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Original Article

Gas Chromatography-Mass Spectrometry (GC-MS) Assay of Bio-Active Compounds and Phytochemical Analyses in Three Species of Apocynaceae

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ABSTRACT

Objective: Gas chromatography coupled with mass spectrometry (GC-MS) was used to analyze for phytochemicals and bioactive compounds in three species of Apocynacae, *Gongronema latifolium, Vincetoxicum rossicum and Marsdenia edulis* commonly found in tropical rainforest vegetation and used as food and traditional medicine by locals. **Methods and Materials:** Phytochemical analysis and GC-MS were carried out using leaf samples of the species following standard protocols. Quantitative phytochemical data were analyzed using analysis of variance (ANOVA) and significance tested at 5% level of probability. Bioactive compounds were identified by comparing the retention times with those of authentic compounds and spectral data obtained from National Institute of Standards and Technology (NIST) library. **Results:** Phytochemical analysis revealed presence of alkaloids, glycosides, tannins, saponins, terpenes, steroids, flavonoids and phenols. Among the three species, *Gongronema latifolium* was highest in flavonoids (28.40 %), *Vincetoxicum rossicum* was highest in steroids (17.25 %) while *Marsdenia edulis* was highest in terpenoids (18.17 %). GC-MS profiling of the species revealed biologically functional compounds with therapeutic properties including linoleic acid, phytol, neophytadiene, n-hexadecanoic acid, squalene, transfarnesol, 5-pentadecen-7-yne, and mercaptoacetic acid. **Conclusion:** The array of bioactive compounds present in the three species especially *Gongronema latifolium,* commonly used as food indicated their utility in pharmacognosy and drug manufacture. This is the first report of GC-MS based metabolite profiling to detect the various bioactive compounds in methanolic extracts of *Vincetoxicum rossicum* and *Marsdenia edulis*. We therefore recommend these species for further study in drug discovery trials.

Key words: *Gongronema latifolium*, *Vincetoxicum rossicum*, *Marsdenia edulis,* Underutilized species, Phytochemical profiling, Drug discovery.

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INTRODUCTION

Tropical rainforests in sub-Saharan Africa is home to a host of plants species, some of whose importance have not yet been exploited. Among these are neglected and underutilized species (NUS) used as food and medicine by local communities in traditional health and nutrition system. Medicinal plants play a crucial role in health care needs of people around the world especially in developing countries[1,2]. Nigeria is richly endowed with indigenous plants which are used in herbal medicine to cure diseases. These plants exhibit a wide range of biological and therapeutic activities including cancer prevention, prevention of inflammation, antidiuretic, laxative, suppression of spasms, prevention of hypertension, hypoglycemic, and anti-microbial functions[3-6]. It is generally assumed that the active medicinal constituents contributing to these protective effects are phytochemicals[7,8]. In historical times, traditional medicine was the only source of health care in Nigeria[9]. In spite of the introduction of western medicine with its attendant cost which often makes it unaffordable to the immediate needs of the poor, the popularity of traditional medicines has not only increased but has become common and remains a viable part of the complex health care system in Nigeria in recent times[9,10].

The family Apocynaceae has attained great significance since the first commercial anticancer drugs of vinblastine, vincristine and their derivatives were developed from *Catharanthus roseus* (periwinkle) [11]. The family is a rich source of drugs that have found use both in traditional and modern medicine. Several species of the family Apocynaceae have been reported to have anti-schizophrenic, anti-hypertensive, antibacterial, anti-inflammatory, antioxidant, antimalarial properties[12-15]. The study species - *Gongronema latifolium*, *Vincetoxicum rossicum* and *Marsdenia edulis* are shrubs of the Apocynaceae family that are found in the tropical forests of Nigeria (Plate 1).

Gongronema latifolium (commonly called "Arokeke" by the Yorubas, "Utazi" by the Ibos, and "Utasi" by the Efiks/Ibibios) has a soft/hairy stem with green leaves that are slightly oval in appearance with a deeply cordate base[16,17]. The plant produces white latex and is characterized by a distinguishable bitter taste especially when eaten fresh[17]. *G. latifolium* has been widely used in folk medicine for maintaining

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(a) (b) (c)

Plate 1: Three species of Apocynaceae. (a) *Gongronema latifolium* (b) *Vincetoxicum rossicum* (c) *Marsdenia edulis*.

healthy blood glucose level.[18] It has also been reported to exhibit various pharmacological properties such as antioxidative, anti-inflammatory, antibacterial, antimalaria, hypoglycemic and hypolipidemic effects[16,19].

