



Volatile active ingredients of *Oxyanthus speciosus* Dc. and its mechanism of actions

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ABSTRACT: Plants are known to synthesize and accumulate a wide variety of chemical compounds which characterizes their color, scent, taste and their applicative functions. In this research, the chemical compounds present in *Oxyanthus speciosus*, a multi indigenously used species was studied. The objective was to possibly explain the mechanism of actions behind their medical and industrial applications. Fresh leaf sample obtained from the wild was processed and extracted using methanol and analyzed using gas chromatography mass spectrometer. The chemical contents were identified according using NIST library. The identified compounds were grouped using their functional groups. Result showed that a total 32 compounds of which ten could not be identified. The identified compounds belong alkaloids (2 members), flavonoid (6 members), phenolic (10 members), and terpenes (2 members). Four compounds {Cycloheptyl cyanide, 2,5- cyclooctadien - 1-ol, cis-1, 3-cyclohexanedicarbonitrile and 1,2 4,5- Tetrazine, 3,6-bis (1-methylethyl)} were reviewed for insecticidal properties while two {Cis- 1,3-cyclohexanedicarbonitrile and Biscyclo[3,1,0] hexan-2- one} were reviewed for anti-microbial actions while others were reviewed as either precursor of known industrial reagents and others inferred as potential precursors for manufacturing of industrial and medical chemicals. Some other active ingredients were determined chemically as capable of been used for food packaging. Based on presence of some active ingredients, the species could also be used in mining and metallurgical practice, as source of anesthesia, enhancement of nutritional qualities, production of plastics and nanoparticles. This validates its high indigenous usage while affirming its potency against plethora of ailments as often promoted by herbal healers. However, the identity of the ten unknown compound is a call for future research while further studies were recommended to isolate purified and incorporate extracts of *O. speciosus* species into the variously mentioned medical and industrial enterprises.

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INTRODUCTION

Plants are undeniably sinks of chemical compounds (Ebigwai *et al.*, 2019). These chemical compounds are called plethora of names such as secondary metabolites Ebigwai *et al.*, 2019, phytochemicals (Lillehoj *et al.*, 2018) or active ingredients (Ebigwai and Enudi *et al.*, 2019). No matter the term applied, plant chemical compounds are natural products produced by biosynthetic pathways (Gadde *et al.*, 2017).

These metabolites are termed secondary because they are not involved in the basic physiological processes of the plants. The fundamental concept in applying plant products to any ecosystem use is anchored on the type of active ingredients it contains (Yuan *et al.*, 2016). There are

four major phytochemical groups. They include terpenes phenolics, nitrogen containing compounds and sulphur containing compounds (Koche *et al.*, 2018).

Quantitatively, the signatures of plant chemical compounds are usually obtained via GCMS OR HPLC analyses (Xiao *et al.*, 2016). The volatile constituents are obtained using the former while the non-volatile constituents are obtained using the latter approach. However, most research concerns on phytochemicals are either skewed towards its application in inhibiting microbial growths, its pharmacological functions (Bremner, 2009) or simply its qualitative values.

These efforts tend to obscure the mechanisms by which the active ingredient acts to perform the

function. Researches outlining the mechanisms of actions by secondary metabolites are scanty to the best of my knowledge.

Plants and plant products have found wide applications in the health, food, pest management, cosmetics and several other human endeavors. The basis for these applications is the natural products they contain (Mashalidis *et al.*, 2019). Researches on plants in Nigeria and other Third World countries are regrettably theoretically focused on their inherent properties rather than on their applicative properties as done in more civilized climes. This difference in approach is further compounded by the huge diversity of plants in the clime that focusses on plants latent character and its rarity in climes that focusses on the value chain obtainable from the natural products.

The duration between designating a plant as a potential candidate for use by human and its eventual application is owed in part to lack of understanding on the mechanisms of actions by the active ingredients. The limit placed on the ecosystem function of a plant natural product is the result of our narrow understanding of the active ingredients mode of action (Yun *et al.*, 2013).

Oxyranthus speciosus Dc had long been credited with an avalanche of medical applications (Aro *et al.*, 2015). In the last couple of years, its natural products have been applied industrially to the production of drugs, insecticides and as anti-microbial etc. (Aro *et al.*, 2015; Nguta *et al.*, 2015). Novel applications of primordially known active ingredients are continually been reported (Gurnani *et al.*, 2014; Adamu *et al.*, 2012; Ebigwai and Ngele, 2020). *Oxyranthus speciosus* is a member of the *Ixoridae* sub family of *Rubiaceae* with huge indigenous uses. In all the studies reporting its efficacy against microbes, only few do show the biochemical mechanisms of action involved in reducing the microbial load.

