



RESEARCH ARTICLE

THE COMPOSITION OF PERINEAL GLAND SECRETION (MUSK) FROM THE AFRICAN CIVET
CIVETICTIS CIVETTA (SCHREBER, 1776)

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ABSTRACT

Hexane extracts of musk from the African civet (*Civettictis civetta*) were analyzed using Gas-chromatography-Mass-spectrometry (GC-MS). A total of 39 volatile compounds were consistently detected in all musk categories. The majority of the identified musk components were from four classes of organic compounds; Carboxylic Acids (38.5%), Alcohols (12.8), ketone (12.8%) and Aldehydes (5.1%). Five musk components (9-Cycloheptadecen-1-one, (Z); Octadecanoic acid; 9-Octadecenoic acid (E); 9-Cycloheptadecen-1-ol; and Cycloheptadecanone) were more abundant than the rest and accounted for 64.62-80.93% of the average percent area of chromatogram across all musk categories. Civetone, the most abundant compound across all age and sex categories, ranges from 54.5-69.71%. 21 of the 39 musk compounds were common to all musk samples. Adult male and female civets comprised the highest number of musk compounds (94.8%). Musk from sub-adult civets lacks some of the lower molecular weight acids, and some higher molecular weight compounds. While two compounds (Oleic acid and Squalene) were unique to adult male civets, two (1,5,9-Undecatrien,2,6,10-trimethyl-(Z) and Heptadecane) were found only in the adult females. Scent marked musk of both dry and wet seasons lack acids of lower molecular weight but contain all of the dominant and most of the higher molecular weight musk compounds.

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INTRODUCTION

Chemo-signaling is a complex odor communication system evolved in a large number of solitary and social animals (Morgan, 2009; Formica et al., 2010). Critical information may be indirectly propagated through chemical signals and effectively used to influence the recipient behaviors. For solitary, cryptic and nocturnal species, chemical signals are used as 'bulletin boards' that relay messages in the absence of the sender (Burger, 2005), whereas social species frequently integrate concurrent visual, auditory, and other behavioral cues (Wood et al., 2005). Most terrestrial viverrids possess specialized perineal glands whose secretions serve for olfactory communications. The African civet, *Civettictis civetta* (Schreber, 1776), is the largest member of the 'true civets' (subfamily viverrinae) that includes the Asian species; the

Malabar civet (*Viverracivettina*), the large-spotted civet (*Viverramegaspila*), Malay civet (*Viverratangalunga*), the large Indian civet (*Viverrazibetha*) and the small Indian civet (*Viverriculaindica*) who possess a specialized perineal glands whose secretions (musk) serve for olfactory communication (Balakrishnan and Sreedevi, 2007a,b). Unlike most mammals, reports on the chemical composition of glandular secretions from most genera of viverrinae are limited (Ruzika et al., 1927; Ding et al., 1988; Wheeler et al., 1998; Weldon et al., 2000; Wood et al., 2002; Formica et al., 2010; Rosell et al., 2010; Duangyod, 2011). These scholars generally report the presence of civetone, muscone, oleic acids, cyclopentadecanone, cyclohexadecanone, cyclic ketones, alcohols, indols, and other classes of compounds in civets of genera *Viverra*, *Viverricula* and *Civettictis*. Both sexes of the African civets possess paired perineal glands which produce a tannish-yellow waxy secretion that is highly pleasant upon dilution (Ray, 1995; Kingdon, 1997; Balakrishnan and Sreedevi, 2007a, b). However, comprehensive reports on the composition and relative proportion of the components of musk for the African civet are lacking. Both male and female

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African civets begin scent marking during the first year of life (Ray, 1995); however, no reports are available on the differences in composition and proportion of musk components between sexes and age categories of these animals. Also, seasonal variation in the perineal gland secretion from the African civet is lacking in the scientific literature although this has been reported for various species of mammals (Burger, 2005). In the present study, musk samples collected from both sexes and age groups were analyzed using GC-MS as part of an effort to conduct a comprehensive analysis of musk components and the compositional variation across sex and age categories and seasons. Gas Chromatography and Mass Spectrometry (GC-MS) is becoming the handiest instrument for the identification and quantification of chemical compositions in complex mixture (Hajslova and Cajca, 2007; Duangyod *et al.*, 2011). It is particularly important for the analysis and component quantification of complex glandular secretions like musk from the African civet.

MATERIALS AND METHODS

The Study Area

The study area was Limmu-Seka district, Oromia Regional State, southwestern Ethiopia. It is geographically located between 89°00' and 98°00' N latitude and between 24°00' and 30°00'E longitude and covers an area of 1777 km², 108 km northwest of Jimma (Fig. 1).