Vincetoxicum rossicum has several common names including swallowwort, pale swallowwort, and dog-strangling vine. There has historically been much confusion about the genus it belongs to with authors placing it within *Vincetoxicum* and others within *Cynanchum*[20-22]. It is native to southern Europe and is a highly invasive plant growing in all of the Eastern United States, in the mid-west, and southern Ontario and Quebec in Canada. Some populations are found in the tropical forests of some parts of Africa including Nigeria. The leaves of the pale swallowwort are oval and contain smooth margins and major veins underneath. The leaves are glossy, dark green and grow opposite on the stem[23]. In parts of Nigeria where the plant grows, according to the locals, it is known to be poisonous and can be mistaken for *Gongronema latifolium* due to their shared morphological characteristics. There is little evidence to show that extracts from the plant exhibit broad spectrum antifungal activity[24].

Marsdenia edulis is native to tropical regions in Asia, Africa, Australia and America. It is a liana with thick green leaves that grow opposite on the stem and are ovate to elliptical[25]. There are no documented reports on the uses of *Marsdenia edulis*, however, the fruits are eaten as a snack among the Ugep people of Cross River state of Nigeria (Personal observation).

Gas chromatography coupled to mass spectrometry (GC-MS) is one of the techniques used to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds among others[26]. Traditionally used medicinal plants have recently attracted the attention of the biological scientific communities. This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations[27].

Natural products that come from medicinal plants are important for pharmaceutical research and for drug development as a source of therapeutic agents. At present, the demand for herbal or medicinal plant products have increased significantly. In this research, we report the GC-MS investigations of methanolic extracts from the leaves of *Gongronema latifolium, Vincetoxicum rossicum* and *Marsdenia edulis* of the family Apocynaceae in Nigeria in order to explore their utility in drugs and medicine.

MATERIALS AND METHODS

Sample preparation for laboratory analysis of phytochemicals

The three species – *Gongronema latifolium*, *Vincetoxicum rossicum* and *Marsdenia edulis* were collected from the forests in Cross River and Imo States in the Southeastern part of Nigeria. The species were authenticated and deposited in the Herbarium and Taxonomy Unit of the Department of Plant and Ecological Studies, University of Calabar. Fresh leaves of the three species were rinsed in running water, air dried at room temperature, milled and stored in plastic bottles in preparation for laboratory analysis.

Quantitative phytochemical analysis in three Apocynaceae species

Quantitative assay was carried out for alkaloids, flavonoids, phenols, saponins, tannins, terpenes, steroids and glycosides.

Determination of total Alkaloids

One gram (1 g) of each sample was weighed into a 250 ml beaker and 100 ml of acetic acid was added, covered and allowed to stand for 24 hours. The solution was filtered and concentrated to one-quarter of the original volume with a rotary evaporator. Ammonium hydroxide was added in drops to the extract until precipitation was complete. The solution was allowed to settle and the precipitate collected afterwards washed with ammonium hydroxide and filtered. The residue (alkaloid) was dried and weighed[28].

Determination of total flavonoids

One gram (1 g) of each sample was repeatedly extracted with 100 ml methanol and allowed to stand for 24 hours at room temperature. The mixture was filtered through a Whatman No 1 filter paper into a pre-weighed 250 ml beaker. The filtrate was transferred into a concentrator and allowed to evaporate to dryness and weighed[29,30]. The percentage flavonoid was calculated as

$$\% \text{ Flavonoid} = \frac{\text{Weight of flavonoid} \times 100}{\text{Weight of sample}}$$

Determination of total phenols

This was determined by colorimetric method using gallic acid as a standard. Approximately 1 g of each of the plant samples was dissolved in 500 ml of water. Approximately 500 ml of the fraction in water was mixed with 2.5 ml of Folin-Ciocalteu reagent (0.2 N). After five minutes, 2 ml of sodium carbonate was added. After 120 minutes standing in the dark, the optical density was measured at 750nm against a blank. The total phenolic content was calculated from the calibration in curve equation in mg / g of the sample[31].

Determination of saponins

One gram (1 g) each of the plant samples was dispersed in 10 ml of 20 % ethanol. The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55 ^{0}C. The mixture was filtered and the residue re-extracted with 10 ml of 20 % ethanol. The combined extracts were reduced to 2 ml in a rotary evaporator. The concentrated sample was transferred into a 10 ml funnel and 5 ml diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. 2 ml of n-butanol was added to the recovered aqueous layer. The solution was washed twice with 3 ml of 5 % sodium chloride solution. The remaining solution was heated in a water bath for evaporation and then dried in an oven to constant weight[28].

Determination of total tannins

This was determined by colorimetry as blue colour formation by the reduction of phosphor tungsto molybdic acid by tannin-like compound in alkaline medium. Approximately 1.1 ml of extract and standard solution of tannic acid (1000 ppm) was made up to 7.5 ml with distilled water. Approximately 0.5 ml Folin-Denis reagent and 1 ml sodium carbonate solution were added and then made up to 10 ml with distilled water. Absorbance was measured at 700 nm. The total tannic acid content was calculated in mg / g[30].

Determination of total terpenoids

Two grams (2 g) of each plant sample were weighed and soaked in 50 ml of 95 % ethanol for 24 hours. The solution was filtered and the filtrate was extracted with petroleum ether at 60 – 80 ^{0}C and concentrated to dryness[32]. The dried ether extract was weighed.

