

Research Article

GC-MS Analysis of Insecticidal Leaf Essential Oil of *Pyrenacantha Staudtii* Hutch and Dalz (Icacinaceae)

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Abstract

Purpose: Insecticides have been known to cause serious toxicological and environmental problems. Hence, the insecticidal activity and chemical composition of a local medicinal plant was investigated.

Methods: Steam distillation of *P.staudtii* leaves was carried out using a Clavenger apparatus in order to obtain the volatile oils. Gas chromatography/mass spectrometric (GC/MS) analyses (DB-5 Optima-5 column) of the essential oil were performed and its insecticidal activity determined.

Results: GC-MS spectrometry showed that the major chemical components of the oil were tetradecanoic acid (22 %), hexanoic acid, α -phellandrene (13 %), and citronellol sp. (7 %). The work also revealed significant insecticidal activity of 80 % and 60 % against *Rhizopertha dominica* and *Tribolium castaneum*, respectively.

Conclusion: The study established the chemical composition and insecticidal activity of the essential oil of the plant leaf. Future formulation studies, toxicity profile and possible mechanism of action may lead to the development of a potential insecticidal product.

Keywords: *Pyrenacantha stautii* leaf, GC-MS, Leaf essential oils, Insecticidal.

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Introduction

The tropical rainforests provide a lot of medicinal plants with potential medicinal properties but the ethno- and phyto-pharmaceuticals of these plants have not been fully exploited¹. Their potential medicinal importance is due not only to the species richness of the tropical flora, but also to the diversity of pathogens, parasites and herbivores against which the plants must provide defensive mechanism. Many of the defense chemicals secreted, because of their metabolic precision, can be used to treat humans and prevent or eradicate other organisms².

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 man³. 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 products³.

The plant *Pyrenacantha staudtii* belongs to the family Icacinaceae and it is a woody climber with green fluorescent flowers. The leaves have various therapeutic uses in traditional medicine as a remedy against dysmenorrhoea, malaria, cancer, intestinal colic, miscarriage and ulcer⁴⁻⁶. Scanty phytochemical information on this plant exists. However, extensive information about the morphology, ethnomedicine, karyology and ecogeographic differentiation of the genus has been accumulated⁷⁻⁸.

The current study is aimed at characterizing the chemical components and insecticidal activity of the essential oil of *P.staudtii* leaf. Therefore, we report for the first time the separation and identification of the components of leaf essential oils with the aid

of GC-MS and other spectroscopic techniques.

Experimental

Plant collection and identification

The fresh leaves of the plant were collected in Benin City. Botanical authentication was done at the Forest Research Institute of Nigeria (F.R.I.N., Ibadan), where a herbarium specimen No. FHT 107624 was deposited.

Extraction and isolation of essential oil

Fresh leaves of *Pyrenacantha staudtii* were obtained in April, 2008. The plant was dried at room temperature, powdered and hydrodistilled for 6 h in a Clavenger apparatus. The oil was dried with anhydrous Na₂SO₄ to remove moisture. The fraction obtained was stored in a refrigerator at - 4°C until use.

Identification of the components

The identification of the chemical constituents was assigned on the basis of comparison of their retention indices and mass spectra with those given in the literature⁹⁻¹².

Gas chromatography

Quantitative and qualitative data were determined by GC and GC-MS, respectively. The oil was injected onto a Shimadzu GC-17A system, equipped with an AOC-20i autosampler and a split/ splitless injector. The column used was an DB-5 (Optima-5), 30 m, 0.25 mm i.d., 0.25 µm *df*, coated with 5 % diphenyl-95 % polydimethylsiloxane, operated with the following oven temperature programme: 50 °C, held for 1 min, rising at 3 °C/min to 250 °C, held for 5 min, rising at 2 °C/min to 280 °C, held for 3 min; injection temperature and volume, 250 °C and 1.0 µl, respectively; injection mode, split; split ratio, 30:1; carrier gas, nitrogen at 30 cm/s linear velocity and inlet pressure 99.8 KPa; detector temperature, 280 °C; hydrogen, flow rate, 50

ml/min; air flow rate, 400 ml/min; make-up (H₂/air), flow rate, 50 ml/min; sampling rate, 40 ms. Data were acquired by means of GC solution software (Shimadzu).

Gas chromatography-mass spectrometry analysis

Agilent 6890N GC was interfaced with a VG Analytical 70-250s double - focusing mass spectrometer. Helium was used as the carrier gas. The MS operating conditions were: ionization voltage 70 eV, ion source 250 °C. The GC was fitted with a 30 m x 0.32 mm fused capillary silica column coated with DB-5. The GC operating parameters were identical with those of GC analysis described above.

Insecticidal activity

The materials used include test insects (*Tribolium castaneum* and *Rhyzopertha dominica*), ethanol (99%), standard insecticide (Permethrin), Petri dish (9 cm diameter), micropipette (1000 µl), growth chamber, test sample, filter paper, glass vials, brush.

