IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, Dec 2022

Is there a connection between species dispersal and its seed size? : A case study in genus *Habenaria* (Orchidaceae)

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Abstract:

The family Orchidaceae is well recognized for its very small, dust-like seeds, which have round to oval endosperm encased in a thin, translucent fusiform testa. These traits demonstrate the sophisticated nature of families and their patterns of development. Since many of the features utilized in seed morphometry have a taxonomic significance, microscopic investigation is used in the current work to examine seeds from the genus *Habenaria*, family Orchidaceae. Quantitative and qualitative data pertaining to the general morphology of the seed is discussed. This includes data about the seed's surface characteristics, such as size, shape, visibility of the embryo, and variation between the seed's length/width ratio at its maximum and minimum. The findings indicate that higher seed volume is the outcome of both bigger length and breadth, according to data on seed volume. Compared to the testa, the embryo's volume is significantly less. Because of this, seeds contain a lot of internal air passages that give them a balloon-like shape. This characteristic helps seeds disperse by air and water currents over great distances. While seeds with modest sizes stay endemic to a particular locality, seeds with maximal length and width disseminate to larger geographical locations. Seed buoyancy is increased by increasing the ratio of seed to embryo air percentage. The current study establishes a favorable link between seed buoyancy and distribution pattern. Because of its longer and flyer-type seeds, *Habenaria digitata* and *H. gibsonii* are extensively dispersed, but *H. grandifloriformis* is still confined to a particular location because of its relatively small seeds.

Keywords: *Habenaria*, scanning electron microscope, seed morphometry, Western Ghats.

Introduction:

The tiny, dusty seeds of the orchid family, orchidaceae, have round, oval, or ellipsoidal endosperm that is encased in a thin, translucent fusiform testa. The elegant nature of the family and its developmental patterns are reflected in this character. The length and breadth of these seeds vary significantly, which is noteworthy from a taxonomic standpoint at the genus and species levels (Arditti & Ghani, 2000). Numerous researchers (Clifford & Smith, 1969; Vij et al., 1992; Rasmussen, 1995; Swamy et al., 2004 and Verma et al., 2013) have shown a clear relationship between orchid seed size and plant habit: epiphytic orchids often have smaller seeds than terrestrial orchids. The information on the numerical and physical properties of orchid seeds provided by Arditti & Ghani

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(2000), however, indicates that this is not a norm. After examining the size of numerous orchid seeds, Molvray & Kores (1995) reached their conclusion that, on average, seed size varies from 0.5 to 0.8 mm depending on the species taken into consideration. According to studies performed by Arditti et al. (1979) and Vij et al. (1992), seeds of advanced Epidendroid orchids (Epidendroideae) vary greatly in shape, with fusiform seeds often showing the least variance among primitive orchids (Cypripedioideae). All other seed forms may have evolved from fusiform seeds, which are found in all of the subfamilies, as a primordial trait (Arditti et al., 1979; 1980; Healey et al., 1980; Rasmussen 1995; Verma et al., 2013). They proposed that a more accurate indicator of orchid seed size should be the volume, not the length or breadth. Additionally, it has been suggested by Arditti et al. (1980) and Augustine et al. (2001) that the length/width ratio might reveal information on the relative degree of truncation of orchid seeds. Linguistically speaking, truncated seeds are those whose L/W value is less than 6.0, whereas elongated seeds are those whose L/W value is greater than 6.0. Using light and scanning electron microscopes, Arditti et al. (1980) and Barthlott and Ziegler (1981) examined a variety of seed characteristics and provided a thorough explanation of the structure and varieties of seeds. The majority of the currently examined species have quadrilateral testa cells. Prior research by Clifford & Smith (1969), Vij et al. (2006), and Verma et al. (2013) revealed that fusiform quadrilateral testa cells are often found in epiphytes and are regularly seen in ground-growing taxa. According to Kurzweil (1993), the seed coat of orchids might consist of variously shaped and decorated cells, which are typically elongated and concave, with straight or slightly undulating anticlinal walls. A correlation between seed size and volume was postulated by Arditti et al. (1979). They contend that there is a direct proportionality between seed size and volume. Higher "seed volume/embryo volume" ratio seeds are predicted to be more buoyant and to belong to more widely spread species than lower ratio seeds. Studies on seed morphometry, according to Augustine et al. (2001), offer incredibly helpful information in resolving various taxonomy, phylogeny, and phytogeographic issues. The majority of the previous researchers concentrated on and talked about the importance of the air space that exists in orchid seeds, which makes the seeds incredibly light and buoyant. Even Augustine et al. (2001) proposed that whereas seeds with less air space exhibit a more constrained and confined distribution, extremely light and buoyant seeds with a higher proportion of air space may get scattered over a broad variety of geographical locations. According to Arditti & Ghani (2000), an increase in the testa's cell length is the cause of the rise in the proportion of air space.