The altitude of the study area ranges between 1338 and 2400 m asl, moderately high ground lying between Ghibe and Didessa river basins. The majority (70%) of the study areas is categorized under moderate and highland agro-climatic zones while the rest is considered lowland. The study area receives over 1550 mm annual rainfall, the peak between June and August (ENMA, 2012). The dry season begins in November and extends up to early May. The district has moderately warm temperature that shows little variation throughout the year with a mean minimum and maximum annual temperature of 13.6 and 26.9°C, respectively (ENMA, 2012).

The study area represents the northern portion of the tropical rain forest belt of the country (Gole *et al.*, 2008; Beenhouwer, 2011) and has typical moist ever green montane forest vegetation characterized by medium to large sized and broad leaved species (Demissew *et al.*, 2004). The undulating landscape of the study area, with over 55% surface forest cover, is among the best forest reserve area of the country, and hence, best habitat for the African civet. Except for the yet accessed intact northern, western and southern jungles, the entire forest of the study area is managed for coffee production (Gole *et al.*, 2008). The district was purposefully selected for this study because of its geographic location (within tropical rainforest), the presence of mixed natural and coffee forest vegetation with mixed crop-livestock agriculture land use system, that favors the abundance of civets (Habtamu and Bekele, 2013, In Press).

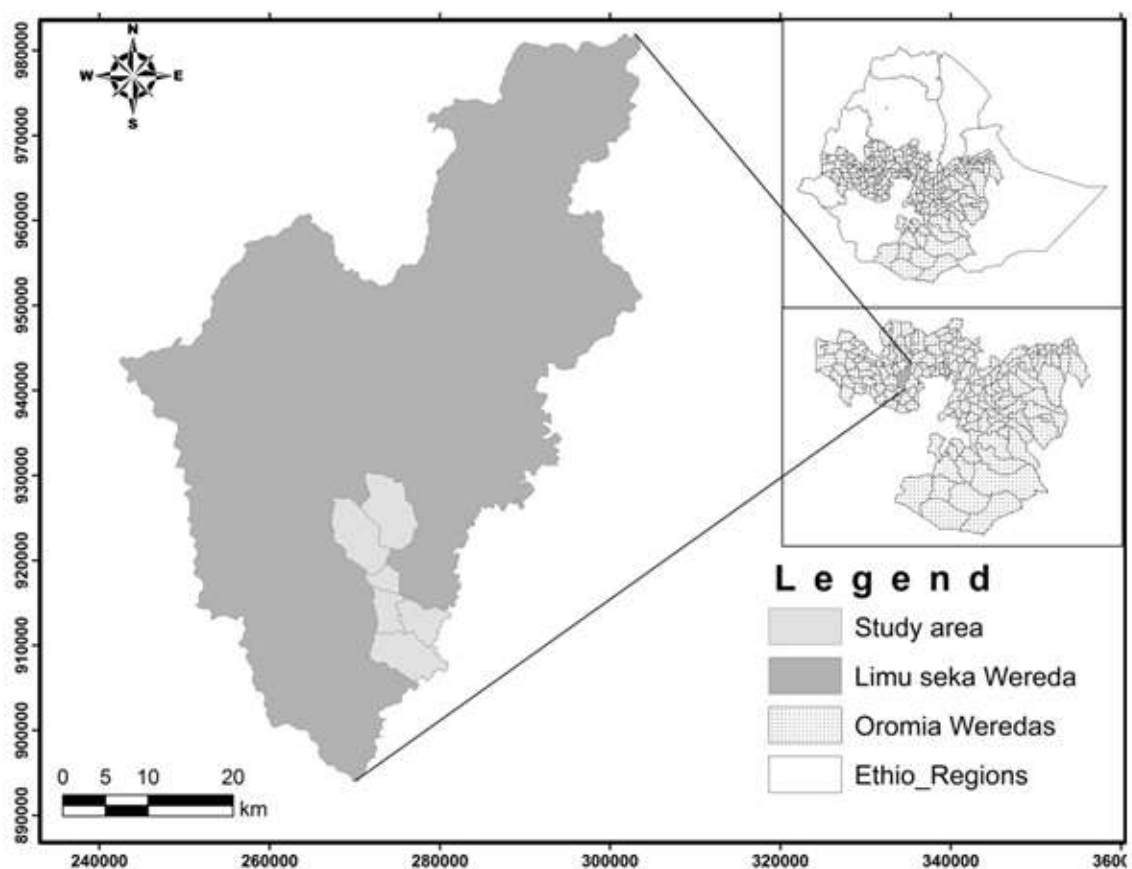


Figure 1. Map of Ethiopia showing the study area

Methods

Sample Collection

Legal permission was obtained from the Federal Wildlife Organization of Ethiopia and the regional Wildlife Authority of Oromiya to trap, transport, research and finally releases the animals into their natural environment. For the collection of musk data from the wild, 6 Farmers Association (FAs) were purposefully selected from among 28 in the district. All accessible musk signposts within the 6 FAs were identified and samples were collected for two seasons (between January and March/2013 for the dry and between July and September/2013 for the wet). Musk samples of various categories (age and sex) were extracted from civets maintained in captivity for feeding and home management research centered in Atinago (capital of the district). Civets of both sexes and all age groups were trapped using padded local snares and kept in individual cage. In total, pooled musk samples of six different categories were collected for this study; 4 samples from the 19 captive civets (for adult and sub-adult civets of both sexes) and 2 pooled musk data scraped from the 83 and 53 environmental signposts for wet and dry seasons respectively. All musk samples were kept in Teflon capped vials and placed in refrigerator (-20°C) until the analysis (Scordato and Drea, 2007). The analysis of the musk samples was performed using a Shimadzu QP2010 GC-MS instrument in the Medicinal/Organic Chemistry Research Laboratory at Elizabeth City State University, USA. Hexane was used as a principal solvent (Wood et al., 2005; Burger et al., 2008) and the reagent level of it was purchased from Sigma Aldrich, PA, USA. The GC was equipped with a 30-m glass capillary column (0.25 mm ID) coated with SE54.

