



GC-MS ANALYSIS OF THE PHYTOCOMPONENTS IN THE EXTRACT OF *PERSEA AMERICANA*

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Abstract

The investigation was conducted to evaluate the overall bioactive components of ethanolic stem bark extract of *P. Americana* using the Gas Chromatography-Mass Spectrometry (GCMS). The stem bark was first sun dried and then coarsely powdered with a blender grinder.

The bioactive components in the extract were determined by Perkin-Elmer GC-MS and the mass spectra of the obtained compounds was matched with National Institute of Standard and Technology (NIST) library data base spectrum. Thus, the qualitative determination of the ethanolic extract of *P. americana* stem bark using GC-MS analysis revealed the presence of various bioactive compounds. From these findings it is evident that the plant of *P.americana* is of phytopharmaceutical significance.

Key words : GC-MS, phytochemicals, *Persea americana*, ethanol extract.

Introduction

The use of plants as a source of medicinal products has been acquired over the years and forms significant component of the health care system. India is the world's largest producer of medicinal herbs and is appropriately referred to as the botanical garden (Ahmedull *et al.*, 2011). Forests have excellent plants (flora), which possess high medicinal value. Until allopathy was discovered, people were relying on Ayurveda and homeopathy as medicinal products that are both plant and herbal. In order to overcome the health requirements, tribes are dependent on the rich diversity of forests for their treatment. Phytochemicals are nutrients with beneficial health effects and are used in pharmacology. (Nisa *et al.*, 2011). Medical science has managed to move from folk medicine and the traditional system of medicine after extensive chemical and pharmaceutical screening. (Boopathi AC and Sivakumar R., 2011). The active phytoconstituents can be found in any part of the plant, such as the leaves, root, bark, flowers, fruit and seeds (Gordon MC and David

JN, 2001). Alkaloids, flavonoids, tannins and phenolic compounds are the most important bioactive secondary metabolites in plants. (Edeoga HO *et al.*, 2005). These plant phytoconstituents are vital for the development and preparation of therapeutic agents in the pharmaceutical industry (Nishak *et al.*, 2011). The phyto constituents present in the medicinal plant has a correlation with its pharmacological activity whereby screening of these active constituents has led to the development of medicinal products which have active constituents that have proved their effectiveness against various diseases. (Sheeja K. and Kuttan G., 2007).

The genus *Persea* belongs to family *Lauraceae* comprising of nearly 80 species (M hameed *et al.*, 1997). The fruit *Persea americana* is locally known as Benne hannu. It is distributed all around the globe and is indigenous to tropical habitats like Central America and the Indian subcontinent. In folk medicine leaves were used in treating convulsions (Ojewole *et al.*, 2006). *P. americana* has been reported to be effective against hepato-toxicity, inflammation, cancer, hypertension, etc

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(Adisa *et al.*, 2011; Adeyemi *et al.*, 2002; Kumar *et al.*, 2016; Imafidon *et al.*, 2010). But there have not been much report on the phytochemical components and biological activity on the stem bark extract of *P. americana*. Hence in this study, the chemical constituents of *P. americana* have been evaluated using GC-MS.

Over the past few years, GC-MS has been increasingly used for analysis of plants with medicinal value. Using this technique, purity of the compounds can be identified even at low concentrations of less than 1 mg. The Gas Chromatography-Mass Spectrometry (GCMS) analysis of the extracts was performed using a GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) fitted with VF-5ms fused silica capillary column of 30 m length, 0.25 mm diameter, and film thickness: 0.25 mm. The electron ionization device used for GC-MS detection had a 70 eV ionisation energy. Helium gas (99.99%) was used at a steady flow rate of 1.51 ml / min as a carrier gas. Temperature of the mass transfer line was set at 200 and 240 °C, respectively. The temperature of the oven was configured at 10 °C / min from 70 °C to 220 °C, kept isothermal for 1 min and increased to 300 °C eventually. 2 ml of water solution from the samples was injected manually in the split less mode. The mass scan was of 50-600 amu and the split ratio was 1:40. GC-MS cumulative running time is 35 min. The relative percentage of each constituent of extracts was expressed as a percentage with normalization of peak area. The mass spectrum of plant extracts was interpreted and was carried out using National Institute of Standard and Technology (NIST) library database with over 62,000 spectral patterns. The compound spectrum was matched with National Institute of Standard and Technology (NIST) library data base spectrum (Omorieg *et al.*, 2015).