It is not unlikely that presently known active ingredient would be reported in the new future as potent candidates for medical and industrial challenges that are currently designated as one with no solutions. The key to predicting and realizing the huge deposits of natural products in plants is undoubtedly our basic understanding of the synergistic interplay of their operational mode of actions. It is in light of these outlines that this research is justified.

The present research was carried out to evaluate the active ingredients present in *O. speciosus* and to determine if possible, the mechanism of actions for each. Specific objectives of the study were to identify the volatile and semi-volatile active ingredients in the extract of *Oxyranthus*

speciosus using GC-MS analysis, to determine the potential and/or Industrial and medical applications of the identified compounds based on understanding of the properties of their functional groups, and to determine the mechanism of actions of the identified compounds as much as possible

METHODOLOGY

Sample collection

The plant sample was obtained from UNICAL Botanical Garden, Calabar Southern Nigeria (N4.952520, E8.343090). The identity of plant was confirmed using both expert recognition by Dr. J. K. Ebigwai and DNA barcoding. The leaves of the plant were harvested during the wet season (August) and washed under running water at Mifor Consult Laboratory, Calabar. The sample was air-dried at room temperature (27°C) for 15 days, then grinded in to powder using electric blender. The powdered plant material was packed in zip-lock bag for extraction and GC-MS analysis.

Sample extraction

Five (5) grams of the sample was weighed with an electronic weighing balance into an extraction thimble of a Soxhlet extractor. Exactly 50 ml of the solvent (methanol diluted with dimethyl sulfuroxide to enable dissolution of the sample) was poured into the round bottom flask; attached to the Soxhlet extractor. This was refluxed thrice. The extract was transferred into the Rotary Evaporator and concentrated to 2 ml. This was transferred further into a Teflon screw capvial well labeled. The extract was cleaned up with 200 mm mesh silica gel and 3 g of anhydrous sodium sulfate in a well packed column ready for analysis with GC-MS.

GC-MS analysis

An agilent 5890 N gas chromatography equipped with an auto sampler connected to an agilent Mass Spectrophotometric Detector was used. One (1) ul of sample was injected in the pulsed spitless mode onto a 30 m x 0.25 mm id DB 5 MS coated fused silica column with a film thickness of 0.15 micrometer. Helium gas was used as carrier gas and the column head pressure was maintained at 20 psi to give a constant of 1 ml/min. Other operating conditions were present. The column temperature was initially held at 55°C for 0.4 min, increased to 200°C at a rate of 25°C/mins, then to 280°C at a rate of 8°C/mins and to final temperature of 300°C at a rate of 25°C/mins, held for 2 mins.

Identification of chemical compounds

The identification of the isolated chemical compounds in the extract was based on retention time

since each of the active ingredients has its unique retention time in the column. Those components with lower retention time were eluted before the ones with high retention time. The detected compounds were identified using National Institute for Standards and Technology (NIST) reference library, which contains spectra that carries the names and the structure of detected compound.

RESULTS

Results on GC-MS analysis

Result of phytochemical screening of *Oxyanthus speciosus* with GCMS was shown in Fig. 1 and 2 and Table 1 and 2.

From Fig 1, a total of 32 mass spectra were detected in the extract, within the retention time of 4.37 - 40.37 minutes. When the spectra were compared with NIST data base, the result presented in Table 1 showed that 20 peaks correspond with compounds on the data base, hence were identified. However, 10 peaks returned no result, hence were not identified.

Some of the compounds identified were: 2,7 - Diocatricyclo decade - 4, one, oxime, 2,5 - Cyclooctadien - 1 - Ol, Cycloheptanol, 2- chloro-trans, Cycloheptyl Cyanide, Cis - 1,3 - cyclohexanedicarbonitrile, 6 - cyano - 1- hexene etc. The most dominant peak was detected at 38.96-minute retention time which returned no result during the query with NIST data bank.

When the identified compounds were grouped according to their functional elements, the result presented in Table 2 showed that phenol with 10 member compounds was the most frequent followed by flavonoid with six (6) members and alkaloid and terpene with two (2) members each.

Table 2 equally showed that some of the compounds [2,5 - cyclooctadien -1-ol, Cycloheptyl cyanide and 1,2,4,5 - Tetrazine, 3,6 - bis (1-methylethyl)] were reviewed to be insecticidal while this compound Bicyclo [3,1,0] hexan - 2-one was reviewed to be antimicrobial against bacteria and fungi.