The injection temperature was programmed at 250°C with split injection mode (1:10 split ratio) (Kumar et al., 2000; Burger et al., 2001; Zhang et al., 2002). Electron impact was used for ionization (methane as a reagent gas) mode at 70eV (electron volt) (Kumar et al., 2000; Duangyod et al., 2011). The carrier gas was He, with pressure 100 kpa, total flow rate of 50ml/m, and with 1.61 ml/m column flow rate (Zhang et al., 2002; Duangyod et al., 2011). Linear velocity of the carrier gas was programmed at 46.3 cm/sec and purge flow rate of 3ml/m. The column oven temperature (at zero time) was adjusted to 60°C with 3m' hold time and increased to 280°C final temperature at a rate of 3°C/m and zero hold time. For the MS, ion source and interface temperature were programmed at 200 and 250°C, respectively (Burger et al., 2001; Zhang et al., 2002). Scan mode with the speed of 1666 scans/s in 0.5s interval time was programmed. Ions with m/z ratio between 40 and 400 were scanned (Rajeswari et al., 2011). Including the 2 minute solvent delay, the entire run lasted 73.33minutes. For the analysis, about 2 mg of pooled musk from each category was dissolved in 2ml Hexane and vortexed (Shimadzu vortex) for a minute. The mixture was then centrifuged, (Eppendorf Centrifuge 5702R) at 4400 rpm for 5 minutes. From the supernatant, two micro-liters (2 µl) were taken using micro-syringe and manually injected for the analysis.

Compound Identification and Data Analysis

Shimadzu GC-MS solution software was used for data acquisition and analysis. The software combines a Real Time

Analysis module for instrument control and data acquisition with a Post Time Analysis module for data analysis and reporting.

For identification of compounds, the mass spectrum of the unknown musk component was compared with the spectrum of the known compounds in the NIST, and Wiley mass spectral databases (Scordato and Deng, 2007; Waterhouse et al., 2008). Peaks were considered positively identified only if the library similarity search result was greater than 90% and the visual inspection of mass spectra of the musk and the library compounds match over 95%. Some compounds that were consistently occurring in most samples, with identical retention time and considerable proportion, but with poor library match results were temporarily named as 'un-identified'. Only peaks with %TIC area greater than 0.1 were automatically picked and considered for analysis.

RESULTS

Musk Compounds

A total of 39 organic compounds were consistently identified from the GC-MS analysis of all musk categories from the African civet (*Civettictis civetta*). The majority of the compounds belong to four major classes of organic compounds: Carboxylic acids, alcohol, ketone and aldehydes (with proportion 38.5%, 12.8%, 12.8%, and 5.1%, respectively). Eight compounds (21%) contained miscellaneous organic functional groups over 62% of which are methyl derivatives. The remaining 4 compounds (10.3%) were temporarily labeled 'un-identified' as further investigation is needed for characterization (Table 1).

