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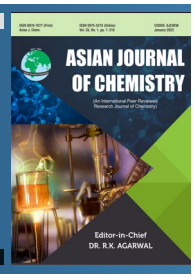

Identification of Phytochemical Constituents in *Phyllanthus acidus* L. Leaf through Gas Chromatography-Mass Spectroscopy as Biostimulant

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The *Phyllanthus acidus* L. belongs to the family phyllanthaceae and possess a wide range of secondary metabolites and phytochemicals in leaves. To siphon off the use of synthetic chemicals in crop production, an alternative like utilizing the natural bio-stimulants could play a crucial role in promoting crop growth and development. The major goal of this study was to employ gas chromatography-mass spectrometry to examine the bioactive compounds present in phyllanthus leaf and to identify and characterize them utilizing dichloromethane as extraction solvent. According to GC-MS analysis, dichloromethane extraction of phyllanthus leaf yielded, 25 phytoconstituents in which ethyl oleate contributed the area percentage of 53.68%, hexadecanoic acid, ethyl ester by 17.47%, octadecanoic acid, ethyl ester by 4.56%, squalene by 1.93% and cyclodecasiloxane, eicosamethyl- by 1.80% were having the largest area coverage percentage. Since most of the phytoconstituents are growth stimulants, it is suggested that phyllanthus leaf extracts be produced on a commercial scale as an exogenous biostimulant for plant growth and development.

Keywords: Bioactive compounds, Biostimulants, GC-MS, Phyllanthus leaf.

INTRODUCTION

The present development in the agricultural system places a greater emphasis on the exploitation of botanical sources, which have the potential to improve the long-term plant production while also addressing the issues of global food security and environmental safety [1]. A wide variety of multi-purpose tree species have received certification as biostimulants for boosting crop growth and productivity in a variety of crops [2]. A biostimulant is any substance or mixture of com-pounds that increases crop quality, abiotic stress resistance and nutritional effectiveness [3]. These multipurpose species have the capacity to contain a wide range of active and diverse phytochemicals in their extracts. In addition to controlling the water-nutrient relations and heat reactions, the phytochemicals appear to be secondary metabolites that help in seed germination, root growth, chlorophyll accumulation, photosynthesis and the transfer of photo-assimilates in plants [4].

Phyllanthus acidus L. Skeels is a tropical tree that belongs to the family Phyllanthaceae [5,6]. The plant is also referred to by other names such as star gooseberry, Malay gooseberry and Otaheite gooseberry [7]. In addition to being utilized for its delicious fruits, phyllanthus is grown in India, Asia, the Caribbean, Central America and South America to treat a variety of illnesses including inflammatory, rheumatism, bronchitis, asthma, respiratory disorders, hepatic diseases and diabetes [8]. The *Phyllanthus acidus* plant has a diverse range of phytochemical compounds with unique biological properties, including antioxidant [9], anti-inflammatory [10], hepatoprotective [11] and antinociceptive properties [12]. The previous studies indicated that *P. acidus* leaf extracts contain some significant phytochemical components, including flavonoids, phenolic compounds, alkaloids, steroids and glycosides [13-15], who isolated flavonoids in *P. acidus* leaf extract [16]. According to Tan *et al*. [8], triterpene, diterpene, sesquiterpene and glycosides were found to be the main groups of bioactive compounds

in the bark, leaves, roots and fruits of *P. acidus*. These phytochemicals present in *Phyllanthus emblica* effectively manage a variety of insect-vector caused diseases of various crop plants [17].

Gas chromatography-mass spectrometry is used to examine the volatile bioactive constituents present in the sample [18]. According to Pino *et al*. [19], totally 77 volatile compounds were identified using GC-MS, in which 45 terpene compounds, 18 ester compounds, seven acids, two phenolic compounds, one alcoholic compound were found in *Grosella*. Though the numerous studies available on medicinal use of *Phyllanthus acidus*, a few scientific studies have been conducted on the GC-MS analysis of fresh *Phyllanthus acidus* leaves used for foliar nutrition. The purpose of the present work is to identify and characterize the GC-MS chromatogram for the assessment of bioactive phytochemicals from the different plant extractions.