With the exception of a few instances of research on the use of seed morphometry, the genus *Habenaria* is either completely disregarded or inadequately investigated for seed characteristics and seed morphometry as a taxonomical tool. Therefore, an effort is made in this work to thoroughly examine the different characteristics of seeds and how they relate to the distribution pattern of species within this underappreciated genus.

Material and Methods:

From 2014 to 2021, mature seeds from eighteen different species of *Habenaria* were gathered from both the Departmental Botanical Garden's germplasm bank and the spontaneously dehiscing capsules found in the wild. Light microscopic investigations and photography studies of 18 species were carried out under an Olympus DM2000 compound microscope. Using light microscopes, investigators examined both qualitative and quantitative data regarding the general morphology of the seed, including seed surface characteristics like size, shape, color, visibility of the embryo, testa structure, curvature, and ridges, ornamentation of the periclinal and anticlinal walls, and variation in the length/width ratio of the seed. The observation table contains the observations that were made. Since the different morphometric characteristics vary greatly within a single species, substantial means have been obtained by observing a large number of seeds. Since the cross section of orchid embryos is elliptical, the formula $4/3\ \pi ab^2$, where a = ½ for length and b = ½ for width, was used to compute the embryos' volume. The terms used here to refer to the morphology of seeds and other finer characteristics in SEM are those recommended by Arditti et al. (1980).

Results and Discussion:

Seed shape **(Table 1 and Plate I)**

Three basic seed shapes are revealed by microscopic studies: (1) spathulate in *Habenaria brachyphylla*, *H. grandifloriformis*, *H. roxburghii*, *H. suaveolens*, and *H. rariflora* (Plate I. a, i, and p-r); (2) fusiform in *Habenaria* commelinifolia, *H. crinifera*, *H. heyneana*, *H. longicorniculata*, *H. longicornu*, *H. marginata*, and *H. plantaginea* (Plate I. b, c, j-m, and o); and (3) filiform in *Habenaria* digitata, *H. foetida*, *H. furcifera*, *H. gibsonii*, and *H. ovalifolia* (Plate I. d, f-h and n). All species have centrally placed, oval to elliptical embryos that are visible.

Seed size (Table 1)

The length of the seeds varies between *Habenaria grandifloriformis* (0.29 ± 0.14 mm) and *H. digitata* (1.69 ± 0.35 mm). *H. commelinifolia* has a width of 0.08 ± 0.02 mm, whereas *H. diphylla* has a width of 0.33 ± 0.02 mm. The species under study may be divided into three different categories according to the length of the seeds: tiny (up to 0.7 mm), intermediate (0.7 to 0.9 mm), and big (0.9 mm to 2.0 mm). Category 1 includes *Habenaria brachyphylla*, *H. diphylla*, *H. grandifloriformis*, and *H. roxburghii* (Plate I. a, e, i, and q), which are species with dorsiventrally flat leaves on the ground. *H. commelinifolia*, *H. crinifera*, *H. heyneana*, *H. longicorniculata*, *H. longicornu*, *H. marginata*, *H. rariflora*, and *H. suaveolens* (Plate I, b, c, e, k-n, p, and r) are included in group (2) *H. digitata*, *H. foetida*, *H. furcifera*, *H. gibsonii*, and *H. ovalifolia* were identified in category (3) (Plate I. d, f-h and n). The bulk of the species investigated in the current study belonged in the intermediate group. The findings align with the findings reported by Molvray and Kores (1995). Therefore, differences in the size and form of seeds in *Habenaria* can serve as an extra taxonomic marker for species identification.