Determination of total steroids

One gram (1 g) of each plant sample was weighed into a beaker and mixed with 20 ml chloroform. One ml of the solution was transferred into 10 ml volumetric flasks. Two (2) ml of 4 N Sulphuric acid and 2 ml 0.5 % Iron (III) chloride were added followed by 0.5 ml of 0.5 % potassium hexacyanoferrate (III) solution. The mixture was heated in a water bath at 7 ^{0}C for 30 minutes with occasional shaking and then diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank[33].

Determination of total glycosides

Glycosides were determined using Baljet reagent.[34-50] One gram (1 g) of each plant sample was dissolved in 20 ml ethanol. Ten (10) ml of the solution was transferred to a 100 ml volumetric flask; 60 ml of water and 10 ml of 12.5 % lead acetate were added, mixed and filtered into a beaker. Fifty (50) ml of the filtrate was transferred into another 100 ml flask and 10 ml of 47 % sodium hydrogen phosphate was added to the precipitate. This was mixed and completed to 100 ml with water and filtered twice to remove excess lead phosphate. Ten (10) ml of purified filtrate was transferred into clean 250 ml flask and treated with 10 ml Baljet reagent (95 ml of 1% aqueous picric acid + 5 ml 10 % aqueous NaOH). This was allowed to stand for one hour for colour development. The colour intensity is proportional to the concentration of the glycoside. The absorbance was measured at 495 nm against blank.

Extraction process with soxhlet extractor

Five grams of each plant sample were weighed into an extraction thimble of a Soxhlet extractor. Fifty (50) ml of the solvent (methanol mixed with dimethyl sulfuroxide to enable dissolution of the sample) was poured into a round bottom flask attached to the Soxhlet extractor. This was refluxed three times. The extract was transferred into the rotary evaporator and concentrated to 2 ml before being further transferred into a well-labelled Teflon screw cap vial. The 2 ml extract was passed through a chromatographic column packed with well baked silica gel and sodium sulfate anhydrous to obtain clean extract and also remove water from the extract.

GC-MS analysis

An Agilent 6890N gas chromatography equipped with an autosampler connected to an Agilent mass spectrophotometric detector was used. Approximately 1 ml of the sample was injected in the pulsed spotless mode unto a GC column 30 m x 0.25 mm ID DB-5MS coated fused silica column with a film thickness of 0.15 mm. 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 preset. The column temperature was initially held at 55 ^{0}C for 0.4 minutes, increased to 200 ^{0}C at a rate of 25 ^{0}C/min, then to 280 ^{0}C at a rate of 8 ^{0}C / min and to final temperature of 300 ^{0}C at a rate of 25 ^{0}C / min, held for 2 minutes. The identification time was based on retention time since each of the components has its separate retention time in the column. The components with lower retention time were eluted before the ones with high retention time.

Data collection and analysis

Data obtained from phytochemical analyses of the three species were analyzed using analysis of variance (ANOVA). Phytochemical compounds were identified by comparing the retention times with those of authentic compounds and the spectral data obtained from National Institute of Standards and Technology (NIST) library. Each determination was carried out in duplicate.

RESULTS

Quantitative phytochemicals in Gongronema latifolium, Vincetoxicum rossicum and Marsdenia edulis

There were significant variations ($P<0.001$) in quantity in all the phytochemicals present in the three species except for alkaloids and tannins which were not significantly different ($P>0.05$) across the three species. *Gongronema latifolium* had the highest concentration of flavonoids (4.68 ± 0.04) and phenols (1.89 ± 0.02), *Vincetoxicum rossicum* had the highest concentration of saponins (1.55 ± 0.01) and steroids (2.30 ± 0.01) while *Marsdenia edulis* had the highest concentration of terpenes (2.18 ± 0.02) (Table 1).

Gas Chromatography-Mass Spectrometry analysis in the three study species

Gongronema latifolium

GC-MS analysis of the active principles in the methanolic leaf extract of *Gongronema latifolium* showed the presence of 18 bioactive compounds (Figure 1). The major compounds identified were n-hexadecanoic acid, phytol, 3-hydroxydodecanoic acid and transfarnesol. 9,12-octadecadienoic acid ($C_{18}H_{32}O_2$) had the highest area peak (%) (17.58%) (Table 2).

Vincetoxicum rossicum

The GC-MS chromatogram analysis of methanol leaf extract of *Vincetoxicum rossicum* (Figure 2) showed the presence of 15 phytocompounds (Table 3). The major compounds identified were Squalene, 5-pentadecen-7-yne, (Z), n-Hexadecanoic acid, Neophytadiene and Phytol. Squalene ($C_{30}H_{50}$) had the highest peak area % (33.04%) followed by 5-pentadecen-7-yne, (Z) ($C_{15}H_{26}$) (15.63%).