EXPERIMENTAL

Plant material: Fresh *Phyllanthus acidus* leaves were collected from the orchards of the Agricultural College and Research Institute, Madurai campus, Tamil Nadu Agricultural University, Madurai campus, India, located at 90.93ºN, 78.12ºE and with altitude of 147 m above mean sea level. The leaves were well cleaned under tap-running water, allowed to air dry in the shade for 2 days and then dried for the following 24 h in a hot air oven at 60 ºC. In order to prepare the dry materials for analysis, they were thoroughly crushed in normal electrical mixer and sieved through 2 mm mesh.

Phytochemical extraction is the first and most accurate procedure in analyzing the profile of phytoconstituents present in plant tissues. Efficiency of extraction is greatly influenced by the kind and concentration of extraction solvents, as well as extraction temperature, time and pressure. The chemical composition and the polarity of phytochemicals were taken into account while choosing the extraction solvents. Most of the studies on phyllanthus leaves used dichloromethane as a solvent to identify the metabolites involved in plant growth and development and to identify the low molecular weight phytochemicals found in the leaves.

Sample extraction: As mentioned above, the powdered phyllanthus leaf sample was extracted using dichloromethane solvent. 1 g of powdered, dried leaf material was extracted in glass vials using 25 mL of dichloromethane over the course of two cycles that lasted 30 min. Whatman No. 40 filter paper was used to filter the extract. The collected filtrate was evaporated using a rotary evaporator at 40 ºC, 250 mbar pressures and 100 rpm. The semi-solid extract was then redissolved in 2 mL of dichloromethane solvent and put into a 2 mL syringe to filter through a Paul membrane filter. After filtration, the material was prepared for GC-MS analysis.

Gas-chromatography-mass spectrometry (GC-MS) analysis: The GC-MS QP 2020 and MSD were combined to perform the GC-MS analysis. A thick Rxi-5 Sil MS fused silica capillary column size 30 m × 0.25 mm, 0.25 µm was used in GC. By using helium as a carrier gas with a constant flow rate of 1 mL/min and maintaining a 250 ºC ion source and injection temperature. The temperature of the gadget was initially set to 70 ºC and maintained for 5 min. The oven temperature was raised to 300 ºC at the end of experiment. Using a detector that worked in scan mode between 40 and 650 *m/z*, the mass spectra of the constituents in the samples were acquired. The MS took 5 min to start and 51 min to finish, with a solvent cut time of roughly 5 min.

RESULTS AND DISCUSSION

The results showed that the composition and chemical nature of phytochemicals varied between extracts and that the most of the components were extract dependent. The GC-MS spectra (Fig. 1) were used to identify 25 phytocomponents from dichloromethane extraction. The area in the chromatogram facilitates the tentative quantification of each compound based on area percentage, as well as the mentioned compound area. The secondary metabolite production in plants is thought to be an adaptive mechanism for overcoming barriers that are difficult to overcome during a difficult and changing growth environment. This mechanism may involve the production of complex chemical forms and interactions in structural and functional strengthening through signalling processes and pathways [20]. Numerous studies have shown that plant secondary metabolism (PSM) has a variety of natural activities, from defence against viruses, insects and herbivores to protection against environmental challenges.

