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EVALUATION OF BIOACTIVE COMPOUNDS IN *Pseudarenthemum tunicatum* LEAVES USING GAS CHROMATOGRAPHY- MASS SPECTROMETRY

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Abstract

Pseudarenthemum tunicatum belongs to the Acanthaceae family and an aqueous infusion of it is taken as a hematinic and for other medicinal purposes. This work was aimed at identifying compounds in dichloromethane/ methanol (1:1v) and methanol extracts of P. tunicatum leaves using Gas Chromatography Mass Spectrometry analysis. Thirty compounds were identified in dichloromethane/methanol extract while thirty five compounds were identified in the methanol extract. The most abundant compounds in the dichloromethane/methanol extract 21-methyl-17-isocholestane-16-[2- [formylthio]ethyl],methyl-5,9,21-octacosatrienoate, 9,19-Cyclolanostan-3-ol, 24-methylene-, (3β.)and n-Hexadecanoic acid which had relative abundance of 13.51%, 12.72%, 10.66% and 10.20% respectively. The most abundant compounds in the methanol extract were 2,5-Furandione- 3-methyl, 5-Hydroxymethylfurfural, 9-Octadecenoic acid, (E) and n-Hexadecanoic acid which had relative abundance of 14.01%, 13.27%, 12.25% and 11.87% respectively. Tetradecanoic acid (i.e myristic acid) and n-Hexadecanoic acid were the only compounds identified in both extracts. Results indicated that polarity of the solvents used in extraction influenced the relative abundance and type of compound extracted. Dichloromethane/ methanol extracted more of the triterpenoids and sterols while methanol extracted more of the flavonoids, fatty acids and fatty acid esters. Various compounds identified are known to have varying bioactivities such as antimicrobial, anticancer, antioxidant and antisickling. As such, P. tunicatum leaves could be useful in the preparation of neutraceuticals.

Key words**:** *Pseudarenthemum tunicatum leaves, Gas Chromatography-Mass Spectrometry, triterpenoids, fatty acids*

INTRODUCTION

Plants serve as source of medicine and an important component of the health care system [10]. Many plants contain compounds which have curative or protective properties against various diseases [16]. These compounds proffer positive health benefit such as being antioxidants which have the ability of “mopping up” reactive oxygen species (ROS) and reactive nitrogen species (RNS) which are implicated in inducing oxidative stress resulting to various types of cancer in different body parts and other ailments associated with it.

There has been an increasing interest on natural product research especially on medicinal plants which seem to have restorative properties [7]. The World Health Organization estimates that approximately 80% of the world population in developing countries relies on traditional plant medicine for primary health care needs, of which a major proportion corresponds to plant extracts or their active principles [20]. They are a source of novel chemical entities that possess beneficial pharmacological and therapeutic properties [11]. *Pseudarenthenum tunicatun* leaves belongs to the family, Acanthaceae [5] [2] reported that plants belonging to Acanthaceae family have been reported to have anti-fugal, cytotoxic, anti-inflammatory, anti-pyretic, antioxidant, insecticidal, hepatoprotective, immunomodulatory, anti-platelet aggregation and anti-viral activities. Red colored infusion of this plant got after boiling the leaves in water is traditionally taken as 'blood tonic' by some human populations in the southern part of Nigeria and in some African countries who have a knowledge of its medicinal usefulness. Nutrient, phytochemical and amino acid profile of the leaves have been elucidated [3]. *P. tunicatum* is a woody herb or under shrub

reaching a height greater than 85cm with red flowers protruding at the tip. This under-shrub grows in evergreen forest and on rocks and near streams. It is found growing from Ghana to West Cameroon and Fernando Po and is wide spread throughout tropical African [5].
The main research interest in plants is aimed at unveiling the presence of some active components in them [17]. Identification and evaluation of these active compounds otherwise known as phytochemicals of uncommonly used plants could help provide information that would be useful in the development of a new drug [1] or in the production of a neutracuetical. It is in view of this that Gas Chromatography- Mass Spectrometry (GCMS) analysis was carried out to identify compounds present in *P. tunicatum* leaves. This will provide information about its usefulness and proposed use as a neutraceutical or as starting material in the synthesis of pharmaceutical drugs.

