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Identification of insecticidal compounds in *Terminalia arjuna* bark extract using gas hromatography and mass spectroscopic technique

S Gomathy, KM Subbu Rathinam
PG and Research Department of Zoology, Rajah Serfoji Govt. College, (Autonomous), Thanjavur, Tamil Nadu, India

Abstract
To identify the insecticidal components of *Terminalia arjuna* bark were evaluated by standard protocol using the equipment Perkin-Elmer Gas Chromatography–Mass Spectrometry. The mass spectrum of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. The GC-MS analysis revealed the presence of various compounds like Phenol, 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl, 2-Methoxy-4-vinylphenol, Benzaldehyde Methoxy, Dodecanoic acid, Tetradecanoic acid, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid and Octadecanoic acid, 4-Hydroxy-3 present in the extract. Among the various compounds, Phenol, 4H-Pyran-4-one, Tetradecanoic acid, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid and Octadecanoic acid identified as insecticidal activity using NIST library. These findings support the use of *Terminalia arjuna* as insecticidal plant.

Keywords: gas chromatography and mass spectroscopy, *Terminalia arjuna*, phytocompounds

Introduction
Food grain losses due to insect infestation during storage are a serious problem, particularly in the developing countries (Talukder *et al.*, 2004) [22]. Losses caused by insects include not only the direct consumption of kernels, but also accumulation of exuviate, webbing and cadavers. High levels of the insect detritus may result in grain that is unfit for human consumption and loss of the food commodities, both, in terms of quality and quantity. Plants are a rich source of secondary metabolites with interesting insecticidal activities (Dubey *et al.*, 2008; de-Fátima *et al.*, 2006) [5, 8]. Different medicinal plants and their medicinal values are widely used for various ailments throughout the world. Various chemical compounds isolated and characterized from Boraginaceous plant species are described. Distinguished examples of these compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides (Shahidi, 2000 and Shahidi, *et al.*, 2008; Meurer-Grimes *et al.*, 1996) [17, 18].
It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations (Mathekaga and Meyer, 1998) [10].
Within a decade, there were a number of dramatic advances in analytical techniques including FTIR, UV, NMR and GC- MS that were powerful tools for separation, identification and structural determination of phytochemicals. Gas Chromatography Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra (Ronald Hites, 1997) [15]. The aim of this study is to identify the insecticidal compounds present in *Terminalia arjuna* (Family: Combretaceae) bark extract with the aid of GC- MS techniques which may provide an insight in its use in insecticidal activity.

Material and Methods
Plant Materials
The *Terminalia arjuna* barks were collected in January 2015 from Karaikudi, Tamil Nadu from a single herb.

Preparation of extracts
The collected *Terminalia arjuna* barks were washed several times with distilled water to remove the traces of impurities from the barks. The barks was dried at room temperature and coarsely powdered. The powder was extracted with methanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used. The extract contained both polar and non-polar phytocomponents of the plant material used.

GC-MS analysis
GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200ºC, then 5ºC/min to 280ºC,

ending with a 9min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. min. 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 chromatograms was a TurboMass Ver 5.2.0 (Srinivasan *et al*., 2013) [21].

Results and Discussion

The invasion of food products by insects and moulds contribute greatly to the loss of quality and quantity. Chemicals and fumigants play a vital role in controlling this problem but they have been known to cause serious toxicological and environmental problems, with the consequent carcinogenic effect on man. The use of plants as an alternative in controlling insects is attracting attention from scientists' worldwide probably due to the non-toxicity, affordability and availability of the products (Atta-ur-Rahman *et al.*, 1997) [14]. Plants are a rich source of secondary metabolites with interesting insecticidal activities. Gas chromatography–mass spectrometry (GC- MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, inorganic, biochemistry and identification of unknown samples. Additionally, it can identify trace in materials that were previously thought to have disintegrated beyond identification. GC-MS has been widely heralded as a "gold standard" for forensic substance identification because it is used to perform a specific test. GC-MS instruments have been used for identification of hundreds of components that are present in natural and biological system (Ronald Hites, 1997) [15].

GC-MS analysis

To identify the insecticidal components of *Terminalia arjuna* bark were evaluated by standard protocol using the equipment Perkin-Elmer Gas Chromatography–Mass Spectrometry. Twenty five compounds were identified in *Terminalia arjuna* by GC-MS analysis. The prevailing compounds were Phenol, 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl, 2-Methoxy-4-vinylphenol, Benzaldehyde Methoxy, Dodecanoic acid, Tetradecanoic acid, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid and Octadecanoic acid, 4-Hydroxy-3 present in the extract. Among the various compounds, Phenol, 4H-Pyran-4-one, Tetradecanoic acid, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid and Octadecanoic acid identified as insecticidal activity using NIST library. These findings support the use of *Terminalia arjuna* as insecticidal plant (Table 1and Fig 1).