Ratio of seed length to width (L/W) (Table 1):

H. commelinifolia, *H. digitata*, *H. foetida*, *H. furcifera*, *H. gibsonii*, *H. ovalifolia*, and *H. rariflora* were found to have elongate seeds in the current study, in accordance with Arditti et al. (1980) and Augustine et al. (2001). In contrast, *H. brachyphylla*, *H. crinifera*, *H. diphylla*, *H. grandifloriformis*, *H. heyneana*, *H. longicorniculata*, *H. longicornu*, *H. marginata*, *H. plantaginea*, *H. roxburghii*, and *H. suaveolens* displayed truncate seeds. *H. gibsonii* had the highest L/W ratio (17.67 ± 3.84), while *H. diphylla* had the lowest ratio (1.75 ± 0.69). The degree of seed truncation is a unique taxonomic characteristic that may be utilized to distinguish across *Habenaria* species.

Seed Volume (Table 1)

The volume and size of the seed have a link, according to Arditti et al. (1979). They contend that there is a direct proportionality between volume and size. Working with the species *Bulbophyllum*, Augustine et al. (2001) discovered that longer testa is not the cause of larger seed volume; rather, it is the wider testa. Identical findings were seen in the current study. The species *H. diphylla* has the highest seed volume (16.97 ± 2.14 mm3 × 10-3) because of its wider seeds (0.31 ± 0.12 mm), whereas the species *H. crinifera* has the lowest seed volume (0.08 ± 0.03 mm) of all the species examined.

Quantative characters of embryo: (Table 2)

In every species under study, variations have been noted in the length, width, and length/width (L/W) ratio of the embryo. differences in breadth are 0.06 ± 0.01 mm to 0.19 ± 0.10 mm, while differences in length range from 0.17 ± 0.09 mm to 0.34 ± 0.17 mm. *H. roxburghii* has the longest embryo (0.34 ± 0.17 mm), while *H. grandifloriformis* has the shortest (0.17 ± 0.09 mm); *H. roxburghii* has the widest embryo (0.19 ± 0.10 mm), while *H. crinifera* has the smallest (0.06 ± 0.01 mm). In *H. diphylla*, the L/W ratio is 1.09 ± 0.09, whereas in *H. crinifera*, it is 5 ± 2.98. Most of the species have an L/W ratio of one to two. According to Healey et al. (1980), the embryo's volume varies from species to genus in orchids, although the embryo's size tends to be consistent within a genus. When working on the genus *Bulbophyllum*, Augustine et al. (2001) provided strong support for this approach. According to Arditti et al. (1980), there may be significant differences in the quantities of seeds and embryos as well as the percentage of air space across several populations within the same species. The current study validates the research conducted by Augustine et al. (2001) and Healey et al. (1980). In *Habenaria*, embryo size is generally consistent within a species, although embryo volume varies widely. According to Augustine et al. (2001), there can often be a significant amount of variance in the volume of the orchid seed and embryo. In most orchid seeds, the embryo only takes up a relatively tiny percentage of the seed; but, in certain *Bulbophyllum* species, the embryo is enormous and takes up a significant portion of the seed. Similar findings were noted in a few *Habenaria* species.

The embryos of *Habenaria grandifloriformis*, *H. rariflora*, and *H. suaveolens* occupy a significant percentage of the seed, contributing to the seeds' rising weight.

Seed volume to embryo volume ratio (Table 2)

According to Augustine et al. (2001), there can often be a significant amount of variance in the volume of the orchid seed and embryo. In most orchid seeds, the embryo only takes up a relatively tiny percentage of the seed; but, in certain *Bulbophyllum* species, the embryo is enormous and takes up a significant portion of the seed. Similar findings were noted in a few *Habenaria* species. The embryos of *H. grandifloriformis*, *H.*, *H. rariflora*, and *H. suaveolens* occupy a significant percentage of the seed, contributing to the seeds' rising weight.

Seed volume to embryo volume ratio (Table 2)

The embryo volume (mm3 × 10-3) varies from 0.4 ± 0.09 in *H. gibsonii* to 16.29 ± 4.36 in *H. diphylla*, while the seed volume (mm3 × 10-3) varies from 0.86 ± 0.12 (*H. crinifera*) to 16.97 ± 2.14 (*H. diphylla*). An increase in embryo volume causes a decrease in air space and a rise in seed weight; conversely, a drop in embryo volume and an increase in seed volume increase the percentage of air space, which causes a decrease in seed weight. *H. gibsonii* and *H. furcifera* were discovered to have the greatest ratios of seed volume to embryo volume (6.63 and 6.57, respectively) in the current investigation, which contributed to their widespread dispersion across India. *H. brachyphylla* and *H. roxburghii*, on the other hand, have the lowest S/E volume ratios (1.2 ± 1.02 and 1.24 ± 0.65, respectively), while having a restricted distribution.

