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Evaluation of insecticidal compounds from *Ficus benghalensis* bark extract using Gas chromatography and mass spectroscopic technique

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Abstract

To identify the insecticidal components of *Ficus benghalensis* bark were evaluated by standard protocol using the equipment Perkin-Elmer Gas Chromatography–Mass Spectrometry (GCMS). 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 9, 12, 15-Octadecatrienoic acid, methyl ester, 9-Octadecenoic acid, 2-Hexadecen-1-ol, 3, 7, 11, 15-tetramethyl-, [R-[R*, R*-(E)] and 9, 12-Octadecadienoic acid identified as insecticidal activity using NIST library. These findings support the use of *Ficus benghalensis* bark extract as insecticidal plant.

Keywords: gas chromatography and mass spectroscopy, *ficus benghalensis*, phytocompounds

1. Introduction

Stored insect pests are a problem throughout the world, because they reduce the quantity and quality of grain. Their damage to stored grains and grain products may amount to 5–10% in the temperate zone and 20–30% in the tropical zone. Such damage may reach up to 40%, in countries where modern storage technologies have not been introduced (Shaaya *et al* 1997) [1]. The use of chemical agents to prevent or control insect infestations has been the main method of grain protection, since it is the simplest and most cost-effective means of dealing with stored product pests. Thus, there is an urgent need to develop safe alternatives to conventional insecticides and fumigants for the protection of grain products against insect infestations. There are increasing efforts to understand indigenous pest control strategies, with a view to reviving and modernizing their use (Belmain *et al* 2001) [2]. Plants are a rich source of secondary metabolites with interesting insecticidal activities (Dubey *et al* 2008; de-Fátima *et al* 2006) [3, 4]. 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) [5, 6, 7].

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) [8]. 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) [9]. The aim of this study is to identify the insecticidal compounds present in *Ficus benghalensis* (Family: Moraceae; Tamil: Aalamaram) bark extract with the aid of GC- MS techniques which may provide an insight in its use in insecticidal activity.

2. Materials and methods

Plant materials

The *Ficus benghalensis* barks were collected in January 2020 from Thanjavur, Tamil Nadu from a single herb.

Preparation of extracts

The collected *Ficus benghalensis* 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 ethanol 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 autosampler 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) [10].

3. 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) [11]. 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) [9].

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 Ethno botanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA Duke's, (2013) [12]. The nature and structure of the compounds were identified at different time intervals using mass spectrometer. The heights 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.

GC-MS analysis

Due to issues concerning the use of synthetic pesticides and the increasing resistance in pest species, pests can be managed by introducing botanical insecticides, especially against soft-bodied insects. To investigate safe alternatives for the management of this pest, the present study was conducted to evaluate the insecticidal effects of crude ethanol extracts *Ficus benghalensis* bark.

Twenty compounds were identified in *Ficus benghalensis* bark extract by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1and Fig 1). The prevailing compounds were 9, 12, 15-Octadecatrienoic acid, methyl ester, 9-Octadecenoic acid, 2-Hexadecen-1-ol, 3, 7, 11, 15-tetramethyl-, [R-[R*, R*-(E)] and 9, 12-Octadecadienoic acid present in the extract. The pharmacological activity of *Ficus benghalensis* represented in table 2. This study explores the goodness of the bark of the plant *Ficus benghalensis* which has a commendable sense of purpose and can be advised as a plant of phytopharmaceutical importance.

The investigation concluded that the stronger extraction capacity of ethanol 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.

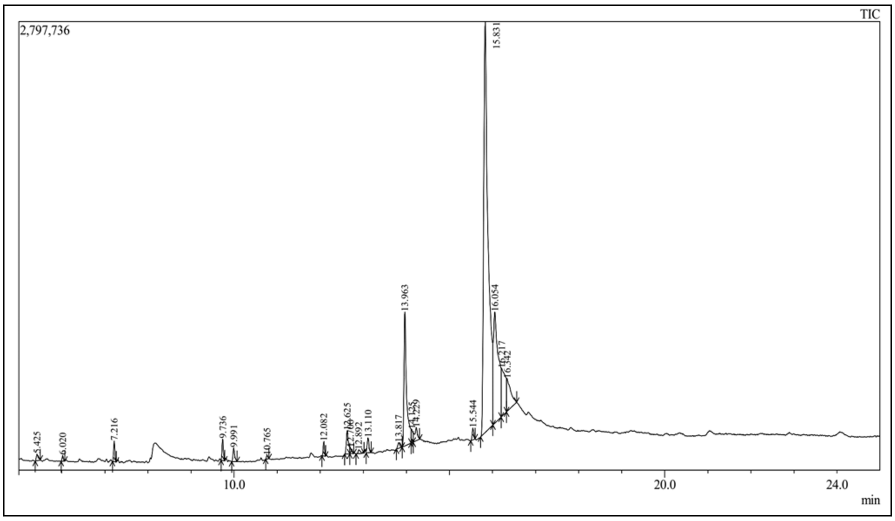

Fig 1: GC MS chromatogram of *Ficus benghalensis* bark extract

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) [13]. Hexadenoic acid has earlier been reported as a component in alcohol extract of the leaves of *Kigelia pinnata* (Grace *et al* 2002) [14] and *Melissa officinalis* (Sharafzadeh *et al* 2011) [15]. Parasuraman *et al.* (2009) [16] 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 (Siddiq Ibraham *et al* 2009) [17]. 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) [18] and *Vitex negundo* (Praveen kumar *et al* 2010) [19].