About 21 (53.8%) of the identified compounds were common to all sampled musk categories (sex, age and seasons). Both adult male (n=7) and female (n=5) civets contained about 94.8% of the identified compounds; however, there are compounds unique to each sex. While Oleic acids and Squalene were recorded only from adult male civets heptadecane and 1,5,9-undecatriene, 2,6,10-trimethyl were identified only from adult female musk. Musk from sub-adult male and female civets contained 64.1 and 61.5% of the identified musk compounds, respectively. In scent marked musk samples, 64.1% and 56.4% of the compounds were observed for the wet and dry seasons, respectively. However, most of the low molecular weight organic acids were absent. Musk component variation among individuals in the same category (e.g. between adult males) was not computed. However, percent component overlap was highest between adult male and female categories (94.4%) and least between adult males and sub-adult females (Fig. 2).

Few major musk components from the African civet (such as 9-Cycloheptadecen-1-one, (Z); Octadecanoic acid; 9-Octadecenoic acid,(E); 9-Cycloheptadecen-1-ol; and Cycloheptadecanone) were more abundant (as a function of %TIC) and contributed between 64.6 and 80.93% of average percent area of chromatogram across all musk categories of which civetone shared the most (between 54.5 and 69.7%) (Table 2). Representative GC-MS chromatograms of musk components from the African civet are shown in Figure 3.

Table 1. Components of musk from the perineal gland secretion of the African civet (*Civettictis civetta*), and the relative proportion of peak area (% total ion concentration (TIC)) of each chromatogram. Musk sources (AM=adult male; AF=adult female; SAM=sub-adult male; SAF= sub-adult female). Seasons (WS= wet season; DS= dry season)

Retention time (minute)	Compounds	AM (n=7)	AF (n=5)	SAM (n=4)	SAF (n=3)	DS (n=53)	WS (n=83)
2.32	Butanoic acid	0.11	0.19	0.16			
3.29	Pentanoic acid	0.13	0.31		0.15		
5.54	Hexanoic acid	0.37	1.60		0.21		
11.67	Octanoic acid	0.82	0.71	0.30	0.43		
13.74	Decanoic acid	2.17	2.17			1.53	0.96
16.39	Benzaldehyde, 2, 4-dimethyl	1.05	1.39				
18.20	Dodecanoic acid	1.04	0.95		0.78	1.12	1.41
19.04	n-Decanoic acid	1.54	1.29		1.28		
19.15	9-Decenoic acid	0.20	0.37				
21.07	1H-Indole, 3-methyl	0.44	0.70	0.63	0.63	0.26	0.36
21.23	Phenol, 2-4-bis (1, 1—dimethyl)	1.95	1.89				
26.07	Heptadecane		1.35			0.72	0.79
30.75	6, 10, 14-Hexadecatrien-1-ol-3, 7, 11, 15, -tetramethyl	0.15	0.16	0.21			0.25
30.77	1, 5, 9-Undecatriene, 2, 6, 10-trimethyl-(Z)		0.29			0.27	
32.86	Tetradecanoic acid	0.73	0.50	0.51	1.94	1.78	1.15
33.03	(E, E)-7, 11, 15-Trimethyl-3methylene hexadeca-1,6,10,14-tetraen	0.26	0.18				
39.04	n-Hexadecanoic acid	1.33	0.94	0.39	1.42		0.99
39.21	Cyclohexadecanone	0.7	0.65	0.96	0.21	0.22	0.91
39.84	8-Cyclohexadecen-1-one	1.83	1.82	1.04	2.04	2.02	1.91
40.36	Cycloheptadecanol (dihydro-civetol)	0.24	0.30	0.62	0.91	0.19	0.32
40.89	9-Cycloheptadecen-1-ol	2.81	3.36	3.51	8.79	2.74	4.31
42.33	Cycloheptadecanone (dihydrocivetone)	5.47	6.09	9.82	4.12	4.20	4.24
43.04	9-Cycloheptadecen-1-one (Z) (civetone)	52.89	43.16	51.2	35.63	49.87	48.24
44.21	9-Octadecenoic acid (E)	6.08	4.39	1.81	8.16	0.64	2.01
44.91	Octadecanoic acid	14.62	12.53	8.45	5.73	17.33	12.67
45.57	Oleic acid	0.14					
47.79	Cyclopentadecanone (exalton)	0.27	0.43	0.47	0.23	0.24	0.36
47.68	Un-identified ^a	0.20	0.29	0.47	0.23	0.24	0.36
48.39	Un-identified ^b	1.86	2.11	1.93	2.04	2.02	1.91
49.93	Eicosanoic acid	2.23	3.30	4.06	1.84		0.98
54.79	Docosanoic acid	1.35	0.67	1.41	1.05		
55.86	2, 6, 10, 14, 18, 22-Tetracosahexaen, -2, 6,10,15 19,23-hexamethyl	0.21	0.32	0.1	3.32		0.24
57.18	Oleyl alcohol	0.12	0.16	0.2			0.19
58.44	Un-identified ^c	0.17	0.15	0.37	1.22	0.19	0.28
62.34	Oxirane-heptadecyl	0.21	0.15	0.5		0.11	
63.24	Un-identified ^d	2.13	1.07	2.10	0.17	2.94	1.41
65.51	Olean-12-en-28-al	0.18	0.40	0.5			
66.04	Squalene	2.73				0.42	0.62
67.16	Cholesterol	1.46	0.19			0.59	1.13

