky MA, Elsayed AM, Oqlah AA. Cytotoxic activity and phytochemical analysis of Arum palaestinum Boiss. Asian Pac J. Trop. Biomed. 2015;5(11):944-947.
10. Taedoumg H, De Block P, Hamon P, Sonké B. *Craterispermum parvifolium* and *C. robbrechtianum* spp. nov. (Rubiaceae) from west central Africa. Nordic. J. Bot. 2012;29(9):700–707.
11. Taedoumg H, hamon p. craterispermum capitatum and *c. gabonicum* (rubiaceae): two new species from the lower Guinean and congolian domains. Blumea J. Plant Taxo. Plant Geo. 2013;57(3):236-242.
12. Iwu MM. Handbook of African medicinal plants. CRC Press; 1993.
13. Ammal RM, Bai GV. GC-MS Determination of bioactive constituents of *Heliotropium indicum* leaf. J. Med. Plants. 2013;1(6):30-33.
14. Kim JY, Suh S, In MK, Paeng KJ, Chung BC. Simultaneous determination of cannabidiol, cannabinol, and gD 9-tetrahydrocannabinol in human hair by gas chromatography-mass spectrometry in human hair by gas chromatography-mass spectrometry. Arch. Pharmacol. Res. 2005; 28(9):1086-1091.
15. Shah I, Al-Dabbagh B, Salem AE, Hamid SA, Muhammad N, Naught DP. A review of bio analytical techniques for evaluation of cannabis (Marijuana, weed, Hashish) in human hair. BMC Chem. 2019;20(9); 13(1):106-126.
16. Syed SU, Maher S, Taylor S. Quadrupole mass filter operation under the influence of magnetic field. J. Mass Spec. 2013; 48(12):1325-1339.
17. (Thermo-Fisher) Thermo Fisher Scientific Website. Gas Chromatography Mass Spectrometry (GC/MS) Information; 2019. Available:https://www.thermofisher.com/ng/en/home/industrial/mass-spectrometry/mass-spectrometry-learning center/gaschromatography-mass-spectrometry-gc-msinformation.html.
18. NIST [National Institute of Standards and Technology]. Chemistry Web Book, SRD 69. Search for species data by chemical name; 2019. Available:https://webbook.nist.gov/chemistry/name-ser/.
19. Aliu YO, Nwude N. Veterinary pharmacology and toxicology experiments. ABU Press, Zaria:1982;104-110.
20. Sharma MD, Rautela I, Sharma, N, Gahlot M, Koshy EP. GC-MS analysis of phytocomponents in juice sample of Indian cane: *Saccharum barberi*. Inter. J. Pharmaceut. Sci. and Res. 2015; 6(12):5147-5153.
21. Indra R, Sharma MD, Sharma N, Kishor, K, Singh K, Sharma N. Comparative GC-MS analysis of leaf and root extract of medicinal plant *Withania somnifera*. World J. Pharmaceu. Res. 2018;7(2):956-972.
22. Clarke EGC, Clarke MI. Factors affecting the actions of poison. In: Veterinary

Toxicology, Bailliere Tindall, London. 1979;9-13.
23. Meenakshi B, Veeresh K, Sali I, Sugumar M, Hannah RV. Neophytadiene from *Turbinaria ornata* suppresses LPS-induced inflammatory response in RAW 264.7 macrophages and sprague dawley rats. Inflam. 2020;43(3):937-950.
24. Bouic PJ. The role of phytosterols and phytosterolins in immune modulation: a review of the past 10 years. Curr. Opin. Clin. Nutr. Metab. Care. 2001;4:471–475.
25. Awad AB, Barta SL, Fink CS, and Bradford PG. β-sitosterol enhances tamoxifen effectiveness on breast cancer cells by affecting ceramide metabolism. Mol. Nutr. Food Res. 2008;52:419–426. 2008/4.
26. Cabral CE, Klein MRST. Phytosterols in the Treatment of Hypercholesterolemia and Prevention of Cardiovascular Diseases. Arq. Bras. Cardiol. 2017;109:475–482. 2017/11.
