

Gas chromatography-mass spectrometry (gc-ms) analysis of bioactive components of *phyllanthus amarus* leaves

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Abstract

Phyllanthus amarus belongs to the family of Euphorbiaceae and it is well known in Nigerian traditional system for its traditional uses. The present investigation was carried out to determine the possible bioactive components of leaves of *P. amarus* using GC-MS analysis. Nine (9) components from *P. amarus* leaves were identified. The prevailing components in the ethanolic extract of leaves were 3,5-di-*t*-butylphenol (1.2%), methyl 14-methyl pentadecanoate (1.4%), palmitic acid (hexadecanoic acid) (11.8%), 10-octadecanoate (5.5%), 9-hexadecenal (9.0%), glycerol 1, 3-dipalmitate (5.7%), 2, 13-octadecadiene-1-ol (8.2%), dioctyl ester (10.1%) and heptanoic acid (9-dece-1-yl ester), (4.6%). The presence of various bioactive compounds confirms the application of *P. amarus* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug or lead compound.

Keywords: *Phyllanthus Amarus* (*P.Amarus*), Traditional Use, Gc-Ms Analysis, Bioactive Compound.

INTRODUCTION

The use and dependence on plants as medicines by man has been in existence since time immemorial and man continues to search for plants as drug for a particular disease within his reach. Herbal medicines are safe than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway (Zaidan et al., 2005). Medicinal plants are expensive gift from nature to human. The approval of traditional medicine as an alternative form of health care and the improvement of microbial resistance to the existing antibiotics has lead researchers to scrutinize the antimicrobial compounds (Parvathi, 2010). Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries, where,

infectious diseases are endemic and modern health facilities and services are inadequate (Zaidan et al., 2005). The medicinal actions of plants unique to particular plant species or groups are consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct (Wink, 1999; Janakiraman et al., 2012).

Screening active compounds from plants has lead to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases including cancer and alzheimer's disease (Sheeja et al., 2007; Mukherjee et al., 2007). Plants remain a vital source of drugs and now-a-days much emphasis has been given to nutraceuticals. *Phyllanthus amarus* (Schumm and Thonn) is one of the medicinally important plants belonging to Euphorbiaceae commonly known as "stone breaker". The plant is locally called gyeron sheffapitu (Marghi) all in Nigerian languages. It is found in forest areas, arid land, Savannah areas, leached or

exhausted soil in many countries including China, India, Nigeria, Cuba and Philippines among others (Bharatiya, 1992; Akah and Nwambie, 1994; Burkill, 1994). The leaves of the plant were used traditionally as antidiarrhoeal, antidiabetic, antioxidant, analgesic, anti-inflammatory, antihypertensive, antimicrobial and hypolipidemic properties (Adeneye, 2006; Odukoya et al., 2007; Odetola and Akojenu, 2000; Anonymous, 2011). It has also widely been reported to offer good treatment for leprosy, hiccup, peptic ulcer, asthma, good laxative, typhoid fever, kidney stones, jaundice, malaria, gonorrhoea, dysentery, hypertension, flu, uterus complaints, tumor, liver protective, astringent, detoxifier, antiviral and antifungal among others (Oluwafemi and Debiro, 2008; Idowu et al., 2009; Akinjogula et al., 2010; Rahaman, 2010; Burkill, 1994). Taking into consideration of the medicinal importance of this plant, the ethanol extract of leaves of *P. amarus* were analyzed for the first time using GC-MS. This work will help to identify the compounds of therapeutic value. A majority of the rich diversity of Nigeria medicinal plants is yet to be scientifically evaluated for such properties. With this background, the present study was aimed to identify the phytoconstituents present in *P. amarus* using GC-MS analysis. GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids esters, alkaloids, steroids, amino acid and nitro compounds (Muthulakshmi et al., 2012).

MATERIALS AND METHODS

Collections of plant materials

Leaves of *P. amarus* were collected in the month of August 2011 from Suleimanti in Jere local government area of Borno state, Nigeria. The plant material was transported in a polythene bag to the Department of Biological science, University of Maiduguri for its identification and the voucher specimen (1360) was deposited at the Postgraduate Research Laboratory, Department of Chemistry.

