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## GC-MS analysis reveals *Dendrobium candidum* is a mosquito-attractant orchid with mosquitocidal compounds

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**Abstract**

Mosquito, the infamous vector organism, is responsible for outbreaks of several deadly epidemics. Semiochemicals or pheromones and some other compounds released from several plants and animals have ability to attract mosquitoes. Heneicosane is one of such compounds reported from some plants that attracts mosquitoes, especially *Aedes* spp. (vector of dengue). Through our experiment based on GC-MS analysis, we report that leaf of *Dendrobium candidum* Wall. ex Lindl, a local orchid of Darjeeling Himalayas, is a source of heneicosane along with several other semiochemicals and odorants that can target mosquitoes. Interestingly, the orchid sample has revealed a number of components having insecticidal activities against mosquitoes which can have made this orchid a complete mosquito-bait. This research invites interest for *Dendrobium candidum* as a mosquito attractant and killer. For this unique property, we recommend this orchid leaf extract as a bait for dengue vector *Aedes* mosquito.

**Keywords:** Dendrobium; orchid; mosquito; dengue; semiochemicals; GC-MS

**1. Introduction**

Mosquitoes are notorious for spreading diseases like dengue, malaria, chikungunya, yellow fever, Zika virus and so on by biting human beings. They also transmit several diseases and parasites to which animals like dogs and horses are very susceptible e.g. dog heartworm, West Nile Virus etc. Mosquito control managements like source reduction, biocontrol, larviciding, adulticiding are followed depending on the situation by using pesticides, biological-control agents, trapping etc. Among them use of biological agents; like plant extracts, larvae eater fishes (e.g. mosquitofish, cyprinids, tilapia etc.), several parasites, pathogens, predators and traps; are well known as eco-friendly or environmentally appropriate methods. Using of orchids as baits in mosquito trap is a new concept where researches are on its peak nowadays. Orchids are famous to lure potential pollinators by offering different types of smells and studies have claimed that orchids emit an odor just like the human body to attract mosquitoes [1, 2]. However, association between orchids (*Platanthera obtusata*) and mosquitos (*Aedes* spp.) are reported, as well as orchid's floral scent compounds that can attract diverse mosquito species [3]. *Dendrobium candidum* Wall. ex Lindl (*Dendrobium candidum*), also called Shihu (in China) and Sekkoku (in Japan), is an orchid found in Japan, Korea, China and the Himalayas which is reported to be used as perfume in Japan and traditional Chinese medicine due to bioactive constituents [4, 5]. Recently, Bhattacharya *et al.* [6] have done a pioneer research on indigenous *Dendrobium* orchids collected from Darjeeling Himalayas to investigate their bioactivities where they reported *Dendrobium candidum* as the most potent free radical scavenger among twelve *Dendrobium* species. But no research on metabolomics has been done with this orchid, so far. In this article we have examined *Dendrobium candidum* leaf extract by GC-MS to get into its metabolomics which insights this orchid's unexplored anti-mosquito activity.

**2. Materials and methods****2.1 Sample collection and preparation**

Following the protocol of Bhattacharya *et al.* [6], few leaves were plucked from a healthy

*Dendrobium candidum* plant [6] from the wilds of Darjeeling Himalayas where utmost care was taken to the plant body during collection because orchids are very rare and restricted in availability. Plucked leaves were collected in a zipper bag and preserved soon in ice packed boxes. Isolated leaves were washed under cold tap water and crushed just before dipping overnight into methanol, a polar and aprotic solvent, to prepare *Dendrobium candidum* leaf extract or DCLE.

## 2.2 Gas chromatography-mass spectrometry analysis

1 µl of DCLE was injected in split mode in the instrument (GCMS-QP2010 Plus). Injection temperature was 260°C and interface temperature was set to 270°C. Ion Source temperature was adjusted to 230°C. Helium was used as carrier gas. Total flow rate was 16.3 ml min<sup>-1</sup> and column flow rate was 1.21 ml min<sup>-1</sup>. Mass spectra were recorded at 5 scan sec<sup>-1</sup> with a scanning range of 40-650 m/z. Quantification of compounds was done on the basis of their peak areas [7]. The data obtained from GC-MS analysis were further analysed by studying available literatures and “The Pherobase” database.

