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Gas Chromatography-Spectrometric Analysis of An African Mistletoe Leaf Extract

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Abstract

The high prevalence of mood disorders and the failings of the current drugs for the treatment of these disorders call for renewed efforts to discover novel chemical entities to meet the disease demand of these clinical conditions. Methanol *Tapianthus globiferus* leaf extract has demonstrated anxiolytic activity in mice. However, reports on specific chemical components of the medicinal plant that may account for the anxiolytic and other biological effects are few. Thus, gas chromatography-mass spectrometric analysis was carried out on methanol *Tapianthus globiferus* leaf extract. The results include acetic acid, oxalic acid, isobutyl amine, N-ethyl formamide, ethanamine, o-allyl hydroxylamine, N, N-dimethyl ethanamine, dimethyl silane, 1-propanol, 2-propenenitrile, carbonyl sulfide, [3, 4-b] pyrazin-5 (4H)-one, 6-(1-pyrrolidinyl)-furanone, thiirane, urea and propenamide. These phytoconstituents are associated with important biological activities and may be responsible for the anxiolytic activity of the extract under investigation and some other pharmacological activities reported for extracts of *Tapinanthus globiferus* and its congeners. These findings are a justification for the traditional uses of *Tapianthus globiferus* for the management of diverse chronic diseases.

Keywords: Azardirachta indica; GCMS; leaf extract; methanol; Tapinanthus globiferus.

I. Introduction

The high prevalence and the huge socio-economic burden of mood disorders, in the face of few chemical classes, toxicity and efficacy liabilities of drugs currently used against these disorders, indicates a need for renewed research to identify and develop new chemical/therapeutic entities which can replace and/or complement the existing drugs against these disorders [1, 2, 3, 4, 5].

Gas chromatography-mass spectrometry (GCMS) remains one of the most effective analytic methods/tools to identify bioactive constituents from natural sources [6]. As its name implies, it is a two-component analytical apparatus operating on the principle that different volatile organic constituents of a crude chemical sample subjected to increasing temperatures within an enclosed space separate at varying points according to their specific molecular masses as the sample traverses through the enclosure. This combined sample-separating analytic technique has been shown to be a rapid and efficient method to identify bioactive compounds in diverse

chemical mixtures including crude medicinal plant extracts [7, 8]. Furthermore, GCMS has also been shown to exhibit robust capacity to identify long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds from natural sources - and has, thus, been previously deployed to analyse bioactive constituents of extracts of medicinal plants such as *Feronia elephantum correa, Evolvulus alsinoides* (L.) L. and *Latouca runcinate* DC. [9, 10, 11]. Certain intrinsic features of this chromatographic procedure, including good analyte peak resolution and analysis speed, high analyte sensitivity, retention time repeatability and reproducibility, accurate analyte quantitation and non-destructive testing of samples are said to confer high compound identification proficiency on GCMS [12] Thus, this analytic procedure is selected for the analysis of methanol leaf extract of the medicinal plant (*Tapinanthus globiferus*) of this study.

Tapinanthus globiferus is one of the several African mistletoes belonging to the family Loranthaceae – to which other hemiparasitic species such as *Tapinanthus banwensis, Tapinanthus dodoneifolius, Tapinanthus oleifolius* and *Tapinanthus coronatus* [13] also belong. Various species in this family have earlier been reported to possess ethnomedicinal efficacies for diverse diseases - including nervous disorders, hypertension, infections, diabetes mellitus, fevers, cancer and epilepsy [14, 15].

Crude methanol and aqueous residue fractions of *Tapinanthus globiferus* leaf extracts have exhibited antidepressant and anticonvulsant activities in rodents in previous studies [16, 17]. Additionally, anxiolytic and antidepressant effects of crude aqueous stem bark extract of a related species, *Tapinanthus dodoneifolius* (DC) Denser have been reported in mice [18].