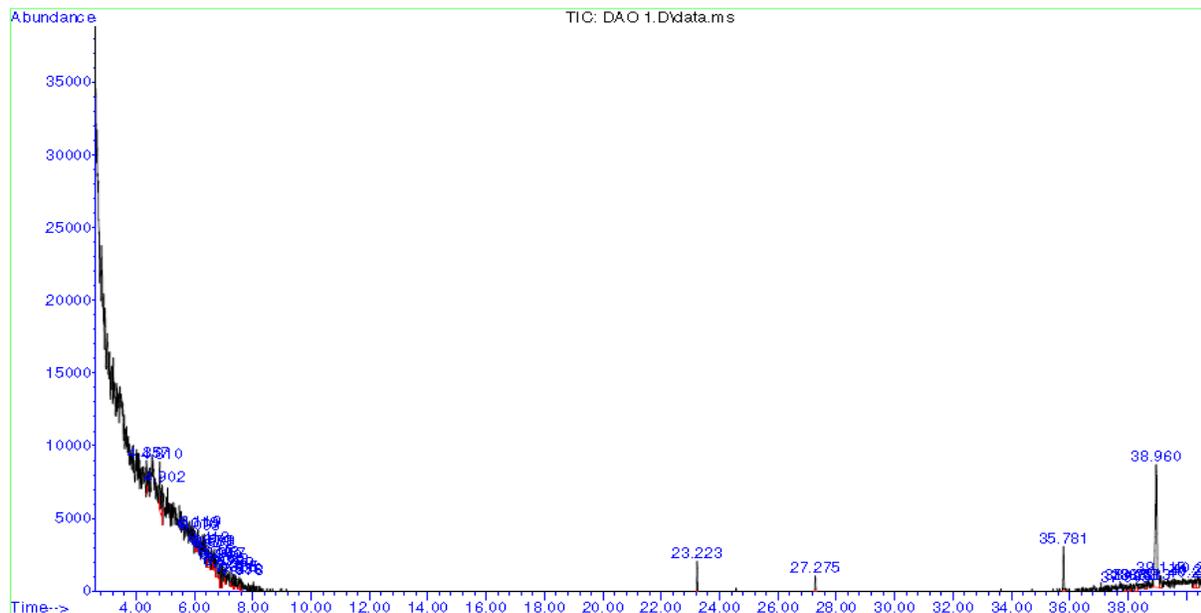


Fig. 2: GCMS chromatogram of *Oxyanthus speciosus*

Table 1. GC-MS screening of *Oxyanthus speciosus*

S/N	Retention time (Min.)	Compound name	Molecular formula	Peak area (%)
1	4.365	2,7-Dioxatricyclo [4.4.0.0(3,8)] decan-4-one, oxime	C ₈ H ₁₁ NO ₃	2.473
2	4.810	2,5-Cyclooctadien-1-ol	C ₈ H ₁₂ O	8.299
3	4.902	Cycloheptanol, 2-chloro-, trans	C ₇ H ₁₃ ClO	3.126
4	6.018	Cycloheptyl cyanide	C ₈ H ₁₃ N	3.072
5	6.093	cis-1,3-Cyclohexanedicarbonitrile	C ₈ H ₁₀ N ₂	1.215
6	6.119	1,1'-Biphenyl, 2,4-dichloro-2',5'-dimethyl	C ₁₄ H ₁₂ Cl ₂	1.922
7	6.412	6-Cyano-1-hexene	C ₇ H ₁₁ N	1.561
8	6.479	Pyrazole-3-carboxylic acid, 4-iodo-1 methyl	C ₅ H ₅ IN ₂ O ₂	2.882
9	6.572	Nickel, bis (1,1-dimethyl- TT-allyl) bis(ethoxy)bis	C ₁₄ H ₂₈ Ni ₂ O ₂	1.509
10	6.588	3,10-Dioxatricyclo [4,3,1,0(2,4)] dec-7-ene	C ₈ H ₁₀ O ₂	2.135
11	6.748	1,6-Heptadiene, 3-methyl	C ₈ H ₁₄	2.166
12	6.840	2-Butynoic acid, 4-(1-pyrrolidiny)-	C ₈ H ₁₁ NO ₂	1.661
13	6.899	9-Thiabicyclo [3.3.1] nonan-3-one 9,9dioxide	C ₈ H ₁₂ O ₃ S	1.570
14	6.957	Pyrimidine, 4-methyl	C ₅ H ₆ N ₂	3.386
15	7.083	Propanal, 2-methyl-oxime	C ₄ H ₉ NO	1.247
16	7.259	1,2,4,5-Tetrazine, 3,6-bis(1-methylethyl)	C ₈ H ₁₄ N ₄	1.242
17	7.326	1,2,4,5-Tetrazine, 3,6-bis(1-methylethyl)	C ₈ H ₁₄ N ₄	1.367
18	7.385	1,2,4,5-Tetrazine, 3,6-bis(1-methylethyl)	C ₈ H ₁₄ N ₄	1.279
19	7.536	Bicyclo[3,1,0]hexan-2-one	C ₆ H ₈ O	1.310
20	7.578	1-Methoxy-2-methyl-3-butene	C ₆ H ₁₂ O	2.118
21	23.223	Ethenamine, N-methylene	C ₃ H ₅ N	2.245
22	27.275	Cyclopropane, 1,1-dimethyl	C ₅ H ₁₀	1.239
23	35.781	Unknown	Unknown	4.790
24	37.903	Unknown	Unknown	1.044
25	38.062	Unknown	Unknown	1.106
26	38.281	Unknown	Unknown	2.049
27	38.583	Unknown	Unknown	1.032
28	38.960	Unknown	Unknown	33.961
29	39.119	Unknown	Unknown	1.956
30	40.227	Unknown	Unknown	1.225
31	40.285	Unknown	Unknown	1.598
32	40.378	Unknown	Unknown	2.213

