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GAS CHROMATOGRAPHY MASS SPECTROMETRY ANALYSIS OF CUTICULAR HYDROCARBONS IN THE ANT TETRAPONERA RUFONIGRA (JERDON) (HYMENOPTERA: FORMICIDAE)

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Abstract. Ants (Hymenoptera: Formicidae) are one of the most diverse and ubiquitous groups of social insects, the largest family under the order Hymenoptera. Cuticular hydrocarbons (CHC) function as chemical cues for the recognition of mates, species, and nest-mates in social insects. The surface of the insects is covered by a complex mixture of CHCs to prevent desiccation. In this study, the CHCs profile of *Tetraponera rufonigra* workers (foragers) were extracted and analyzed in Gas Chromatography and Mass Spectrometry (GC-MS). The result showed that *Tetraponera rufonigra* CHCs profile was composed of straight- and branched-chain alkanes and alkenes of 19 to 35 carbons atoms. A total of forty (40) CHCs were identified in the workers of *Tetraponera rufonigra*. The major CHCs that were recorded include DI-N-Decylsulfone ($C_{20}H_{42}O_2S$), 15-Methyltritriacontane ($C_{34}H_{70}$); 6-Fluoro-2-trifluoromethylbenzoic acid, Eicosyl ester ($C_{28}H_{44}O_2F_4$), 13-Methylhentriacontane ($C_{32}H_{66}$), Octadecane, 9-ethyl-9-heptyl ($C_{27}H_{56}$), Eicosane, 9-octyl ($C_{28}H_{58}$) and

Stearic acid, 3-(Octadecyloxy) propyl ester ($C_{39}H_{78}O_3$). These CHCs act as a chemical messenger between the ant colony, nest and nest-mate recognition of the ant *Tetraponera rufonigra*.

Keywords: Hymenoptera, Ant, Cuticular hydrocarbons, Kairomones and GC-MS.

1. INTRODUCTION

Ants belong to a single large family Formicidae of the order Hymenoptera. It is represented by 26 extant subfamilies with 14,711 valid species and 428 valid genera out of which, 152 species are listed by IUCN and from India, 10 subfamilies are represented by 100 genera with 828 species [9]. In India, Himalaya and the Western Ghats harbor a large number of ant species, 656 species from 88 genera were recorded from Himalaya, and 455 species from 75 genera were recorded from the Western Ghats, In Tamil Nadu, 184 species from 51 genera were recorded [3, 4]. The genus *Tetraponera* is composed of arboreal ants with large eyes and slender bodies. These ants are generalist inhabitants of dead twigs and branches. The genus is distributed throughout the Paleotropics [34] and is currently represented by 94 species and 19 subspecies [5, 9]. Ten species of *Tetraponera* have been reported till date from India [4, 6]. Ants are amongst the most dominant animals in the world and employ complex forms of chemical communication [16]. Over 100 exocrine glands have been described in social insects with more than half of these found in ants [7]. These glands are believed to produce a vast array of signals that encode information about an individuals species, sex, age, caste, status and relatedness, in addition to alarm and trail pheromones [18]. It has long been believed that nest-mate discrimination signals are encoded in cuticular lipids, particularly hydrocarbons that coat all insects [29].

Biochemical investigations have shown that insect cuticular hydrocarbons (CHCs) are synthesized internally in oenocytes [19]. CHCs have been intensely investigated in social insects as they are known to be involved nestmate recognition [33]. Semio-chemicals remain the primary means of ant communication. The ability to recognize other individuals as nestmates is achieved through the exchange of chemical signals, in particular, CHCs [18]. These long-chain hydrocarbons (linear and branched alkanes and alkenes) cover the external layer of the cuticles and beyond their communicative role, they mainly serve to avoid desiccation and protect insects against biotic and abiotic stress [18]. Martin and Drijfhout (2009) found a total of about 1000 different CHCs occurring in peculiar, species-specific mixtures, irrespective of species phylogeny. Most abundant are n-alkanes followed by monomethylalkanes, dimethylalkanes, alkenes, dienes and, more rarely, trimethylalkanes. methylalkenes, methylalkadienes, trienes and tetramethylalkanes are seldom produced by very few ant species [24]. CHCs are synthesized in the oenocytes, which are cells associated with fat bodies or epidermis, and later transported to the target tissues by lipid carriers (lipophorin) through the hemolymph [27]. The