Similarly, crude aqueous and methanol extracts as well as fractions obtained from the methanol *Tapiannthus globiferus* leaf extract under the present study have demonstrated significant in-vivo anxiolytic activity in mice in earlier studies [19, 20, 21]. The same crude methanol extract has been previously shown to exhibit significant antioxidant activities comparable to ascorbic acid; and to possess important phytochemicals including saponin, terpenoids, cardiac glycosides, flavonoids and alkaloids in addition to several vital cellular metal elements like Zinc, Magnesium, Manganese, Calcium, Ferric iron, Copper and Potassium [22].

However, despite the scientific reports of important biological activities of extracts from *Tapinanthus globiferus* and of the presence of multiple bioactive phytochemicals and metal elements in these extracts, there is paucity of scientific studies specifically aimed at identifying the active biochemicals responsible for the observed biological effects of extracts from this medicinal plant. This study, therefore, is an attempt to identify the probable bioactive constituents of the methanol leaf extract of *Tapinanthus globiferus* that are likely to be responsible for its anxiolytic and, potentially, other biological activities, using gas chromatography-mass spectrometric analysis.

II. Materials and Methods

A. Collection, Identification and Extraction of Plant Materials

Leaves of *Tapinanthus globiferus* were collected from an *Azadirachta indica* (Neem) tree at Shuni road, Mabera, Sokoto, Sokoto State, Nigeria. Its specimen vouchers (UDUH/ANS/0135) were deposited, after

authentication, at the herbarium of Botany Department, Faculty of Science, Usmanu Dan Fodiyo University, Sokoto, Nigeria. Following collection, the plant material was briskly cleaned off with clean water to remove any dirt or contaminants and was then air-dried under shade for days till their weights stagnated. The plant material was then finely powdered with an electric blender and stored under room temperature till use. Seventy per cent (70%) crude methanol extract of the plant leaves was obtained by soaking 250 g of its fine dry powder in 1 L of methanol/distilled water (70/30) for 24 hours; the macerate was subsequently Whatman's paper (150 mm)-filtered and evaporated in rotatory water bath at 45-50^{O}C to produce a dry greenish extract (31.45 g).

B. Gas Chromatography-Mass Spectrometric Analysis of Methanol *Tapinanthus globiferus* Leaf Extract

The procedure was carried out using Agilent Technologies Machine model 78808 and Agilent Technologies Mass Spectrum Detector model 5977A. The procedure to identify compounds in the crude methanol extract was initiated by completely dissolving 0.10 g of it in 10 ml of absolute methanol for 4 hours. Three (3) µl of this stock solution was then auto-injected into the GC-MS machine inlet and the sample was run for a total of 67 minutes with the oven temperature initially set at 50 °C to increase at 3 °C every minute to a maximum of 300 °C. The column used was HP-5ms Ultra Inert (30 m x 250 µm x 0.25 µm) set at an initial temperature of 50 °C and a maximum temperature of 325 °C. The initial and post-run pressures in the column were 4.4867 psi and 20.0000 psi, respectively. The flow rate through the column was 0.73677 microlitre/min at an average velocity of 31.282 cm/minute. The carrier/eluant gas was helium set at 1 ml/minute. The retention times, the fragmentation patterns and the peaks attained on the chromatograms were used to determine the structural characteristics/probable identities of the compounds present in the extract. Computer searches were made using Mass Hunter INT: TIC S2D mass spectrum database.

III. Results

Table 1: Gas Chromatography-Mass Spectrometric Analysis of Methanol Tapinanthus globiferus Leaf Extract.

Chemical compound	Functional group	Molecular formula	Molecular weight (g/mol)
Acetic acid	Carboxylic acid	CH_3COOH	60.05
Oxalic acid	Dicarboxylic acid	$C_{10}H_{16}O_4$	200.23
Isobutyl amine	Amine	$(CH_3)_2CHCH_2NH_2$	73.14
N-Ethylformamide	Amide	C3H7NO	73.09
Ethanamine	Amine	C6H13N	99.17
o-Allyl hydroxylamine	Amino-oxy	C3H7NO	109.55
N, N-dimethyl Ethanamine	Amine	C4H11N	73.14
Dimethyl silane	Hydrocarbon	C_2H6Si	58.15
1-propanol	Fatty alcohol	C3H8O	60.10
2-propenenitrile	Nitrile	C3H3N	53.06
Carbonyl sulfide	Ketone	COS	60.07
Furazano [3,4-b] pyrazin-5(4H)-one, 6-(1-pyrrolidinyl)-	Ketone	$C_8H_9N_5O_2$	207.19
Thiirane	Organosulphur	C_2H_4S	60.12
Urea	Amide	CH_4N_2O	60.09
Propanamide	Amide	CH3CH2C=O(NH2)	73.09