Discussion

Discovery of potential lead compounds and their advancement towards bioactive molecule development involves routing examination of plant extracts to yield a single bioactive compound. Plants

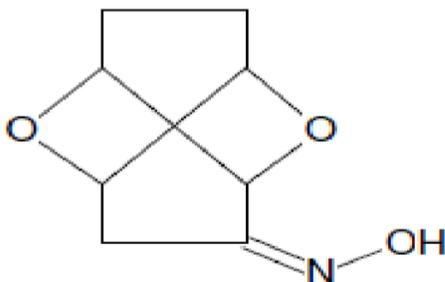
are naturally gifted at the synthesis of bioactive compounds, whose characterization has led to discovery of new, bioactive products with high therapeutic, antibiotic and other industrial potentials (Huie, 2002; Cheng et al., 2014).

The bioactive compounds contained in *O. speciosus* are discussed in relation to their medical and industrial applications.

2,7-Dioxatricyclo [4.4.0.0(3,8)] decan-4one, oxime

Fig. 5.1: molecular structure of 2,7-Dioxatricyclo [4.4.0.0(3,8)] decan-4 one, oxime

The functional group consists of a hydroxyl group directly bonded to an unsaturated nitrogen atom. It belongs to the imines, with general formula $RR'C=NOH$; where R and R' are alkyl groups. There exist two ether linkages in the compound. It is one of the several functional groups in this species which confers anti-bacterial and anti-fungus properties (Luo et al., 2012) on the species. This compound acts on bacteria by the inhibition of β -ketoacyl-(acyl-carrier-protein) synthase III (FabH) (Luo et al., 2012). The compound also acts by reversibly binding to the bacterial 50S ribosomal sub units and by blocking nascent proteins' progression through their exit tunnel in bacterial protein biosynthesis (Dubravko and Antolović, 2016). Industrially, the functional group is responsible for the use of this species in the production of an organic compound called Caprolactam, which is a precursor for the



polymer called Nylon 6 (Herzog et al., 2013). The compound confers on this species its uses as organic catalyst (Herzog et al., 2013), antidotes as nerve agents (Herzog et al., 2013), as precursor of perillaldehyde used as artificial sweetener (Bielman, 2000), precursor of Methyl ethyl ketoxime used as an additive for preventing the skin burns from chemicals, and also as de-oxidant or a corrosion inhibitor which lowers toxicity (Bielman, 2000). The compound has also been used in the synthesis of other organic compounds such as cobalt and ketones.

Hydroxy 2, 5 – Cyclooctadiene

This compound is a cyclic hydroxylated unconjugated diene, since the two double bonds in its structure are separated by more than two single bonds.

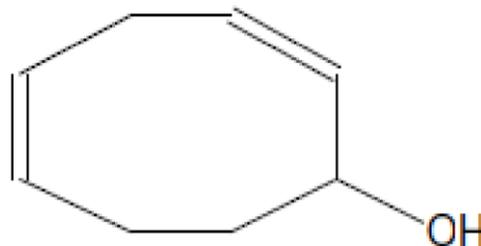


Fig. 5.2: molecular structure of hydroxy 2, 5 – cyclooctadiene

Hydroxy 2, 5-cyclooctadiene forms variety of useful chemicals; many complexes with different metals. It has been applied as a molecular weight modifier in the manufacture of polybutadiene which is used in the production of high-impact polystyrene for food-contact packages (Lautens, and Larin 2019). It is also used industrially in metallurgy for metal ore preparation, casting and surface treatment of metals (Zhao et al., 2014) manufacturing of plastics and plastic additives (Zherikova et al 2009), as raw materials used in a variety of products and industries (e.g. in cosmetics, chemical manufacturing, production of metals (Tillack et al 2000) and as accelerators, activators and oxidizing agents. Despite, its diverse industrial use, no study to the best of my knowledge has conferred these uses on this plant. Pharmaceutically, it acts to reduce the total liver glutathione in the body (Sies 1999) by neutralizing oxygen rich species.