cuticular chemical profiles of insects are composed primarily of straight-chain and branched-chain alkanes and alkenes of 1935 carbon [8, 24].

The epicuticle of insects is protected against desiccation and pathogen attack by a hydrophobic layer of lipids [15]. This layer is typically composed of a complex mixture of straight-chain and methyl-branched alkanes and alkenes (commonly referred to as cuticular hydrocarbons, CHCs) [8, 19] as well as a number of high polar compounds like wax esters, long-chain fatty alcohols and aldehydes. Apart from their protective function, cuticular lipids are also involved in the communication of many insect species. The non-polar CHCs have been extensively investigated over the past three decades with respect to their role as semiochemicals [17, 21, 29].

2. MATERIALS AND METHODS

2.1. **ANT COLLECTION.** The ant *Tetraponera rufonigra*, belongs to the family Formicidae, subfamily Pseudomyrmecinae and order Hymenoptera. The worker ants *Tetraponera rufonigra* (n=150) were manually collected from the Neem trees (*Azadirachta indica*) in the Department of Zoology, Pachaiyappas College for Men Campus (PACM), Kanchipuram (KPM) and immediately transported to the laboratory for cuticular extractions. Kanchipuram is situated on the northern East Coast of Tamil Nadu and is adjacent to Bay of Bengal and Chennai (Latitude: 12.8341735°N and Longitude: 79.7036402°E) India.

FIGURE 1. Ant *Tetraponera rufonigra* workers collected from Pachaiyappas College for Men campus, Kanchipuram, Tamil Nadu.

2.2. **Extraction of Cuticular Hydrocarbons (CHCs).** The workers of *Tetraponera rufonigra*, whole body were freeze killed for 20 min and submerged (1:1 ratio, 10 no's/10 mL), in non-polar solvent n-hexane (HPLC grade; Sigma-Aldrich, Mumbai, India) for 48 hrs at 25°C for the extraction of CHCs. The ant *Tetraponera rufonigra* extract (CHCs) was filtered in Whatman No.1. filter paper, and the filtered extract (CHCs) was directly injected to Gas Chromatography and Mass Spectrometry (GC-MS).

2.3. **Gas Chromatography and Mass Spectrometry analysis (GC-MS).** The *Tetraponera rufonigra*, workers CHCs were analyzed by Gas Chromatography and Mass Spectrometry (GC-MS). Gas Chromatography and Mass Spectrometry [JEOL GCMATE II-GC-MS] system equipped with a quantitative analysis by SIM mode detector, An HP5 Column 30.0m $\times$ 250 μm and the 0.25 μm film thickness were used. The oven was programmed from an initial temperature 60°C (hold for 2 min) to the final temperature 300°C at the rate of 20 (36.0 minutes). The final temperature hold up time was 6 minutes. Helium at the rate of 1 ml/min was used as the carrier gas in constant flow mode (Oven: Initial temp 60°C for 2 min, ramp 10°C/min to 300°C, hold 6 min, Inj A auto=250 °C, Split=10:1, Carrier Gas=Helium, Solvent Delay=2.00 min, Transfer Temp=240°C, Source Temp=240°C and Scan: 10 to 600 Da). NIST version 2 library search was used for CHCs analysis.