Materials and Methods

Collection of plant material and preparation of the extract

P. americana stem bark in the month of April-May was procured from the district of Kodagu, Karnataka. The stem bark was confirmed and authenticated by Dr. Noeline Pinto, Department of Botany, St Agnes College, Mangalore Karnataka, India. A voucher specimen (No.16PH007R) was deposited at NGSIM Institute of pharmaceutical Sciences Derlakatte, Mangalore.

P. americana's fresh bark had been sun-dried and cut into small pieces, coarsely powdered using a dry grinder. The powdered bark was subjected to maceration. Ethanol was used as the solvent for maceration and kept for a period of seven days with stirring occasionally. The complete solvent extract was distilled off after 7 days

and the concentrate was evaporated to a syrupy consistency on a water bath and evaporated until dry. The extract was also preserved for further use in a desiccator. Preliminary phytochemical analysis was performed by standard procedure for testing the various chemical groups present in the ethanolic extract (Devasagayam TPA *et al.*, 2004). Further GC-MS analysis was carried out at Vellore Institute of Technology.

GC-MS analysis

GC-MS analysis was carried out at Vellore Institute of Technology, Tamil Nadu State. GC-MS study of the bioactive compounds in the stem bark of the ethanolic extract of *P. americana* was conducted

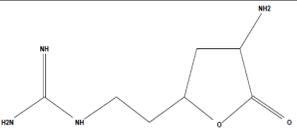
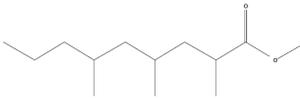
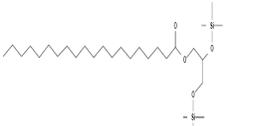
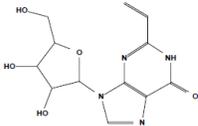
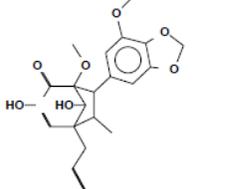
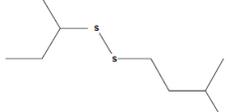
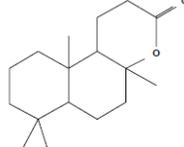
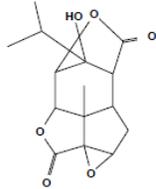
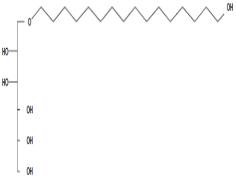
Results and Discussion

Ten bioactive compounds were identified in the GC-MS chromatogram of ethanolic extract of *P. americana* stem bark (Fig. 1). The X-axis in the given chromatogram denotes the retention time of the identified compound in minutes and the Y-axis denotes the percentage peak area of the identified compound at the corresponding retention time. The mass spectra of the ten compounds were identified by comparing with the NIST library mass spectra. The identified compounds with their Retention time in min, Molecular formula, molecular weight, Peak area %, chemical structure and the name of the compound are presented in table 1.

The different phytochemicals which are responsible in contributing to the medicinal activities of the plant are shown in table 2. The mass spectra of identified compounds from ethanolic extract of *p. americana* stem bark are presented in (Fig. 2). Of the ten phytochemicals identified in this fraction, the most major compounds were Nonanoic acid methyl ester, N-hexadecanoic acid, Ambreinolide and D-mannitol, 1-o-(16-hydroxyhexadecyl) with peak area 17.157%, 28.75%, 12.62% and 10% respectively. The other constituents which were identified in minor proportion were Eicosanoic acid, 2, 3-bis [(trimethylsilyl) oxy] propyl ester (5.16%), 2-vinyl-9-[.beta.-d-ribofuranosyl] hypoxanthine (8.37%) Sec-butyl isopentyl disulphide (5.17%), etc.

