



Research Article

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Morphological characteristics of flowers and pollen, Preliminary Phytochemical Evaluation and Thin layer chromatography of ethanol extracts of five species of genus *Balanophora* J.R. & G. Forst collected in Viet Nam

Nguyen Thanh Tung^{1*}, Nong Phuong Anh¹, Ho Thu Trang¹, Nguyen Van Quan¹, Le Ngoc Van¹, Bui Thi Thao¹,
Nguyen Viet Than¹, Nguyen Quang Hung²

¹ Department of Pharmacognosy, Hanoi University of Pharmacy, 13-15 Le Thanh Tong street, Hoan Kiem Distr., Hanoi, Viet Nam.

² Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Viet Nam.

ABSTRACT

The objective of this study is to determine the morphological characteristics of flowers and pollen, preliminary phytochemical evaluation and thin layer chromatography of five species of genus *Balanophora* J.R. & G. Forst collected in Viet Nam: *Balanophora fungosa* J.R. & G. Forst ssp. *indica* (Arn.) Hansen var. *indica*, *Balanophora fungosa* J.R. & G. Forst ssp. *indica* (Arn.) Hansen var. *globosa* (Jungh.) Hansen, *Balanophora latisejala* (Tiegh) Lecomte, *Balanophora laxiflora* Hemsl, *Balanophora subcupularis* P.C. Tam. The morphological characteristics of male flowers, female flowers and spadicules, pollen grain of five species were described. Results of preliminary phytochemical evaluation of ethanol extracts of five species showed that all of five species have tannins, flavonoids, coumarins, sterols. Thin layer chromatography of ethanol extracts of five species were developed and base on TLC chromatogram, it is easy to differentiate these species.

Keywords: *Balanophora*, Pollen morphology, Preliminary phytochemical evaluation, Thin layer chromatography.

INTRODUCTION

Genus *Balanophora* J.R. & G. Forst, belonging to family Balanophoraceae, comprised about 19 species in worldwide, 12 species in China [1]. The extracts and the isolated compounds of this genus have strong free radical scavenging activities, and some other effects such as inhibiting HIV effects, hypoglycemic effects, anti-inflammatory and analgesic effects [2]. Of genus *Balanophora* in Viet Nam, *B. fungosa* ssp. *indica* var. *indica* and *B. fungosa* ssp. *indica* var. *globosa* are two varieties of *B. fungosa* ssp. *indica* were recently recorded appearing in Ha Giang, Viet Nam [3]. These two have some similar morphological characteristics: dioecious plants, actinomorphic male flowers, female flowers on main axis of inflorescence and basal of spadicules, leaves spirally arranged. Specially, it was very difficult to distinguish dry samples of these two species. *B. laxiflora* is listed in the 2007 Red Data Book of Viet Nam and research works on genus *Balanophora* J.R. & G. Forst in Viet Nam are only focus on this species. *B. latisejala* is a rare species and dry sample of this species is sometime difficult to distinguish with *B. laxiflora*. *B. subcupularis* is a new record species for Flora of Viet Nam [4]. Species of genus *Balanophora* J.R. & G. Forst have diverse and complex morphological characteristics, especially it is difficult to distinguish dry sample of them. This paper provided database on pollen morphology, preliminary phytochemical evaluation and Thin layer chromatography of ethanol extracts of five species which could be helpful to differentiate these species.

MATERIALS AND METHODS

Collection and identification of plants

The fresh samples of five species of genus *Balanophora* J.R. & G. Forst were collected at different locals in Viet Nam. Comparative morphological methods were used to identify plants. The voucher specimens were deposited at the Herbarium of Museum of Biology, Faculty of Biology, VNU University of Science, Vietnam National University, Hanoi.

*Corresponding author:

Nguyen Thanh Tung

Department of Pharmacognosy,
Hanoi University of Pharmacy, 13-
15 Le Thanh Tong street, Hoan
Kiem Distr., Hanoi, Viet Nam
Email:

thanhtungng.pharmacist[at]gmail.com

Table 1: Information of samples of five *Balanophora* species collected

No.	Scientific names	Places and times of collecting	Voucher specimens numbers
1	<i>Balanophora fungosa</i> J.R. & G. Forst ssp. <i>indica</i> (Arn.) Hansen var. <i>indica</i>	Ha Giang province December 2017	HNU 022615
2	<i>Balanophora fungosa</i> J.R. & G. Forst ssp. <i>indica</i> (Arn.) Hansen var. <i>globosa</i> (Jungh) Hansen	Ha Giang province October 2016	HNU 022607
3	<i>Balanophora latisejala</i> (Tiegh.) Lecomte	Lam Dong province September 2017	HNU 022612
4	<i>Balanophora laxiflora</i> Hemsl.	Ha Giang province September 2016	HNU 022608
5	<i>Balanophora subcupularis</i> PC. Tam	Lam Dong province November 2016	HNU 022609



