Comparison of different analytical methods for the identification of teak leaf volatiles

Bindu K. Jose, V. V. Sudheendrakumar, T. V. Sajeev

Forest Entomology Division, Kerala Forest Research Institute, Peechi, 680653, Thrissur, Kerala, India, Email: bindukjose@gmail.com, vv.sudheendrakumar@gmail.com, tvsajeev@gmail.com

ABSTRACT

Solvent extraction followed by gas chromatography mass spectrometry (GCMS) and solvent free extraction technique of head space combined with GCMS were employed in the analysis of teak leaf volatiles. Fresh and shade dried teak leaves of same maturity from a single tree in Nilambur locality were taken for the analytic studies. A comparative study was conducted on the volatile outputs of teak leaves produced through different identification techniques. GCMS analysis of tender teak leaves gave eight different volatiles (2-hexenal, amyl vinyl carbinol, 3- Hexene- 1-ol, aetate bicyclol [7.2.0] undec-4-ene, 1,6 cyclodecadiene, (E,E)-7,11,15,trimethyl-3-methylene and (E,E,E) 3,7,11,15-tetra methyl hexa deca). The tender dried teak leaf powder had two compounds (Amyl vinyl carbinol and 3- Hexene- 1-ol, aetate). Head space combined with GCMS of tender teak leaf produced six compounds like ethanol, 2-methoxy, 1R-α pinene, β- phellandrene, sabinene, caryophyllene and alpha caryophyllene and mature teak leaf had two compounds ethanol, 2-methoxyand and 1R-α- pinene.

Keywords: GCMS, Head space combined with GCMS, Teak leaves, Volatiles

INTRODUCTION

To choose the right experimental deign and technology for testing a hypothesis is the rationale behind the success of an experiment [4]. There exist several extraction and testing methods for characterization of plant's volatile. The freshness of collected plant parts, collection time are also important factors for the listing of volatile compound [3]. According to Cao [1] different extraction techniques used for the extraction of volatiles from the natural products exhibit different efficiencies. Quantitation errors in volatile analysis can happen without using appropriate testing methods [2].

A comparative study was undertaken of different extraction methods for the isolation of volatile organic compounds from tender and mature teak leaves. The volatile profile of teak will help to decipher the mechanism of host plant selection by teak defoliators. The aim of this study was to compare different methods for the analysis of teak volatile compounds. The techniques studied include gas chromatography mass spectrometry (GCMS) of fresh and dried teak leaves, and the solvent free extraction technique of head space combined with GCMS.

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MATERIALS AND METHODS

Two types of identification methods were applied to detect teak leaf volatiles. First one was the GCMS analysis of tender and mature teak leaves. Fresh and dried teak leaves of different maturity were used for this study. Both the leaf samples were collected from a single tree. The second one was the Head Space GCMS analysis, in which fresh leaves were employed for the examination.

GCMS analysis of tender and mature teak leaf

Initially volatile analysis of teak leaves of different maturity (first positioned tender) and fifth positioned mature) leaves in a branch from a single tree) was carried out using GCMS analysis. Volatile extraction had done by using n- hexane [3]. Leaf samples for the study was collected from Nedunkayam teak plantation, Nilambur Forest Division, Malappuram district Kerala. Leaf samples were collected from a single tree.

Fresh and shade dried teak leaves were used for the analysis. Five grams each of the samples (chopped fresh leaves and shade dried leaf powder) were dipped in 50 ml n- hexane and kept for 24 hours with continuous shaking. This solution was filtered using normal filter paper and the filtrate passed through sodium sulphate in order to remove the aqueous part. Then it was concentrated to 1/10 of the total volume and subjected for GCMS analysis.

The column used for GCMS analysis was DB 5. The carrier gas was He at 1.0ml/minute. Oven temperature was 60^0c – 210^0c at 3^oc/minute, injector temperature was 210^0c, injection size-0.1µl, split ratio- 1.40 and MS were taken at 70ev, m/2 – 40-450.

Head space GCMS analysis of tender and mature teak leaves

Fresh leaves of tender and mature teak leaves were collected between 9.30 am and 10 am from lower branch of a single teak tree growing at Nilambur. Leaf volatile listing was done using Head Space GCMS. Maturity of the leaves was decided according to their position in the branch. The 1st and 5th positioned leaves were treated as tender and mature respectively. The leaves were plucked from the petioles by minimum damage and placed in the glass bottles with rubber caps, supplied with the head space GCMS, and were sealed and transported immediately to the laboratory.

The collected leaf materials were subjected to volatile analysis using Head Space GCMS Varian MS #1. The column used was VF5MS. Following was the program configuration selected for the volatile analysis. Injection mode – GC Head space, syringe – 1ml headspace, syringe temperature - 35^0C, sample agitator - agitate and heat, agitator temperature - 50^0C, incubation time 0.25 min, incubation rpm – 250, agitator on – 10 sec, agitator off – 2sec, plunger fill speed – 100.000ml/sec, Fill srokes – 3, viscosity delay – 1.00sec, injector- front, preinjection delay – 0.500sec, plunger injection speed – 250.000µl/sec, post injection delay – 0.500 sec, syringe flush time – 30 sec, and GC cycle time – 0.30 min.