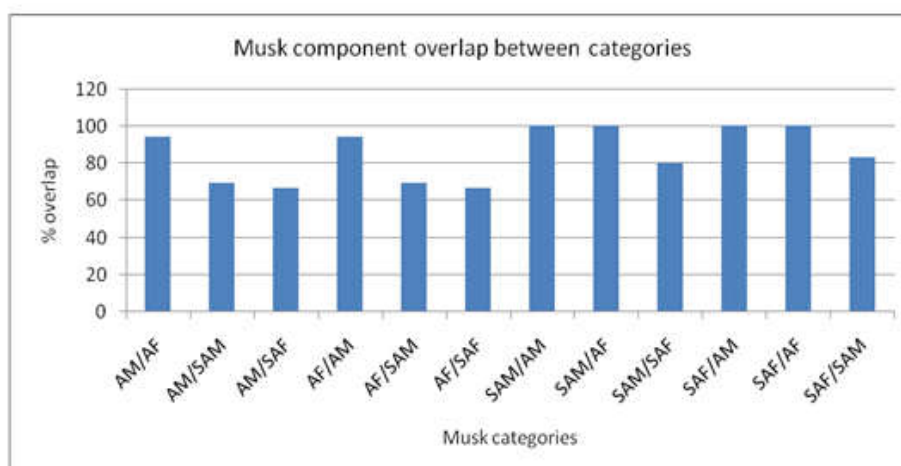


Figure 2. Percent components overlap among pooled musk of different categories. (AM=adult male; AF=adult female; SAM=subadult male; SAF= subadult female, and the slash (/) is to show the overlap of the top to the bottom

Table 2. Contribution (%TIC) of the five principal components in musk from the African civet (MAC) (*Civettictis civetta*) and the proportion of civetone in the representative musk categories (AM=adult male; AF=adult female; SAM=subadult male; SAF= subadult female; WS= wet season; DS= dry season)

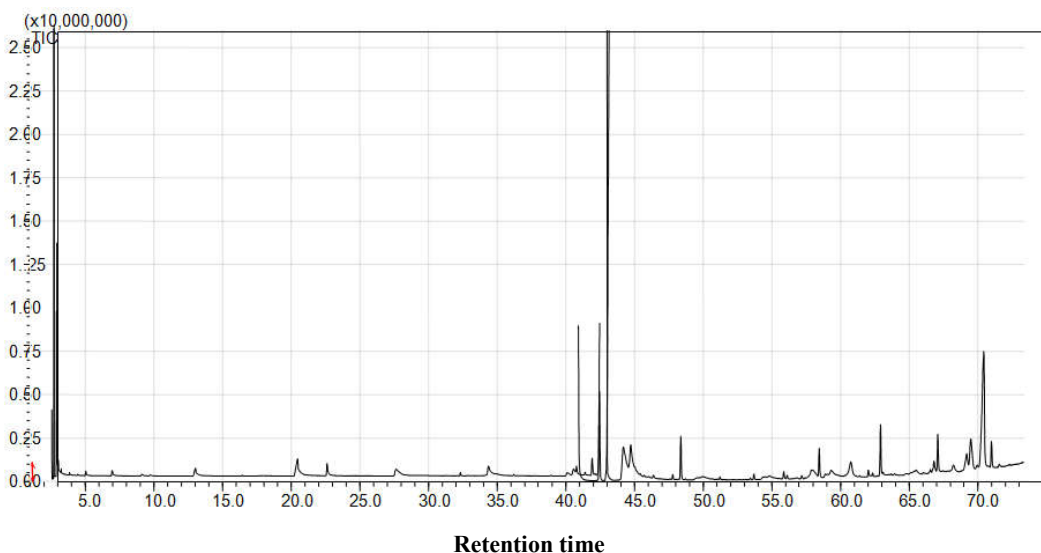
	Musk categories					
	AM (n=7)	AF (n=5)	SAM (n=4)	SAF (n=3)	WS (n=14)	DS (n=16)
Average total % TIC of the 5 -principal components	80.9	78.9	75.3	64.6	69.3	74.1
Proportion of civetone	63.4	54.7	68.1	54.5	69.7	67.3

Except for the abundance (%TIC) of few major compounds, the species specific chromatogram profile is similar along the categories (Fig. 3). The highest peak is for civetone and it is by far the most abundant compound in all musk categories (sex, age and seasons). Adult male civets comprised the highest proportion of civetone (52.89 %TIC) and followed by the sub-adult males. Sub-adult females comprised the least (35.63%TIC). %TIC of Civetone in scent marked musk, for both seasons, lie between the two extremes (Table 1).

