GC-MS and proximate analysis of the hydromethanol extract of C*raterispermum schweinfurthi* leaves.

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

Result: 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.

Key words: C*raterispermum 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 and Ethical Approval

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 3000mg/kg body weight. 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 (20).

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 (21-22).

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 3000mg/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 (23), 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%.

Figure 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 (24). According to Bouic, 2001: Awad *et al.,* 2008: Cabral and Klein, 2017: Lesma *et al*., 2018 (25-28), phytosterol has a wide range of biological and pharmacological effects including: anticarcinogenic, immunomodulatory, 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 (29).

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

Figure 1: GC-MS spectra, showing retention time and intensity counts

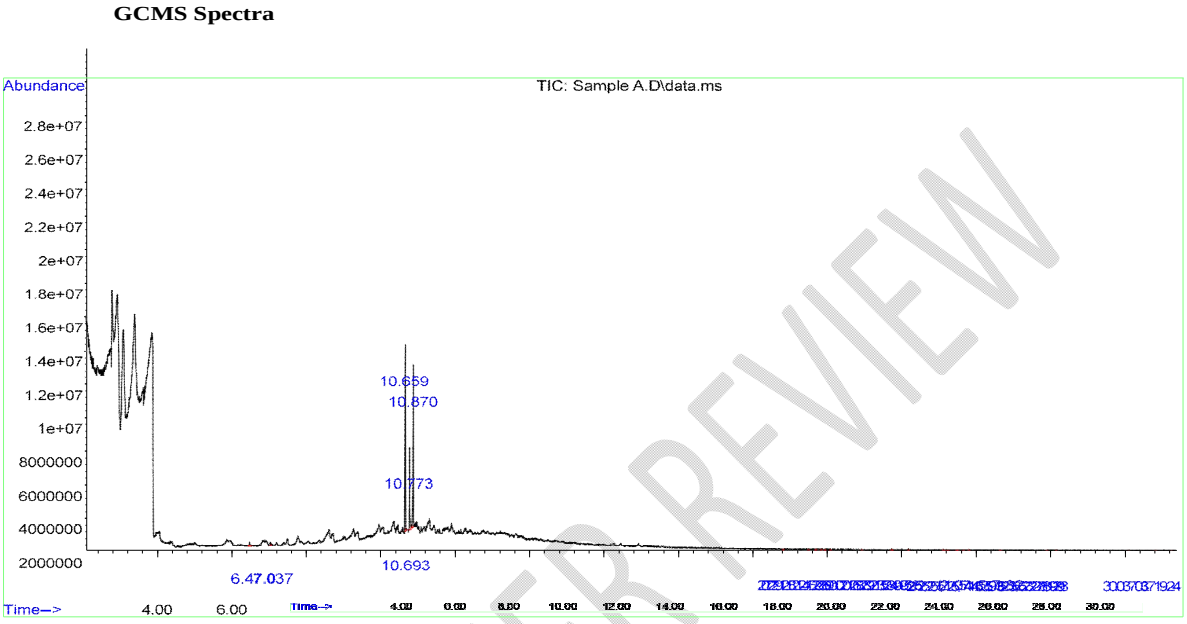

Table 1: Chemical formular, molecular weight and percentage composition of Identified Compounds in hydromethanol extract of leaves of *Craterispermum schweinfurthii* using GC-MS

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-dioxide	$C_{16}H_{11}NO_2S$	281	0.27
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 (24).
Phytosterol	Phytosterol has a wide range of biological and pharmacological effects including: anticarcinogenic, immunomodulatory, aphrodisiac, anti-inflammatory, anti-diabetic, antioxidant and anticholesterol properties (25-28).
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 (29).
1,3-Butadiene-carboxylic acid	Most butadiene is used to make synthetic rubbers for the manufacture of tyres, grommets and elastic bands (30). Toxic to human beyond trace level (31)
Hexasiloxane, 1,1,3,5,5,7,7,9,9,11,11-dodecamethyl-	Antimicrobial, Antiseptic, Hair Conditioning agent, Skin- Conditioning Agent, emollient, Solvent (32)
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) (33).
Cyclodecasiloxane, eicosamethyl-	Emollient, in personal care products, lubricant and de-foaming agent (34).
Carbamic acid, (3-ethylphenyl)-, ethyl ester	Some carbamate esters have use as muscle relaxants (35).
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 (36-40).
Imidazo [1,2-a] pyridine-2(3H)-one	Anti-ulcer and cardioprotective effects (41-42).
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 (43).
6,7-Benzo-phenothiazine-5,5-dioxide	Possess psycholeptic, sedative, hypnotic, anxiolytic, anticonvulsant, muscle relaxant, and amnesic actions (44-45).
2,6-Dihydroxybenzoic acid, 3TMS derivative	Occurs naturally in Phyllanthus acidus and in the aquatic fern Salvinia molesta. A human xenobiotic and plant metabolite (46).
Cholestan-3α-ol acetate	Main flavor component of mushrooms (47).
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 (48).
Phenol,6-methyl-2-[(4morpholinyl) methyl]-	Phenol is widely used as an antiseptic to treat sore throat (49). An active ingredient in some oral analgesics (50).
Phosphinic acid, diethyl-, (3-trifluoromethylphenyl) amide	Acts as a pH adjuster in cosmetics and skin-care products (51) and as a sanitizing agent in the dairy, food, and brewing industries (52).
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 (53-54).
9-Desoxo-9-x-acetoxy-3-desoxy-7,8,12-tri-O-acetylingol-3-one	Improves vision and yeast production (55).
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 (56).
3H-Pyrazol-3-one, 2,4-dihydro-2,4,4,5-tetramethyl-	Used in people with familial adenomatous polyposis (57) and in the treatment of osteoarthritis (58).

Thiazole, 4-(4-iodophenyl)-2-(3-tolylamino)-	The thiazole ring is notable as a component of the vitamin thiamine (B1) (59).
Pseudosarsasapogenin	Lowered blood sugar and reversed diabetic weight gain in experimental mice (60). Halted the decline in muscarinic acetylcholine receptors (mAChRs) in animal models of Alzheimer's disease (61).

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.

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