Many of the compounds identified have been reported, to be biologically relevant as shown in (Table 2). Nonanoic acid methyl ester and N-hexadecanoic acid are reported to have Antioxidant, antibacterial and antifungal activities (Chandrasekaran *et al.*, 2011). D-mannitol, 1-o-(16-hydroxyhexadecyl) is reported for its use for acute traumatic brain injury (Wakai A *et al.*, 2013). The presence of various bioactive compounds in the *P. americana* supports traditional practitioner's use of the plant for various ailments. GC-MS research is the first

Table 1: GC-MS analysis of the ethanolic extract of *P.americana* stem bark.

S.N.	RT (min)	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area (%)	Structure
1.	18.30	Threo-4-hydroxy-l-homoarginine lactone	C ₇ H ₁₂ N ₄	186	1.850	
2.	20.135	Nonanoic acid, 2,4,6-trimethyl-, methyl ester, (2r,4s,6r)-(-)-	C ₁₃ H ₂₆ O ₂	214	17.157	
3.	21.291	N-hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	28.755	
4.	22.821	Eicosanoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester	C ₂₉ H ₆₂ O ₄ Si ₂	530	5.196	
5.	23.382	2-vinyl-9-[.beta.-d-ribofuranosyl] hypoxanthine	C ₁₂ H ₁₄ O ₅ N ₄	294	8.373	
6.	23.962	Bicyclo[3.2.1]oct-3-en-2-one, 3,8-dihydroxy-1-methoxy-7-(7-methoxy-1,3-benzodioxol-5-yl)-6-methyl-5	C ₂₁ H ₂₄ O ₇	388	5.431	
7.	24.527	Sec-butyl isopentyl disulphide	C ₉ H ₂₀ S ₂	192	5.177	
8.	25.307	Ambreinolide	C ₁₇ H ₂₈ O ₂	264	12.621	
9.	26.748	3,6-methano-8h-1,5,7-trioxacyclopenta [ij]cycloprop[a]azulene-4,8(3h)-	C ₁₅ H ₁₈ O ₆	294	5.373	
10.	27.218	D-mannitol, 1-o-(16-hydroxyhexadecyl)-	C ₂₈ H ₅₈ O ₇	506	10.016	

Sl.no	Name of the compound	Activity
1	<u>Nonanoic acid methyl ester</u>	Antioxidant, antibacterial and antifungal activities
2	<u>N-hexadecanoic acid</u>	Antioxidant, anti-inflammatory, antibacterial
3	<u>D-mannitol, 1-o-(16-hydroxyhexadecyl</u>	Acute traumatic brain injury

Table: 2 Phyto constituents identified by GC MS analysis in the *P. americana* bark extract, reported to have biological activity and cited in PUBMED