Figure 1: Five species of genus *Balanophora* J.R. & G. Forst

Study on morphological characteristics of flowers and pollens

Male flowers of five species were observed and taken pictures with camera Nikon D80 while female flowers and spadices were observed and taken pictures using Zoom Stereomicroscope with camera Nikon.

Microscopical investigations of pollens of five species were done by using microscope with camera Nikon DS-Fi2. The pollens of five *Balanophora* species were size measured, taken picture in polar view and equatorial view.

Preliminary Phytochemical Evaluation

Preliminary phytochemical examination of ethanol extracts of samples were carried out for tannins, coumarins, flavonoids, alkaloids, sterols, anthranoid, acid amin etc. according to standard procedure [6, 7].

Thin layer chromatography

Thin layer chromatography techniques were carried out using ethanol extracts of five species. The same amount of ethanol extracts of five species (5 μ l) were applied on the HPTLC plates silica gel 60 F 254 (Merck) using Linomat 5 (CAMAG). Compatible solvent system was used to develop Thin layer chromatography (TLC). After development, the plates were taken image in Visualizer (CAMAG) at $\lambda = 254\text{nm}$ and $\lambda = 366\text{nm}$. The plate were derivatized with reagent Vanilin/Sulfuric acid and taken image. The chromatographic evaluation were done with VisionCATs software.

RESULTS

Morphological characteristics of flowers

B. fungosa ssp. *indica* var. *indica*: Male flowers actinomorphic, perianth

lobes 4-5, ovate-elliptic, apex acute. Female flowers on main axis of inflorescence and lower part of spadices. Spadices 1200 – 1600 μm long with an obtuse or truncate top part.

B. fungosa ssp. *indica* var. *globosa*: Male flowers actinomorphic with perianth lobes 4-6(-7), elliptic-lanceolate. Female flowers on main axis of inflorescence and lower part of spadices. Spadices 1000 – 1100 μm long, subclavate.

B. latisejala: Male flowers zygomorphic, normally 4 merous with two narrow, ovate, acute tepals and two wide, nearly square, truncate

median tepals. Female flowers on main axis of inflorescences as well as on spadices.

B. laxiflora: Male flowers: subsessile, zygomorphic; anthers were broken up into many locelli, dehiscent by short slits. Perianth lobes 4-6, suborbicular to ovate, 2-3 mm, apex acute to obtuse. Female inflorescences ovoid-speroid to oblong-ellipsoid, apex acuminate.

B. subcupularis: Male flowers: at basal of androgynous inflorescences, nearly actinomorphic; perianth lobes usually 4, widely ovate, apex truncate, less than 1.5 mm. Female flowers: at basal of spadices and main axis of inflorescences.



Figure 2: Male flowers of five species of genus *Balanophora* J.R. & G. Forst. 1. *B. fungosa* ssp. *indica* var. *indica*, 2. *B. fungosa* ssp. *indica* var. *globosa*, 3. *B. latisejala*, 4. *B. laxiflora*, 5. *B. subcupularis* (a. Male flower, b. Female flowers and spadices)

Pollen morphology

Pollens of five species of genus *Balanophora* J.R. Forst & G. Forst are small grains with length of the longest axis ranged from 10-20 μm . Most of pollens are type of monad. The shape of the pollen grains varies from species to species in genus *Balanophora* J.R. Forst & G. Forst. Pollen

grains were described by the shape of their outline both in polar and equatorial views. Pollen were divided in to different shape classes according to method of G. Erdtman (1952) which base on the ratio of polar axis (PA) and equatorial diameter (ED) [7]. The pollen grain of *B. latisejala* have the thickest exine (approximate 2 μm).