MATERIALS AND METHODS

Psuederathemum tunicatum leaves were harvested from nearby farms in Abia State University, Umuahia location in Umudike, Ikwuano Local Government Area in the month of January, 2017. It was identified by a taxonomist in the Department of Plant Science and Biotechnology, Abia State University Uturu, Nigeria. Specimen sample of the leaves were deposited in the herbarium. The leaves were de-stalked, washed in clean tap water and drained off the water. Subsequently, the leaves were dried under a shade for 5 days after which they were pulverized in a blender. Bioactive compounds were extracted according to the method described by [21]. Methanol and dichloromethane/methanol (1:1v/v) were used to extract bioactive compounds. 20g of the pulverized leaf sample was put into two labeled conical flasks respectively. 400ml dichloromethane/methanol and 400 ml methanol were added to the conical flasks containing the samples respectively and shaked vigorously. Each flask containing the sample and solvent were covered using aluminum foil and allowed to stand for 24h at room temperature before the sample mixtures were filtered through whatman filter paper No.1 respectively. The respective extracts were concentrated by evaporating excess solvent by boiling in a water bath. Hence, two extracts of *P. tunicatum* leaves were obtained. These extracts were subjected to gas chromatography-mass spectrometry (GCMS) analysis for the separation and identification of compounds respectively. This was done using GCMS (Model QP 2010 series, Shimadzu, Japan) equipped with Optima 5ms fused capillary column of 30mm length, 0.25 mm diameter and 0.25 mm film thickness. Helium (99.99%) was used as carrier gas. The temperature programming was set with initial column oven temperature of 60^0C hold time of 2mins by 120^0Cmin to a final temperature of 300^0C with hold time for 2mins. 2.0 µl of the *P. tunicatum* leaf extracts were injected using a Hamilton syringe into the GC for total ion chromatographic analysis with split injection technique (3:1) respectively. The injector temperature was 250ºC, ion source temperature was 200ºC with an interface temperature of 280ºC and recorded over a scan range of 45 to 650m/z with electron impact ionization energy of 70ev. Total running time of GC-MS for the methanol extract was 29 min, while for the dichloromethane/methanol extract was 23min. The relative percentage of each extract constituents were expressed as a percentage with peak area normalization. Compounds were identified by mass spectroscopy. This was done by comparing retention indices and mass spectra fragmentation patterns of the compounds with those stored on the computer library of the National Institute of Standard Technology (NIST/EPA/NIH Mass Spectral Library, Version 2.0). Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

RESULTS AND DISCUSSIONS

GC-MS analysis revealed the presence of 30 compounds in dichloromethane/methanol extract and 35 compounds in methanol extract of *Pseuderathemum tunicatum* leaves. Peak

number, retention time, compound name, molecular weight, molecular formula and relative abundance are stated in their various tables. Table 1 shows compounds identified in dichloromethane/methanol extract of *P. tunicatum* leaves which represented the lipophilic fraction. Four compounds were identified as the major bioactive compounds.

They are 21-methyl-17-isocholestane-16-[2-[formylthio]ethyl] and it had a relative abundance of 13.51% and eluted as represented at peak 26.

Methyl-5,9,21-octacosatrienoate had a relative abundance of 12.72% and eluted as represented at peak 19; 9,19-Cyclolanostan-3-ol, 24-methylene- (3β.) had a relative abundance of 10.66% and eluted as represented at peak 29 and n-Hexadecanoic acid (i.e. palmitic acid) had a relative abundance of 10.20% and eluted as represented at peak 17.

Table 1. Bioactive Compounds indentified in Dichloromethane/ methanol extract of *Pseuderathernum tunicatum* leaves