Marsdenia edulis

The GC-MS chromatogram analysis of methanol leaf extract of *Marsdenia edulis* (Figure 3) showed the presence of six phytocompounds (Table 4). Of the six compounds identified, the most prevailing were Squalene, Oxirane tetradecyl, 1-chloro nonadecane and 1,10-decanediol. Squalene ($C_{30}H_{50}$) had the highest peak area % (62.22%) followed by Oxirane tetradecyl ($C_{16}H_{32}O$) (18.08%).

Bioactivity of identified phytocompounds

Ten major compounds were identified in the three study species. Among the compounds, seven were reported to have antimicrobial, antioxidant, anticancer and anti-inflammatory activities and no activity was reported in three compounds (Table 5).

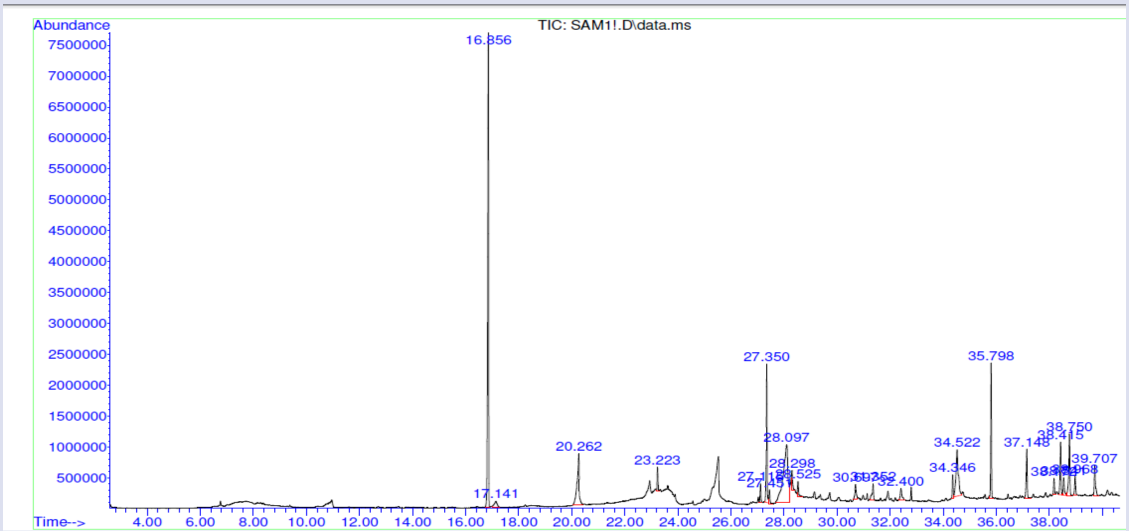


Figure 1: GC-MS chromatogram of Methanolic leaf extract of *Gongronema latifolium*.

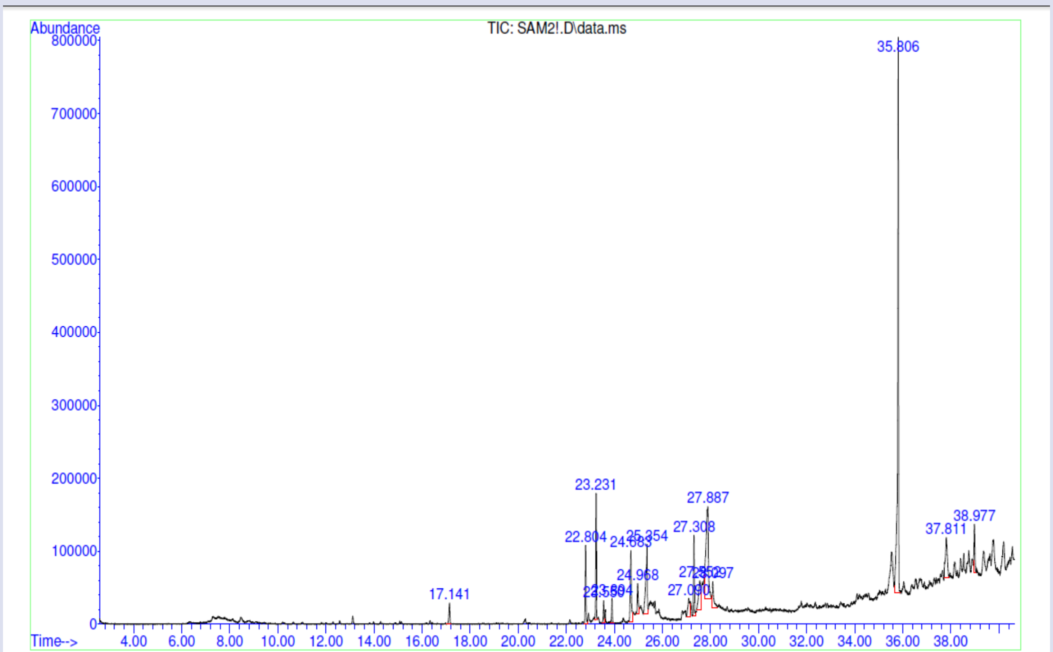


Figure 2: GC-MS chromatogram of methanolic leaf extract of *Vincetoxicum rossicum*.