The insecticidal property of *P. staudtii* essential oil was investigated against *Rhyzopertha dominica* and *Tribolium castaneum* in comparison with commercial insecticide Permethrin and ethanol (negative control). In evaluating the insecticidal activity of the volatile oils, a preliminary test was carried out in which several doses, ranging from those having no killing effect on the experiment population to those with a minimum of 100 % killing of this population, were assessed. Using a micropipette (Rainin magnetic assist), the precise volume (2 mL) of essential oil was added to ethanol (2 mL) (negative control) and diluted to 5 mL. From this, 0.5 mL of solution was uniformly applied to a 9 cm disk of filter paper (Whatman No.1) and placed in a Petri dish. Insects used for the test were reared in glass jars at 27 ± 1°C and 60 ± 5% relative humidity (RH) at the laboratory of the H.E.J Research Institute of Chemistry in Karachi, Pakistan.

Twenty adult insects, less than 1 month old, were introduced into the dish 5 min later and the dish was covered. A control with ethanol alone (2 mL) was made. For each preparation, five replicates were made. The number of dead insects was determined 24 h after application. Mortality (%) was calculated as in Eq 1.

$$\text{Mortality (\%)} = \left(\frac{\text{No. of insects alive in test}}{\text{No. of insects alive in control}} \right) (100) \dots\dots\dots 1$$

Statistical analysis

Data are expressed as means ± s.e.m. Statistical analysis was by one way analysis of variance (ANOVA) and Student's t –test, using SAS 1994, Version 8.2 (SAS Institute, Inc., Cary, NC, USA). The statistical level of significance was p<0.05.

Results

The chemical composition of the leaf essential oil of *P. staudtii* is presented in Table 1. The yield of the essential oil was 1.40 (w/w) %. It was found to be a mixture of mono- and sesqui-terpenoids, and fatty acids. A total of 19 components were identified by their retention indices RI, as well as by GC-MS and other spectroscopic techniques, accounting for 95 % of the oil. Six (6) of the components accounted for the 80 % of the oil (Table 1). The oil comprised of fatty acid derivatives (52 %), sesquiterpene hydrocarbons (32 %) carophyllene (6 %), and oxygenated sequiterpenes such as carophyllene oxide (5 %). Monoterpenes hydrocarbons such as phallenden (12 %), pinene (2 %), thujene sp. (2 %), terpinene (4 %) were also present in the essential oil. Hexadecanoic (30 %) and tetradecanoic (22 %) acids were major components. The essential oil showed 80 and 60 % insecticidal activity, respectively, (see Table 2) against *Rhyzopertha dominica* and *Tribolium castaneum*.

Table 1: Composition of essential oil of *Pyrenacantha staudtii* leaf

Compound	Content (%)	RI
Thujene sp.	0.5	923
Camphene	0.67	945
α - Pinene	0.5	970
β -Myrcene	3.26	984
α -Phellandrene	12.45	995
Δ^3 -Carene	trace	1005
<i>p</i> -cymene	2.42	1012
Limonene	1.25	1019
Terpene	3.57	1049
β - Caryophyllene	5.57	1464
Camphor	0.23	1130
β -Terpinol-4-ol	0.80	1161
α - Terpineol	trace	1172
Methylsalicylate	1.35	1193
Citronellol sp.	7.00	1212
α - Terpenylacetate	trace	1260
β - Caryophyllene oxide	5.54	1565
Hexadecanoic acid	28.32	1963
Tetradecanoic acid	20.60	1787

The identification was performed by calculation of retention index (RI) on a DB-5 column Trace < 0.1%

Table 2: Insecticidal activity of essential oil of *Pyrenacantha staudtii* leaf

Treatment	Conc. ($\mu\text{g}/\text{cm}^3$)	Insecticidal activity (%)
Essential oil	1019.10	60 \pm 1.44 (TC)
Essential oil	1019.10	80 \pm 1.22 (RD)
Permethin(positive control)	237.5	100 \pm 0.00
Ethanol (negative control)	-	-

TC = *Tribolium castaneum*; RD = *Rhizopertha dominica*; - = no effect

Discussion

The insecticidal activity of the essential oil would be dependent on the active chemical constituents and the gross sensitivity of the target pest to the active chemical principles¹³. The volatile oil of *P. staudtii* contains limonene which is reported to be an active insecticidal constituent, that exerts a toxic effect on Coleopterans¹⁴. The insecticidal activity can be explained, in part, by the high concentration of fatty acids and minor components such as limonene and phellandrene. Thus, the large proportion of

fatty acids might have contributed to the activity of the oil against *Rhizopertha dominica*. This is because, hexadecanoic acid is known to possess a variety of medicinal and pharmacological properties, such as larvicidal effect, on *Plasmodium falciparum*¹⁵, and a sex pheromone¹⁶.

Pesticides are used extensively in agricultural farms, homes and other public places. Resistance to one or more pesticides has occurred in most major pest species. Relentless development of resistance is a serious threat to the future use of these

materials, and consequently, there is an urgent need to develop affordable, safe and sound pest control agents and techniques. The essential oil of *P. staudtii* demonstrated some potentials as a new plant insecticide.

Conclusion

We have determined the chemical composition of the essential oil of leaf of *P. staudtii* leaf by a GC-MS analysis, and have evaluated its insecticidal activity. The results showed that the essential oil was active against the two insects - *Tribolium castaneum* and *Rhyzopertha dominica* - tested. However, further studies will need to be undertaken to ascertain fully its bioactivity, toxicity profile, effect on the ecosystem and agricultural products. This will help to establish the synergistic effect of mono- and sesquiterpene hydrocarbon components of the essential oil and its suitability as an insecticide.

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