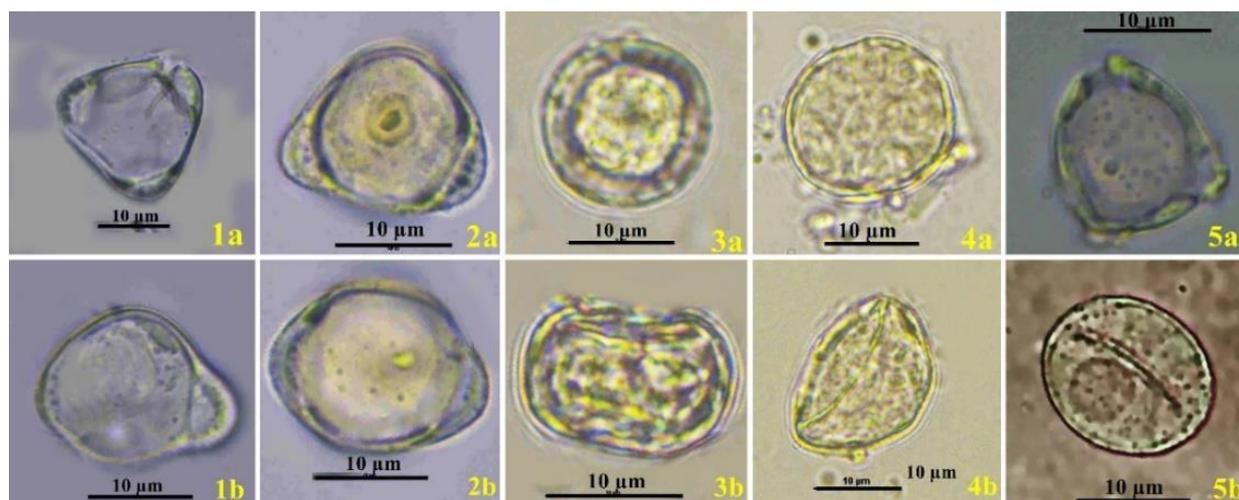


Figure 3: Pollen morphology of five species of genus *Balanophora* J.R. & G. Forst. 1. *B. fungosa* ssp. *indica* var. *indica*, 2. *B. fungosa* ssp. *indica* var. *globosa*, 3. *B. latisejala*, 4. *B. laxiflora*, 5. *B. subcupularis* (a. Polar view, b. Equator view)

Table 2: Characteristics of pollen of five *Balanophora* species

Species	Characteristics			
	Size	Shape in polar view	Shape in equator view	Pollen shape class
<i>B. fungosa</i> ssp. <i>indica</i> var. <i>indica</i>	16 – 18 µm	obtuse straight triangular	obtuse acuminate elliptic	sub-oblate
<i>B. fungosa</i> ssp. <i>indica</i> var. <i>globosa</i>	17 – 19 µm	obtuse straight triangular	obtuse acuminate elliptic	sub-oblate
<i>B. latisejala</i>	14 – 16 µm	circular	truncate elliptic	sub-prolate
<i>B. laxiflora</i>	14 – 17 µm	circular	acute acuminate elliptic	prolate
<i>B. subcupularis</i>	12 – 15 µm	obtuse convex triangular	circular	sub-prolate

Preliminary Phytochemical Evaluation

The results showed that ethanol extracts of five species have some

phytochemical constituents such as Flavonoids, Coumarins, Tannins, Sterols. (Table 3).

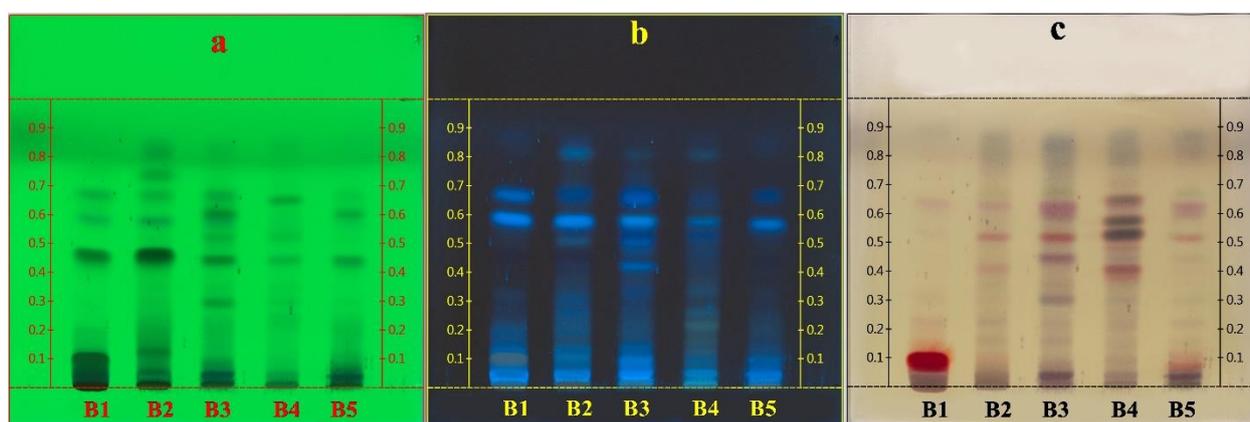
Table 3: Phytochemical screening of 90% ethanol extracts of five species