Peak No	RT (mins)	Name of Compound	Molecular formula	Molecular weight	Relative abundance (%)
1	3.709	n-Octanoic acid	$C_8H_{16}O_2$	144	0.12
2	3.900	2-Butanone, 4-hydroxy-3-methyl	$C_5H_{10}O_2$	102	0.26
3	5.857	1,8-Nonadien-3-ol	$C_9H_{16}O$	140	0.16
4	6.120	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	$C_{10}H_{20}O$	156	0.91
5	6.336	Methylene chloride	CH_2Cl_2	84	0.07
6	6.700	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, (2S-trans)-	$C_{10}H_{18}O$	154	0.34
7	6.941	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, trans-	$C_{10}H_{18}O$	154	0.17
8	10.694	2-Decanynoic acid	$C_{10}H_{16}O_2$	168	0.10
9	11.173	Z,Z-4,16-Octadecadiene-1-ol acetate	$C_{20}H_{36}O_2$	308	0.16
10	11.273	5-Aminoimidazole-4-carboxylic acid, methyl ester.	$C_5H_7N_3O_2$	141	0.15
11	11.726	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	0.23
12	12.057	Z,Z-4,16-Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$	308	0.34
13	12.286	Z,Z-4,16-Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$	308	0.34
14	12.434	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.67
15	12.746	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3β, 17β)	$C_{22}H_{32}O_2$	328	0.41
16	13.558	Oxacyclododecan-2-one	$C_{11}H_{20}O_2$	184	0.70
17	13.682	n-Hexadecanoic acid	$C_{16}H_{32}O_2$		10.20
18	14.581	Phytol	$C_{20}H_{40}O$	296	3.70
19	14.798	Methyl 5,9,21-octacosatrienoate	$C_{29}H_{52}O_2$	432	12.72
20	14.904	2,4,6,8-Tetramethyl-1-octacosanol	$C_{32}H_{66}O$	466	7.03
21	15.144	Triamcinolone acetonide	$C_{24}H_{31}FO_6$	434	3.41
22	15.837	Olean-12-en-3-one	$C_{30}H_{48}O$	424	4.70
23	15.933	Olean-12-en-3-one	$C_{30}H_{43}O$	424	4.19
24	16.279	9,19-Cyclo-9β-lanostane-3β,25-diol	$C_{30}H_{52}O_2$	444	7.93
25	16.532	Ergosta-7,22-dien-3-ol acetate (3β, 5α)	$C_{30}H_{48}O_2$	440	4.92
26	16.838	21-Methyl-17-isocholestane 16-[2-[formylthio]ethyl]-	$C_{30}H_{52}OS$	460	13.51
27	17.081	D:B-Friedo-B':A'-neogammacer-5-en-3-ol, (3β) (i.e Simiarenol)	$C_{30}H_{50}O$	426	8.19
28	17.284	Isocitronellol	$C_{10}H_{20}O$	156	1.43
29	17.689	9,19-Cyclolanostan-3-ol, 24-methylene-, (3β)-	$C_{31}H_{52}O$	440	10.66
30	19.967	α-Tocopherol	$C_{29}H_{50}O_2$	430	1.39
31	20.651	Cyclopentane, 1,1'-hexadecylidenebis-	$C_{26}H_{50}$	362	0.90

Source: Own findings.

These represented 47.09% of the compounds present in the lipophilic fraction. These compounds consists of a stanol ester, unsaturated fatty acid, a triterpene and a

saturated fatty acid. Stanol esters are a heterogenous group of phytosterol esters with a saturated ring structure which are known to reduce the low density lipoprotein cholesterol when ingested [9]. n-Hexadecanoic acid has been reported to have activities like being antioxidant [12] which is about 68% antioxidant activity [8] as well as having cytotoxic activity [14], anti-inflammatory, anti spasmodic and antiviral [15]. 9,19-Cyclolanostan-3-ol, 24-methylene- (3β.) has been reported to have antimicrobial activity [18]. It has been reported that plant sterols and stanols are of good therapeutic options for the management of hypercholesterolemia [23] while triterpenes have been reported to have cytotoxic activity [24]. The presence of 21-methyl-17-isocholestane-16-[2-[formylthio]ethyl] which is a derivative of stanols, 9,19-Cyclolanostan-3-ol, 24-methylene- (3β.) which is a triterpene and n-Hexadecanoic acid a saturated fatty acid found in appreciable quantities therefore suggests the usefulness of *P. tunicatum* leaves in human nutrition.