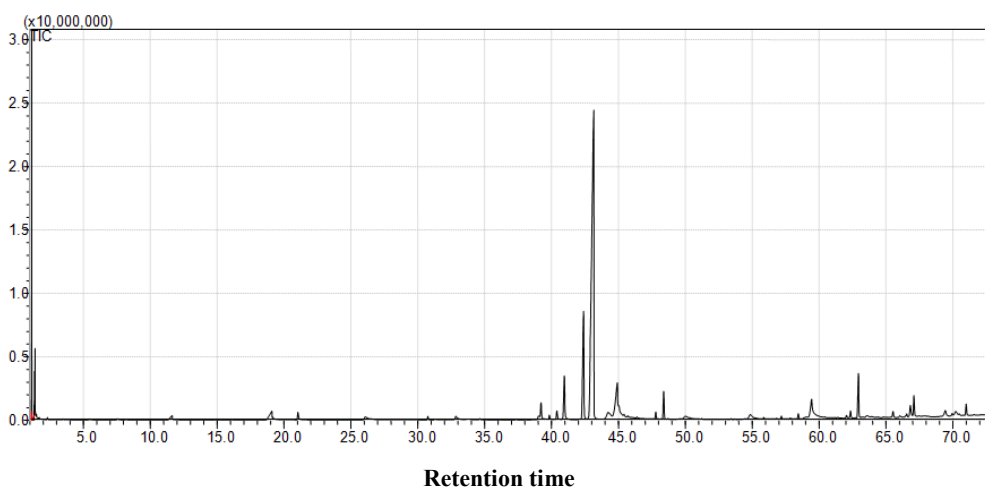
DISCUSSION

Ruzica *et al.* (1927) reported cyclic ketones, and non-specific free fatty acids, alcohols, indols and other compound classes in the musk from the three civet genera, *Civettictis*, *Viverra*, and *Viverricula*. Later, these were also reported by other workers (Jacob and Schliemann, 1983; Ding *et al.*, 1988). Wheeler *et al.* (1998) and Buesching *et al.* (2000) reported