	<i>B. fungosa</i> ssp. <i>indica</i> var. <i>indica</i>	<i>B. fungosa</i> ssp. <i>indica</i> var. <i>globosa</i>	<i>B. latisejala</i>	<i>B. laxiflora</i>	<i>B. subcupularis</i>
Flavonoids	+	+	+	+	+
Coumarins	+	+	+	+	+
Saponins	-	-	-	-	-
Tannins	+	+	+	+	+
Alcaloids	-	-	-	-	-
Anthranoids	-	-	-	-	-
Fatty acids	-	-	-	-	-
Sterols	+	+	+	+	+

Notes: (+): positive (-): negative

TLC Chromatogram analysis

Solvent system (I): Chloroform – Ethylacetate – Acid formic (5:5:1) were used for TLC studies.



- Track B1: *B. fungosa* ssp. *indica* var. *indica*
- Track B2: *B. fungosa* ssp. *indica* var. *globosa*
- Track B3: *B. latisejala*
- Track B4: *B. laxiflora*
- Track B5: *B. subcupularis*

Figure 4: TLC chromatogram of ethanol extracts of five species developed with solvent system (I) observed at: a. $\lambda = 254\text{nm}$, b. $\lambda = 366\text{nm}$, c. Derivatized with vanillin/acid sulfuric reagent.

Chromatograms of extracts of five species of genus *Balanophora* have some similarities and dissimilarities when observing at $\lambda = 254\text{nm}$, $\lambda = 366\text{nm}$ and after derivatization:

When observing at $\lambda = 254\text{nm}$, chromatogram of B1 (*B. fungosa* ssp. *indica* var. *indica*) had a dark track with R_f -value = 0,11 which turned into

orange red after derivatization. This track wasn't observed in chromatograms of four other species.

When observing at $\lambda = 366\text{nm}$: All chromatogram of five species had two light blue fluorescence tracks with R_f -value = 0,59; 0,65; respectively. Beside, chromatogram of B2 (*B. fungosa* ssp. *indica* var. *globosa*), B3 (*B.*

latisejala) and B4 (*B. laxiflora*) had a blue fluorescence track with R_f -value = 0,81. Chromatogram of B3 (*B. latisejala*) had a blue fluorescence track with R_f -value = 0,42 while chromatogram of B4 (*B. laxiflora*) didn't have an equivalent track.

After derivatization, chromatogram of B3 (*B. latisejala*) had a purple blue track with R_f -value = 0,30 while chromatogram of B4 (*B. laxiflora*) didn't have an equivalent track. In contrast, chromatogram of B4 (*B. laxiflora*) had three track with R_f -value = 0,40; 0,57; 0,64; respectively while chromatogram of B3 (*B. latisejala*) didn't have equivalent tracks.