Other compounds found in appreciable quantities include 2,4,6,8-Tetramethyl-1-octacosanol (7.03%), Olean -12-en-3-one (8.89%), 9, 19-cyclo-9β-lanostane-3β-20-diol (7.93%), D:B-Friedo-B':A'-neogammacer-5-en-3-ol, (3β)- (i.e Simiarenol) (8.19%). Results therefore indicated the presence of both tetracyclic and pentacyclic triterpenoids of dammarane, oleanane, cyclolartane and friedoursane type which constitutes about 36.08% of the compounds present in dichloromethane/methanol extract of *P. tunicatum* leaves. It has been reported that simiarenol is present in the root of *Rhododen rondayrium* and may have in vitro leishmanicidal activity against leishmania donovani promastigoes [4]. Some other compounds identified in this extract which has been reported to have positive health benefits include phytol (3.7%) and ∝ -tocopherol (ie vitamin E 1.39%). Phytol has been reported to have activities such as antimicrobial, anti-cancer, anti-inflammatory, anti-diuretic, immune-stimulatory and anti-diabetic activities [19]. There are four tocopherol isoforms namely ∝, β, γ,δ-tocopherols among which ∝-tocopherol was found in dichloromethane/methanol extract. It is known to have the highest vitamin E activity amongs the isoforms [6]. ∝ -Tocopherol has some biological activities such as being antioxidant, anti-inflammatory, anti microbial, radical scavenging and anti-spasmodic [19].

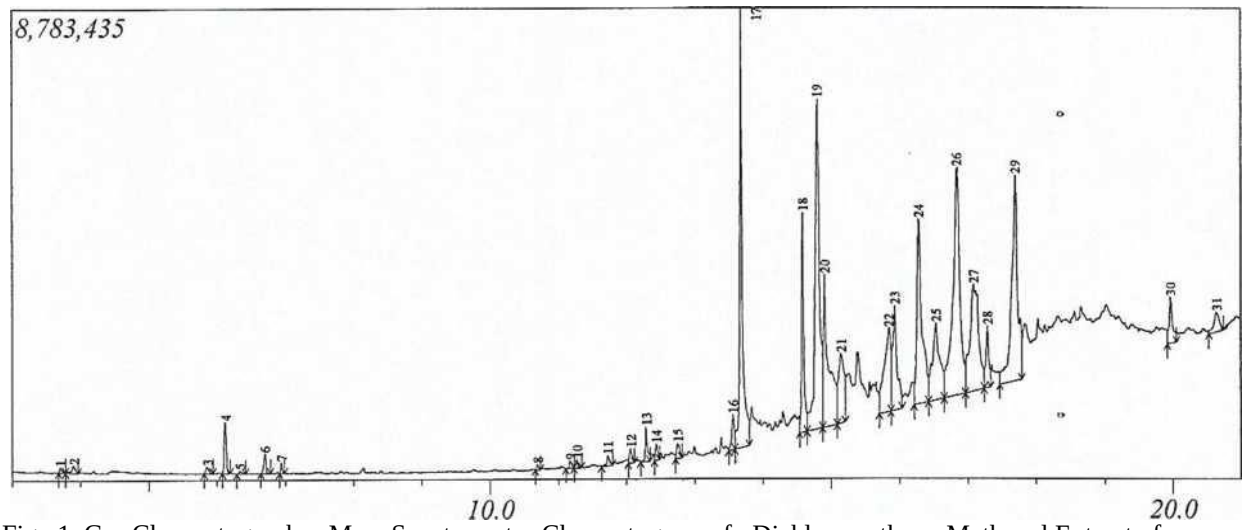

Fig. 1. Gas Chromatography- Mass Spectrometry Chromatogram of Dichloromethane-Methanol Extract of *Pseuderanthemum tunicatum* leaves.
Source: Own findings.

Table 2 shows results on compounds present in the methanol extract of *Pseuderanthemum tunicatum* leaves.The major compounds identified include 2, 5 – furandone, 3- methyl which had relative abundance of 14.01% and eluted as peak 4; 5-hydroxymethly furfural

had a relative abundance of 13.27% and eluted as peak 15; n – hexadecanoic acid (i.e. palmitic acid) had a relative abundance of 11. 87% and eluted as peak 30 and 9-octadecenoic € (i.e. oleic acid) had a relative abundance of 12.25% and eluted as peak 34.

