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

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INTRODUCTION

Medicinal plants play a crucial role in health care needs of people around the world especially in developing countries. Nigeria is richly endowed with indigenous plants which are used in herbal medicine to cure diseases. The family Apocynaceae is a rich source of drugs that have found use both in traditional and modern medicine. 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.

(a):***Gongronema latifolium***

(b): ***Vincetoxicum rossicum***

(c): ***Marsdenia edulis***

MATERIALS AND METHODS

1. 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, air dried at room temperature, milled and stored in plastic bottles in preparation for laboratory analysis.

2. Quantitative phytochemical analysis in three Apocynaceae species

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

3. Extraction process with soxhlet extractor for GC-MS analysis using methanol

4. GC-MS analysis

An Agilent 6890N gas chromatography equipped with an autosampler connected to an Agilent mass spectrophotometric detector was used. 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.

5. 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.

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. 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 %) (Table 1).

Gas Chromatography-Mass Spectrometry analysis in the three study species

GC-MS profiling of the species revealed biologically functional compounds with therapeutic properties (Fig. 1, 2, 3 and Table 2)

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

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

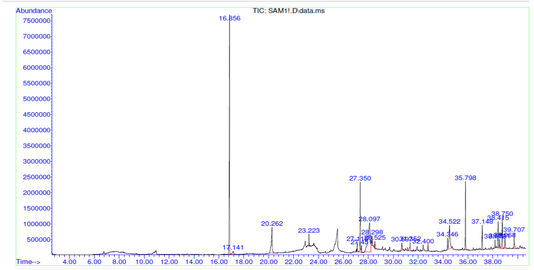

Fig. 1: GC-MS chromatogram of methanolic leaf extract of *G. latifolium*

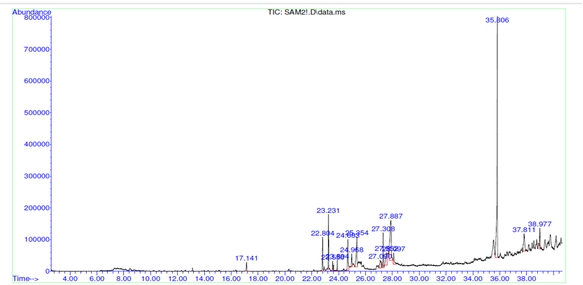

Fig. 2: GC-MS chromatogram of methanolic leaf extract of *V. rossicum*

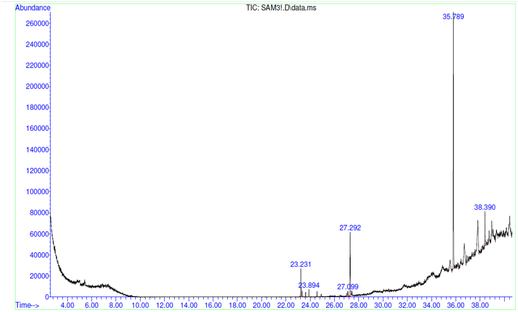

Fig. 3: GC-MS chromatogram of methanolic leaf extract of *M. edulis*

Table 2: Compounds of importance identified in the three species of Apocynaceae

Compound	Class	Identified in	Importance/activity
n-hexadecanoic acid (Palmitic acid)	Saturated fatty acid	*G. latifolium* *V. rossicum*	Antimicrobial, anti-inflammatory, hypocholesterolemic, antioxidant, nematicidal, hemolytic
Phytol	Cyclic diterpene	*G. latifolium* *V. rossicum*	Antimicrobial, anticancer, antioxidant, diuretic, anti-inflammatory
9,12-octadecadienoic acid (Linoleic acid)	Polyunsaturated omega-6 fatty acid	*G. latifolium*	Anti-inflammatory, hypoglycemic, serum insulin elevation
Transfarnesol	Sesquiterpene	*G. latifolium*	Antibacterial
Squalene	Linear triterpene	*V. rossicum* *M. edulis*	Antioxidant, antistatic, antibacterial, anticancer, antitumor.
5-pentadecen-7-yne, (Z)	Alkaloid	*V. rossicum*	
Neophytadiene	Sesquiterpene	*V. rossicum*	Analgesic, anti-inflammatory, antimicrobial, antioxidant.
Oxirane, tetradecyl	Oxirane	*M. edulis*	
Nonadecane, 1-chloro	Alkane hydrocarbon	*M. edulis*	
1,10-decanediol	Diacrylate	*M. edulis*	Essence, perfumes and pharmaceuticals, flexible dental materials.
Mercaptoacetic acid	Alpha-mercapto carboxylic acid	*G. latifolium*	oil and gas, cosmetics, cleaning, leather processing, metals, fine chemistry and polymerization.
Cis-vaccenic acid	Trans-fatty acid	*G. latifolium*	May down regulate gluconeogenesis and liver fat accumulation.

CONCLUSIONS

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

PUBLICATION

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