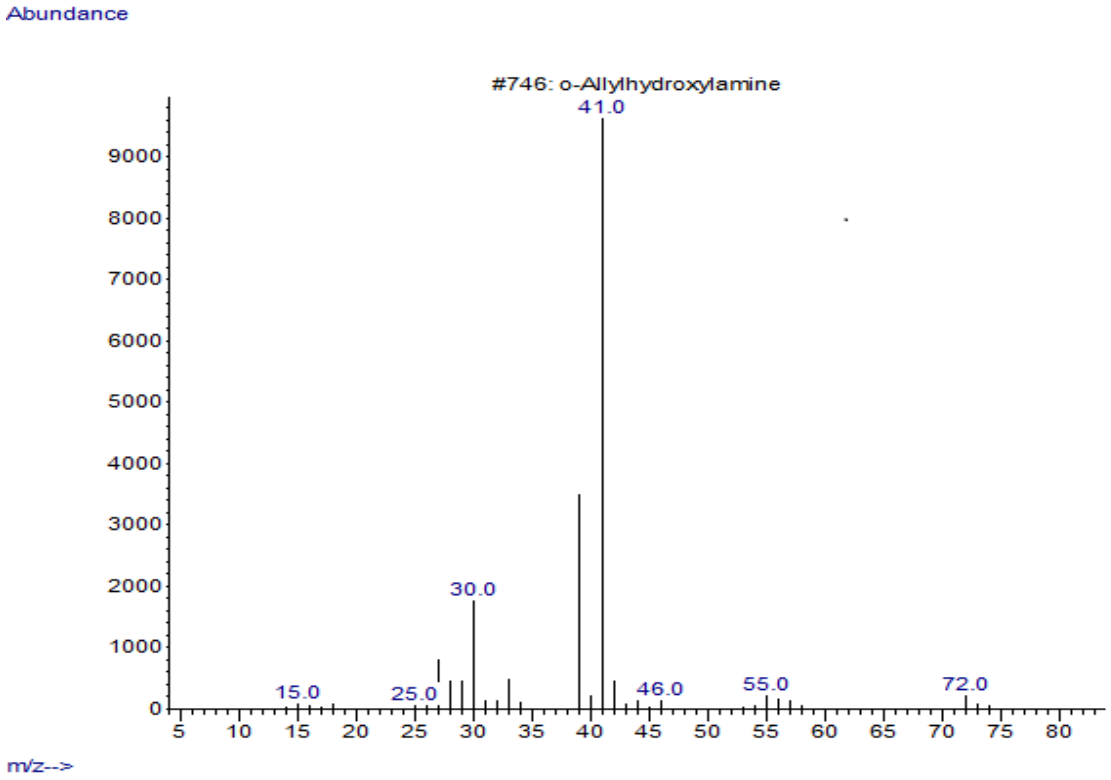

Figure 1: Chromatogram of methanol *Tapinanthus globiferus* leaf extract showing α-Alkyl hydroxylamine.

IV. Discussion

GCMS analysis of methanol *Tapinanthus globiferus* leaf extract yielded fifteen bioactive phytochemicals belonging to ten different functional groups. Identification and characterization of existing and novel pharmacophores and their functional groups in complex mixtures, including plant extracts, as in this study, and by extension, their documented/potential/unknown biological activities, have been established as key beneficial applications of this analytic tool [11, 12].