This compound conferred on the species insecticidal properties. Injection of the produced poisonous parasporal crystal by herbivorous insects interact with larval midgut epithelial cells and destroy membrane integrity, ultimately leading to insect death.

The compound confers stress management on this species as heavy metals chelating agents as the compound is bioactive substance against host of pathogenic organisms (Haynes, 2015).

Trans-2-chlorocycloheptanol

This compound is an alcohol derivative, having a chlorine atom substituted at the trans orientation of the vicinal position to the hydroxyl locant.

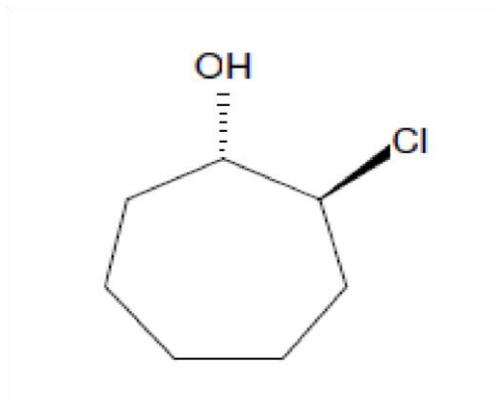


Fig. 5.3: molecular structure of Trans-2-chlorocycloheptanol

The compound confers on the species anti mycotic, anti-neoplastic and immunostimulant activities. Trans-2-chlorocycloheptanol is also a precursor of numerous compounds. Pimelic acid is produced by the oxidative cleavage of cycloheptanone. Several microorganisms, including *Mucor plumbeus*, *Mucor racemosus*, and *Penicillium chrysogenum*, have been found to reduce cycloheptanone to cycloheptanol (NCBI 2020).

Cycloheptyl cyanide

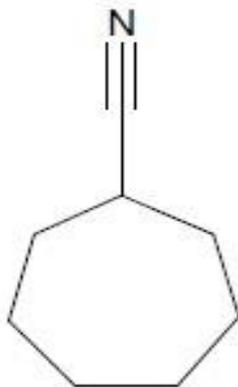


Fig. 5.4: molecular structure of Cycloheptyl cyanide
Cycloheptyl cyanide, like every other nitride is very toxic. It generally irritates the eyes and skin, and is poisonous if accidentally ingestion.

The compound confers on the species the histoxic hypoxia (ATSDR, 2006) property. Inhalation of the compound causes inhibition of the mitochondrial enzyme cytochrome c oxidase which ultimately results in the inability of cells to take up or use oxygen from blood stream Anseuw et al., 2013; Hamel, 2011; Dorland, 2012). Cycloheptyl cyanide is a precursor for other natural compounds such as 2-

amino - 2- thiazoline - 4 -carboxylic acid, thiocyanate [(anion) and thiocyanic acid. Industrially, Cycloheptyl cyanide is used in a number of processes, including mining, metallurgy, manufacturing, electroplating and photography, due to its ability to form stable complexes with a range of metals (ATSDR, 2006).

Cis-1,3- Cyclohexanedicarbonitrile

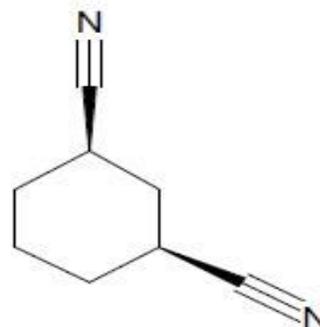


Fig. 5.5: Molecular structure of Cis-1,3-Cyclohexanedicarbonitrile

The compound confers on the species the anesthetic and anti-inflammatory properties (Grub and Loser, 2012). Also known as 1,3-Butadiene, Cis-1,3-Cyclohexanedicarbonitrile is used medically as propellant in aerosol spray medications. It is important industrially as a monomer in the production of synthetic rubber such as automobile tires, nylon, chloroprene, sulfolane and adiponitrile (Grub and Loser, 2012). Butadiene is also used in the industrial production of 4-vinylcyclohexene via a Diels Alder dimerization reaction (Feller and Craig, 2009).