These constitute 51.40% of compounds present in the hydrophilic fraction of *P. tunicatum* leaves. 2, 5- furandione, 3 –methyl has been reported to have anti-cancer activity [15]. 5-hydroxy methyl furfural is a sugar derivative and is a heterocydic aldehyde widely present in foods. It is produced though the degradation of hexoses via Maillard reaction during heat treatment of foods containing reducing sugars and amino acids in an acid environment [25]. 5 – hydroxyl methyl furfural has been reported to increase enzyme activities such as superoxide dismutase and glutathione peroxidase as well as potential therapeutic agent for the treatment of Alzheimer's disease [13].

n-Hexadecanoic acid (i.e palmitic acid), 9-Octadecenoic acid (i.e oleic acid) and octadecanoic acid (i.e stearic acid) were the major fatty acids present in the methanol extract of *P. tunicatum* leaves.

Table 2. Bioactive compounds identified in Methanol extract of *Pseuderathernum tunicatum* leaves

Peak No	RT (mins)	Name of Compound	Molecular Formula	Molecular weight	Relative abundance (%)
1	3.382	1,3-Dioxan-5-ol,4,4,5 trimethyl	$C_7H_{14}O_3$	146	0.09
2	3.625	3-methoxy-3-methyl-tetrahydro-pyran-2-one	$C_7H_{12}O_3$	144	0.20
3	4.314	2-Furancarboxaldehyde, 5-methyl	$C_6H_6O_2$	110	0.16
4	4.932	2,5-Furandione, 3-methyl-	$C_5H_4O_3$	112	14.01
5	5.062	1H-Azonine, octahydro-1-nitroso-	$C_8H_{16}N_2O$	156	0.92
6	5.638	Butanedoic acid, monomethyl ester	$C_5H_8O_4$	132	0.45
7	5.806	2-Furancarboxylic acid, methyl ester	$C_6H_6O_3$	126	1.12
8	6.051	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	1.62
9	6.139	2(3H)-Furanone,5-ethyenyldihydro-5-methyl.	$C_7H_{10}O_2$	126	1.14
10	6.274	2,5-Furandione, dihydro-3-methylene	$C_5H_4O_3$	112	0.87
11	6.593	Heptanoic acid, 3-hydroxy-methyl ester.	$C_8H_{16}O$	160	0.17
12	6.783	Levoglucosenone	$C_6H_6O_3$	126	0.40
13	7.057	Butanedioic acid, methylene-, 4-methyl ester	$C_6H_8O_4$	144	1.56
14	7.205	1α, 2 α, 4α, 5β-cyclohexanetetrol	$C_6H_{12}O_4$	148	0.64
15	8.096	5-Hydroxymethyl furfural	$C_6H_6O_3$	126	13.27
16	9.900	1,3-Dioxolane, 4,5-diethenyl-2,2-dimethyl	$C_9H_{14}O_2$	154	9.01
17	10.056	2-Furanmethol	$C_5H_6O_2$	98	0.66
18	10.802	Citric acid, trimethyl ester	$C_9H_{14}O_7$	234	5.11
19	10.904	β-D-Glucopyranose-1,6-anhydro	$C_6H_{10}O_5$	162	2.28
20	11.212	Octanedioic	$C_8H_{14}O_4$	174	0.46
21	11.607	2-Hydroxypropane-1,2,3-tricarboxylic acid, dimethyl ester	$C_8H_{12}O_7$	220	4.71
22	11.685	Undecanoic acid	$C_{11}H_{22}O_2$	186	2.43
23	12.005	Nonanedioic acid	$C_9H_{16}O_4$	188	0.90
24	12.307	n-Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.97
25	12.956	9-undecenol-2,10-dimethyl	$C_{13}H_{26}O$	198	1.27
26	13.035	9-undecenol-2,10-dimethyl	$C_{13}H_{26}O$	198	0.47
27	13.000	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.43
28	13.383	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.36
29	13.578	cis-9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	2.24
30	13.723	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	11.87
31	14.107	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.37
32	14.304	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.75
33	14.488	6-Octadecenoic acid, methyl ester (Z)	$C_{19}H_{36}O_2$	296	0.80
34	14.823	9-Octadecenoic, (E)-	$C_{18}H_{34}O_2$	282	12.25
35	14.932	n-Octadecanoic acid	$C_{18}H_{36}O_2$	284	7.22
36	18.469	Hexadecanoic acid, (3-bromoprop-2-ynyl) ester	$C_{19}H_{33}BrO_2$	372	0.46
37	18.658	3α-(Trimethylsiloxy)cholest-5-ene	$C_{30}H_{54}OSi$	458	0.40

Source: Own findings.