27. Lesma G, Luraghi A, Bavaro T, Bortolozzi R, Rainoldi G, Roda G, Viola G, Ubiali DS. Phytosterol and γ-Oryzanol Conjugates: Synthesis and Evaluation of their Antioxidant, Antiproliferative, and Anticholesterol Activities. J. Nat. Prod. 2018;8(1):2212–2221.
28. Shearer MJ, Okano T. Key Pathways and Regulators of Vitamin K Function and Intermediary Metabolism. Annual Rev. Nutr. 2018;38 (1):127–151.
29. Kangning L, Wei-lin M, Wen-li X. Studying on the addition reaction mechanism of ethylene and butadiene based on density functional theory. Res. Square. 2021;10(3) 1-12.
30. Schulz, RP, Blumenstein J, Kohlpaintner, C. Crotonaldehyde and crotonic acid. Ullmann's Encyclopedia of Industrial Chemistry. Available:http://onlinelibrary.wiley.com/doi/10.1002/14356007.a08_083.pub2/summary. 25 October, 2019.
31. (EC) Environment Canada. Screening Assessment for the Challenge, Dodecamethylcyclohexasiloxane. Nov 2008. Ottawa, Ontario, Canada: Environment Canada, Health Canada.
32. Mak P, Jeffrey YW, Xu W, Weijun T, Fairlie P, David P. *Peptidomimetics I* (PDF). Topics in heterocyclic chemistry. Spring. Ber. Heid. 2015;48:235–266.
33. MacDonald, M. Your body:the missing manual. O'Reilly media, Inc. 2009.
34. Block W, John H, Beale V, John M. eds. Central nervous system depressant. Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry. Philadelphia, PA: Lippincott, Williams & Wilkins. 2004;495.
35. Kumari A, Singh R, Rajesh K. Medicinal chemistry of indole derivatives: Current to future therapeutic prospectives. Bioorg. Chem. 2019;8(9):1030-1042.
36. Thanikachalam P, Veeraveedu M, Rahul K, Garg V, Monga V. An insight into the medicinal perspective of synthetic analogs of indole:A review. Eur. J. Med. Chem. 2019;180(8):562–612.
37. Qin H, Liu J, Fang W, Ravindar L, Rakesh KP. Indole-based derivatives as potential antibacterial activity against methicillin-resistance *Staphylococcus aureus* (MRSA) . Eur. J. Med. Chem. 2020;19(4):112-124.
38. Ramesh D, Joji A, Vijayakumar B, Gowrivel S, Aiswarya M, Maheswaran K, Tharanikkarasu H. Indole chalcones: Design, synthesis, in vitro and in silico evaluation against Mycobacterium tuberculosis. Eur. J. Med. Chem. 2020; 198(15):112-123.
39. Jia Y, Wen X, Gong Y, Wang X. Current scenario of indole derivatives with potential anti-drug-resistant cancer activity. Eur. J. Med. Chemistry. 2020;200(5):112-119.
40. Juanli W, Hong W, Guiting S, Donglin Y, Jiuhong H, Xiaofang Y, Hongxia Q, Zhong Zhu C, Zhigang X, Chuan X. A novel imidazopyridine derivative exerts anticancer activity by inducing mitochondrial pathway-mediated apoptosis. Biomed. Res. Int. 2020; (20)2:49-55.
41. Sihang L, Can H, Jane P, Amanda T, Serge C, Robert B, Gabrielle P. Pharmacokinetics and pharmacodynamics of loprinone hydrochloride and ropinirole in patients with prolactinomas. Br. J. Clin. Pharmacol. 2019;85(2):366-376.
42. Herndon F, James W, Laurent SE. (η^6-Benzene) tricarbonylchromium, in Encyclopedia of Reagents for Organic Synthesis, John Wiley & Sons, Chichester; 2008.