RESULTS AND DISCUSSION

The result of dried and fresh tender teak leaves by GCMS and head space GCMS were different. The solvent assisted extraction and solvent free extraction studies on volatiles by GCMS and Head space GCMS showed marked variation.

Tender leaf (GCMS)

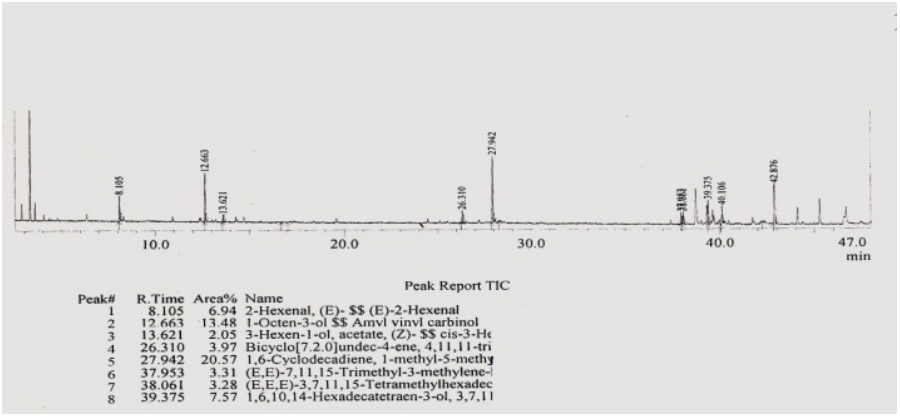

Figure 1. Volatiles of fresh tender teak leaf by GCMS.

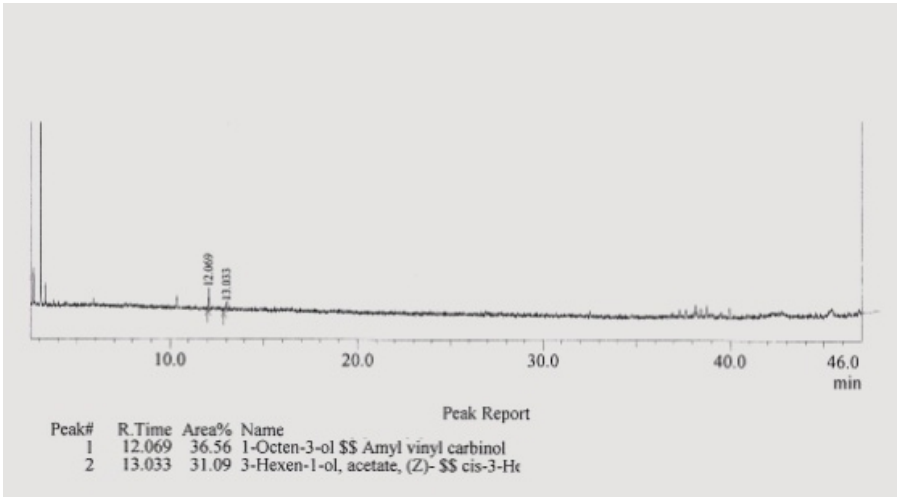

Figure 2. Volatiles of dry tender teak leaf by GCMS.

Fresh tender teak leaf (Figure 1) had eight different types of volatiles (2-hexenal, amyl vinyl carbinol, 3- Hexene- 1-ol, aetate bicyclol [7.2.0] undec-4-ene, 1,6 cyclodecadiene, (E,E)-7,11,15,trimethyl-3-methylene and (E,E,E) 3,7,11,15-tetra methyl hexa deca) (Figure 1) while the dried powder of tender teak leaves (Figure 2) had only two volatile (Amyl vinyl carbinol and 3-Hexene- 1-ol, aetate) compounds. All the other six compounds that the fresh leaf holds was absent in the dried form. It indicates that even though the drying was done in shade, most volatiles were lost from the leaf.

Mature leaf (GCMS)

Alpha Pinene, Bicyclol [7.2.0], α-Caryophyllene and n-Hexonic acid were presented in mature teak leaves by GCMS analysis. Volatile profiles of mature teak leaves were different from tender fresh and dry leaves.

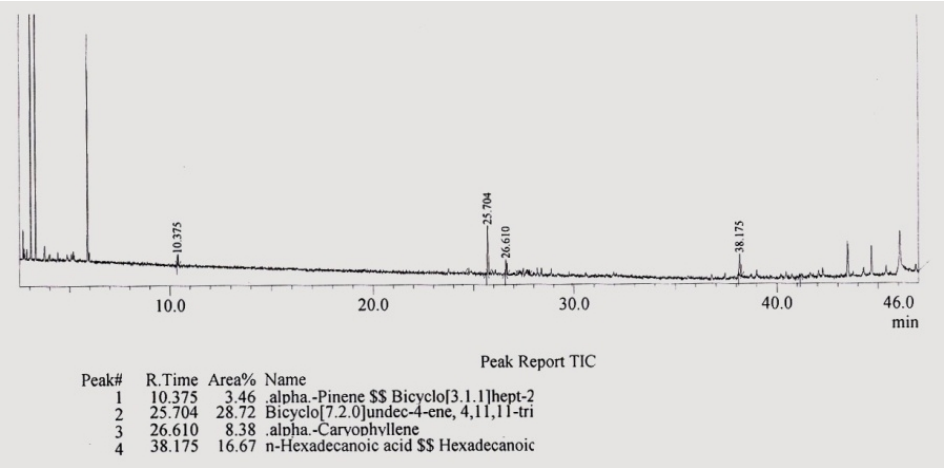

Figure 3. Volatiles of fresh mature teak leaf by GCMS.