Preparation of powder and extract

The leaves of *P. amarus* were washed in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The plants were shade dried and pulverized to powder using a pestle and a mortar. Required quantity of powder of leaves were weighed and transferred to the soxhlet apparatus for extraction where the pulverized powder was extracted exhaustively with 99% ethanol. The crude extract obtained was concentrated to dry between 40°C to 45°C using water bath. The final residue thus obtained was then subjected to GC-MS analysis. 2µl of the

ethanolic extract of *P. amarus* was employed for GC-MS analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis were carried out on a GC-MS-QP 2010 Plus Shimadzu system and Gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1 fused silica capillary column (30m x 0.25mm 1D x µl df, composed of 100% dimethyl polysiloxane). For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2µl was employed (Split ratio of 10:1) injector temperature-250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (Isothermal for 2 min.) with an increase of 10°C / min to 200°C then 5°C / min. to 280°C / min, ending with a 9 min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5s and fragments from 40 to 550Da. Total GC running time was 36 minutes. The relative percentage amount of each component was calculated, by comparing its average peak area to the total areas, Software adopted to handle mass spectra and chromatogram was a turbomass. The detection employed the NIST Ver. 2.0 year 2009 library.

Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National institute of Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS

GC-MS analysis

The compounds present in the ethanolic extract of leaves of *P. amarus* were identified by GC-MS (Figure 1).

The active principles with their retention time (RT), molecular formula, molecular weight (MW) and percentage composition in the ethanolic extract of leaves of *P. amarus* is presented in Table 1. Nine (9) compounds were identified in the ethanolic extracts of leaves of *P. amarus*. The prevailing compounds were 3, 5-di-t-butyl phenol (1.2%), methyl 14 -methyl pentadecanoate (4.1%), palmitic acid (Hexadecanoic