## 3. Results

GC-MS of DCLE revealed twenty one different compounds (Table 1) in the chromatogram with twenty nine peaks (Figure 1). Among them seventeen compounds, sharing a total of 94.61%, are directly or indirectly associated with mosquito (Table 1) where twelve are attractants of different insects including mosquitoes, known as semiochemicals (cumene, mesitylene, sabinene, durenene, dodecane etc.) and seven compounds (including two of the abundant compounds; tetradecane and pentadecane) with 45.46% of share in total extract are known mosquitocides, either larvicides or adulticides; shown in Table 1. Furthermore, sabinene, dodecane, germacrene D, neophytadiene and methyl linoleate; these compounds have both mosquito baiting and mosquitocidal activities. Esters of linoleic acid are reported as *Aedes* spp. pheromones and odor active chemicals [8] and, in our result, we have found one of its derivative methyl linoleate which also exhibit the same role. Thus, it is supposed to be a compound with all mosquito-associated properties (Semiochemical, odorant and mosquitocidal) just like dodecane (Table 1).

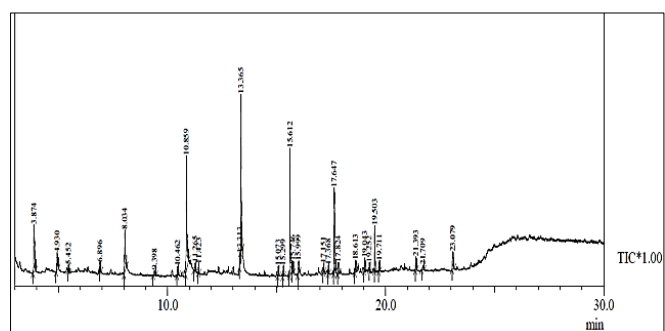
The major compound associated with this extract was heneicosane (Figure 2) because of its abundance (with 28.82% peak area) as well as reported oviposition attractant pheromone of *Aedes* spp. larvae [9], the mosquito responsible for diseases like dengue, Zika virus and chikungunya. Table 1 is presented in order to show and compare the amount of each compound and each type of compound regarding their association with mosquitoes as described. Furthermore, semiochemicals are not the only reason behind mosquito baiting properties of this orchid but compounds like 3-hexen-1-ol, propanoate, (z)-; 1,2-benzenedicarboxylic acid, diethyl ester; and 1,2-benzenedicarboxylic acid, diisooctyl ester; can also be accepted in this category because carboxylic acids, lactic acids and other acidic volatiles are responsible for human odors which elicit both electrophysiological and behavioural responses in mosquitoes [10]. Moreover, dodecane, tetradecane, pentadecane and heneicosane, the four major previously mentioned mosquito-associated-compounds of *Dendrobium candidum* leaf can also exhibit human odor properties [11] to attract mosquitoes. All together there are

eight compounds occupying 74.67% area in GC-MS chromatogram of DCLE, are marked as significant odorant chemical that can attract mosquitoes (Table 1).

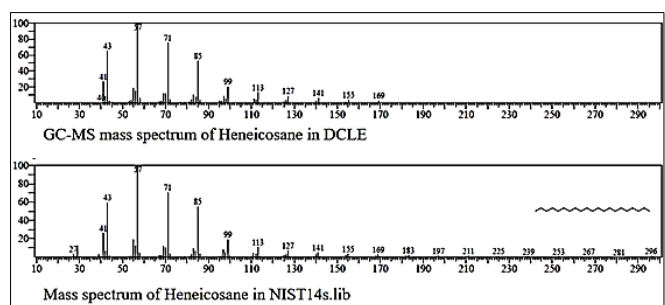
## 3.1 Tables and Figures

**Table 1:** DCLE compounds and activities on the basis of their association with mosquito; S= semiochemicals or attractants; M= mosquitocides; O= odorant and Oa=oviposition-attractant.

Amounts	Name	Mosquito associated activities
7.86	Cumene	S [12]
2.97	Mesitylene	S [12]
0.85	Sabinene	S [12]; M [13]
0.95	Durenene	S [14]
6.03	Dodecane	S [12]; M [15]; O [11]
0.53	3-hexen-1-ol, propanoate, (z)-	O (being a carboxylic acid)
1.09	Octane, 3-ethyl-2,7-dimethyl-	S [12]
17.41	Tetradecane	M [16]; O [11]
1.42	Germacrene D	S [12]; M [17]
0.59	Diethyl phthalate	O (being a carboxylic acid)
17.2	Pentadecane	M [16]; O [11]
1.73	Octane, 2-methyl-	S [12]
1.79	Sulfurous acid, 2-ethylhexyl isohexyl ester	S [18]
28.82	Heneicosane	Oa [9]; O [11]
2.25	Dodecane, 2-cyclohexyl-	No activity reported
1.28	Neophytadiene	S [12]; M [19]
1.26	Cyclopentane, 1-hexyl-3-methyl-	No activity reported
1.27	Methyl linoleate	S [8]; M [20]; O [8]
1.41	Octadecane, 5-methyl-	No activity reported
0.48	Cyclohexane, eicosyl-	No activity reported
2.82	Diisooctyl phthalate	O (being a carboxylic acid)