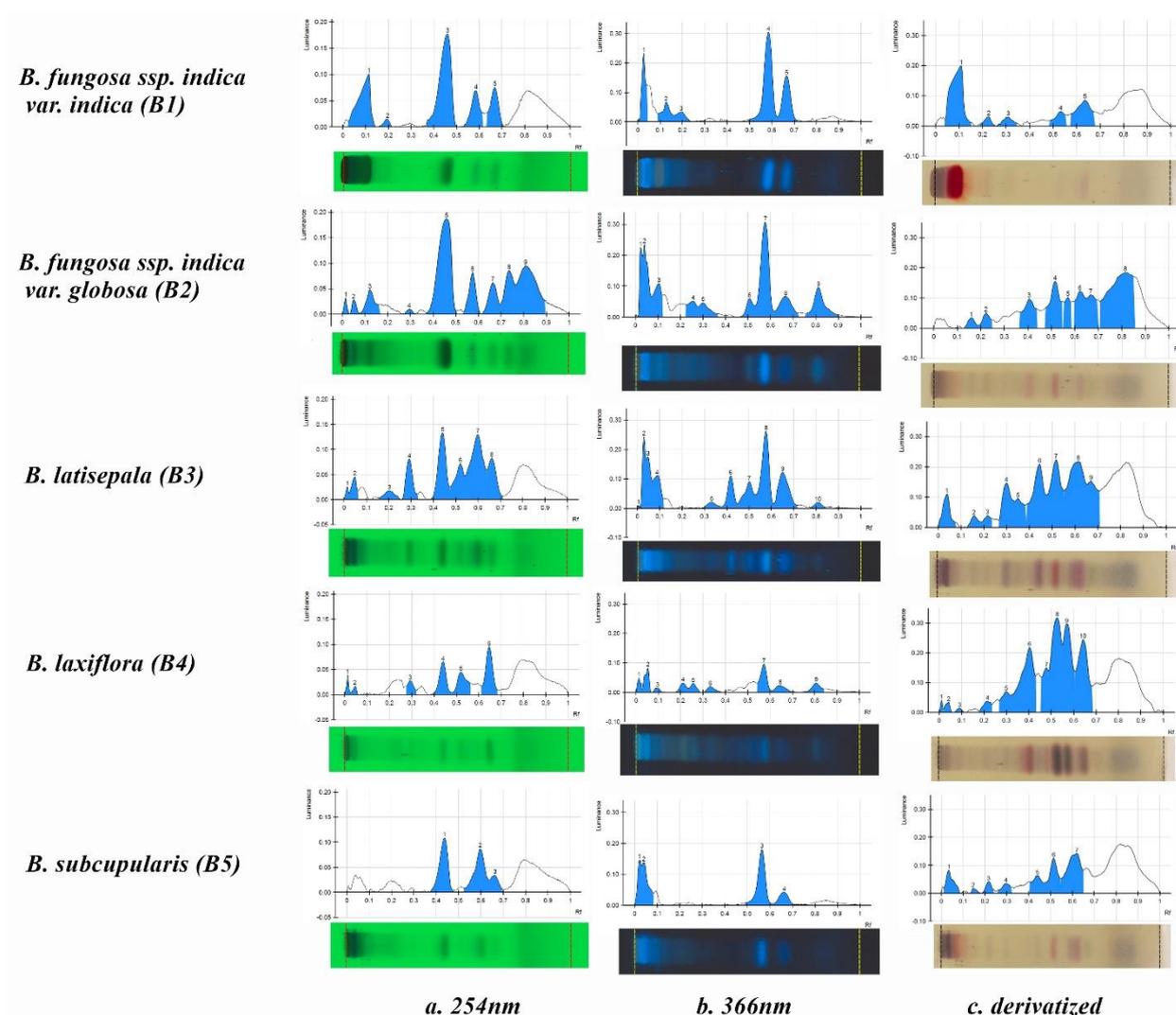


Figure 5: TLC profile (densitogram and chromatogram) of extracts of five species of genus *Balanophora* J.R. & G. Forst

DISCUSSION

The morphological characteristics of flowers and pollen of five species of genus *Balanophora* J.R. & G. Forst were described and compared together. Preliminary phytochemical examination of five species showed that all of them had Flavonoids, Coumarins, Tannins, Sterols.

The Thin layer chromatography of five species were also developed to provide chromatogram which could help to differentiate dry samples of *Balanophora* species:

B. fungosa ssp. indica var. indica and *B. fungosa ssp. indica var. globosa* were two varieties of *B. fungosa ssp. indica* so that they have some similarities in morphological characteristics of male flowers, female flowers and spadicles, pollen grain. It is difficult to distinguish dry sample of two species base on morphological characteristics but chromatogram of two species had clearly dissimilarities and that is basis to distinguish them.

B. latisejala and *B. laxiflora* could be distinguished base on pollen morphology (pollen of *B. latisejala* had a thick exine) and TLC chromatogram.

B. subcupularis was new record species for Flora of Viet Nam and this species is different from other species: Male flowers at basal of androgynous inflorescences, perianth lobes less than 1.5 mm.

This is the first study about the morphological characteristics of pollen of species of genus *Balanophora* J.R. & G. Forst in Viet Nam. It's also the first time chromatography of *Balanophora* species in Viet Nam was developed.

CONCLUSION

Taxonomically, five of the plants belong to the same genus *Balanophora* J.R. & G. Forst (family Balanophoraceae). They have some similarities and dissimilarities in morphology of flowers and pollen and in TLC chromatogram. This study is helpful not only for further research on

genus *Balanophora* in Viet Nam but also for distinguishing species of this genus.

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