The investigation concluded that the stronger extraction capacity of methanol could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat insect.

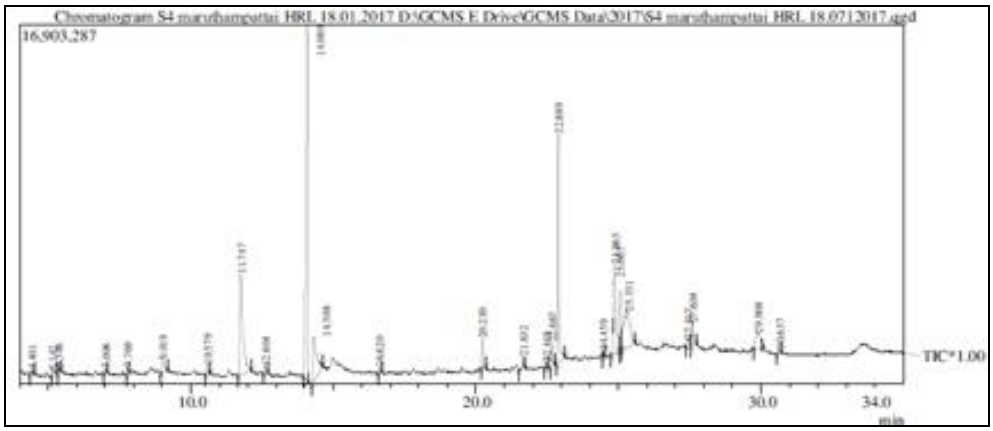

Fig 1: GC MS chromatogram of *Terminalia arjuna* bark extract

Hexadecanoic acid, ethyl ester is recommended to be a saturated fatty acid and it might as act as an Antioxidant, hypocholesterolemic, anti-androgenic, hemolytic and alpha reductase inhibitor (Sermakkani, 2012) [16]. Hexadenoic acid has earlier been reported as a component in alcohol extract of the leaves of *Kigelia pinnata* (Grace *et al.*, 2002) and *Melissa officinalis* (Sharafzadeh *et al.*, 2011) [19]. Parasuraman *et al.* (2009) [12] identified 17 compounds with n- Hexadecanoic acid and Octadecanoic acid as the major compounds in the leaves of *Cleistanthus collinus.* GC-MS analysis of ethyl acetate extract of *Goniothalamus umbrosus* revealed the presence of n-Hexadecanoic acid (Siddig Ibrahim *et al.*, 2009). Hexadecanoic acid, Phytol, 9, 12 - Octadecadienoic acid, 9, 12, 15- Octadecatrienoic cidand Squalene were Identified in the ethanol leaf extract of *Aloe vera* (Arunkumar and Muthuselvam, 2009) [1] and *Vitex negundo* (Praveen kumar *et al.*, 2010). Squalene has earlier been reported as antimicrobial, antioxidant, anticancer, Neutralize different xenobiotics, anti-inflammatory, antiatherosclerotic and anti-neoplastic, role in skin aging and pathology and Adjuvant activities and cosmetics as a natural moisturizer (Ponnamma and Manjunath, 2012) [13]. Devi *et al.* (2009) [4] reported that *Euphorbia longan* leaves mainly contained n-hexadecanoic acid and Octadecadienoic acid. These reports are in accordance with the result of this study.

Table 1: GC-MS analysis revealed the presence of Phytochemical component in bark of *Terminalia arjuna*