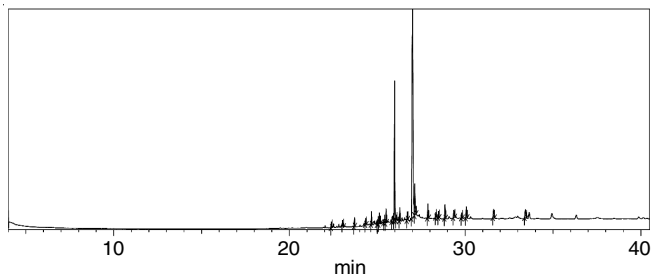
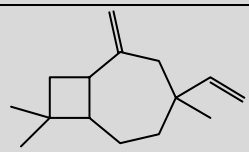
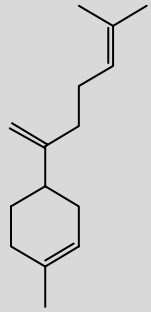
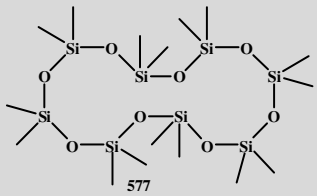
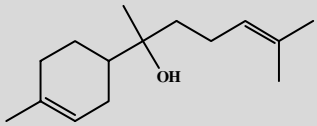
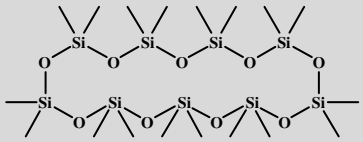
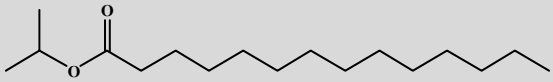
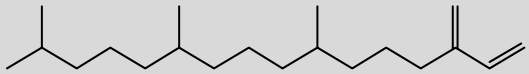
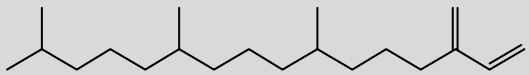
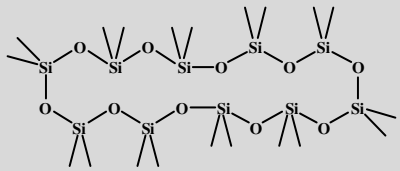
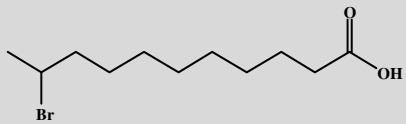
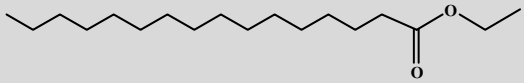
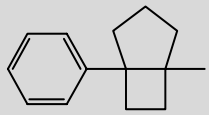
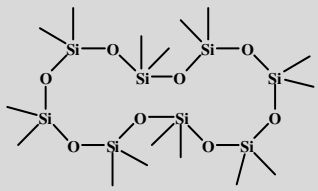
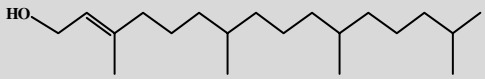
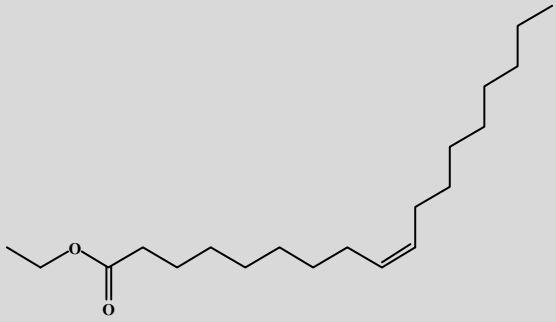
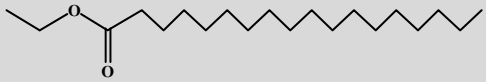
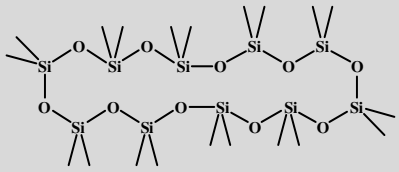
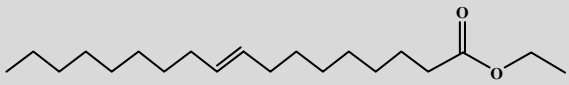
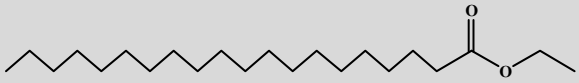
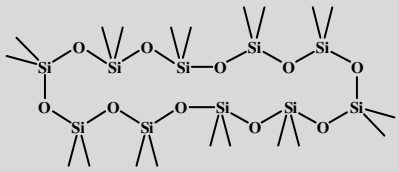
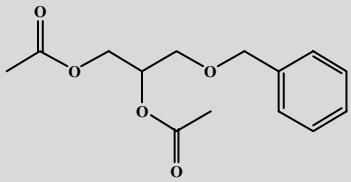
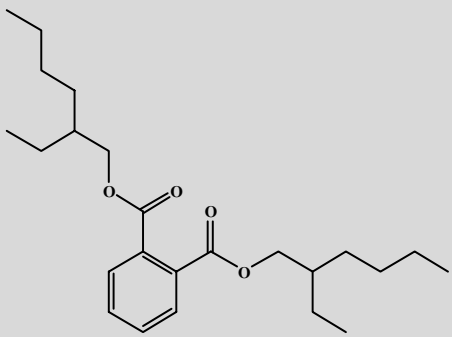