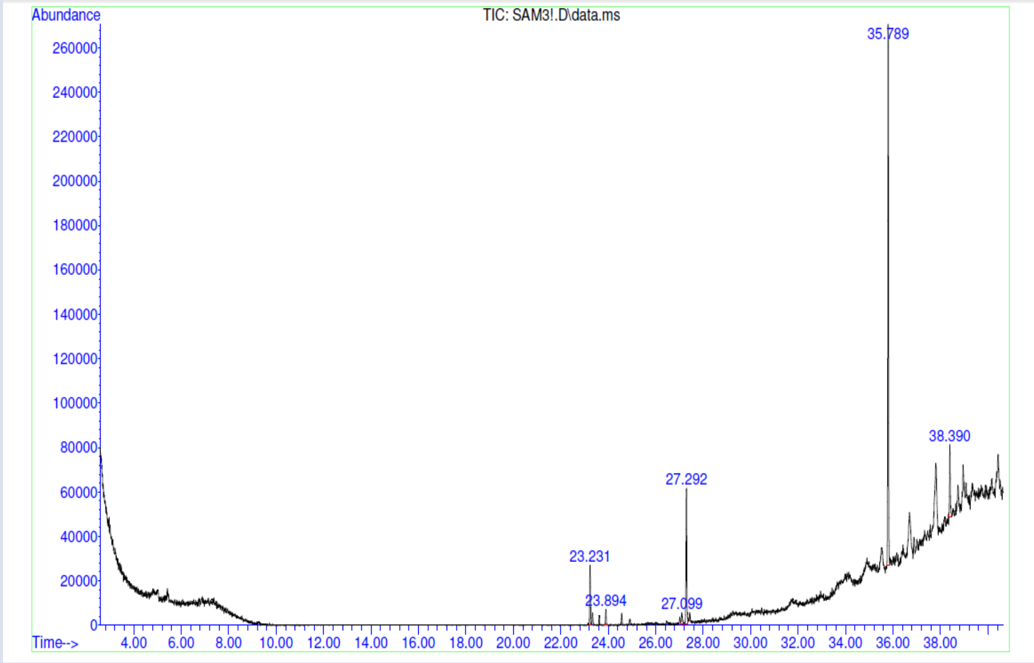


Figure 3: GC-MS Chromatogram of methanolic leaf extract of *Marsdenia edulis*.

Table 1: Means and Standard Errors (SE) as well as Percentages of Phytochemicals in three species of Apocynaceae.

Phytochemical (mg / g)	Gongronema latifolium	Vincetoxicum rossicum	Marsdenia edulis	LSD (p = 0.05)
Alkaloids	$3.67^{a} \pm 0.02$ (22.26)	$2.49^{a} \pm 0.03$ (18.67)	$3.08^{a} \pm 0.06$ (25.67)	NS
Tannins	$1.98^{a} \pm 0.02$ (12.00)	$2.22^{a} \pm 0.01$ (16.65)	$2.16^{a} \pm 0.01$ (18.00)	NS
Saponins	$0.98^{c} \pm 0.02$ (5.94)	$1.55^{a} \pm 0.01$ (11.63)	$1.47^{b} \pm 0.01$ (12.25)	0.002
Flavonoids	$4.68^{a} \pm 0.04$ (28.40)	$2.35^{b} \pm 0.01$ (17.63)	$1.22^{c} \pm 0.02$ (10.17)	0.056
Terpenes	$1.54^{b} \pm 0.01$ (9.34)	$1.57^{b} \pm 0.02$ (11.78)	$2.18^{a} \pm 0.02$ (18.17)	0.028
Steroids	$1.41^{b} \pm 0.05$ (8.56)	$2.30^{a} \pm 0.01$ (17.25)	$1.29^{c} \pm 0.01$ (10.75)	0.013
Glycosides	$0.33^{a} \pm 0.01$ (2.00)	$0.33^{a} \pm 0.02$ (2.48)	$0.25^{b} \pm 0.03$ (2.08)	0.041
Phenols	$1.89^{a} \pm 0.02$ (11.50)	$0.52^{b} \pm 0.02$ (3.90)	$0.35^{c} \pm 0.01$ (2.92)	0.025

Means ± SE are based on three replications. Different lower-case letters in each row denote significant differences among the means based on the Least Significant Difference test at 5% probability level. Values in brackets are the means expressed as percentages of the total phytochemicals present in each species.

Table 2: Phytochemicals identified in the methanolic leaf extract of *Gongronema latifolium*.