Air space (Table 2):

The relevance of air space in orchid seeds—which contributes to their extreme lightness and buoyancy—has been covered by Arditti et al. (1980), Arditti & Ghani (2000), and Augustine et al. (2001). Arditti and Ghani (2000) proposed that a rise in the testicular cell length is the cause of the proportion of air space increasing. In this study, the highest proportion of air space in seeds was found in *H. gibsonii* (84.91 ± 11.34%), followed by *H. furcifera* (84.77 ± 9.45%), while the lowest percentage of air space was found in *H. grandifloriformis* (0.86 ± 0.46%).

Conclusion:

It has been established via the current morphometric investigations on *Habenaria* seeds that the different traits investigated have diagnostic significance and can be utilized for both the Phytogeographical studies of species and the taxonomical separation between diverse species. According to the L/W ratio, seven species have truncated seeds, whereas the remaining species have elongated seeds. The long-distance dispersal of the species is facilitated by longer seed lengths, a higher ratio of seed to embryo volumes, and a higher percentage of air space. Higher seed volume is the outcome of both bigger length and breadth, according to data on seed volume. Compared to the testa, the embryo's volume is significantly less. Because of this, seeds contain a lot of internal air passages that give them a balloon-like shape. This characteristic helps seeds disperse by air and water currents over great distances. While seeds with modest sizes stay endemic to a particular locality, seeds with maximal length and width disseminate to larger geographical locations. Seed buoyancy is increased by increasing the ratio of seed to embryo air percentage. The current study establishes a favorable link between seed buoyancy and distribution pattern. Because of its longer and flyer-type seeds, *Habenaria digitata* and *H. gibsonii* are extensively dispersed, but *H. grandifloriformis* is still confined to a particular location because of its relatively small seeds.

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Legends

Plate – I: Seed Diversity in *Habenaria* species.

a- *Habenaria brachyphylla*, b- *H. commelinifolia*, c- *H. crinifera*, d- *H. digitata*, e- *H. diphylla,* f- *H. foetida*, g- *H. furcifera*, h- *H. gibsonii*, i- *H. grandifloriformis*, j- *H. heyneana*, k- *H. longicorniculata*, l- *H. longicornu*, m- *H. marginata*, n- *H. ovalifolia*, o- *H. plantaginea*, p- *H. rariflora*, q- *H. roxburghii*, r- *H. suaveolens* (Scale bar = 100 µm)