Although this study may be the first report of propenamide, acetic acid, isobutylamine, N-ethyl formamide, thiirane and Ethanamine as phytoconstituents of methanol *Tapinanthus globiferus* leaf extract, these compounds have also been previously identified in methanol/dichloromethane Moringa oleifera flower extracts [23]. Again, even though no specific biological activities have been established for three of the identified compounds in this study i.e., propanamide, thiirane and ethanamine, their derivatives have been reported to have modulatory effects in animal models of anxiety and depression. For instance, propanamide, which has also been previously reported as a phytoconstituent in methanol *Mangifera indica* stem bark [24], has derivatives such as (S)-WAY 100135 or (S)-N-tert-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenyl-propanamide dihydrochloride – a 5-hydroxytryptamine subtype 1A (5HT-1A) antagonist [25], N-[(8α)-dimethylergoline-8-yl]-2,2-dimethylpropanamide (SDZ208911) - a partial benzodiazepine agonist [26] and N-[3-(1-{[4-(3,4-difluorophenoxy) phenyl methyl} (4-piperidyl))-4-methylphenyl]-2-methyl propanamide (SNAP 94847) - a melanin-concentrating hormone (MCH) antagonist [27] that have all been shown to exhibit anxiolytic and antidepressant activity in rodent anxiety models. Similarly, SB-699551-A (N - [2 - (dimethyl amino) ethyl] – N -

[4'-[(2-phenylethyl) amino] methyl] [1,1'-biphenyl]-4-yl] methyl] cyclopentane propanamide dihydrochloride is another propanamide derivative that has been shown to be 5HT-5A antagonist [28]. This compound and its highlighted derivatives could account, partly, for the anti-anxiety effect of this same extract reported in earlier studies [19, 20, 22] as well as diverse biological activities previously reported for *Tapinanthus globiferus* extracts in other related studies [13, 14, 15, 16, 17].

Thiirane is another compound identified in the extract under the current investigation and this study may be the first to report it as a phytoconstituent of methanol *Tapinanthus globiferus* leaf extract even though thiirane has been previously identified in the Moringa oleifera flower extracts [23] and its derivatives identified as chemical components of *Allium hookeri* root/leaf [29], *Allium sativum* [30] and *Allium cepa* [31]. Thiirane has been characterized as a heterocyclic ethylene sulfide and the smallest and simplest sulfur-containing episulfide [32]. This compound has been reported to be a potent and selective inhibitor of certain matrix metalloproteinases that act as promoter enzymes to diverse disease processes – including central nervous system, cardiovascular, musculosketal and carcinogenic pathologies [33; 34]. Additionally, thiirane-containing derivatives have been reported to have anti-phobic, nephro-stimulant, anticancer and antiviral and anticonvulsive effects [35, 36, 37, 38, 39]. In view of the health-enhancing property of the medicinal plant extracts mentioned in the foregoing – all of which contain thiirane, it is therefore suggestive thiirane and its derivatives may be contributory to the anxiolytic activity earlier reported [19, 20, 22] for the methanol *Tapinanthus globiferus* leaf extract being presently investigated as well as the diverse biological effects of extracts from this same medicinal plant reported previously in other studies [13, 14, 15, 16, 17].

N, N-dimethyl ethanamine and ethanamine identified in this study represents first of such finding of these compounds being chemical components of methanol *Tapinanthus globiferus* leaf extract. However, related ethanamine derivatives such as N-ethyl-N-nitroso ethanamine, N-methyhlene ethanamine, N, N-dimethyl 2, 2'-oxybis ethanamine and, 2, 2'-oxybis ethanamine have been previously identified in Moringa oleifera leaves [43], olive oil [68], Calotropis procera leaf [105] and *Eucalyptus camaldolehsis* leaf extract [106], respectively. Most of the afore-mentioned medicinal plant extracts from which these ethanamine derivatives were identified and isolated have been shown to exhibit significant antioxidant activity [43, 68, 78, 105, 106]. Ethanamine and its derivatives are known to be associated with important biological activities. For instance, N, N-Dimethyl-5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indole-3-ethanamine benzoate is an orally bio-available potent 5-HT1B and 5-HT1D agonist that has demonstrated effectiveness in migraine treatment [38, 39] and in alleviating calcitonin gene-related peptide-induced light aversion in mice. Similarly, 2-Ethyl-5-methoxy-N, N-dimethyl-1H-indole-3-ethanamine oxalate – a potent selective 5-HT6 agonist and adenylate cyclase activator has been shown to exhibit antidepressant activity [40, 41]. This finding may be the first report of ethanamine and its derivatives being phytochemical constituents of *Tapinanthus globiferus* leaf extracts. The potential antidepressant activity of the above-stated ethanamine derivatives may explain, partly, the reported antidepressant activity of *Tapinanthus* spp in previous studies [15, 16].