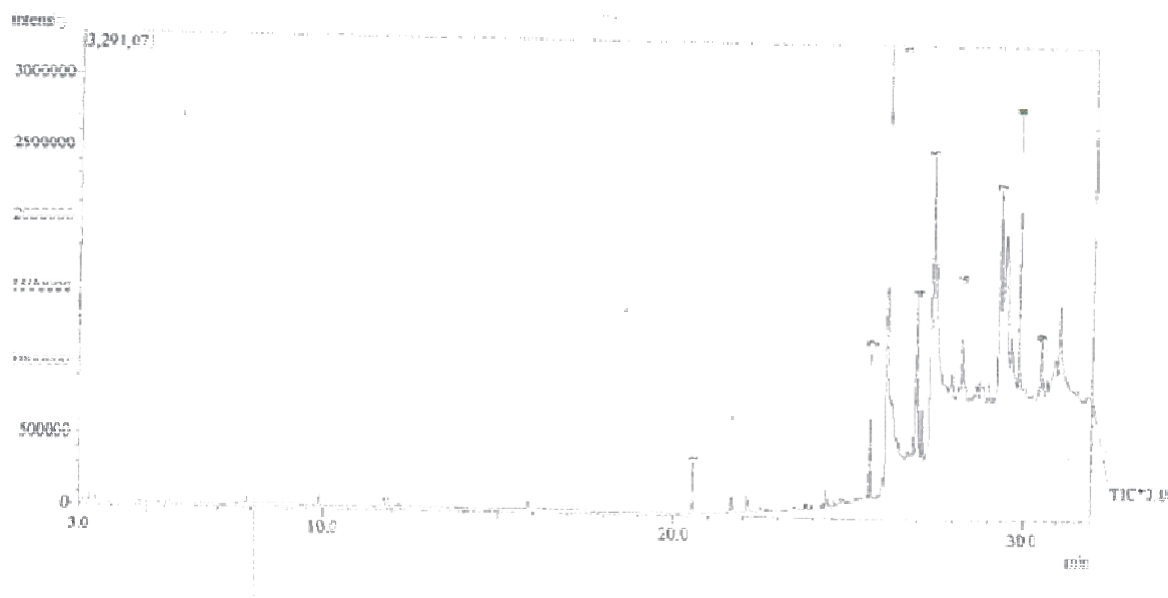


Figure 1. GC-MS Chromatogram of ethanol Extract of leaves of *P. amarus*

Table 1. Components detected in *P. amarus* leaf extract

S/N	RT	Name of compound	Hit quality	Molecular formula	MW	Percentage composition
1	20.5	3,5-di-t-butyl phenol	86	C ₁₄ H ₂₂ O	206	1.2
2	25.6	Methyl 14-methyl pentadecanoate	94	C ₁₇ H ₃₄ O ₂	270	4.1
3	26.1	Palmitic acid (Hexadecanoic acid)	90	C ₁₆ H ₃₂ O ₂	256	11.8
4	26.9	10-octadecanoate	92	C ₁₉ H ₃₆ O ₂	296	5.5
5	27.3	9-hexadecenol	90	C ₁₆ H ₃₂ O	238	9.0
6	28.2	Glycerol 1,3-dipalmitate	82	C ₃₅ H ₆₈ O ₅	568	5.7
7	29.3	2, 13-Octadecadiene-1-ol	86	C ₁₈ H ₃₄ O	266	8.2
8	29.8	Diocetyl ester	88	C ₂₄ H ₃₈ O ₄	390	10.1
9	30.0	Heptanoic acid (9-dece-1-yl ester)	64	C ₁₇ H ₃₂ O ₂	268	4.6
		Unidentified compounds				39.9
		Total % composition				100

acids (11.8%), 10-octadecanoate (5.5%), 9-hexadecenal (9.0%), glycerol 1, 3-dipalmitate (5.7%), 2, 13-octadecadiene-1-ol (8.2%), Dioctyl ester (10.1%) and Heptanoic acid (9-dece-1-yl ester) (4.6%). Major Phytocompounds and its biological activities obtained through the GC-MS study of leaves of *P. amarus* have been tabulated (Table 2). The activity of phytocompounds identified in *P. amarus* by GC-MS is based on Dr. Duke's phytochemical and ethnobotanical databases.

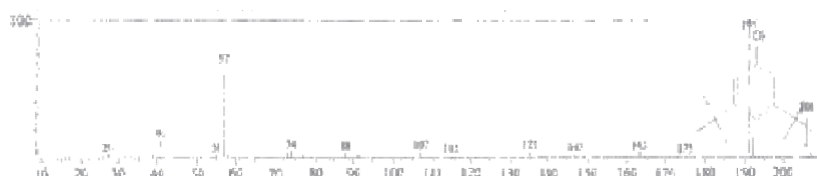
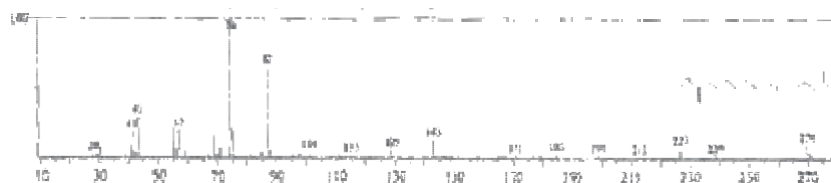
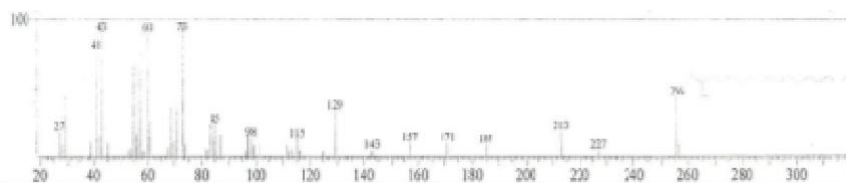
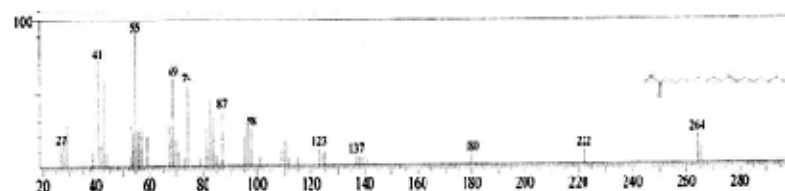
The spectrum profile of GC-MS confirmed the presence of nine major components with the retention