Tender leaf (Head space GCMS)

Tender teak leaf collected during July months had had six compounds - ethanol, 2-methoxy, 1R-α pinene, β- phellandrene, sabinene, caryophyllene and alpha caryophyllene were found in the leaves.

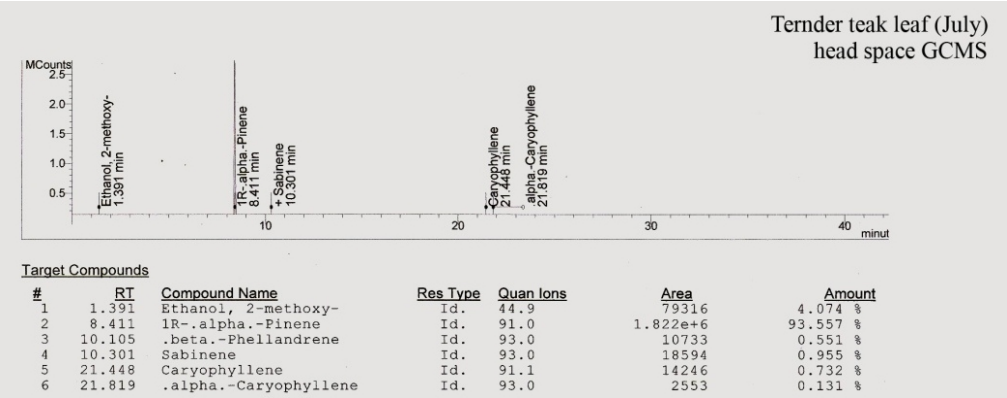

Figure 4. Volatiles of tender teak leaf by Head space GCMS.

Mature teak leaf (Head space GCMS)

In the month July, teak leaf contain (Figure 5) ethanol, 2-methoxy- (85.848) and 1R-α-pinene (14.152).

CONCLUSION

The volatile profiles of tender and mature teak leaf were different dependent on the analytical techniques and method of preparations used. In this work the dried and fresh teak leaves exhibited large variation in their volatile content even when they were identified by similar methods. In solvent extraction and analysis by GCMS , the tender teak leaves hold eight different volatiles (2-hexenal, amyl vinyl carbinol, 3- Hexene- 1-ol, aetate bicyclol [7.2.0] undec-4-ene, 1,6

cyclodecadiene, (E,E)-7,11,15,trimethyl-3-methylene and (E,E,E) 3,7,11,15-tetra methyl hexa deca). While the tender dried teak leaf powder had only two compounds Amyl vinyl carbinol and 3-Hexene- 1-ol, aetate). Loss of volatiles was clearly recorded in drying process. Loss of volatile compounds in drying of mature leaf was also observed in the study.

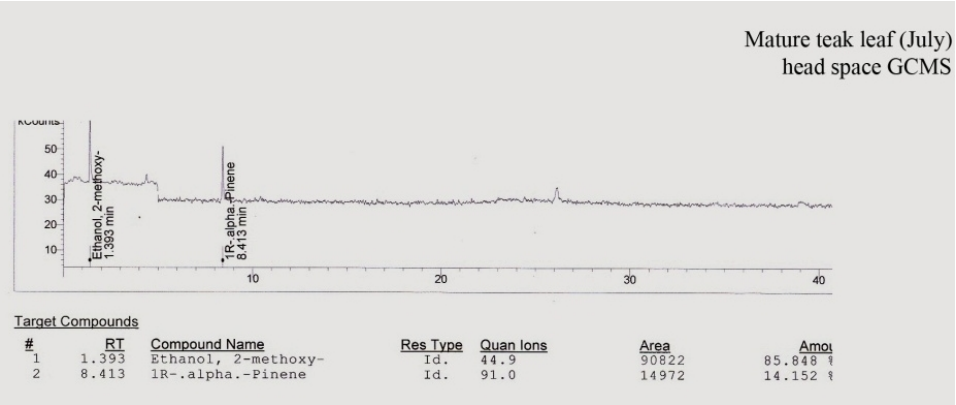

Figure 5. Volatiles of mature tender teak leaf by head space GCMS.

The different techniques studied showed that solvent extraction of fresh leaves produced more compounds than the other two. The compounds obtained in solvent extraction analysis (GCMS) and solvent free analysis (GCMS with Head space) was different in tender and mature teak leaves. Hence comparison among the process requires further detailed study. The study revealed that at present there is no single optimal method that exists for the extraction of volatile organic compounds and further research needed for standardization of the technique.

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