Furazano [3,4-b] pyrazin-5(4H)-one, 6-(1-pyrrolidinyl)-, identified as one of the components of methanol *Tapinanthus globiferus* leaf extract under the present investigation, belongs to a group of phytoconstituents collectively referred to as furazano pyrazines whose specific biological activities have not been significantly

elucidated. The finding of this novel chemical compound demonstrates one of the key uses of GC-MS as a versatile tool in identifying and, thereby, generating novel pharmacophores [7, 8] which may engender further scientific probes to unravel their biological activities in the body. In light of this, furazano pyrazines have been characterized as weak acids that act as small-molecule transmembrane proton transfer facilitators in the mitochondria. This transcellular transfer is linked to mitochondrial matrix pH dissipation, which in turn, results in the inhibition of ATP synthetase-independent mitochondrial oxidative stress [42]. Furazano phytoconstituents have been reported to exhibit structure-activity correlation for the afore-mentioned biological activities and to possess potential therapeutic efficacy in mitochondrial oxidative stress-related pathologies such as Parkinson's disease, obesity, heart failure, insulin-resistance diabetes mellitus, ageing and ischemia-reperfusion injuries [42; 44, 45]. Although, the specific biologic activities of furazano [3,4-b] pyrazin-5(4H)-one, 6-(1-pyrrolidinyl)- moiety and its derivatives have not been established, previous studies have identified it in significant amounts in extracts obtained from *Baccopa monnieri* [45] and *Polycarpaea corymbosa* [46, 47]. These medicinal plant extracts have been shown to exhibit significant antioxidant, antimicrobial, anamnestic, anxiolytic and antidepressant effects in animal and human studies [44, 45, 46, 47, 48]. The observed biological activities may be due to the presence of Furazano [3,4-b] pyrazin-5(4H)-one, 6-(1-pyrrolidinyl)- and its derivatives in these medicinal extracts.

Dimethyl silane is another phytoconstituent identified in the methanol *Tapinanthus globiferus* leaf extract under the current study whose biological activity is largely yet unknown. However, related silane derivatives – trimethyl silanes have been previously identified not only as phytochemical components of extracts of *Amomum nilgiricum* and *Allium hookeri* but also that these silane compounds exhibited significant anti-inflammatory, neuroprotective, anti-lipidemic and antioxidant, antiviral antifungal activities [49, 50, 51]. The presence of silane derivatives in these medicinal plants may be contributory to the reported biological activities of these plant extracts and dimethyl silane reported herein may therefore be contributory to the biological activities earlier reported for *Tapinanthus globiferus* leaf extracts [19, 20, 21, 22] under the current and other previous investigations [14, 15, 16, 17, 18].

Oxalic acid, acetic acid and o-Allyl hydroxylamine are small molecular phytochemicals identified in the present study. Antimicrobial/antibacterial and antineoplastic/cytotoxic activities are biological activities commonly associated with these phytoconstituents and their derivatives. Oxalic acid, arguably the strongest acid in plants, exists as oxalates of calcium, potassium, magnesium, manganese, zinc, etc., in a concentration range of less than ten percent of dry mass in edible, and higher concentrations in non-edible (toxic), plants [52, 53]. Physiologically, oxalic acid is thought to be concerned with plant intracellular cation/anion balance, plant defence and phyto-remediation [54, 55, 56, 57]. This acid and its oxalates have been identified, previously, in seed and fruit pulp of extracts of *Passifora foetida* L. [58], stem extracts of *Combretodendron macrocarpum* and *Glyphaea brevis* [59], *Ludwigia stolonifera* aerial and root extracts [60] and