No	*RT	Compound	Mol. Formula	Mol. Weight	Peak Area %
1	23.223	2-penten-1-ol, (Z)-	$C_5H_{10}O$	86.134	1.20
2	27.115	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.487	0.99
3	27.350	Phytol	$C_{20}H_{40}O$	128.171	7.57
4	27.451	Methyl Stearate	$C_{19}H_{38}O_2$	298.511	0.84
5	28.097	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280.446	17.58
6	28.298	Cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.461	1.15
7	28.525	1-trimethylsilyl-2-ethene	$C_{12}H_{28}Si_2$	228.526	1.00
8	30.697	7-nonenamide	$C_{19}H_{17}NO$	155.241	1.45
9	31.352	12-methyl-E, E-2,13-Octadecadien-1-ol	$C_{19}H_{36}O$	280.488	1.43
10	32.400	Glycerol-1-Palmitate	$C_{19}H_{38}O_4$	330.503	1.21
11	34.346	D-Xylulose, tetrakis (trimethylsilyl) ether, pentafluorobenzyloxime (Isomer 2)	$C_{17}H_{42}O_5Si_4$	438.85	1.23
12	34.522	19,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280.446	6.67
13	35.798	Trans-Farnesol	$C_{15}H_{26}O$	222.366	6.09
14	38.172	4-Hydroxybenzoxazolone	$C_7H_5NO_3$	151.121	0.88
15	38.415	Mercaptoacetic acid, 2TMS derivative	$HSCH_2CO_2H$	92.120	3.19
16	38.541	1,2-15,16-Diepoxyhexadecane	$C_{16}H_{30}O_2$	354.408	1.22
17	38.750	Cholesta-6,22,24-triene, 4,4-dimethyl	$C_{29}H_{46}$	394.687	4.29
18	38.968	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	430.71	1.18

*Retention time

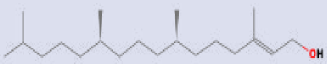
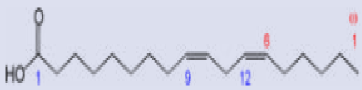
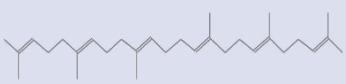
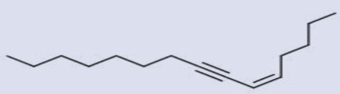
Table 3: Phytochemicals identified in methanol extract of *Vincetoxicum rossicum*.

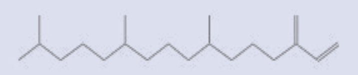
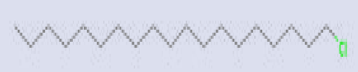
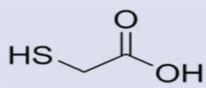
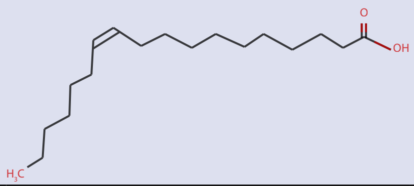
N0	RT	Compound	Mol. Formular	Mol. Weight	Peak Area %
1	22.804	Neophytadiene	$C_{20}H_{38}$	278.524	4.39
2	23.231	Neophytadiene	$C_{20}H_{38}$	278.524	5.91
3	23.550	2-Cyclopenten-1-one, 3-(1-methylethyl)-	$C_8H_{12}O$	124.180	1.12
4	23.894	6-methyloctahydrocoumarin	$C_{10}H_{16}O_2$	168.236	1.28
5	24.683	Cyclobutane, (1-methylethylidene)	C_7H_{12}	96.173	5.93
6	24.968	Methyl (methyl-4-0-methyl-alpha.-d-mannopyranoside) urinate	$C_9H_{16}O_7$	236.220	2.20
7	25.354	n-hexadecanoic acid (palmitic acid)	$C_{16}H_{32}O_2$	256.430	7.06
8	27.090	1,2-15,16-diepoxyhexadecane	$C_{16}H_{30}O_2$	254.408	2.20
9	27.308	Phytol	$C_{20}H_{40}O$	128.171	4.46
10	27.552	Z, Z-6,13-octadecadien-1-ol acetate	$C_{20}H_{36}O_2$	308.506	4.52
11	27.887	5-pentadecen-7-yne, (Z)-	$C_{15}H_{26}$	206.373	15.63
12	28.097	Tridecanal	$C_{13}H_{26}O$	198.350	2.40
13	35.806	Squalene	$C_{30}H_{50}$	410.730	33.04
14	37.811	1-Methoxy-3-(2-hydroxyethyl) nonane	$C_{12}H_{26}O_2$	202.338	5.43
15	38.977	dl.-alpha. -Tocopherol	$C_{29}H_{50}O_2$	430.717	2.92

Table 4: Phytochemicals identified in methanolic leaf extract of *Marsdenia edulis*.