S.No	R. Time	Area%	Molecular Formula	Name of the Chemical compound
1.	4.401	0.33	$C_5H_6O_2$	2-Cyclopenten-1-one, 2-hydroxy-
2.	5.142	0.55	C6H6O	Phenol
3.	5.346	0.37	$C_4H_6O_3$	2-Hydroxy-gamma-butyrolactone
4.	7.006	0.31	$C_6H_{12}O$	Oxetane, 2-Propyl-
5.	7.769	0.40	C6H8O4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydro
6.	9.019	1.25	$C_6H_6O_3$	5-Hydroxymethylfurfural
7.	10.579	0.65	$C_9H_{10}O_2$	2-Methoxy-4-vinylphenol
8.	11.747	12.96	$C_9H_{12}O$	Phenol, 4-propyl-
9.	12.604	0.57	$C_8H_8O_3$	Benzaldehyde, 4-Hydroxy-3-Methoxy-
10.	14.089	26.55	$C_8H_8O_3$	Methyl 4-Hydroxybenzoate
11.	14.308	7.19	$C_8H_8O_3$	Methyl 4-Hydroxybenzoate
12.	16.620	0.37	$C_{12}H_{24}O_2$	Dodecanoic acid
13.	20.230	1.62	$C_{14}H_{28}O_2$	Tetradecanoic acid Myristic acid
14.	21.632	0.68	$C_{15}H_{30}O_2$	Pentadecanoic acid
15.	22.448	0.29	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
16.	22.667	1.39	$C_{15}H_{28}O_2$	Cyclopentadecanone, 2-hydroxy-
17.	22.889	13.33	$C_{16}H_{32}O_2$	n-Hexadecanoic acid
18.	24.459	0.26	$C_{19}H_{36}O_2$	9-Octadecenoic Acid, Methyl ester
19.	24.863	9.83	$C_{16}H_{30}O$	cis-9-Hexadecenal
20.	25.067	4.19	$C18H36O_2$	Octadecanoic acid Stearic acid
21.	25.331	9.88	$C_{16}H_{28}N_2$	Pyrazine, Tetrakis(1-Methylethyl)-
22.	27.417	1.01	$C_{20}H_{40}O_2$	Eicosanoic acid
23.	27.604	2.14	$C_{18}H_{35}NO$	9-Octadecenamide
24.	29.908	3.55	$C_{29}H_{50}O_2$	Vitamin E
25.	30.637	0.33	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The 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. The biological activities listed (Table 2) are based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA (Duke's, 2013) [6]. The nature and structure of the compounds were identified at different time intervals using mass spectrometer. The bheights of the different peaks indicate the relative concentration of the different components present in the sample. The finger prints of the compound which can be identified from NIST library database. different xenobiotics, anti-inflammatory, antiatherosclerotic and anti-neoplastic, role in skin aging and pathology and Adjuvant activities and cosmetics as a natural moisturizer (Ponnamma and Manjunath, 2012) [13]. Devi *et al.* (2009) [4] reported that *Euphorbia longan* leaves mainly contained n-hexadecanoic acid and Octadecadienoic acid. These reports are in accordance with the result of this study.

Table 2: Biological activity of phyto-components identified in the methanolic extract of the *Terminalia arjuna* bark by GC-MS.

S. No	Compound name	Biological activity**
1.	Phenol	Antioxidant, anticarcinogenic, anti-inflammatory, Fungicide Insecticide,
2.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy	Antimicrobial
3.	2-Methoxy-4-vinylphenol	Antimicrobial, Anti-inflammatory
4.	Benzaldehyde, 4-Hydroxy-3-Methoxy-	Antimicrobial
5.	Dodecanoic acid	Antimicrobial, increase HDL level,
6.	Tetradecanoic acid	Antioxidant, cancer preventing, nematicide, Larvicidal and repellent activity
7.	Hexadecanoic acid, methyl ester	Antioxidant, Pesticide hypocholesterolemic, Anti androgenic, hemolytic, Alpha reductase inhibitor, Antibacterial and antifungal
8.	n-Hexadecanoic acid	Antioxidant, Pesticide, Flavor, 5Alpha Reductase inhibitor, Antifibrinolytic, Hemolytic, Lubricant, Nematicide, Antialopecic
9.	Octadecanoic acid	Antimicrobial activity

****Source**: Dr.Duke's phytochemical and ethnobotanical databases [Online database].

Uraku (2015) [23] investigated the Chemical Compositions of *Cymbopogon citrates* Leaves by Gas Chromatography-Mass Spectrometry (GC-MS) Method. Six compounds were identified in the methanol leaf extract and they include; hexadecanoic acid (8.11%), hepta-9,10,11-trienoic acid (17.43%), octadecenoic acid (8.41%), 2-ethenyltetradecan-1-

ol 13.28%), eicosane aldehyde (37.56%) and 1-ethoxyoctadecane (15.20%) as the major chemical constituents. Das and Sudhakar Swamy (2016) determined the bioactive compounds by GC-MS in fruit methanol extracts -a comparative analysis of three *Atalantia* species from south India. Twenty seven compounds were identified from the mass spectra obtained. 1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid was the major compound.

Uraku (2016) [24] examined the Bioactive Constituents of Methanol Fraction of *Spilanthes uliginosa* (Sw) Leaves. The major phytocompounds identified in the leaf extract are hexadecanoic acid (8.68%), hepta-9, 10, 11-trienoic acid (19.36%), octadecenoic acid (8.14%), 5-hydroxylmethyl heptadecane (14.02%), docosane aldehyde (41.72%) and 1-ethoxyoctadecane (8.08%).

Conclusion

The present study characterized the phytochemical profile of the *Terminalia arjuna* bark extract using GC-MS. The chromatogram shows the comparative concentration of different components getting eluted as a purpose of retention time. The heights of the different peaks indicates the relative concentration of the compounds exist in the methanolic extract of *Terminalia arjuna* bark. The mass spectrometer analyses the compounds which were eluted at different time intervals to recognize the nature and structure of the compounds. These spectrum are finger print of the compound which can be identified from the NIST library. The identification of various bioactive compounds confirms the insecticidal application of *Terminalia arjuna* bark for a variety of insect. Further research is in progress for the evaluation of insecticidal activity in *Terminalia arjuna* bark.

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