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FEMORAL GLAND SECRETION OF GEKKONIDS *HEMIDACTYLUS AQUILONIUS* AND *HEMIDACTYLUS FLAVIVIRIDIS* FROM ASSAM, INDIA

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ABSTRACT

Lizards have been around since the time of the dinosaurs, but climate change poses a threat to their existence on earth as they are highly sensitive to temperature fluctuations. They feed on nocturnal insects and occasionally are cannibalistic, eat rice grain, appreciate sugar, and at times seeds of wild plants and rarely hibiscus flowers. The present study reports the femoral gland secretion of two species of Gekkonids *Hemidactylus aquilonius* and *Hemidactylus flaviviridis* from Assam using gas chromatography and mass spectrometry techniques. In *Hemidactylus aquilonius* esters (35%) and alkanes (25%) were predominant whereas alkanes (53%), esters (21%) and halides (11%) were the major constituents of femoral gland secretion of *Hemidactylus flaviviridis*. In conclusion, this is the first report on the femoral gland secretion from the genus *Hemidactylus* in Assam.

Keywords: house gecko, *Hemidactylus aquilonius*, *Hemidactylus flaviviridis*, femoral gland secretion, alkanes, esters

INTRODUCTION

Chemoreception is an important characteristic in lizards. The males usually possess the precloacal femoral glands on the ventral surface. These glands are epidermal structures present on the ventral surface of the thighs which secrete chemical cues (**Smith, 1935**). Their location suggests that the secretion is usually deposited on a substratum as the lizard moves about. These cues may be the implication of home range, social status or competitive ability of the male or information on the quality of male for mating choice by the female (**Martın and Lopez, 2000; Aragon *et al.*, 2001; Lopez and Martin, 2002**). The secretions of these glands are usually certain organic compounds or pheromones. Analysis of secretion of femoral glands in lizards such as Lacertids and Iguanids (**Chauhan, 1986; Alberts, 1990; Weldon *et al.*, 1990; Alberts *et al.*, 1992; Escobar *et al.*, 2001, 2003; Lopez and Martin, 2005**) suggested the presence of lipids, carboxylic acids and alcohols. Despite their immense importance in territory making by the males of the species as well as attracting females during mating, studies on the femoral gland secretion of most lizards have not been carried out so far.

The genus *Hemidactylus* or house geckos occupy a wide range of habitats ranging from household walls to tree barks to leaf litters. From the Indian northeastern state of Assam, five species of these geckos have been described. Although taxonomic studies and the bioinventory have been carried out (**Purkayastha et al., 2011**), studies related to components of femoral gland secretion of lizards is lacking from this region. Hence, the aim of the present work was to study the pre-anal femoral gland secretion of two species of house geckos *Hemidactylus aquilonius* and *Hemidactylus flaviviridis* from Assam.

MATERIAL AND METHODS

Sample collection

H. aquilonius and *H. flaviviridis* males were captured by hand during the months of July till October, 2014 (figure 1). The lizards were weighed, their body length measured and the femoral pore number was counted. After capture, secretions from femoral glands of the male

A

B

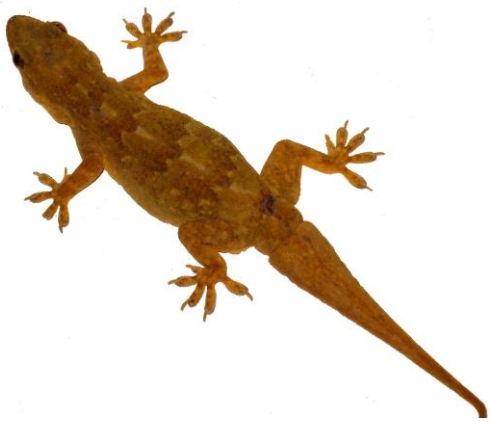

Figure 1 Two species of house geckos in the present study (**A**) *Hemidactylus aquilonius* and (**B**) *Hemidactylus flaviviridis*.