time 20.5, 25.6, 26.1, 26.9, 27.3, 28.2, 29.3, 29.8 and 30.0 min, respectively (Figure.1). The individual fragmentation patterns of the components were illustrated in Figures 2a- i. The mass spectrum of the compound with retention time 20.5 min (1.2%) gave 10 major peaks (M/Z) at 191, 57, 206, 41, 107, 74, 163, 147, 27 and 135 (Figure. 2a). The mass spectrum of the compound with retention time 25.6 min (4.1%) gave 10 major peaks (M/Z) at 74, 87, 43, 41, 57, 143, 270, 227, 129 and 28 (Figure. 2b). The mass spectrum of the compound with retention time 26.1 min (11.8%) gave 10 major peaks (M/Z) at 60, 73, 43, 41, 256, 129, 213, 85, 27 and 98

Table 2. Activity of phytocomponents identified in the ethanol leaf extra of *P. amarus*

S/N	Name of the compound	Nature of compound	Activity
1	3, 5-di-t-butyl phenol	Phenolic compound	Antimicrobial Antioxidant Anti-inflammatory
2	Methyl 14-methylpentadecanoate	Ester	Antifungal
3	Palmitic acid (Hexadecanoic acid)	Fatty acid	Antimicrobial Antidiarrhoeal
4	10-octadecanoate	Ester	Antimicrobial
5	9-hexadecenal	Aldehyde	Antimicrobial
6	Glycerol 1, 3-dipalmitate	Ester	Antimicrobial
7	2, 13-octadecadiene – 1 –ol	Alcoholic compound	Antimicrobial
8	Dioctyl ester	Ester	Antimicrobial
9	Heptanoic acid (9-dece-1-yl ester)	Ester	Antimicrobial

**Activity source: Duke, (2012)

**Figure 2_a.** Mass spectra of 3, 5-di-t-butylphenol**Figure 2_b.** Mass spectra of methyl 14-methyl pentadecanoate**Figure 2_c.** Mass spectra of palmitic acid (hexadecanoic acid)**Figure 2_d.** mass spectra of 10-octadecanoate