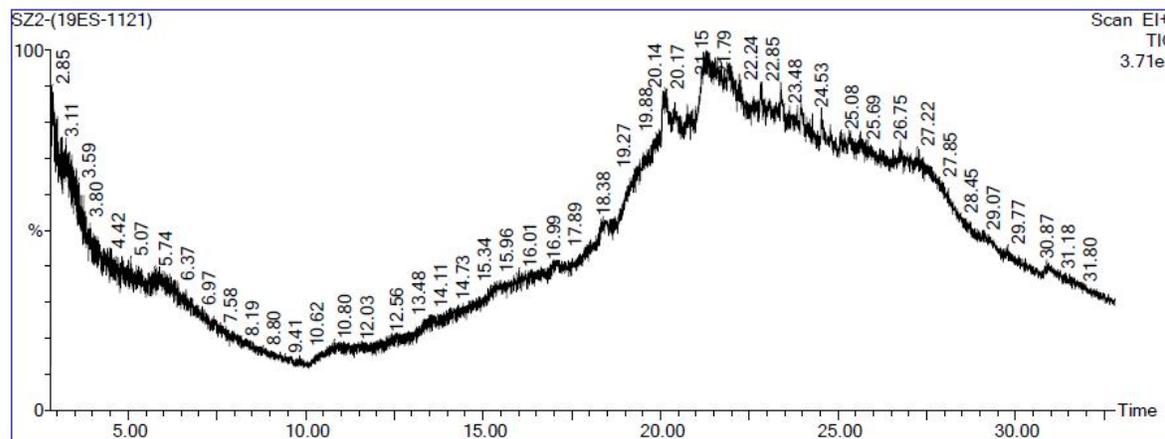
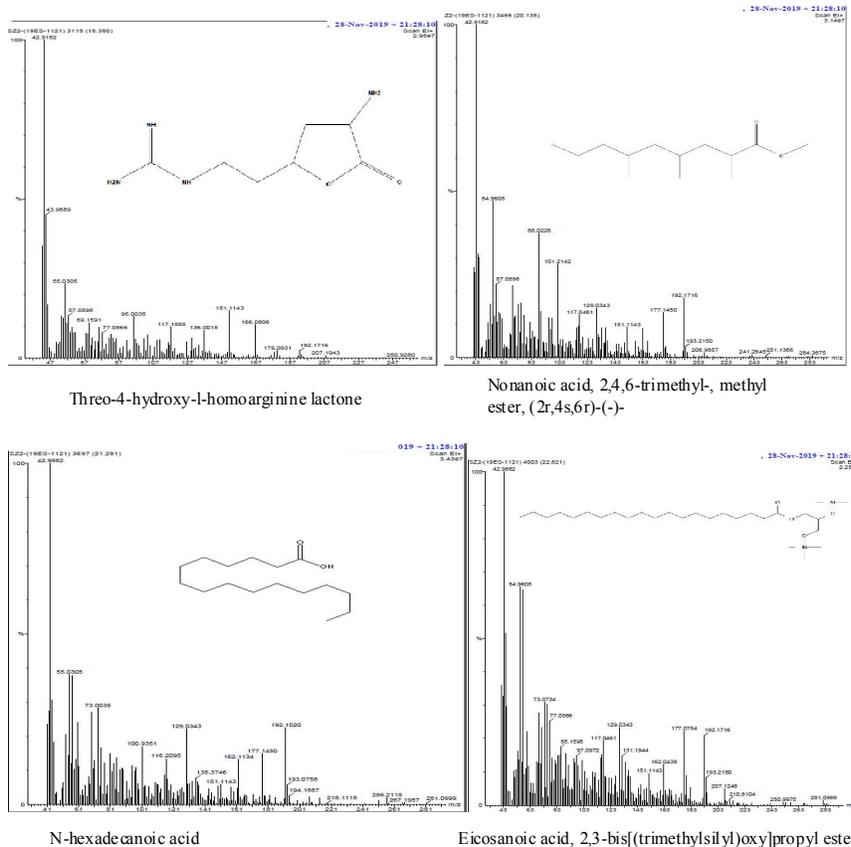


Fig. 1: GC-MS chromatogram of ethanolic extract of *P.americana*.