Lizards were extracted by gently pressing around the femoral pores with forceps, and the secretion was directly collected into glass vials followed by closure with teflon-lined stoppers. The vials were stored at -20^{0}C until further analysis. The lizards were then immediately released to their capture sites.

Gas chromatography/mass spectrometry

Samples were analyzed using Perkin Elmer make Claurus 680 Gas Chromatograph and Claurus 600C Mass spectrometer. Elite-5MS capillary column (length 60 m, ID 0.25 mm, maximum program temperature 350°C) was used with phase reference of 5% diphenyl and 95% dimethyl polysiloxane (low bleed). 5µl of sample was dissolved in 10 ml of HPLC grade n-hexane and injected in 0:1 splitless mode. The gas chromatograph was programmed to an initial oven temperature of 50^0C for 10 minutes, increased to a final temperature of 280^0C at the rate of 8^0C per minute and kept for 10 mins. The carrier gas used was helium. The injector temperature was 250^0C. Impurities identified in the solvent and/or the control vial samples were not reported. The relative amount of each component was determined by the total ion current (TIC). Library search was carried out against the most relevant peaks.

RESULTS AND DISCUSSION

Gas chromatography/mass spectrometry analyses of the femoral gland secretions of male house geckos *H. aquilonius* and *H. flaviviridis* gave total ion chromatograms that were qualitatively as well as quantitatively different (figure 2). A total of 35 compounds were identified from the femoral gland secretion of the house geckos, 16 from *H. aquilonius* 19 from *H. flaviviridis*. In *H. aquilonius* the highest retention time was showed by the compound dichloroacetic acid, 2,2-dimethylpropyl ester followed by hexacosane; cyclohexane, 1,1'-[1,2-bis(1,1-dimethylethyl)-1,2-ethanediyl]bis-, (r*,r*)-(.; D-mannitol, 1-o-(22-hydroxydocosyl)-; and sulfurous acid, butyl heptadecyl ester (Table 1). In *H. flaviviridis* the compounds such as sulfurous acid, butyl tetradecyl ester demonstrated the highest retention time followed by ; hexacosanol, acetate; pentatriacontane; octatriacontane, 1,38-dibromo-; and heptacosanoic acid, 25-methyl-, methyl ester thereby confirming their absolute presence followed (Table 2).

Table 1 Individual constituents present in the femoral gland secretion from the house gecko *Hemidactylus aquilonius*

Sl. No.	Constituent	Retention time (minutes)	Molecular weight
1	2-propenoic acid, methyl ester	21.76	86
2	1-butene-3-ethoxy	22.34	100
3	Cyclobutanecarboxylic acid, pent-2-en-4-ynyl ester	23.61	164
4	Oxalic acid, cyclobutyl pentadecyl ester	35.58	354
5.	Octadecane, 1-chloro-	36.83	288
6	1-dodecanol, 2-methyl-, (s)-	37.05	200
7	Heptadecane, 2,6,10,15-tetramethyl-	38.43	296
8	Methyl 2-hydroxy-eicosanoate	38.54	342
9	Docosanoic acid, docosyl ester	39.71	648
10	Tetracontane	39.78	562
11	1-hexene, 6-phenyl-4-(1-phenylethoxy)-	40.32	280
12	Sulfurous acid, butyl heptadecyl ester	41.27	376
13	D-mannitol, 1-o-(22-hydroxydocosyl)-	41.90	506
14	Cyclohexane, 1,1'-[1,2-bis(1,1-dimethylethyl)-1,2-ethanediyl]bis-, (r*,r*)-(.	42.47	306
15	Hexacosane	42.94	366
16	Dichloroacetic acid, 2,2-dimethylpropyl ester	44.89	198

Table 2 Individual constituents present in the femoral gland secretion from the house gecko *Hemidactylus flaviviridis*