1,1'- Biphenyl- 2,4- dichloro-2', 5' – dimethyl

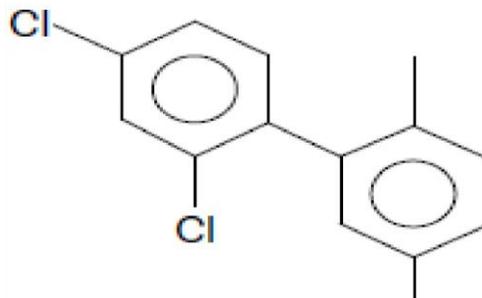


Fig. 5.6: Molecular structure of 1,1'- Biphenyl- 2,4- dichloro-2', 5' – dimethyl

1,1'- Biphenyl- 2,4- dichloro-2', 5' – dimethyl is a representative of chlorinated biphenyls (CBs). Polychlorinated biphenyls have been employed commercially due to their non-flammability, chemical stability, high boiling point and electrical insulating properties in electrical and heat appliances, as plasticizers and pigments in surfaces. But, due to their persistence and toxicity in the environment, their use is halted in the USA (EPA web archive, 2017). The Environmental Protection Agency published that PCBs exhibits carcinogenic and neurological effects on contact with biological systems.

Pyrazole - 3- carboxylic acid, 4 - iodo - 1 methyl

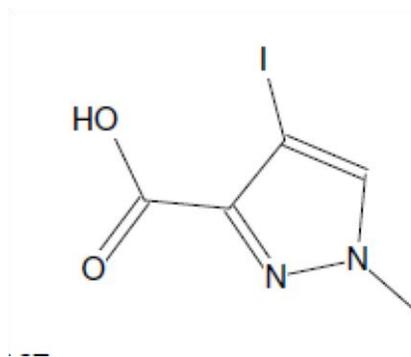


Fig 5.7: Molecular structure of Pyrazole - 3- carboxylic acid, 4 - iodo - 1 methyl

Aromatic organic heterocycle containing pyrimidine scaffolds possesses two nitrogen atoms of Five-membered ring. This Pyrazole skeleton confers analgesic, antipyretic, anticancer, antiviral, anti-inflammatory, antioxidants, antimicrobial, anti-diabetic, anticonvulsant, anti-arrhythmic activities on this species. The pyrazole derivative: Diphenyl-1H-pyrazole-4-carboxylic acid demonstrated anti-proliferative effects by inhibiting a range of cyclin-dependent kinases (Sun et al., 2013). Other derivatives such as pyrazole carbonyl thiourea and pyrazolyl thiourea derivatives are effective against different types of human cancer cells (Nitulescu et al., 2013; Nitulescu et al., 2010). Industrially, pyrazole is applied in agro-chemical industries as herbicides.

Nickel, bis (1,1-dimethyl- TT-allyl) bis(ethoxy) [bis (Ni(C₅H₄CH₃)₂)].

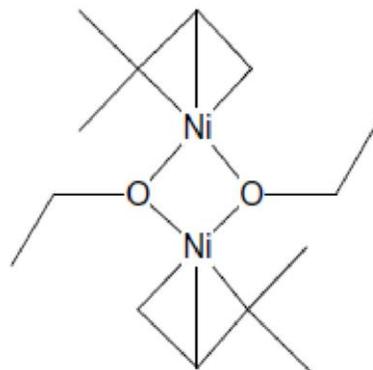


Fig. 5.9: Molecular structure of Nickel, bis (1,1-dimethyl- TT-allyl) bis(ethoxy) bis (Ni(C₅H₄CH₃)₂).

This compound confers on this species as a volatile precursor for the controlled growth of nickel nanoparticles on silicon substrates. The potential of this species as an ingredient for the production of high-density storage media is owed to the presence of this compound. It has been reported that nickel shows a unique reactivity which enables the chemists to apply this to entirely different sets of chemical transformations.

3, 10- Diocatricyclo [4,3,1 0(2,3)] dec-7- ene

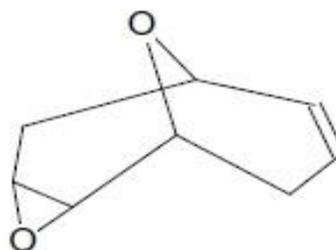


Fig 5.10: Molecular structure of 3, 10- Diocatricyclo [4,3,1 0(2,3)] dec-7- ene

This compound possesses oxo groups imbedded in a decene structure. Decene is an alkene with the formula C₁₀H₂₀. Decene contains a chain of ten carbon atoms with one double bond. There are many isomers of decene depending on the position and geometry of the double bond. Dec-1-ene is the only isomer of industrial importance. As an alpha olefin, it is used as a monomer in copolymers and is an intermediate in the production of epoxides, amines, oxo alcohols, synthetic lubricants, synthetic fatty acids and alkylated aromatics. The epoxide groups of

3, 10- Diocatricyclo [4,3,1 0(2,3)] dec-7-ene can react via a ring opening mechanism to give poly alcohols through stepwise polymerization.