Plate - I

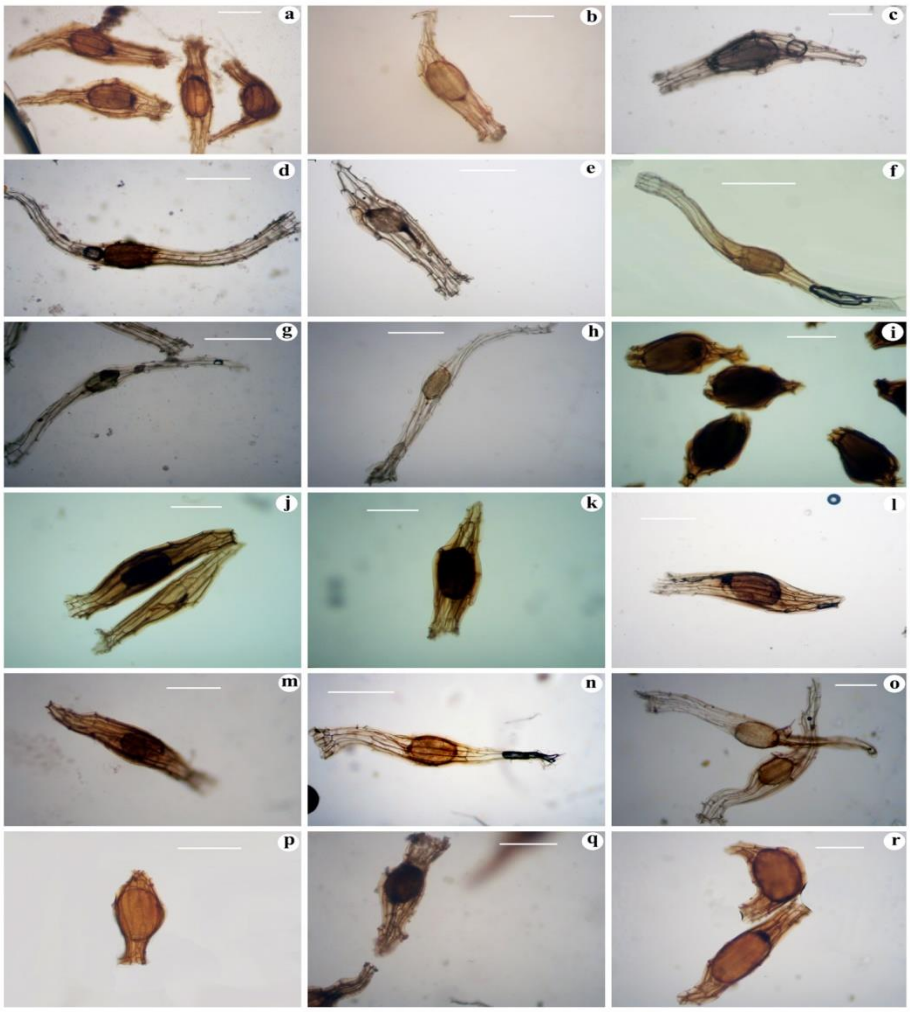

Table -1. Comparative features of the seeds in *Habenaria* species

Sr. No.	Name of the species	Length (L) (mm)	Width (W) (mm)	Length/ Width ratio of seed (L/W)	Volume of Seed (S) (mm^3 X 10^{-3})
1	H. brachyphylla	0.39 ± 0.16	0.11 ± 0.08	3.50 ± 1.06	1.26 ± 0.50
2	H. commelinifolia	0.75 ± 0.22	0.08 ± 0.02	9.00 ± 1.23	1.36 ± 0.62
3	H. crinifera	0.47 ± 0.16	0.08 ± 0.03	5.67 ± 2.56	0.86 ± 0.12
4	H. digitata	1.69 ± 0.35	0.11 ± 0.05	15.25 ± 3.54	5.48 ± 0.95
5	H. diphylla	0.58 ± 0.24	0.33 ± 0.02	1.75 ± 0.69	16.97 ± 2.14
6	H. foetida	1.44 ± 0.34	0.11 ± 0.04	13.00 ± 2.56	4.67 ± 0.74
7	H. furcifera	1.44 ± 0.54	0.14 ± 0.05	10.40 ± 2.69	7.29 ± 1.49
8	H. gibsonii	1.47 ± 0.28	0.09 ± 0.03	17.67 ± 3.84	2.68 ± 0.86
9	H. grandifloriformis	0.29 ± 0.14	0.16 ± 0.04	1.82 ± 0.54	1.85 ± 0.74
10	H. heyneana	0.54 ± 0.28	0.14 ± 0.06	3.86 ± 1.25	2.77 ± 0.95
11	H. longicorniculata	0.53 ± 0.32	0.14 ± 0.05	3.80 ± 1.65	2.66 ± 0.80
12	H. longicornu	0.67 ± 0.31	0.14 ± 0.04	4.80 ± 1.36	3.37 ± 0.94
13	H. marginata	0.47 ± 0.21	0.11 ± 0.02	4.25 ± 2.11	1.53 ± 0.85
14	H. ovalifolia	0.97 ± 0.46	0.11 ± 0.06	8.75 ± 2.25	3.14 ± 0.86
15	H. plantaginea	0.72 ± 0.40	0.17 ± 0.05	4.33 ± 1.23	5.25 ± 1.06
16	*H. rariflora*	0.78 ± 0.35	0.11 ± 0.04	7.00 ± 1.68	2.51 ± 0.65
17	*H. roxburghii*	0.61 ± 0.24	0.22 ± 0.08	2.75 ± 0.098	7.90 ± 1.24
18	*H. suaveolens*	0.47 ± 0.21	0.14 ± 0.06	3.40 ± 1.56	2.38 ± 0.56

(± SD, n= 10)