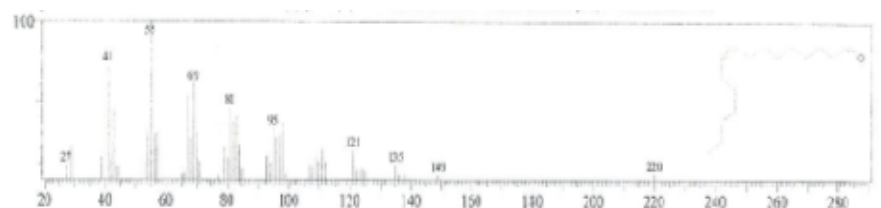


Figure 2e. Mass spectra of 9-hexadecenal

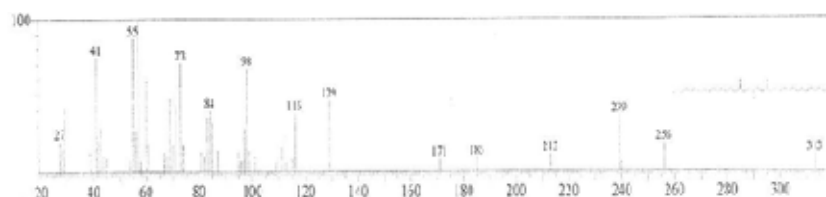


Figure 2f. Mass spectra of glycerol 1, 3-dipalmitate

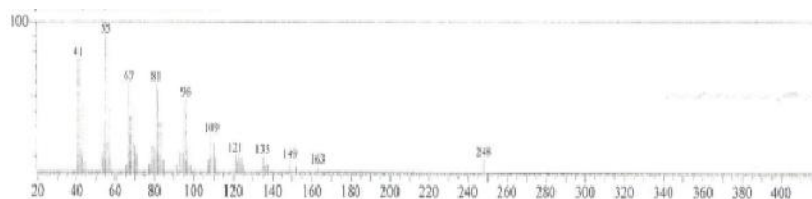


Figure 2g. Mass spectra of dioctyl ester

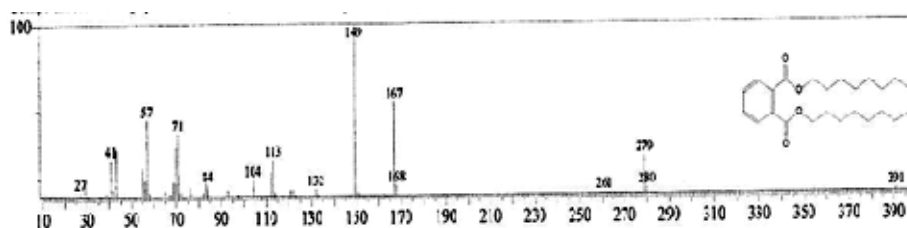


Figure 2h. mass spectra of 2, 13-octadecadiene -1-ol

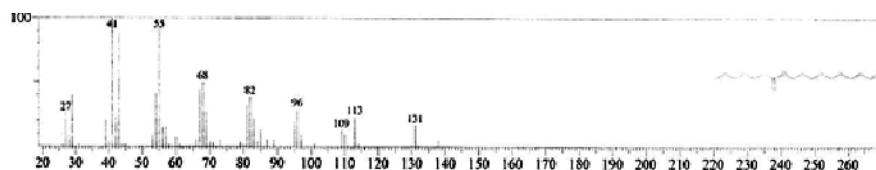


Figure 2i. Mass spectra of heptanoic acid (9-dece-1-yl ester).

Identified components from the spectra

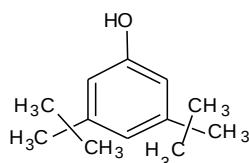


Figure 2a. 3, 5-di-t-butylphenol

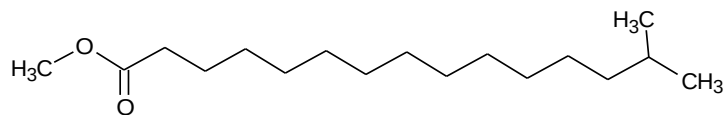


Figure 2_b. methyl 14-methyl pentadecanoate

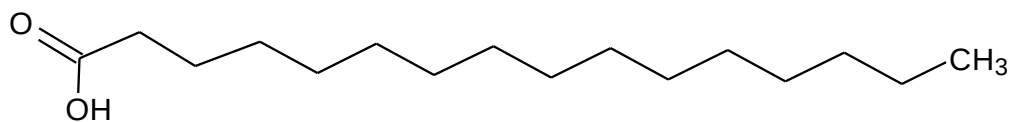


Figure 2_c. Palmitic acid (Hexadecanoic acid).

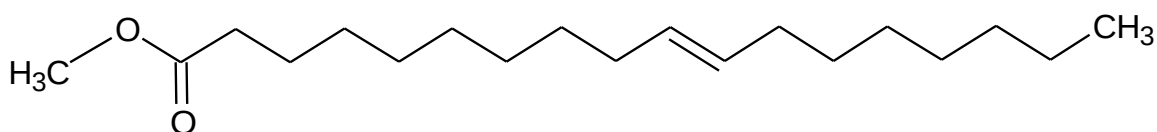


Figure 2_d.10-octadecenoate

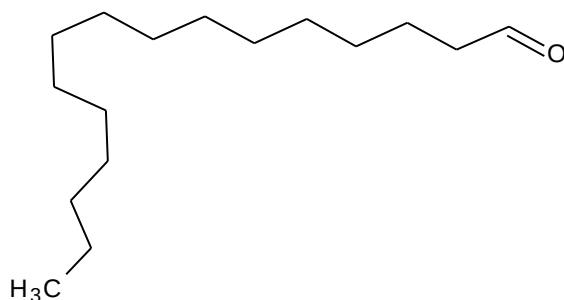


Figure 2_e. 9-hexadecenal

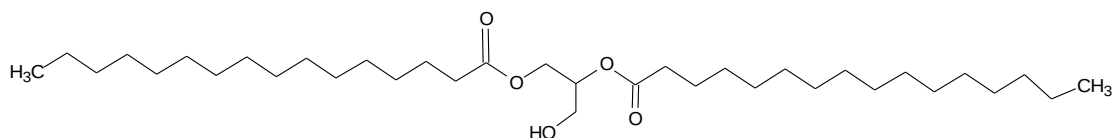


Figure 2_f. Glycerol 1, 3-dipalmitate.