Dec- 1-ene has been detected to be an initial product in the microbial degradation of n-decane .1-Decene (C10) is equally used in the production of plasticizer alcohols and poly alpha olefin (PAO) mainly is used as high-performance synthetic lubricant for automatic transmission engines, crankcase, aviation, industrial gear systems and marine applications. It can also be used in organic synthesis of flavors, perfumes, pharmaceuticals, dyes, oils, and certain resins. Decane is a major component of diesel and is also used in paint manufacturing as a hydrocarbon solvent (Ophardt, 2003).

Hept-6-ene Nitrile

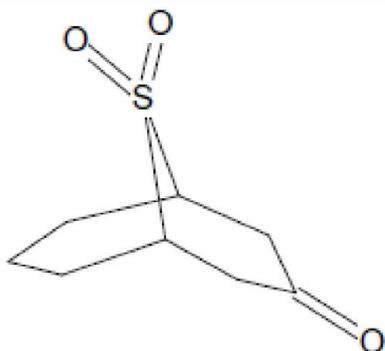


Figure 5.11: Molecular structure of Hept-6-ene nitrile It is an unsaturated alkyl cyanide. Like other cyanides, hept-6-ene nitrile is a skin irritant. Based on its olefin (C=C) nature, it can be polymerized into a plastic product (poly hept-6-ene nitrile)

4-(1-pyrrolidinyl)-2-butynoic acid

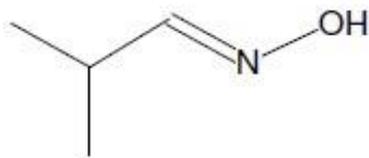


Figure 5.12: Molecular structure of 4-(1-pyrrolidinyl)-2- Butynoic acid

2-Butynoic acid, which can be derived from 4-(1-pyrrolidinyl)-2-butynoic acid, has been employed as synthon in a variety of reactions, including cycloacylation of phenols to flavones and chromones, and cyclization to γ -butyrolactones. It was also used in synthesis of Z-trisubstituted olefins via γ -alkylation.



9-Thiabicyclo (3.3.1) nonan-3-one-9, 9-dioxide

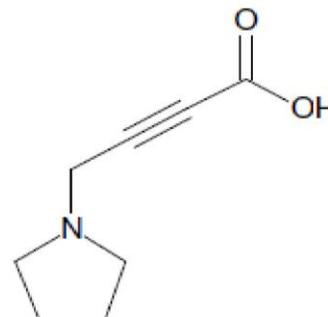


Fig.5.13: Molecular structure of 9-Thiabicyclo (3.3.1) nonan-3-one-9, 9-dioxide

This compound is a sulphate, hence should possess oxidative properties. The presence of the carbonyl functionality will also diversify its reactivity. No literature on its medical and industrial use is reported yet.

4-methylpyrimidine

Fig 5.14: Molecular structure of 4-methylpyrimidine 4-methylpyrimidine reacts with metal ions to give both poly-nuclear and discrete complexes (Shimizu *et al.*, 2007). This compound confers on the species, the free radical attack property (Brown *et al.*, 1994). Derivatives of 4-methylpyrimidine such as 2-amino-4-chloro-6-methylpyrimidine and piperazinyl pyrimidines are potential medicinal agents due to the presence of electronegative nitrogen which confers antimicrobial properties. Standard drugs like Chloramphenicol and fluconazole have structural analogues of synthesized piperazine derivatives (Thrivani *et al.*, 2014). Some compounds prepared from pyrimidines have been used in the treatment of inflammation, hypertension, anxiety, depression, or cancer (Chowell *et al.*, 2015).

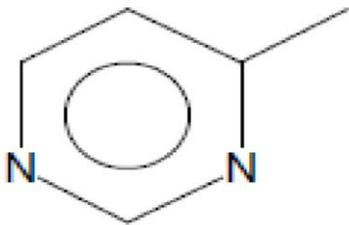
Propanal -2 - methyl – oxime

Fig. 5.15: Molecular structure of Propanal -2 - methyl – oxime

This compound confers antibacterial property on the species. Propanal -2 - methyl - oxime acts on bacteria by the inhibition of β -ketoacyl-(acyl-carrier-protein) synthase III (FabH) (Luo et al., 2012). The compound also acts by reversibly binding to the bacterial 50S ribosomal sub units and by blocking nascent proteins' progression through their exit tunnel in bacterial protein biosynthesis (Dubravko and Antolović, 2016). Industrially, the functional group is responsible for the use of this species in the production of an organic compound called Caprolactam, which is a precursor for the polymer called Nylon 6 (Herzong et al., 2013).