43. Page C, Michael C, Sutter M, Walker M, Hoffman BB. Integrated pharmacology (2nd ed.). C.V. Mosby; 2002.
44. Olkkola KT, Ahonen J. Midazolam and other benzodiazepines. In Schüttler J, Schwilden H (eds.). Modern Anesthetics.

Handbook of Experimental Pharmacology. 2008;182. 335–360.
45. Niyogi P, Pattnaik S, Maharana L. Quantitative Identification of Major and Minor Constituents of Aerial Parts of Mollugo pentaphylla Linn. Using GC-MS. Asian J. Chem. 2016;28(10):2335-2338.
46. (1-octen-3-ol*) thegoodscentscompany.com*. Retrieved 2015-05-31.
47. Mitchell A, Stephen C, Waring R, Rosemary H. Aminophenols. Ullmann's Encyclo. Indust. Chem. 2000;10(6):88-96.
48. Naresh K, Nidhi G. Phenolic acids:Natural versatile molecules with promising therapeutic applications. Biotechnol. Rep. 2019;20(5):24-29.
49. Frank HK, Stadelhofer ZC. Some facts about phytochemicals and oral analgesics. UC Cooperative Extension Center for Health and Nutrition Research Nutrition and Health Info Sheet. Available:https://nutrition.ucdavis.edu/outreach/nutr-health info-sheets/pro-phytochemical.\. 2019.
50. (ID) Ingredient dictionary. Cosmetic ingredient dictionary. Paula's Choice. Archived from the original on 18 January 2008. Retrieved 16 November 2007.
51. Star-San DF. Five Star Chemicals. Archived (PDF) from the original on 8 February 2016; 2016.
52. Janeš D, Kantar D, Kreft S, Prosen H. Identification of buckwheat (*Fagopyrum esculentum* Moench) aroma compounds with GC–MS. Food Chem. 2009;112(1):120-124.
53. Jeong JB, Hong SC, Jeong HJ, Koo JS. Anti-inflammatory effect of 2-methoxy-4-vinylphenol via the suppression of NF-κB and MAPK activation, and acetylation of histone H3. Arch. Pharma Res. 2011; 34(12):2109-2116.
54. Brennan MB, Struhl K. Mechanisms of increasing expression of a yeast gene in Escherichia coli. J. Mol. Biol. 1980;136 (3):333-338.
55. Gattermann L, Skita A. Eine Synthese von Pyridin-Derivaten. A synthesis of pyridine derivatives]. Chemische Berichte. 1916;49(1):494–501.
56. (CMP) Celecoxib Monograph for Professionals. Drugs.com. American Society of Health-System Pharmacists. 11 November 2019; 2019. Archived from the original on 20 May 2019.
57. Zhang W, Moskowitz RW, Nuki G, Abramson S, Altman RD, Arden N. OARSI recommendations for the management of hip and knee osteoarthritis, part I:critical appraisal of existing treatment guidelines and systematic review of current research evidence. Osteo. Cart. 2007;15(9):981–1000.
58. Eicher T, Haunptmann S. The chemistry of heterocycles: Structure, reactions, synthesis and applications. J. Chem. 2003; 10(6):12-23.
59. Fabiolla R, Santos P, Heitor-Gomes A, Brenda SM, Saravanan S, Adriano A, Souza A, Jackson-Roberto G, Silva A, Parimelazhagan T, Lucindo-José Q J, Jullyana de-Souza SQ. Anti-inflammatory and modulatory effects of steroidal saponins and sapogenins on cytokines: A review of pre-clinical research. Phytomedicine. 2022;96,16(8):153-162.
60. Hu Y, Yaer X, Xia Z, Zongqin V, Sun Q, Orsi A, Rees D. A new approach to the pharmacological regulation of memory: Sarsasapogenin improves memory by elevating the low muscarinic acetylcholine receptor density in brains of memory-deficit rat models. Brain Res. 2005;1060(1–2):26–39.
61. National Research Council (US). Committee for the Update of the Guide for the Care and Use of Laboratory Animals.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/99246