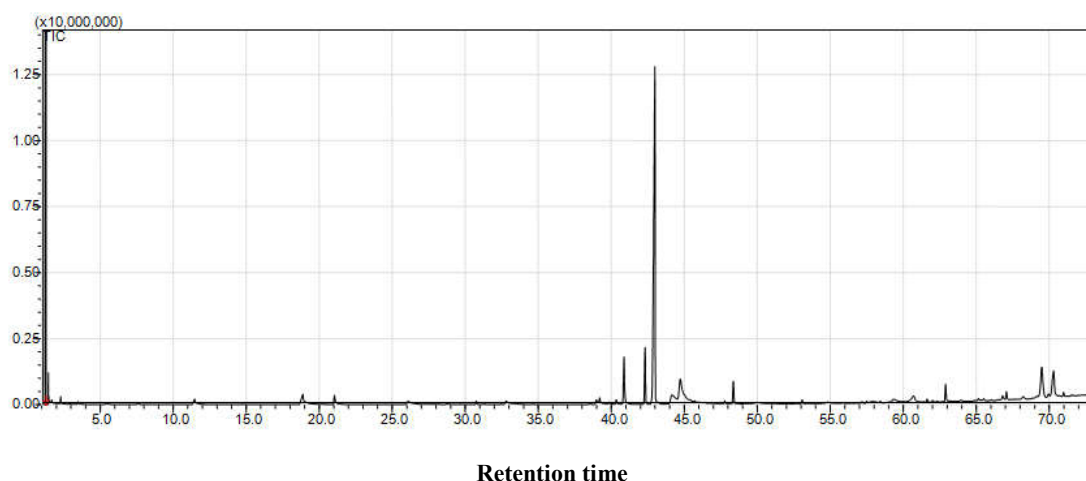
3a/ Adult male



3b/ Adult female



3c/ Sub-adult male



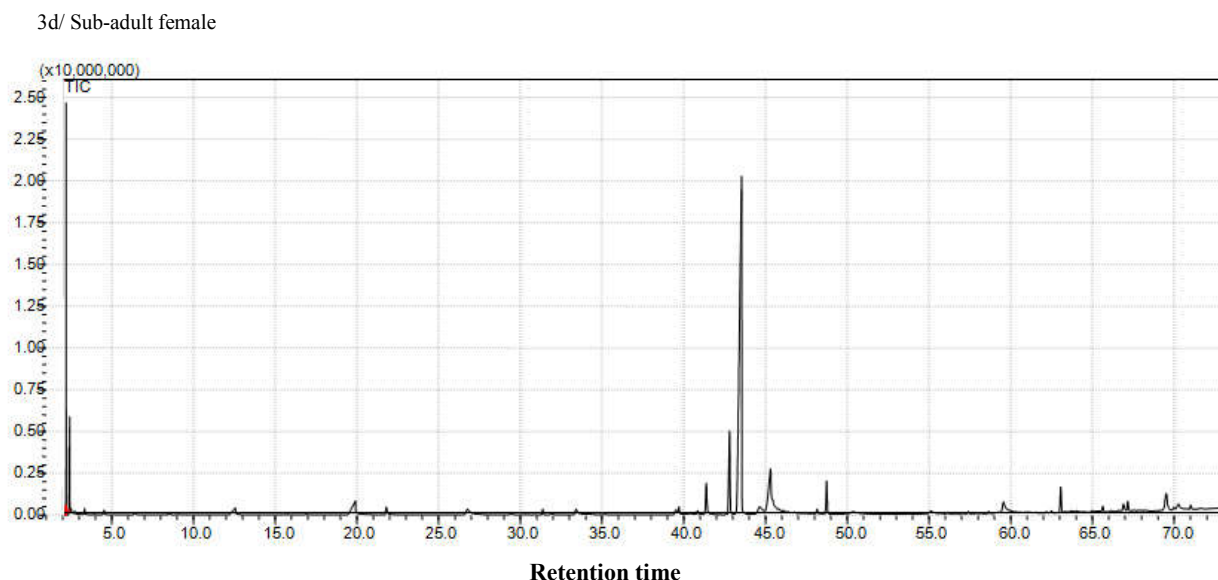


Figure 3. Representative chromatogram of musk for different sex and age categories of the African civet (*Civettictis civetta*). (3a= adult male, 3b=adult female, 3c=sub-adult male, 3d=sub-adult female); The Y-axis is for %TIC and X-axis represents retention time. 3a/ Adult male

Cycloheptadecanone (dihydrocivetone), 2-undecanone, 2-tridecanone, 2-pentadecanone and 2-heptadecanone as the principal components of musk from the palm civet (*Pagumalarvata*). Recently, Duangyod *et al.* (2011) reported Civetone, Dihydrocivetone and Normuscone as the three principal components of musk from captive small Indian civet (*Viverricula indica*). The present study is the first comprehensive report of the chemical composition of the African civet musk. In this study, over 65 different compounds were recorded using GC-MS. However, only 39 compounds met the minimum criteria, i.e., consistent occurrence in all samples with abundance (% TIC) greater than 0.1% (Table 1). Previously reported major musk components from all civet genera were also found to be dominant in the present study, however, none of the four straight chained ketones (2-undecanone, 2-tridecanone, 2-pentadecanone and 2-heptadecanone) (Wheeler *et al.*, 1998; Buesching *et al.*, 2000) were recorded in musk from the African civet. Most acids appeared at relatively lower retention time on the chromatogram. Organic acids are quite abundant in secretions of a wide variety of mammals (e.g. Knapp *et al.*, 2006; MacDonald *et al.*, 2007; Scordato *et al.*, 2007; Rosell *et al.*, 2010), however, acids in the African civet musk (39.5%) are more abundant and relatively heavier, with molecular weight ranging from 88 to 284 (for Butanoic and Octadecanoic acids, respectively). The absence of low molecular weight acids from scent marked musk samples indicates their fast volatility. Fast volatile compounds in scent marks serve as a long range scent attraction for the conspecifics (Muller-Schwarze, 2006).

Hence, early eluting low molecular weight acid compounds in musk from the African civets may have similar behavioral functions. Long-chain alcohols are rare in many mammalian exocrine secretions (Burger, 2008). Musk from the African civet, however, contains a high molecular weight macro-cyclic alcohol with molecular weight ranging between 252 (9-Cycloheptadecen-1-ol) and 382 (Cholesterol), probably contributing for the special musky flavor.

Muller Schwartze (2006) reported the abundance of such long chain heavy alcohols in Musk-ox. Cyclic ketones in musk from the African civet are moderately heavy, with molecular weight ranging from 224 (Exalton) to 252 (Dihydrocivetone). Short to medium straight chain ketones have been reported for a number of mammals (Burger *et al.*, 2001). Cyclic ketones, however, are rare (Ruzika *et al.*, 1927; Ding *et al.*, 1988; Wheeler *et al.*, 1998; Duangyod *et al.*, 2011). Musk from the African civet, however, is rich in cyclic ketones. In mammalian secretions, compounds with higher molecular weight are known to form greasy mixtures, which were reported to serve as fixatives and delay the volatility of the lighter components of scent marks (Burger *et al.*, 2008; Rosell *et al.*, 2010). In this study, relatively higher concentration of cyclic ketones has been observed in samples collected from environmental signposts and may serve similar scent delaying functions in the African civet to which adult individuals are more responsible.