**Fig 1:** GC-MS chromatogram of DCLE



**Fig 2:** GC-MS mass spectrum of major DCLE compound heneicosane

## 4. Discussion

After studying components of DCLE on their association with mosquitoes, *Dendrobium candidum* orchid leaf is almost

ready to grab the crown of a complete anti-mosquito biological agent; as its compounds can attract mosquitoes; can kill their larvae and/or adults; and some can exhibit both properties. Interestingly, among these active compounds; dodecane (6.03%), tetrad cane (17.41%), pentad cane (17.2%) and heneicosane (28.82%); are four abundant compounds which are structurally long chains of alkanes, known to be biosynthesized in cutin, suberine and wax biosynthesis pathway. So, this plant has a way to release those volatile semiochemicals and odorants easily to attract mosquitoes without depending upon photosynthesis or respiration or any other physiological processes as the lion share in the chromatogram is comprised of cuticular hydrocarbons (compounds from outer parts of leaves).

Compound heneicosane (28.82% in our CJLE) is well established as one of the vital *Aedes* spp. pheromone which attract them to lay eggs in the fresh stagnant water [21] and might regulate the outbreaks of dengue or other *Aedes* borne diseases but there was no report on it, so far. After extending our study on this compound and its connection with mosquitoes, we have found *Periploca laevigata* Aiton, Hort. Kew. 1: 301 (1789), locally called cornicabra (Spanish) was the only plant, reported till date, to contain heneicosane as the major compound in its leaves (38.2%) and other aerial parts (11.6% in branch; 24.1% in flower, 9.4% in fruit) [22]. This plant is native to “Macaronesia ecoregion”, more specifically Canary Islands, Savage Islands of Madeira [23] and Cape Verde [24]. Interestingly, these places are reported with significant spread of *Aedes* spp. and their diseases as large dengue epidemics were seen on three of those Macaronesian islands e.g.; Cape Verde (Dengue virus-3), Madeira (Dengue virus-1) and Canary Island [25, 26, 27]. Moreover, places of Macaronesia ecoregion are reported with dengue-4 virus [28], Zika virus [29], yellow fever and chikungunya [25] also.

According to scientists, climate change and introduction of infected people, are only valid reasons behind transmission and out breaks of these mosquito-borne diseases [27], no doubt, but plants like *Periploca laevigata* or *Dendrobium candidum* rich in *Aedes* mosquito attractant compounds like heneicosane are also able to play a considerable role in increasing the probability of spreading epidemics, such as dengue. And, this hypothesis has to be taken considered as a recent report states that *Aedes aegypti* from those places, mentioned before, are able to spread diseases like yellow fever, chikungunya and Zika virus also, pinpointing life threatening risks [30]. This study suggests that orchids may be playing the major role to spread dengue by attracting *Aedes* mosquitoes in this region (Darjeeling Himalayas and sub-Himalayan plains of North Bengal in India). *Dendrobium candidum* and other heneicosane rich plants of their native places can lead the same life threatening risks if proper mosquito control management is not taken. But, in this case, *Dendrobium candidum*, it can exhibit the solution of this problem, unlike other heneicosane rich plants, its leaves are rich in anti-mosquito compounds as well which can be used as biological mosquitocide too. Moreover, being eco-friendly, mosquitocides with plant extract is a good option and in a recent study [31] it was seen that orchids combined with bacteria can be source of novel nanoinsecticides for its efficacy against dengue, malaria and filariasis mosquitoes.

## 5. Conclusions

There is an urgent need to develop new anti-mosquito active

products. Odor-based traps and bio-controlling agents are in demand as mosquito vectored diseases are increasing and getting severe by changing epidemiology. In tropical countries, diseases spread by mosquito, dengue in particular, is a vital problem causing number of deaths on post monsoon periods on every year. Furthermore, in our study on source of pheromone like heneicosane, we have interpreted that not only climate change and human interference are responsible for dengue related diseases but also mosquito attractant native plants have much role to play in influencing lifecycle of vectors to spread diseases. At last but not least, in some places these orchids are very rare and restrictive, so, *in-situ* conservation, *in vitro* micropropagation techniques like tissue culture for *ex-situ* conservation etc. are needed, along with establishing germplasm banks, so that, more experiments beyond metabolomics can be done to implement orchids in bio-controlling of mosquitoes.

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