Sl. No.	Constituent	Retention time (minutes)	Molecular weight
1	Oxirane, (2-methylbutyl)-	21.77	114
2	2-butene, 1-butoxy-, (e)-	22.35	128
3	Cyclobutanecarboxylic acid chloride	23.61	118
4	L-norleucine, n-ethoxycarbonyl-, octadecyl ester	33.82	455
5	Tridecane, 2,2,4,10,12,12-hexamethyl-7-(3,5,5-trimethylhexyl)-	34.05	394
6	Dodecane, 1-fluoro-	35.60	188
7	Tritetracontane	37.06	604
8	Dotriacontane	38.44	450
9	Tridecane, 6-cyclohexyl-	38.54	266
10	Pentacosane	39.79	352
11	Sulfurous acid, butyl octadecyl ester	40.87	390
12	Hentriacontane	41.28	436
13	Meso-4,5-dicyclohexyl-2,7-dimethyloctan	41.89	306
14	Eicosane	42.96	282
15	Heptacosanoic acid, 25-methyl-, methyl ester	43.48	438
16	Octatriacontane, 1,38-dibromo-	44.36	690
17	Pentatriacontane	44.91	492
18	Hexacosanol, acetate	45.09	424
19	Sulfurous acid, butyl tetradecyl ester	47.27	334

A
B

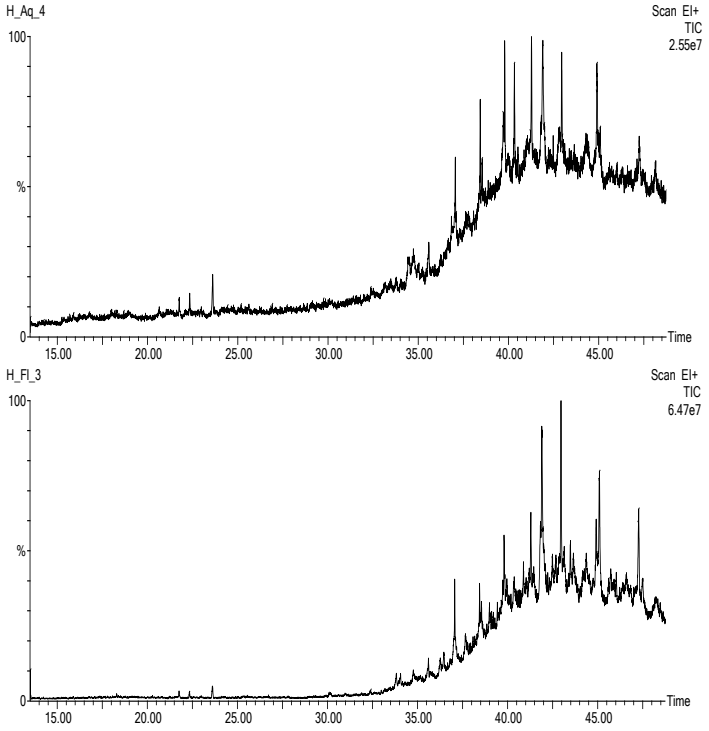

Figure 2 Total ion chromatogram of an extract of (**A**) *Hemidactylus aquilonius* and (**B**) *Hemidactylus flaviviridis*.

Overall, the present study revealed the presence of various type organic compounds in the femoral gland secretion of the house geckos, including alkanes (25% in *H. aquilonius* versus 53% in *H. flaviviridis*), esters (31% in *H. aquilonius* versus 21% in *H. flaviviridis*) and halides (6% in *H. aquilonius* versus 11% in *H. flaviviridis*) as the major ones. Contents of alcohols (19% in *H. aquilonius* versus 5% in *H. flaviviridis*) and carboxylic acids (13% in *H. aquilonius* vs 10% in *H. flaviviridis*) were also moderate in the femoral gland secretion from both the species, whereas alkenes (6%) were present only in the femoral gland secretion from *H. aquilonius* (figure 3).