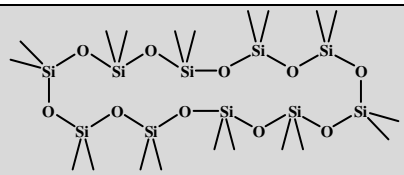
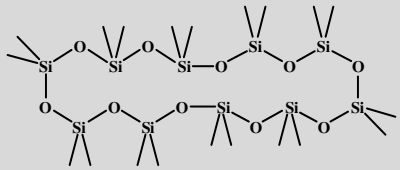
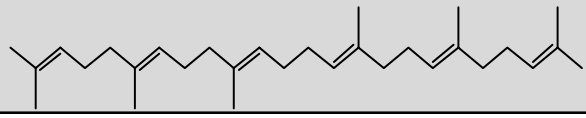
Fig. 1. Chromotogram of dichloromethane extraction of *Phyllanthus acidus* leaf

Dichloromethane extraction: Phyllanthus leaf extraction with dichloromethane produced about 25 phytoconstituents in which ethyl oleate have the highest percentage of 53.68% followed by hexadecanoic acid, ethyl ester (17.47%), octadecanoic acid, ethyl ester (4.56%), squalene (1.93%) and cyclodecasiloxane, eicosamethyl- (1.80) at the retention time of 27.012, 25.986, 27.139, 33.430 and 28.851 min, respectively (Table-1). The results showed that organoheterosilanes contributed 32% of the total amount of metabolites to plant growth and development, followed by fatty acid esters by 28%, sesquiterpenoids by 16%, terpenoids by 8% and other compound derivatives by 16% (Fig. 2). Ethyl oleate is the dominant bioactive compound present in phyllanthus leaf and it serves as an acaricide and a plant metabolite and it is coming under the fatty acid group organic compounds. Fatty acid is an important energy storage molecule that plays a major role in the plant system for maintaining membrane integrity [21]. The second predominant compound was *n*-hexadecanoic acid, which poss-

TABLE-1
IDENTIFIED BIOACTIVE COMPOUNDS USING DICHLOROMETHANE EXTRACTION OF *Phyllanthus acidus* THROUGH GC-MS

R.T. (Area, %)	Compound name	m.f. (m.w.)	Molecular structure	Chemical classification
22.389 (0.92)	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	$C_{15}H_{24}$ (204)		Unsaturated hydrocarbons
23.021 (0.64)	β-Bisabolene	$C_{15}H_{24}$ (204)		Sesquiterpenoids
23.698 (0.79)	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$ (592)		Organoheterosilanes
24.331 (0.78)	α-Bisabolol	$C_{15}H_{26}O$ (222)		Sesquiterpenoids
24.660 (1.15)	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$ (666)		Organoheterosilanes
25.015 (0.55)	Isopropyl myristate	$C_{17}H_{34}O_2$ (270)		Fatty acid esters
25.121 (1.24)	Neophytadiene	$C_{20}H_{38}$ (278)		Sesquiterpenoids
25.375 (0.33)	Neophytadiene	$C_{20}H_{38}$ (278)		Sesquiterpenoids
25.498 (1.26)	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$ (740)		Organoheterosilanes
25.879 (1.20)	Undecanoic acid, 10-bromo-	$C_{11}H_{21}O_2Br$ (264)		Fatty acids and conjugates
25.986 (17.47)	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$ (284)		Fatty acid esters
26.085 (1.03)	5-Methyl-1-phenylbicyclo[3.2.0]heptane	$C_{14}H_{18}$ (186)		Benzene and substituted derivatives