Aloe vera extracts [61] in [62]. The finding that oxalic acid-/oxalate-containing *Passifora foetida* L. extracts exhibited phytoremediation [58],and that Ludwigia spp. extracts demonstrated detoxifying [63], hypoglycemic [64], anticancerous [65] and hepato-protective [66] effects indicate oxalic acid/oxalate component of the methanol *Tapinanthus globiferus* extract in this study [19, 20, 21, 22] and of *Tapinanthus globiferus* extracts in

other studies [14, 15, 16, 17, 18] may partly account for the observed diverse biological activities of these extracts.

Acetic acid identified in the leaf extract under the present study has also been previously identified as a phytoconstituent of extracts obtained from Moringa oleifera [23, 43], gallic [31] and Syzygium alternifolim (Wt.) Walp. [67]. While acetic acid has been associated with antibacterial, acidifying, vericolytic and mucolytic biological activities [67], indole-3-acetic acid isolated from endophytic *streptomyces spp*. has been reported for its plant growth-promoting effect [67], and serverogenin acetate isolated from *Amomum nilgiricum* has been shown to exhibit potent hypoglycemic, bactericidal, antioxidant, antiviral and antifungal effects [49]. If most, if not all, of the aforementioned-biological effects of these acetic acid/acetate-containing medicinal plants are shared with *Tapinanthus globiferus*, it then suggests that acetic acid and perhaps, its derivatives in *Tapinanthus globiferus* and its congeners may be partly contributory to the reported pharmacological effects of these plant extracts [14, 15, 16, 17, 18].

O-Allyl hydroxylamine is yet another chemical compound identified in the present study will be the first time it is being reported as a phytoconstituent of *Tapinanthus globiferus* extracts. Literature search indicates, apart from its detection as olive oil [68], the current investigation is one of the few reports of this compound as a phytochemical component of any medicinal plant extracts. Its specific biologic actions may not have been known, but it has been reported to exhibit significant antiplasmodial and antimicrobial effects [69]. If *Tapinanthus* species have been reported for their antiplasmosdial activity [70], and o-Allyl hydroxylamine, on its part, has been associated with significant antiplamodial effect, it then implies this compound, o-Allyl hydroxylamine identified in methanol *Tapinanthus* globiferus extract in this study may account, in part, for the reported antiplasmodial activity of *Tapinanthus* species.

Dimethyl silane is also yet another phytoconstituent identified in the methanol *Tapinanthus globiferus* leaf extract under the current study whose biological activity has not been known. However, this compound has been previously identified in *Simmondsia chinensis* L. seed extract [71] – and trimethyl silanes identified as phytochemical components of extracts of *Amomum nilgiricum* [49] nd *Amaranthus Viridis* [72] – with these silane compounds reported as exhibiting significant antioxidant and antimicrobial activities [49, 71, 73]. The fact that these afore-mentioned medicinal plant extracts share dimethyl silane and other silane derivatives with methanol *Tapinanthus globiferus* leaf extract in the current study and that both groups of medicinal plants have been shown to exhibit antioxidant and antimicrobial activities is an indication the silane derivatives in these plant extracts are likely partially responsible for the observed biological effects in them.

Isobutylamine is another phytoconstituent identified in this study. This is the first report of this small molecular chemical entity as a phytoconstituent of methanol *Tapinanthus globiferus* leaf extract. Although its biological activity has not been specified, it has been previously identified in extracts from Moringa oleifera parts [23, 74, 75], olive oil [68[and Crataegus oxyacantha [76 & 77 in 78] – all of which have been shown to exhibit significant cardioprotective activity. Heart failure and hypertension are some of the common conditions for which *Tapinanthus spp*-derived extracts have been traditionally used [13, 14, 15] and their cardioprotective efficacy may be related to the presence of cardiotropic amines (e.g., isobutyl amine) and other cardiotropic

phytochemicals in these medicinal plants.