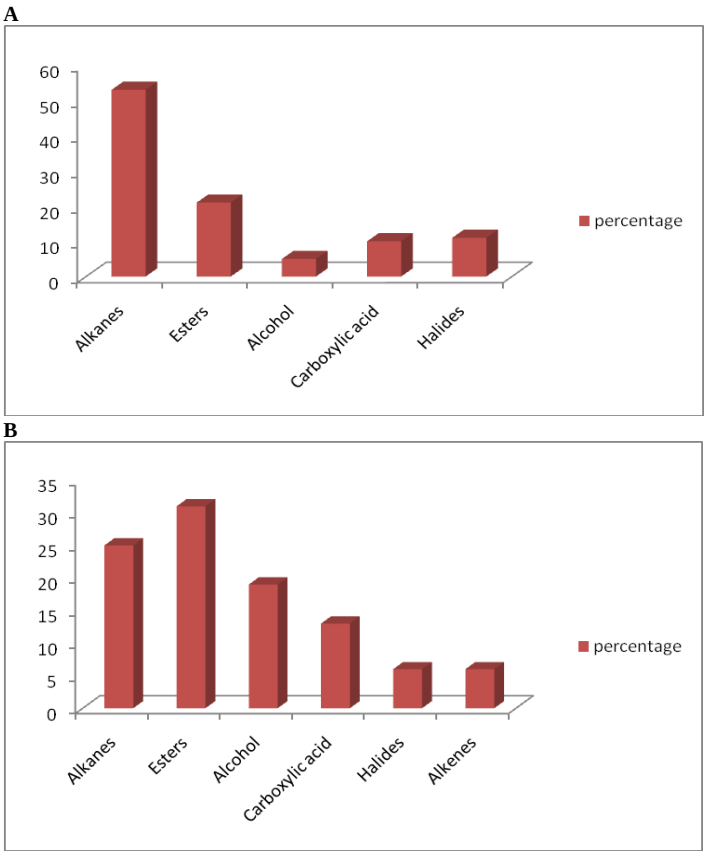

Figure 3 Major components present in the femoral gland secretion of (**A**) *Hemidactylus aquilonius* and (**B**) *Hemidactylus flaviviridis*.

Femoral gland secretion in lizards plays a major in chemoreception apart from reproduction. It is believed that the secretion of femoral gland is deposited in the home range of a male and functions to mark its territory. The secretion is odoriferous, and facilitates pairing of the sexes in the breeding season (**Cole, 1966a**). Differences between the chemical cues in various males of the same as well as different species could help females of the species to discriminate between them and thus affect their choice of males for mating. The secretion protruding from the pores in the breeding season also act as a tactile stimulus to females to quieten them in courtship or copulation (**Noble and Bradley, 1933**). In the present study, the secretions from femoral glands of *H. aquilonius* and *H. flaviviridis* were predominated by alkanes, esters, alcohols and carboxylic acids. **Alberts *et al.* (1992)** reported that in most cases such secretions are of lipid origin. However, in desert iguana *Dipsosaurus dorsalis* the main constituents of the femoral gland secretion were proteins and lipids. Volatile lipids are believed to act as olfactory cues for the detection of femoral secretions (**Alberts, 1990)**. In other lizard species, these constituents belong to three main classes of alkanes, carboxylic acids, and steroids (**Chauhan 1987; Alberts 1990; Alberts et al., 1992**). A possible explanation of the differences in chemical composition of the secretions might be the consequence of different diets, or different available food sources in the island and in the mainland in regards to the habitat which the lizard species occupies. Also, such differences might simply result from genetic differences between populations and species. However, testing such hypothesis would require detailed behavioural study of the house geckos. In conclusion, this is the first report on the femoral gland secretion from the genus *Hemidactylus* in Assam, India.

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