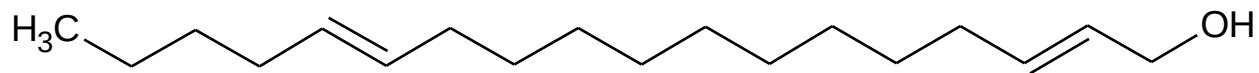


Figure 2_g. 2, 13-octadecadiene-1-ol.

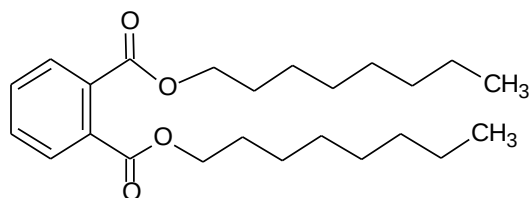


Figure 2_h. Dioctyl ester

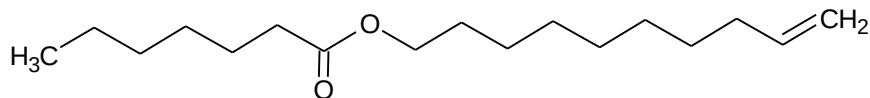


Figure 2i. Heptanoic acid (9-decen-1-yl ester)

(Figure. 2c). The mass spectrum of the compound with retention time 26.9 min (5.5%) gave 11 major peaks (M/Z) at 55, 41, 69, 74, 87, 98, 264, 123, 222, 180 and 137 (Figure. 2d). The mass spectrum of the compound with retention time 27.3 min (9.0%) gave 10 major peaks (M/Z) at 55, 41, 68, 81, 95, 121, 135, 149 and 220 (Figure. 2e). The mass spectrum of the compound with retention time, 28.2 min (5.7%) gave 10 major peaks (M/Z) at 55, 41, 73, 98, 129, 84, 239, 116, 256 and 27 (Figure. 2f). The spectrum of the compound with retention time 29.8 min (10.1%) gave 9 major peaks (M/Z) at 149, 167, 57, 71, 279 113, 41, 104 and 390 (Figure. 2g). The mass spectrum of the compound with retention time 29.3 min (8.2%) gave 10 major peaks (M/Z) at 55, 41, 67, 81, 96, 109, 248, 121, 135 and 149 (Figure. 2h). The mass spectrum of the compound with retention time 30.0 min (4.6%) gave 9 major peaks (M/Z) at 41, 55, 68, 82, 96, 27, 113, 131 and 109 (Figure. 2i).

DISCUSSION

3, 5-di-t-butyl phenol is a phenolic compound and it may be employed as an antioxidant, antimicrobial, antifungal and anti-inflammatory agents (Duke, 2012). Hexadecanoic acid (palmitic acid) is a fatty acid and it may be an active antimicrobial and antidiarrhoeal agent. 2, 13- octadecadiene-1-ol is suggested to be an aliphatic alcoholic compound and it employed as an antimicrobial (Duke, 2012). The present study results were confirmed by the antibacterial inhibition against the selected pathogens. By interpreting these compounds, it is found that *P. amarus* posses various therapeutical application. In the present study we characterized the chemical profile of *P. amarus* using GC-MS analysis. The chromatogram showed the relative concentration of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometer analyses the compound eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different M/Z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. This report is the first of its kind to analyze the chemical constituents of *P. amarus* using GC-MS analysis. In addition to this the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant. The result of the

present study supported and supplemented the previous observations (Janakiraman et al., 2012; Muthulakshmi et al., 2012; Wu et al., 2010; Vohra and Kaur, 2011; Sangeetha and Vijayalakshmi, 2011; Roy et al., 2010).

GC-MS analysis showed the existence of various compounds with different chemical structures. The presence of various bioactive compounds confirms application of *P. amarus* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug or a lead compound.

CONCLUSION

In the present study, nine components from leaves of *P. amarus* were identified by GC-MS analysis. The presences of various bioactive compounds justify the use of this plant for its various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. It could be concluded that *P. amarus* contains various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

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