FIGURE 2. Collection and extraction of cuticular hydrocarbons (CHCs) from *Tetraponera rufonigra*

3. RESULTS AND DISCUSSION

3.1. **Cuticular hydrocarbon profiles.** In this study, the ant *T. rufonigra* CHCs extract was composed mainly of straight-chain and branched-chain alkanes and alkenes. A total of forty (40) CHCs were identified in the workers of *T. rufonigra* (Table 1). The CHCs of *T. rufonigra* were a complex mixture of alkanes, alkenes, and methyl-branched alkanes ranging from C_{11} to C_{39} carbon atoms such as DI-N-Decylsulfone($C_{20}H_{42}O_2S$), 15-Methyltritriacontane ($C_{34}H_{70}$); 6-Fluoro-2-trifluoromethylbenzoic acid, Eicosyl ester ($C_{28}H_{44}O_2F_4$), 13-Methylhentriacontane ($C_{32}H_{66}$), Octadecane, 9-ethyl-9-heptyl ($C_{27}H_{56}$), Eicosane, 9-octyl ($C_{28}H_{58}$) and Stearic acid, 3-(Octadecyloxy) propyl ester ($C_{39}H_{78}O_3$) etc. The identified *T. rufonigra* CHCs were mostly a mixture of n-alkanes, monomethyl alkanes, and dimethyl alkanes with linear chain lengths varying from C_{11} to C_{39} carbons (Table 1 & 2). Table 1 & 2. Cuticular hydrocarbons (CHCs) profile of the ant *T. rufonigra*

Six major CHCs were present in the social insects such as n-Alkanes, Olefins (alkenes and alkadienes), methyl alkanes (monomethylalkanes, dimethylalkanes and trimethyl alkanes),

TABLE 1

S.No	CUTICULAR HYDROCARBONS(CHCS)	MOLECULAR FORMULA
1.	DI-N-DECYLSULFONE	$C_{20}H_{42}O_2S$
2.	2,6-LUTIDINE 3,5-DICHLORO-4-DODECYLTHIO-	$C_{19}H_{31}NCl_2S$
3.	3,3-DIBUTOXY-1,1,1,5,5,5-HEXAMETHYLTRISILOXANE	$C_{14}H_{36}O_4Si_3$
4.	4-[4-CHLOROPHENYL]-1-METHYL-.ALPHA.-[3-[2-METHYL-2-BUTOXY]PROPYL]-4-PIP	$C_{21}H_{34}O_2NCl$
5.	6-HYDROXY-7-N-DOCOSYLMERCAPTO-5,8-QUINOLINEDINONE	$C_{31}H_{49}O_3NS$
6.	URIDINE, 5-HEPTAFLUOROPROPYL-	$C_{12}H_{11}O_6N_2F_7$
7.	1-(BETA.-D-RIBOFURANOSYL)-5-FLUORO-4-O-DIFLUOROMETHYLURACIL	$C_{10}H_{11}O_6N_2F_3$
8.	5-FLUORO-1-RIBOFURANOSYLIMIDAZOLE-5-CARBOXYLIC ACID, ETHYL(ESTER)	$C_{11}H_{15}O_6N_2F$
9.	3-METHOXY-2,4,5-TRIFLUOROBENZOIC ACID, NONADECYL ESTER	$C_{27}H_{43}O_3F_3$
10.	1,2,3-PROPATRIOL, 1-INDOL-4-YL(ETHER)	$C_{11}H_{13}O_3N$
11.	15-METHYLTRITRIACONTANE	$C_{34}H_{70}$
12.	OCTADECANE, 9-ETHYL-9-HEPTYL	$C_{27}H_{56}$
13.	HENEICOSANE, 3-METHYL-	$C_{22}H_{46}$
14.	EICOSANE, 9-OCTYL-	$C_{28}H_{58}$
15.	HEXADECANE, 8-HEXYL-8-PENTYL	$C_{27}H_{56}$
16.	OCTADECANE, 3-ETHYL-5-(2-ETHYLBUTYL)-	$C_{26}H_{54}$
17.	HEPTADECANE, 8,8-DIPENTYL-	$C_{27}H_{56}$
18.	NONAHEXACONTANOIC ACID	$C_{69}H_{138}O_2$
19.	2-METHYLDOCOSANE	$C_{23}H_{48}$
20.	DI-N-DECYLSULFONE	$C_{20}H_{42}O_2S$
21.	13-METHYLHENTRIACONTANE	$C_{32}H_{66}$
22.	DOCOSANE, 2,4-DIMETHYL-	$C_{24}H_{50}$
23.	1-BROMOEICOSANE	$C_{20}H_{41}Br$
24.	OCTADECANE,1,1'-[1,3 PROPANEDIYLBIS(OXY)]BIS-	$C_{39}H_{80}O_2$
25.	STEARIC ACID, 3-(OCTADECYLOXY)PROPYL ESTER	$C_{39}H_{78}O_3$
26.	1,6;3,4-DIANHYDRO-2-DEOXY-.BETA.-D-RIBO-HEXOPYRANOSE	$C_6H_8O_3$