Table -2. Comparative features of the Embryo in Habenaria species seeds

Sr. No.	Name of the species	Length L (mm)	Width W (mm)	Length/ Width ratio (L/W)	Volume of Seed (S) (mm^3 X 10^{-3})	Embryo Volume (E) (mm^3 X 10^{-3})	Seed Volume/ Embryo Volume ratio (S/E)	Air Space (%)
1	H. brachyphylla	0.28 ± 0.16	0.08 ± 0.02	3.33 ± 2.13	1.26 ± 0.50	1.01 ± 0.26	1.24 ± 0.65	19.67 ± 4.62
2	H. commelinifolia	0.28 ± 0.18	0.08 ± 0.02	3.33 ± 2.16	1.36 ± 0.62	1.01 ± 0.28	1.35 ± 0.86	25.95 ± 6.65
3	H. crinifera	0.28 ± 0.14	0.06 ± 0.01	5.00 ± 2.98	0.86 ± 0.12	0.45 ± 0.08	1.91 ± 0.98	47.73 ± 8.52
4	H. digitata	0.28 ±	0.08 ±	3.33 ±	5.48 ±	1.01 ±	5.42 ±	81.56

		0.13	0.03	2.42	0.95	0.30	2.85	±11.23
5	H. diphylla	0.33 ± 0.19	0.31 ± 0.12	1.09 ± 0.09	16.97 ± 2.14	16.29 ± 4. 36	1.04 ± 0.24	4.00 ± 2.56
6	H. foetida	0.31 ± 0.17	0.08 ± 0.04	3.67 ± 2.54	4.67 ± 0.74	1.11 ± 0.22	4.20 ± 2.13	76.21 ± 9.59
7	H. furcifera	0.31 ± 0.16	0.08 ± 0.03	3.67 ± 2.64	7.29 ± 1.49	1.11 ± 0.26	6.57 ± 2.45	84.77 ± 9.45
8	H. gibsonii	0.25 ± 0.14	0.06 ± 0.02	4.50 ± 2.78	2.68 ± 0.86	0.40 ± 0.09	6.63 ± 3.45	84.91 ±11.34
9	H. grandifloriformis	0.17 ± 0.09	0.14 ± 0.09	1.20 ± 1.06	1.85 ± 0.74	1.83 ± 0.45	1.01 ± 0.19	0.86 ± 0.46
10	H. heyneana	0.26 ± 0.12	0.12 ± 0.08	2.17 ± 1.64	2.77 ± 0.95	1.96 ± 0.69	1.41 ± 0.90	29.27 ± 8.36
11	H. longicorniculata	0.19 ± 0.14	0.11 ± 0.07	1.75 ± 1.26	2.66 ± 0.80	1.26 ± 0.31	2.12 ± 1.20	52.86 ± 6.53
12	H. longicornu	0.28 ± 0.16	0.11 ± 0.08	2.50 ± 1.84	3.37 ± 0.94	1.79 ± 0.40	1.88 ± 1.56	46.68 ± 6.24
13	H. marginata	0.22 ± 0.15	0.08 ± 0.04	2.67 ± 1.95	1.53 ± 0.85	0.81 ± 0.16	1.89 ± 1.65	47.08 ± 8.95
14	H. ovalifolia	0.22 ± 0.15	0.08 ± 0.03	2.67 ± 1.90	3.14 ± 0.86	0.81 ± 0.20	3.89 ± 1.59	74.29 ±11.23
15	H. plantaginea	0.25 ± 0.16	0.14 ± 0.09	1.80 ± 1.32	5.25 ± 1.06	2.52 ± 0.89	2.08 ± 1.24	51.94 ± 8.26
16	H. rariflora	0.22 ± 0.14	0.08 ± 0.03	2.67 ± 1.87	2.51 ± 0.65	0.81 ± 0.22	3.11 ± 1.69	67.87 ± 6.35
17	H. roxburghii	0.34 ± 0.17	0.19 ± 0.10	1.71 ± 1.20	7.90 ± 1.24	6.60 ± 2.12	1.20 ± 1.02	16.50 ± 4.62
18	H. suaveolens	0.22 ± 0.15	0.11 ± 0.09	2.00 ± 1.56	2.38 ± 0.56	1.44 ± 0.67	1.66 ± 1.13	39.78 ± 6.52

(± SD, n= 10)