N-ethyl formamide, chemically known as N-Formyl ethylamine is another chemical compound that is being reported for the first time as a constituent of methanol *Tapinanthus globiferus* leaf extract. Literature review shows it has only been reported as phytochemical component of olive [68] and little is known of its biological activity – except that a chemically related compound, N-methyl formamide has been reported for antitumour activities [79]. The finding of N-ethyl formamide as a phytochemical is expected to trigger scientific research on its biologic functions and activities.

2-propenenitrile is another compound identified in the present study and is the first to report it as a phytochemical obtainable from *Tapinanthus globiferus* leaves. However, a number of 2-propenenitrile derivatives, 3-Fluoro-2-propenenitrile [80], 2-(4-chlorophenylsulfonyl)-3-cyclohexylamino-propenenitrile [81], 3-phenyl-2-Propenenitrile [82] and, 3– (3.4- dimethoxyphenyl) –2–Propenenitrile [83] have been previously identified in extracts from *Citrullus lanatus* (Watermelon) Seeds, Ehretia laevis stem bark, *Reseda muricata* and Feronia elephantum Correa (Rutaceae) stem bark, respectively. Although, no specific in-vivo biological activity has been established for 2-Propenenitrile as a chemical compound, the fact that 2-Propenenitrile derivative from Ehretia laevis stem bark extract is thought to confer antioxidant activity on it [84] and the essential oil of Feronia elephantum Correa which main phytoconstituent is 3– (3.4- dimethoxyphenyl) –2–Propenenitrile has demonstrated potent antioxidant and cytotoxic activities [85] indicates 2-Propenenitrile compound identified in this study may be partly responsible for antioxidant activity of methanol *Tapinanthus globiferus* leaf extract in an earlier study [22] and its antioxidant and anticancer effects reported in other studies [13, 14].

1-Propanol (synonyms: 1-propyl alcohol, propanol, propan-1-ol, n-propyl alcohol, n-propanol; isomer: 2-propanol) is a lower molecular weight aliphatic alcohol that is yet another chemical molecule identified in the plant extract under the present investigation. The present report is the first of such to identify 1-Propanol as a phytochemical component of methanol *Tapinanthus globiferus* leaf extract. Previously, this same compound has been identified as a phytoconstituent of methanol Cissus vitiginea (wild) [85] and methanol Nicotiana tabacum [86] extracts. Other studies have also shown derivatives of this compound, such as 2-methyl-1-Propanol [87], 3-methoxy-2-(methoxymethyl)-2- methyl-1-Propanol [88] and hexahydro-1(2H)-naphthalenylidene)-1-propanol [89] have been previously identified in Alysicarpus glumaceus, Senna tora and Ruellia prostrata, and Ziziphus spina-christi extracts, respectively. Research indicates propanol, like other lower alcohols, exhibits antiseptic, bactericidal, and central nervous system-depressant and anti-arrhythmic effects [90, 91]. If propanol possesses the just afore-mentioned properties on its own merit as a chemical compound and the propanol-possessing Ziziphus spina-christi [92], Cissus vitiginea [93], and Nicotiana tabacum [94] extracts also exhibit antibacterial, antioxidant and cardio-protective activities, it can be reasonably suspected that the 1-propanol and its derivatives present in the methanol *Tapinanthus globiferus* under the present study may be contributory to the antimicrobial and cardio-protective traditional uses of *Tapinanthus* species [13, 14, 15].