TABLE 2

S.No	CUTICULAR HYDROCARBONS(CHCS)	MOLECULAR FORMULA
27.	DI-N-DECYLSULFONE	$C_{20}H_{42}O_2S$
28.	3-BUTOXY-1,1,1,5,5,5-HEXAMETHYL-3-(TRIMETHYLSILOXY)TRISILOXANE	$C_{13}H_{36}O_4Si_4$
29.	2,6-LUTIDINE 3,5-DICHLORO-4-DODECYLTHIO-	$C_{19}H_{31}NCl_2S$
30.	5-FLUORO-3-TRIFLUOROMETHYLBENZOIC ACID, EICOSYL ESTER	$C_{28}H_{44}O_2F_4$
31.	3,3-DIBUTOXY-1,1,1,5,5,5-HEXAMETHYLTRISILOXANE	$C_{14}H_{36}O_4Si_3$
32.	6-FLUORO-2-TRIFLUOROMETHYLBENZOIC ACID, EICOSYL ESTER	$C_{28}H_{44}O_2F_4$
33.	2-FLUORO-3-TRIFLUOROMETHYLBENZOIC ACID, EICOSYL ESTER	$C_{28}H_{44}O_2F_4$
34.	5-FLUORO-3-TRIFLUOROMETHYLBENZOIC ACID, NONADECYL ESTER	$C_{27}H_{42}O_2F_4$
35.	4-FLUORO-3-TRIFLUOROMETHYLBENZOIC ACID, EICOSYL ESTER	$C_{28}H_{44}O_2F_4$
36.	2-FLUORO-5-TRIFLUOROMETHYLBENZOIC ACID, EICOSYL ESTER	$C_{28}H_{44}O_2F_4$
37.	2-FLUORO-3-TRIFLUOROMETHYLBENZOIC ACID, OCTADECYL ESTER	$C_{26}H_{40}O_2F_4$
38.	4-FLUORO-2-TRIFLUOROMETHYLBENZOIC ACID, EICOSYL ESTER	$C_{28}H_{44}O_2F_4$
39.	3-METHOXY-2,4,5-TRIFLUOROBENZOIC ACID, NONADECYL ESTER	$C_{27}H_{43}O_3F_3$
40.	TRIMETHYL(4-TERT.-BUTYLPHENOXY)SILANE	$C_{13}H_{22}OSi$

Methylolefins (methyl alkenes and methyl alkadienes) [25].