26.277 (1.25)	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$ (592)		Organoheterosilanes
26.708 (0.93)	Phytol	$C_{20}H_{40}O$ (296)		Diterpenoids
27.012 (53.68)	Ethyl oleate	$C_{20}H_{38}O_2$ (310)		Fatty acid esters
27.139 (4.56)	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$ (312)		Fatty acid esters
27.876 (1.67)	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$ (740)		Organoheterosilanes
28.327 (0.58)	(*E*)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$ (310)		Fatty acid esters
28.488 (0.81)	Eicosanoic acid, ethyl ester	$C_{22}H_{44}O_2$ (340)		Fatty acid esters
28.851 (1.80)	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$ (740)		Organoheterosilanes
29.358 (1.35)	1,2-Propanediol, 3-benzyloxy-1,2-diacetyl-	$C_{14}H_{18}O_5$ (266)		Alkyldiacylglycerols
29.804 (0.81)	*Bis*(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$ (390)		Benzoic acid esters

30.066 (1.70)	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$ (740)		Organoheterosilanes
31.619 (1.60)	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$ (740)		Organoheterosilanes
33.430 (1.93)	Squalene	$C_{30}H_{50}$ (410)		Triterpenoids

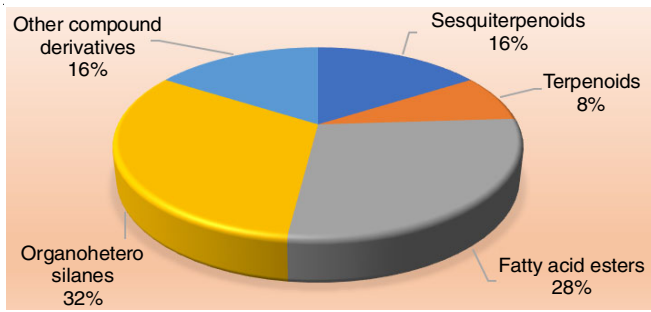


Fig. 2. Composition of phytochemical constituents present in dichloromethane extraction of *Phyllanthus acidus* leaf

esses pesticide, namaticide, antioxidant and hypocholesterolemic properties [22]. *n*-Hexadecanoic acid is also known as palmitic acid, which was found in significant amounts in wheat mitochondrial membranes and its level increased even more during cold stress [23]. Nishu & Kumar [11] also identified *n*-hexadecanoic acid in methanolic extract of moringa leaf.

The results indicated that sesquiterpenoids alone contributes 16% of total identified compounds that play a major role in the below ground interaction of plant systems. According to several workers [24-26] reported that most of the sesquiterpenes are volatile, while some compounds undergone significant modification may be semi-volatile. These volatile organic molecules frequently play a part in long-range communication or signalling between organisms in the underground because they can travel long distances by advected transport in gas or aqueous phases or by diffusion. Plant-plant and plant-insect communication has long been thought to rely on the volatile bouquets of plants produce. Terpenoids contribute 8% of total compounds, terpenes and their derived terpenoids are the largest class of secondary metabolites found in plants. They have basic roles in growth and development as well as more specialized roles in interactions between plants and their environment, resistance to environmental stresses and defence against predators and pathogens [27,28]. Triterpenes are biosynthesized in the cytosol, while diterpenes and sesterterpenes are produced in the plastids. While some of these are non-volatile terpenes are constitutively produced as part of normal growth and development, others are phytoalexins that are only produced in response to pest, disease or elicitor challenge. Many of these non-volatile root terpenes are secreted from plant roots, where they act as the first line of defence for the plant and mediate many biological processes [29]. A number of terpenoids have important functions in plant defence against biotic and abiotic stress or as signal molecules to attract pollinating insects [30-32].

Conclusion

The GC-MS analysis of the present study supported that the phyllanthus leaves contain a wide range of bioactive metabolites. Botanicals are considered as a biostimulant since they are an alternative to synthetic chemicals and are widely available, environmentally friendly and relatively inexpensive. Crop enhancement with phyllanthus leaf extract is an economical and environmentally responsible way to boost crop production. In addition to various nutritional and biological purposes, it is utilized as a green fertilizer, natural pollination, plant-insect signalling and biopesticides. Nevertheless, more research is required to completely determine their bioactivities.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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