N0	RT	Compound	Mol. Formular	Mol. Weight	Peak Area %
1	23.231	1,10-Decanediol	$C_{10}H_{22}O_2$	174.284	7.30
2	23.894	1-Octadecyne	$C_{18}H_{34}$	250.470	1.94
3	27.099	Oxirane, 2,2 – (1,4-butanediyl) bis-	$C_{10}H_{18}O_4$	202.247	2.07
4	27.292	Oxirane, tetradecyl-	$C_{16}H_{32}O$	240.431	18.08
5	35.789	Squalene	$C_{30}H_{50}$	410.730	62.22
6	38.390	Nonadecane, 1- chloro-	$C_{19}H_{39}Cl$	302.966	8.39

Table 5: Compounds of importance identified in the three species of Apocynaceae.

Compound	Structure	Class	Identified in	Importance/activity
n-hexadecanoic acid (Palmitic acid)		Saturated fatty acid	*Gongronema latifolium* *Vincetoxicum rossicum*	Antimicrobial, anti-inflammatory, hypocholesterolemic, antioxidant, nematicidal, hemolytic [35-37].
Phytol		Cyclic diterpene	*Gongronema latifolium* *Vincetoxicum rossicum*	Antimicrobial, anticancer, antioxidant, diuretic, anti-inflammatory [37-40].
9,12-octadecadienoic acid (Linoleic acid)		Polyunsaturated omega-6 fatty acid	*Gongronema latifolium*	Anti-inflammatory, hypoglycemic, serum insulin elevation [42].
Transfarnesol		Sesquiterpene	*Gongronema latifolium*	Antibacterial [43].
Squalene		Linear triterpene	*Vincetoxicum rossicum* *Marsdenia edulis*	Antioxidant, antistatic, antibacterial, anticancer, antitumor [35,44].
5-pentadecen-7-yne, (Z)		Alkaloid	*Vincetoxicum rossicum*	

Neophytadiene		Sesquiterpene	*Vincetoxicum rossicum*	Analgesic, anti-inflammatory, antimicrobial, antioxidant [45].
Oxirane, tetradecyl		Oxirane	*Marsdenia edulis*	
Nonadecane, 1-chloro		Alkane hydrocarbon	*Marsdenia edulis*	
1,10-decanediol		Diacrylate	*Marsdenia edulis*	Essence, perfumes and pharmaceuticals, flexible dental materials [46, 47].
Mercaptoacetic acid		Alpha-mercapto carboxylic acid	*Gongronema latifolium*	oil and gas, cosmetics, cleaning, leather processing, metals, fine chemistry and polymerization [48,49].
Cis-vaccenic acid		Trans-fatty acid	*Gongronema latifolium*	May down regulate gluconeogenesis and liver fat accumulation [50].

DISCUSSION

The phytochemical analysis carried out in this study revealed the presence of alkaloids, glycosides, flavonoids, tannins, steroids, saponins, terpenoids and phenols in *Gongronema latifolium, Vincetoxicum rossicum* and *Marsdenia edulis*. These secondary metabolites are reported to have many biological and therapeutic properties[51]. Alkaloids have been reported to demonstrate adverse array of pharmacological actions including analgesia, local anesthesia, cardiac stimulation, vasoconstriction, muscle relaxation and toxicity[52,53]. All the species studied were rich in alkaloids. The findings are in line with Trease and Evans[54], who reported that the order Gentiales (of the three study species) of the Apocynanceae family is one of those found to be rich in alkaloids.

Flavonoids are a class of water-soluble plant pigments. They are found to be better antioxidants with multiple biological activities including vasodilatory, antitumor, anti-inflammatory, antibacterial effects[55]. In this study, *Gongronema latifolium* leaves were high in flavonoids and phenols which may be responsible for its antioxidant, hypoglycemic, analgesic properties[51,56-57].

Vincetoxicum rossicum leaves were high in steroids, alkaloids, tannins and flavonoids indicating that the species could have biologically useful and therapeutic properties.

Marsdenia edulis leaves showed the presence of terpenes, alkaloids, tannins and saponins. Terpenes have desirable properties for use in food as additives such as capsanthine, in cosmetics and perfumery for fragrance due to their volatile nature. Geraniol and linalool are common terpenoids used in perfumes. Terpenes are also used in the pharmaceutical industry to reduce inflammation, relief pain and aid sleep. In biotechnology industries, terpenes have been discovered to be useful as biofuels and can also be turned into suitable chemical feed-stocks[58,59].