The 8 miscellaneous organic compounds contributed about 20.5% towards the total number of compounds in the African civet musk. The four 'unidentified' groups have 10.3% compound share (Table 1). These compounds were uniformly recorded in chromatogram of most musk samples; however, their mass spectral data could not be matched with the compounds in the library with certainty. For scent marking mammals, most compound groups are common to all members and serve as a species scent signature (Palagi and Dapporto, 2006). Likewise, the 21 compounds (53.8%) found in samples from all sex and age categories of civets, may serve in signaling species-specific information. However, some specific compounds occur as an individual fingerprint, coding for and establishing individual differences (Buesching *et al.*, 2000). This may result from the presence or absence of specific compounds, variation in relative abundance of common compounds or from the complex interactions of these (Zhang *et al.*, 2002).

In the present study, components of musk from adult male African civets were considered as a base line to compare all other musk categories. With two compounds unique to each sex (Squalene and Oleic acid for male and Heptadecene and 2, 6, 10-trimethyl-(Z) for female), most musk compounds recorded during this study were present in their compound list. The two unique compounds may signal information specific to the sexes. The sub-adult members of the African civets have relatively less musk components (Table 1). Sub-adult males lack many of the lower molecular weighted organic acids and late eluting heavy compounds. The long range scent communication and scent delaying in African civets may develop with age. However, like the adult males, the %TIC of major musk components and the concentration of civetone were significantly higher for sub-adult males than sub-adult females (Table 1). Quite surprisingly, the %TIC of civetone in musk was significantly higher in sub-adult male than both in adult females ($\chi^2=23.04$, 1df, $P<0.01$) and sub-adult females ($\chi^2=23.14$, 1df, $P<0.01$). Sub-adult female civets have relatively higher proportion of two compounds (9-Cycloheptadecen-1-ol and 9-Octadecenoic acid (E)). The behavioral significance of the abundance of these compounds is not known, however, sub-adult females have relatively the least amount proportion of civetone.

The other distinguishing features of musk from the African civet was the presence of exceptionally high proportion of few compounds accounting between 64.6 and 80.9% total %TIC, of which civetone is the highest (between 54.5 and 69.7%) in all musk categories (Table 2). Some studies reported the variation in principal musk components between sexes (Duangyod *et al.*, 2011). In this study, civetone was the principal musk component and its proportion varies between sexes, less for adult and sub-adult females. The presence of several cyclic compounds is also the other unique feature of musk from the African civet. The change in patterns of scent marking and the associated behavioral responses are reported to follow the seasonal pattern of reproductive physiology (Drea, 2007). Musk samples from captive African civets were not collected on seasonal basis. However, the two season musk data from environmental signposts showed no distinct seasonal variation in the chemical profiles. Most major musk compounds were recorded but with relatively fewer components (25 for the wet and 22 for the dry season). It was observed that, most organic acids of lower molecular weight were absent for both, but more during the dry season. Ketones, alcohols, some acids and most of the higher molecular weight musk compounds, however, were all recorded from both seasons. The early eluting low molecular weight organic acids were reported to be lost at ambient temperature (Drea, 2007), and high ambient temperature enhances the rate of volatility of scent marks (Muller-Schwartz, 2006).

The present observation was in line with these reports. On the contrary, the concentration of the musk principal component (Civetone) was relatively high in musk sampled from environmental sign posts (Table 1). This high concentration may have resulted from the loss of excess moisture and fast volatile compounds. Variation in the rate of scent marking during different seasons was reported for several species (Svendsen, 1980; Rosell *et al.*, 2002). In the present study,

from the specified and permanently visited musk sign post sites, the number of observed scent marks were significantly higher during the wet season than the dry season (83/53) ($\chi^2=6.18$, 1df, $P<0.05$). Two probable reasons for this may be the rapid wash away of fresh scent marks by the heavy summer rain of the area, and low volatility of musk components due to reduced ambient temperature. In both cases, duplicated marks, in shorter spatial distance, may be the solution. Chemical communication is the only channel to convey basic information between nonspecific of the solitary and extremely secretive larger African viverrid. The seasonality and the specific role of the major musk compounds in altering the reproductive and other behavioral repertoires need detailed studies both in the wild and in captivity.

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