1-(1-propenyl)-aziridine is yet another chemical compound identified in the methanol *Tapinanthus globiferus* leaf extract under the present investigation and it is the first report of this molecule being a phytoconstituent of the plant. Although literature search shows the same molecule has not been identified in any other plant and has

not been associated with specific biological activities, structurally related aziridine derivatives i.e., 1-(2-buten-1-yl)-aziridine [95], 2,2-dimethyl-aziridine [68], and miraziridine [96, 97] previously identified in extracts obtained from *Oldenlandia corymbose*, olive oil and red marine sponges (*Thenonella spp*.), respectively – have been shown to exhibit significant antimicrobial and anticancer activities. These biologic activities of aziridine derivatives are viewed to be due to their broad-spectrum protease inhibition – including potent cathepsin B inhibition [96, 97, 98, 99]. The fact that these structural relatives of 1-(1-propenyl)-aziridine have been reported to possess antimicrobial/anticancer effects suggests this compound that has just been identified in the extract under the current study may contribute, partly, to the antimicrobial and antitumour efficacy of this and other extracts obtained from *Tapinanthus* spp. Infections and cancer management are two common indications for which extracts of *Tapinanthus globiferus* and its congeners are traditionally deployed [13]. The foregoing finding may thus justify these traditional uses of extracts obtained from *Tapinanthus globiferus* and its congeners for these indications.

Finally, the finding of carbonyl sulfide and urea amongst the yields of methanol *Tapinanthus globiferus* leaf extract under the current investigation poses some intellectual challenge. This is because any of the compounds is viewed either not to occur naturally, toxic or does any play any beneficial biologic role to warrant its existence as a phytoconstituent of an extract obtained from a highly efficacious medicinal plant as *Tapinanthus globiferus*. For instance, carbonyl sulfide (syn.: carbon oxysulfide, carbon oxide monosulfide & carbon oxide sulfide) – the most abundant gas on the earth's atmosphere is said to be toxic in high concentration; its specific biologic/physiologic/pharmacological activities yet unestablished apart from its suspected regulatory role in the metabolism and recycle of biologically reactive Sulphur species [100]. This study is the first time it is being reported as a phytoconstituent of *Tapinanthus globiferus* and literature search indicates only one study has reported it as a phytoconstituent of a medicinal plant (olive oil) [68]. Urea is the last of the phytochemical compound identified in the extract of the current investigation, and it is the first of its kind to identify urea as a phytoconstituent of *Tapinanthus globiferus.* Urea and its derivatives have been previously identified in root of *Moringa* [43], olive oil [68], *Nicotiana Tabacum* L. [86] and *Ziziphusspina Christi* leaves [89]. Urea has hitherto been thought of as bereft of any significant biological activity in-*vivo*. However, emerging evidence indicates a role for both renal and extrarenal urea transporter proteins as potential therapeutic targets for fluid and salt homeostasis [102, 103] – with urea and its analogues reported to have been safely and cost-effectively deployed in the treatment euvolemic hyponatremia [103, 104]. Diuresis is key requirement to offset the fluid overload often seen in hypertension and heart failure, two clinical conditions for which *Tapinanthus globiferus* and congeners have been traditional used in alleviating. The presence of osmosis-inducing urea and urea derivatives in the methanol *Tapinanthus globiferus* leaf extract being investigated suggests these phytochemicals could partly account for the efficacy of this medicinal plant in the afore-mentioned health disorders.

Of course, this study is constrained by a few limitations. One, the fact that gas chromatography-mass spectrometry is largely well-suited for separating volatile and thermally stable chemical compounds which may be only a small fraction of the total number of phytochemicals present in the extract means a substantial proportion of the chemical constituents that are not volatile and thermally unstable could be missed. Two, the usually suboptimal molecular ionization and mass spectral isomer and structural information for which GCMS

is generally known may have been at play and probably leading to submaximal sample identification in the extract under investigation [107, 108]. Together, these factors may have contributed to the paucity of the chemical entities yielded in this study despite reports of diverse biological activities of extracts from this highly medicinal plant [13, 14, 15, 16, 17, 18].

V. Conclusion

The identified chemical compounds and their derivatives highlighted above may be responsible for the multiple biological activities reported for *Tapinanthus globiferus* extracts. These findings are a justification for the traditional use of extracts of *Tapinanthus globiferus* and its congeners for the treatment of infections, cancers, hypertension, heart failure and mood disorders. There may be need to further investigate the phytochemistry of this medicinal plant using high performance liquid chromatography or a combination of gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry to engender greater sample identification in the extract.

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