The CHCs of *Myrmecia gulosa* are a complex mixture of alkanes, alkenes and methyl-branched alkanes ranging from C-23 to C-39 [35]. High abundance of n-alkanes on their cuticle of ant *M.eumenoides* and *P.barbatus*. Soroker and Hefetz [31], reported 17 hydrocarbons in the desert ant Cataglyphis niger. The ant C. niger CHCs were 13,11-Methylheptacosane, 7-Methylheptacosane, 5-Methylheptocosane, 3-Methylheptocosane, n-Nonacosane, 5-Methylnonacosane and 3- Methylnonacosane [31]. Smith [30] reported that the CHCs of *O.haematodus* were Tricosane, 11-Methyltetracosane, Pentacosane; 5-Methylp-entacosane, 3-Methylpentacosane,

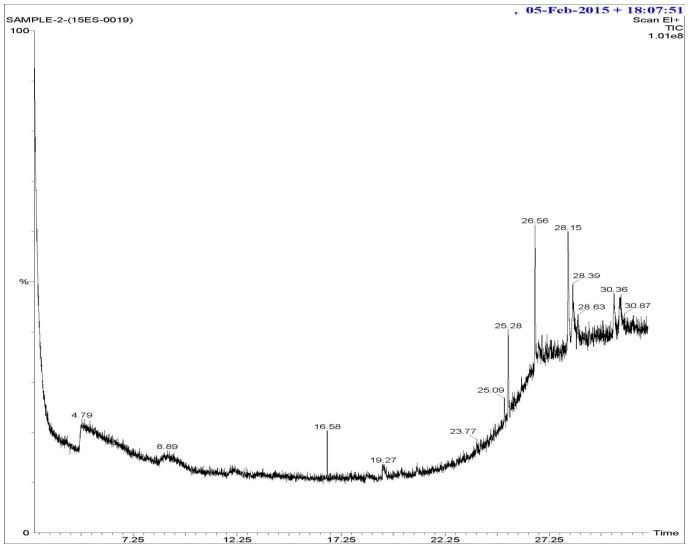

FIGURE 3. GC-MS chromatograms of cuticular hydrocarbons (CHCs) of ant *T. rufonigra* worker.

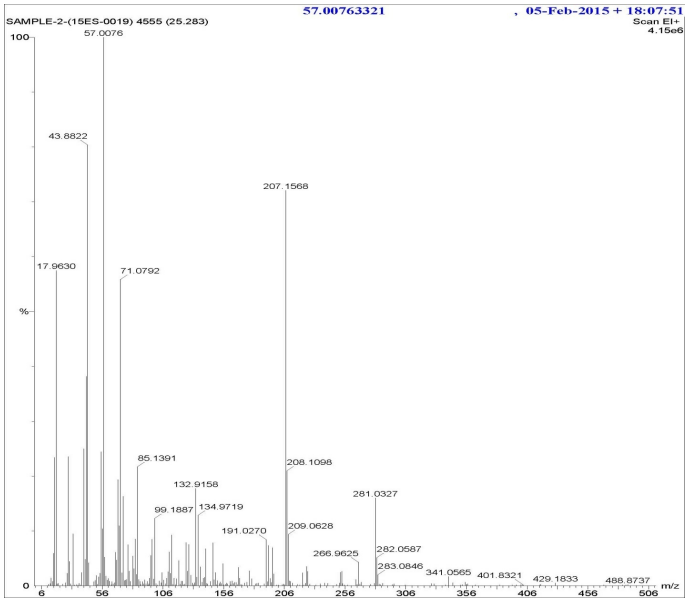

FIGURE 4. GC-MS chromatograms of cuticular extracts (CHCs) of ant *T. rufonigra* worker.

Hexacosane, 2-Methylhexacosane, Heptacosane, Octacosane, Nonacosane and Hentriacontane [30].