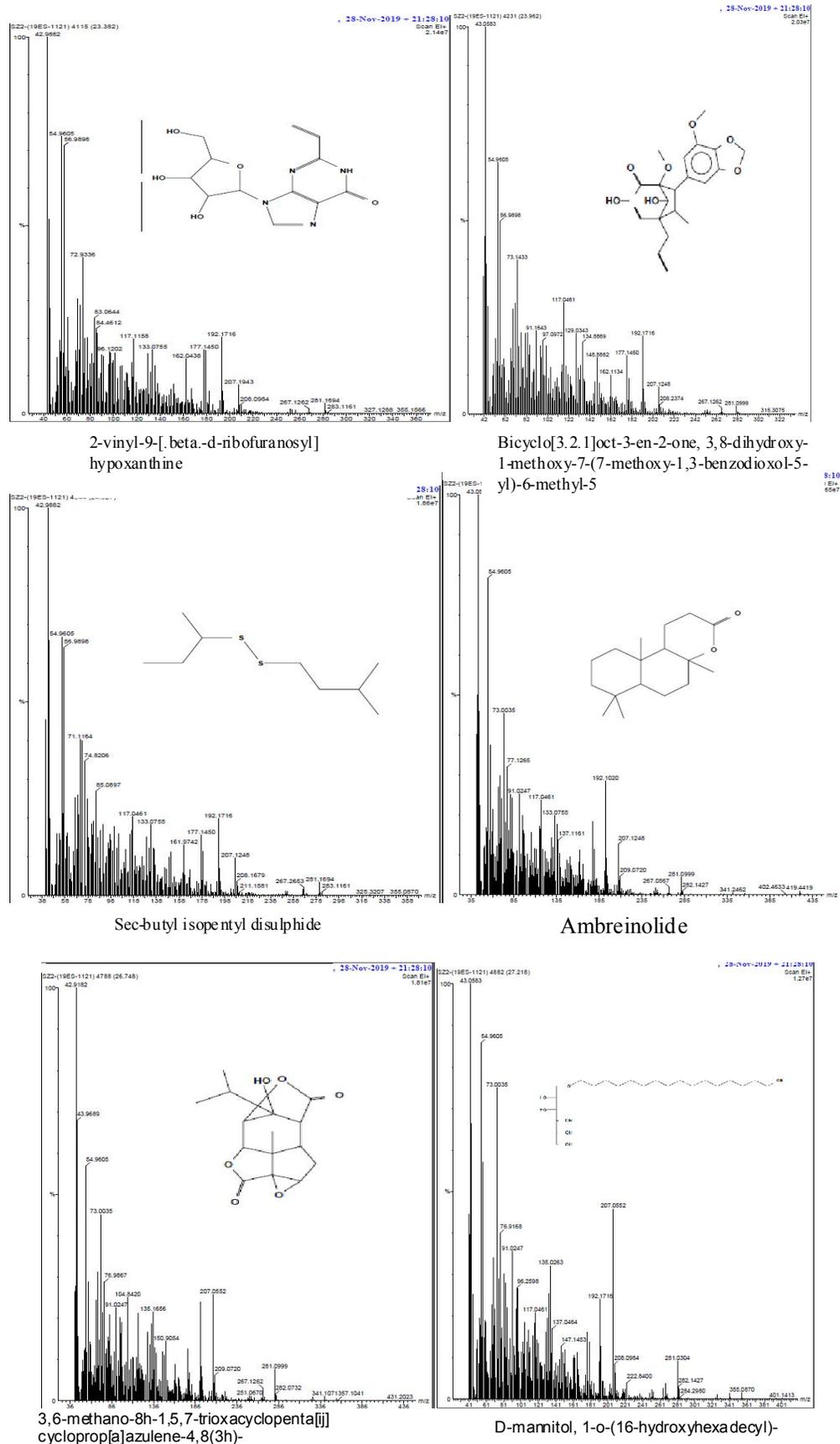


Fig. 2: Mass spectrum and structure of phytochemicals identified by GC-MS in the ethanolic extract of *P.americana*.

step towards understanding the value of medicinal properties in this medicinal plant and this method of study would be useful for further study. Nevertheless, the isolation and subjection of individual phytochemical constituents to biological activity would certainly produce fruitful results. It could be inferred from the findings that *P. americana* contains various bioactive compounds and hence can be considered as a plant of phyto-pharmaceutical importance. Research work is underway to establish biological activity of bark extract of *P. americana* and to enhance its pharmacological profile in the arena of traditional medicine.

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