They have been reported to have antioxidant activities. However, [8] reported n-hexadecanoic acid to have 68% antioxidant activity while 9-Octadecenoic acid has only

moderate activity and n-Octadecanoic acid had poor antioxidant activity. Mostly identified compounds were ketones, dibasic acids, fatty acids, fatty acid esters, sterols, stanols, flavonoids, nitrogen compounds and triterpenoids. The polarity of the respective solvents played a major role to extract more specific compounds. This was confirmed by the isolation of phyto-compounds on polarity based extraction [22]. Dichloromethane methanol extracted more of sterols, stanols and triterpenoids while methanol extracted more of flavonoids, aldehydes, nitrogen compounds , fatty acids and fatty acid esters. It was observed that some compounds were common to both dichloromethane/methanol and methanol extracts of *P. tunicatum* leaves but in varied relative abundance. They are tretradecanoic acid (myristic acid) and n-Hexadecanoic acid (Palmitic acid).

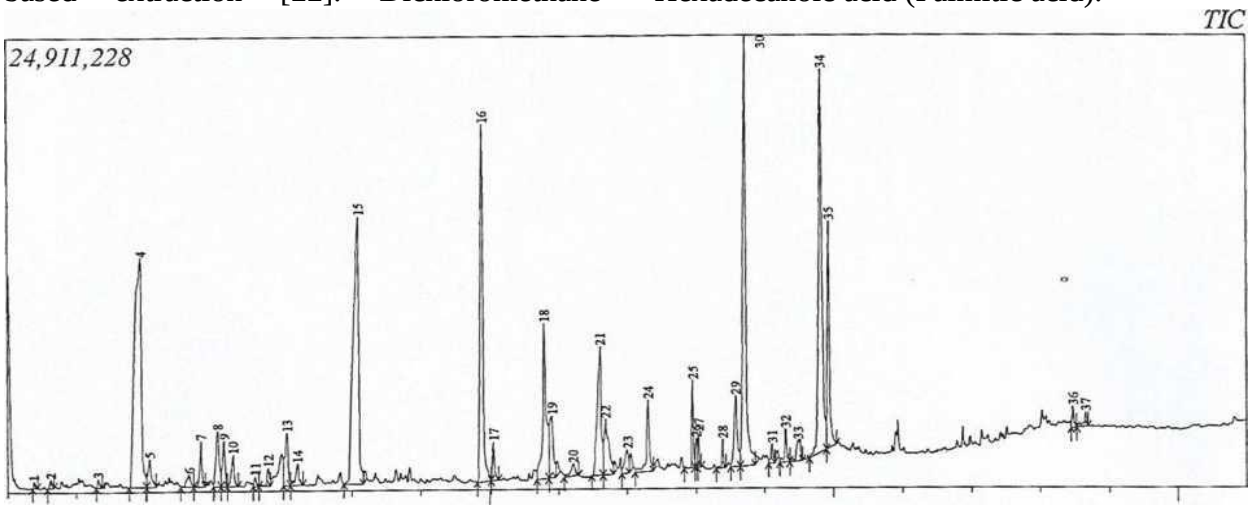

Fig. 2. Gas Chromatography- Mass Spectrometry - Chromatogram of Methanol Extract of *Pseuderanthemum tunicatum* leaves.
Source: Own findings.

CONCLUSIONS

In the present study 30 compounds from dichloromethane/methanol extract and 35 compounds from methanol extract were identified in *Psuederanthenum tunicatum* leaves. The polarity of solvents used for extraction resulted to variations in compounds extracted. The dichloromethane/methanol extract revealed that *P. tunicatum* leaves has good quantities of triterpenoids of the dammarane, oleanane, cyclolartane and friedoursane type. 21-methy 1-17 isocholestane – 16- [2-[formylthio] ethyl] which is a stanol was the most abundant compound in the dichloromethane/methanol extract while 2,5- furandienee – 3- methy (flavonoid) was the most abundant compound in the methanol extract of *P. tunicatum* leaves.

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