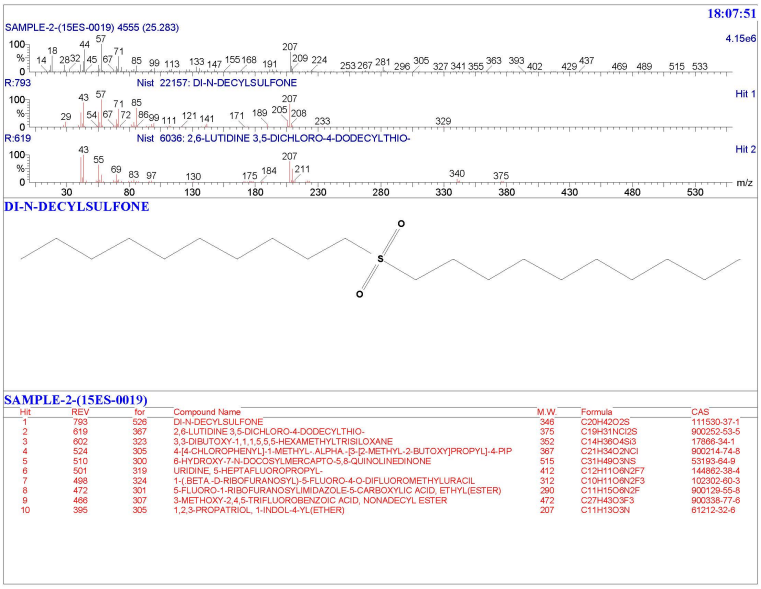

FIGURE 5. GC-MS chromatograms of cuticular hydrocarbons Di-N-Decylsulfone present in the ant *T. rufonigra* worker.

FIGURE 6. GC-MS chromatograms of cuticular hydrocarbon 15-Methyltritriacontane present in the ant *T. rufonigra* worker.

The evolutionary success of organisms relies on their adaptability to various environmental conditions. The integument represents the outer, functional and structured interface between an organism and its habitat [10, 22]. It functions as a physical barrier to pathogens or predators,

provides protection against wounding and injuries [12, 13] and [28], regulates the exchanges of water, primarily, but also plays a role in respiration, determination of inner temperature, body movements [14, 20, 26], intra- and inter-specific communications or sensing [2, 23]. Valadares et al. [32] reported that there is a variation in the cuticular hydrocarbon composition of *A. sexdens* workers. Similar results were found in the weaver ant *Camponotus textor* where only a single compound occurred exclusively in the cuticle of different size workers (Campos et al. [11].

Azhaguraj et al. [1] studied the weaver ant *O. smaragdina* CHCs and reported n-alkanes such as Hexadecane, Octacosane, Heptacosane, Eicosane,9-octyl, Pentatriacontane, Hexacosane 9-octyl, Nonadecane, Heneicosan, and Heptadecane. The cuticular hydrocarbons profile of *O. smaragdina* was composed primarily of straight-chain and branched-chain alkanes and alkenes. It may have acted as a chemical messenger between the ant colony and nest recognition of the weaver ant *O. smaragdina*. Hence, the present study has produced important information on ant *Tetraponera rufonigra* workers cuticular hydrocarbons (CHCs) profiles.

4. Conclusion

The cuticular hydrocarbons profiles of the *Tetraponera rufonigra* were extracted and analyzed by GC-MS studies. A total of forty (40) CHCs were identified in the workers of *T. rufonigra*. *Tetraponera rufonigra* CHCs extract were composed primarily of straight-chain and branched-chain alkanes and alkenes. In this study, the following epicuticular hydrocarbons, DI-N-Decylsulfone($C_{20}H_{42}O_2S$), 15-Methyltritriacontane ($C_{34}H_{70}$); 6-Fluoro-2-trifluoromethylbenzoic acid, Eicosyl ester ($C_{28}H_{44}O_2F_4$), 13-Methylhentriacontane ($C_{32}H_{66}$) Octadecane, 9-ethyl-9-heptyl ($C_{27}H_{56}$), Eicosane, 9-octyl ($C_{28}H_{58}$) and Stearic acid, 3-(Octadecyloxy) propyl ester ($C_{39}H_{78}O_3$) were present in *T. rufonigra*. These cuticular hydrocarbons may have acted as a chemical messenger between the ant colony and nest recognition of the Ant species *T. rufonigra*.

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