Results obtained from biochemical profiling of the three study species using GC-MS revealed the existence of various bioactive compounds of biological and therapeutic importance. These compounds included linoleic acid, phytol, neophytadiene, n-hexadecanoic acid, squalene, 5-pentadecen-7-yne, (Z)-, transfarnesol, mercaptoacetic acid. Similar occurrences of these compounds have also been reported in many medicinal plants[37,60,61]. Linoleic acid identified in *G. latifolium* possesses anti-inflammatory, hypoglycemic properties and also elevates serum insulin [42]. Phytol identified in *G. latifolium* and *V. rossicum* is an important diterpene that possesses antimicrobial, anticancer, antioxidant, diuretic and anti-inflammatory activities[37-40]. Al-Hindi *et al.*[62] also reported the presence of phytol and palmitic acid in the ethanolic extract of *G. latifolium*. Neophytadiene identified in *V. rossicum* is a good analgesic, anti-inflammatory, antimicrobial and antioxidant[45]. Hexadecanoic acid is known to exhibit strong antimicrobial, anti-inflammatory and hemolytic activity[35-37,61]. Squalene identified in *V. rossicum* and *M. edulis*. is a triterpene that acts as a natural antioxidant. The European commission for health and consumers catalogued squalene as an ingredient for cosmetics. According to this institution, squalene has several functions including, antistatic, emollient, hair conditioning, refatting and skin conditioning[63]. 5-pentadecen-7-yne was identified in *V. rossicum* and is used as material for coating, also in flavoring drinks and spirits[41]. Some useful minor compounds were also identified and they included cis-vaccenic acid and mercaptoacetic acid. Cis-vaccenic acid has been reported by Weir *et al.*,[50] as an omega-7 monounsaturated fatty acid that may down regulate gluconeogenesis and liver fat accumulation. Mercaptoacetic acid was identified in *G. latifolium* and is a high-performance chemical containing mercaptan and carboxylic acid functionalities. Reports indicate its use in industries and applications as wide as in oil and gas, cosmetics, cleaning, leather processing, metals, fine chemistry and polymerization. It forms powerful complexes with metals that give it specific characteristics sought after for the assisted recovery of ore as well as for cleaning and corrosion inhibition[48,49].

This is the first report of GC-MS based metabolite profiling to detect the various bioactive compounds in methanolic extracts of *Vincetoxicum rossicum* and *Marsdenia edulis*. The presence of the various bioactive compounds in *Gongronema latifolium* justifies the use of the plant for food and medicine and their presence in *Vincetoxicum rossicum* and *Marsdenia edulis* indicated that they could be isolated and subjected to biological activity making these plants more useful.

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CONFLICTS OF INTEREST

None.

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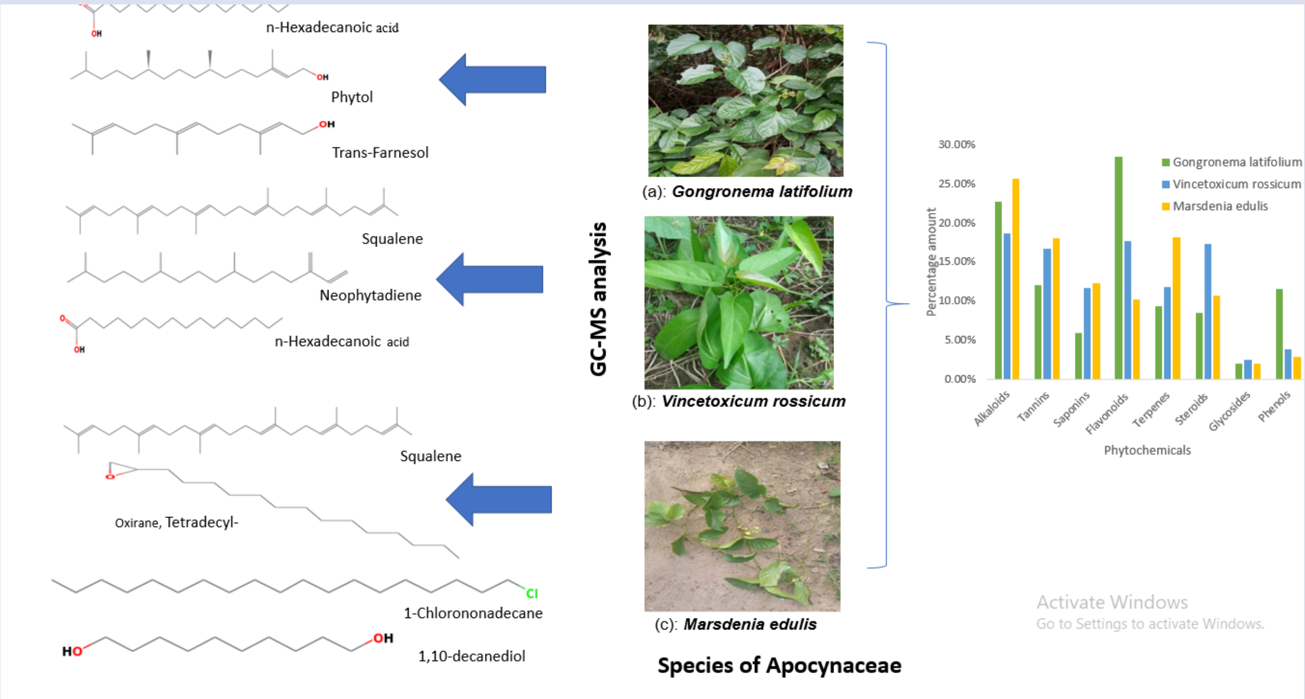
GRAPHICAL ABSTRACT



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