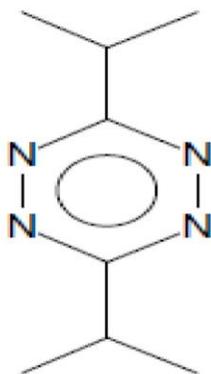
3,6-bis (1-methylethyl)-1,2,4,5-Tetrazine

Fig. 5.16: Molecular structure of 3,6 -bis (1-methylethyl)-1,2,4,5-Tetrazine

The name "Tetrazine" refers to a family of organic compounds having 4 nitrogen atoms fused in an aromatic ring. This compound is a derivative of this group. Tetrazine cyclo-additions have been proven to be a new bio-orthogonal carbon coupling tool (Devaraj and Weissleder, 2011). Tetrazine cyclo-addition as one of the bio orthogonal covalent reactions have found widespread use in chemical biology (Prescher and Bertozzi, 2005). Its application

includes the tracking of metabolite analogs, activity-based protein profiling, target guided synthesis of enzyme inhibitors, and imaging small molecules in live cells and animals (Speers et al., 2003).

A variety of covalent reactions have been used as irreversible chemo-selective coupling tools. Notable examples include the Staudinger ligation and the copper catalyzed or strain-promoted azide-alkyne cycloaddition. The interest is to utilize such orthogonal reactions to assemble imaging agents, nanomaterials, and therapeutics in the presence of live cells both in vitro for microscopy and diagnostic application, drug delivery, as well as in vivo.

Industrially, Tetrazine ligation has found numerous applications in fluorescent imaging, positron emission computed tomography (PET) and single photon emission computed tomography (SPECT) scanning, radionuclide therapy, and radiochemistry or drug target identification among several others (Laughlin et al., 2008).

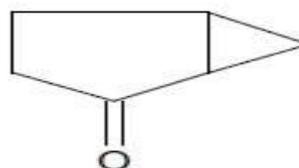
Bicyclo [3, 1, 0] hexan -2-one

Fig. 5.17: Molecular structure of Bicyclo [3, 1, 0] hexan -2-one

This compound belongs to the ketone group due to the presence of the carbonyl functionality. It has a clear oily appearance and belongs to the category of a building block for other important chemicals. Bicyclo [3, 1, 0] hexane 2-one is an intermediate in synthesis (+) - sabinene, a component of juniper berry oil having antibacterial and antifungal activity (Marino et al., 1999). This compound acts on fungi by directly affecting the feed palatability, it reduces the feed nutrient, value, and cause livestock and poultry poisoning (Kolossova and Stroka, 2012).

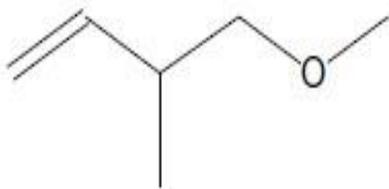
1- Methoxy - 2- methyl - 3 – butane

Fig. 5.18. Molecular structure of 1-Methoxy- 2-methyl-3-butane

This is an unsaturated ether compound. Ethers have been applied in medicine as anesthetics and solvents, though they are highly flammable.

Due to the presence of the C=C bond in the molecule, it can be applied as a vinyl monomer for polymerization process to get plastics of diverse properties.

N- Methyl ethyl Ethenamine

5.19: Molecular structure of N-methyl ethyl Ethenamine

This compound belongs to the class of organic compounds known as imines. These are compounds containing an imine functional group, with the general structure $RN=CR_2$ ($R = H$, hydrocarbyl). This compound is reported to use in the production of H1 and H2 receptors.

Cyclopropane, 1,1 dimethyl

Fig. 5.20: Molecular structure of 1,1 dimethyl Cyclopropane,

This compound is a cycloalkane consisting of a cyclopropane ring substituted with two methyl groups attached to a geminal carbon atoms. Medically, it's has an excellent pest control effect and is therefore used as an active ingredient in controlling pest. It's mode of actions is by targeting systems or enzymes of the pest (Ifeanacho, 2019). Industrially, through this species has been reported in induction of anaesthesia, it has not been available or clinical use since the mid-1980.

CONCLUSION

This research was undertaken to characterize chemical compounds present in *Oxyanthus speciosus* and to determine mechanism of actions behind known and inferred industrial and medical applications. Results showed that a total of 20 compounds were identified in the extract. The identified compounds belong to the phenol, flavonoid, alkaloids and Terpenes phytochemical groups. Some of these compounds were reviewed or inferred to possess insecticidal activities, precursor of industrial reagents/plastic production while some compounds were said be active against micro-organisms. The mechanism of actions was shown in most of the compounds as possible. Further studies are recommended to isolate, purify and incorporate relevant extracts of this species into the variously mentioned industrial and medical endeavors.

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