Squalene has earlier been reported as antimicrobial, antioxidant, anticancer, Neutralize different xenobiotics, anti-inflammatory, anti-atherosclerotic and anti-neoplastic, role in skin aging and pathology and Adjuvant activities and cosmetics as a natural moisturizer Ponnamma and Manjunath (2012) [20]. Devi *et al.* (2009) [21] reported that *Euphorbia longan* leaves mainly contained n-hexadecanoic acid and Octadecadienoic acid. These reports are in accordance with the result of this study.

Uraku (2015) [22] 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) [23] 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.

Table-1: GC-MS analysis revealed the presence of phytochemical component in bark of *Ficus benghalensis*

Peak#	R. Time	Area%	Height%	Molecular weight	Molecular formula	Molecular Name
1	5.425	0.37	0.72	168	$C_{12}H_{24}$	Cyclohexane, hexyl
2	6.020	0.22	0.63	170	$C_{11}H_{22}O$	2-Undecanone
3	7.216	0.82	2.25	224	$C_{16}H_{32}$	3-Hexadecene, (Z)-
4	9.736	0.88	2.35	252	$C_{18}H_{36}$	9-Octadecene, (E)
5	9.991	0.72	1.52	330	$C_{20}H_{26}O_4$	1,2-Benzoldicarbonsaeure, di-(hex-1-en-5-yl-ester)
6	10.765	0.15	0.39	100	$C_6H_{12}O$	1-Penten-3-ol, 4-methyl
7	12.082	0.54	1.59	280	$C_{20}H_{40}$	3-Eicosene, (E)
8	12.625	1.41	2.53	278	$C_{20}H_{38}$	9-Eicosyne
9	12.700	0.30	0.68	112	$C_7H_{12}O$	3-Methoxy-1-cyclohexene
10	12.892	0.31	0.42	222	$C_{16}H_{30}$	1-Hexadecyne (CAS)
11	13.110	0.77	1.63	336	$C_{22}H_{40}O_2$	19,19-Dimethyl-8,11-Icosadienoic Acid
12	13.817	0.37	0.63	292	$C_{19}H_{32}O_2$	9,12,15-Octadecatrienoic acid, methyl ester
13	13.963	10.54	14.64	282	$C_{18}H_{34}O_2$	9-Octadecenoic acid
14	14.125	0.63	1.45	232	$C_{12}H_{12}N_2O_3$	2-([(1-Cyano-1-Methylethyl) Amino]Carbonyl)Benzoic Acid
15	14.229	1.14	1.46	587	$C_{36}H_{75}O_3P$	Phosphonic acid, dioctadecyl ester
16	15.544	0.55	1.30	296	$C_{20}H_{40}O$	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-
17	15.831	54.80	44.88	280	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid
18	16.054	16.12	12.13	282	$C_{18}H_{34}O_2$	Oelsauere
19	16.217	5.64	5.23	238	$C_{15}H_{26}O_2$	N-Terpinenyl ester of n-pentanoic acid
20	16.342	3.73	3.56	110	C_8H_{14}	2-methylmethylenecyclohexan

Table 2: GC-MS analysis revealed the presence of phytochemical component in bark of *Ficus benghalensis* and their biological activities

S. No	Name of the Compounds	Biological Activities**
1	9,12,15-Octadecatrienoic acid, methyl ester	Flavour, Insecticide, Anti –inflammatory, Nematicide
2	9-Octadecenoic acid	Cancer preventive, Flavor Hypocholesterolemic, 5-Alpha reductase inhibitor, Anti-androgenic, Perfumery Insectifuge, Anti-inflammatory Anemiagenic, Dermatitigenic, Choleretic
3	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]	Antimicrobial, Anti-inflammatory, Cancer-Preventive, anti-diuretic, Antioxidant
4	9,12-Octadecadienoic acid	Anti-inflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, anti-histaminic anti-eczemic, antiacne, 5-Alpha reductase inhibitor, anti-androgenic, anti-arthritic, anti-coronary.

***Source:** Dr. Duke's phytochemical and ethno botanical database (online database)

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%).

4. Conclusion

The present study characterized the phytochemical profile of the *Ficus benghalensis* 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 ethanolic extract of *Ficus benghalensis* bark. The identification of various bioactive compounds confirms the insecticidal application of *Ficus benghalensis* bark for a variety of insect. Further research is in progress for the evaluation of insecticidal activity in *Ficus benghalensis* bark.

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