The 7th International Conference on Sustainable Agriculture and Environment (ICSAE-7)

Surakarta, Indonesia. 27 August 2020
https://icsae.id; e-mail: 7thicase@gmail.com

Letter of Acceptance

We are pleased to announce that your abstract with the following identity

Paper ID: #47

Tittle: #The Morphology and Density of Pasak Bumi (Eurycoma longifolia, Jack) Leaf Trichomes in Several Natural Populations in Indonesia.

Author(s): #Zulfahmi Zulfahmi*; Parjanto Parjanto; Edi Purwanto; Ahmad Yunus

Affiliation (first or corresponding author): #Universitas Islam Negeri Sultan Syarif Kasim (UIN SUSKA) Riau

is **accepted** for **Oral (Recorded Video)** presentation in ICSAE-7.

Please proceed to the conference payment and finish the administrative-related work. The committee requires the submission of your full paper prior to the deadlines. Conference details, guidelines and deadlines are updated at the conference's site and notification e-mails are sent to the primary contact of the above mentioned abstract.

31 July 2020
Surakarta – Indonesia

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Dr. Mithat Direk

ICSAE-7

7th International Conference on Sustainable Agriculture and Environment

CONFERENCE GUIDE AND BOOK OF ABSTRACTS

7th International Conference on Sustainable Agriculture and Environment

August 27, 2020
Surakarta - Indonesia

THE 7th INTERNATIONAL CONFERENCE ON SUSTAINABLE AGRICULTURE AND ENVIRONMENT (ICSAE-7)

Conference Guide and Book of Abstracts

SURAKARTA - INDONESIA

AUGUST 27, 2020

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Preface

Contemporary events shows us that we humans are very vulnerable to economic and environmental fluctuations. As humanity progress, produce and consume like it will exist until infinity. Sustainability has always been the case, is accepted as life guidance but until then we; researchers, companies and students should try to work on this concept so that next generation can benefit from our efforts.

Sustainable way of dealing with environment and agriculture is not easy. The benefits from sustainable agriculture vary and sustainable agriculture doesn't have harmful effects on environment or sources. In agricultural activities have been performed for chaining the environment or utilizations of environment as sources with maximum benefits. If we do not success that, it is impossible for us to talk about modern agriculture. Under poor management of agriculture, biodiversity and soil properties can be damage, contaminate and some problems limiting efficient agricultural activities may be occur.

The main objective of farming is to produce food for human being, but unfortunately some parts of the farmlands have used also as settlements areas. By this way, some natural resources have been deteriorated; consequently size of farmland has reduced day by day. In that regard, it is possible to say that we are in going in a circle. More than one-third of food supplies is as form of wastes due to the over buying than required amounts of food by costumers.

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Now, I would like to thank whole people being at Scientific Board, Managerial Board, authors or participants by supporting their valuable works and particularly Prof. Ahmad Yunus from Universitas Sebelas Maret Indonesia and his team, for all their contributions on continuity of these conference series since it was held, for the 1st, at 2013 in Surakarta Indonesia, then move around the globe and at 7th ICSAE its back again in Surakarta. I do appreciate all the efforts given to prepare this event. This is the first digital conference of ICSAE series which could be reach wider and bigger participant around the world.

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Conference Guide

As informed, ICSAE-7 will be held by online due to current Covid-19 pandemic circumstances. Considering time constraints and to avoid various technical problems (such as lagging internet connection, different time zone, etc) during the conference, the conference is held by online.

There are two different sessions in this conference, namely pararel session and general assembly. The pararell session can be accessed through conference website (https://icsae.id), all the digital format of paper presentation (recorded video and poster) are listed and opened to all participants. The generall assembly, invited speakers talk, will be performed through live meeting. Both stages can be enjoyed by all participants which are listed and/or invited with the following time frame:

1. Parallel session in digital venue : August 25-27, 2020

2. General assembly : August 27, 2020

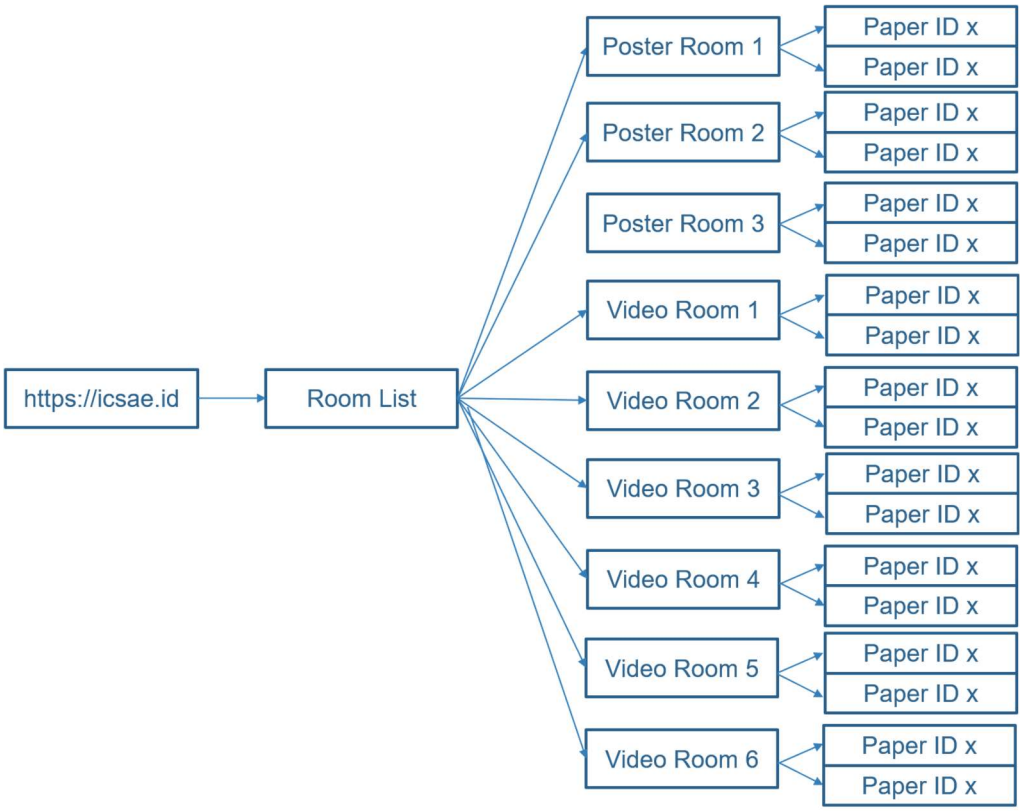
ICSAE-7th Stages

Parallel Session Guide

The following is the guide to access the digital venue for pararell session:

1. Please visit ICSAE-7 Website (https://icsae.id)
2. Click **'ICSAE Parallel Session'** button. You will be redirected to the room list, there are 3 poster rooms and 6 video rooms
3. Click on the presentation type and the preferred topic in specific room
4. Click the presentation title
5. The abstract, and presentation media can be viewed accordingly
6. Leave any comments in the form for Q & A, please write your name and email for further contact.
7. The presenter is able to reply every the comments.

Flowcart Parallel Session ICSAE-7



General Assembly Rundown

Time (GMT+7; AM)	Activities	Person in Charge
07.30-08.00	Registration and preparation	Committee
08.00-08.10	Opening	MC
08.10-08.25	Opening Remarks (**Prof. Jamal Wiwoho**)	UNS-Rector
08.25-08.30	Preparation for Session 1 (2 Invited Speakers) Announcements	MC
08.30-08.55	Invited Speaker 1 (**USA – Prof. Henry Utomo**)	Moderator
08.55-09.20	Invited Speaker 2 (**UNS – Prof. Ahmad Yunus**)	Moderator
09.20-09-30	Q n A	Moderator
09.30-09.35	Preparation for Session 2 (2 Invited Speakers) Announcements	MC
09.35-10.00	Invited Speaker 3 (**USA – Prof. Taifo Mahmud**)	Moderator
10.00-10.20	Invited Speaker 4 (**UNS – dr. Betty Suryawati**)	Moderator
10.20-10.30	Q n A	Moderator
10.30-10.35	Preparation for Session 3 (2 Invited Speakers) Announcements	MC
10.35-11.00	Invited Speaker 5 (**Turkey – Dr. Mithat Direct**)	Moderator
11.00-11.20	Invited Speaker 6 (**UNRAM – Prof. M. Sarjan**)	Moderator
11.20-11.30	Q n A	Moderator
11.30-11.45	Closing remarks	MC
11.45-11.55	Announcements	MC

Link for live meeting via Zoom will be distributed exclusively to the listed participants and/or listeners via e-mail, prior the General Assembly.

Video Room 5

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Paper ID: 47

THE MORPHOLOGY AND DENSITY OF PASAK BUMI (*EURYCOMA LONGIFOLIA*, JACK) LEAF TRICHOMES IN SEVERAL NATURAL POPULATIONS IN INDONESIA.

Zulfahmi[1,4*], Parjanto[2], Edi Purwanto[2], A. Yunus[2,3]

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Abstract

Eurycoma longifolia Jack is one of the important medicinal plants in Indonesia and is used in many traditional as well as modern medicine. Until now, there is no information regarding the trichome characteristics of *E. longifolia* and its relationship with environmental factors is unknown. The purpose of this study was to investigate the morphology and density of the leaf trichomes of *E. longifolia*, and observed its correlation with geographic and climate factors of population. The morphology and density of the leaf trichome of six populations of *E. longifolia* were investigated using a light microscope. Analysis of variance, correlation analysis, principal component analysis (PCA), and clustering of the population were performed. The results of this study detected two types of glandular trichome in the leaf of *E. longifolia*, namely peltate and capitate. Analysis of variance showed a significantly different density of trichome among populations. Inter-population variation in trichome density may be caused by different micro-environmental of each population and genetic factors of plant individuals within population. We confirmed that geographic and climate factors of the population have significant positive/negative correlations with types and density of trichomes. The PCA analysis exhibited that trichomes density could be used as a distinguishing characteristic among populations. Analysis of PCA and UPGMA divided population studies into two groupings.

Keywords*: correlation, environmental factors, leaf trichome, population differentiation*

CERTIFICATE

THIS IS TO CERTIFY THAT

Zulfahmi

HAS PARTICIPATED AS

SPEAKER

at the 7th International Conference
on Sustainable Agriculture and Environment (ICSAE-7)

August 27, 2020
Surakarta - Indonesia

Vice Rector of Academic Affairs

Prof. Dr. Ir. Ahmad Yunus, MS
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The Morphology and Density of Pasak Bumi (Eurycoma longifolia, Jack) Leaf Trichomes in Several Natural Populations in Indonesia

AUTHOR(S)

Zulfahmi, Parjanto, Edi Purwanto, Ahmad Yunus

has been presented at the ***7th International Conference on Sustainable Agriculture and Environment (ICSAE-7)***

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Preface

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Preface

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The current ICSAE-7 at 2020 was held during global pandemic situation, for that the committee decided to shift from offline to online meeting. It's not an easy task by the way, there were travel banned, different time zone, and internet connection are factors need to be overcome during the organization of this event. Question may be arising, why the ICSAE is not postponed. Well, it is the commitment of scientist to communicate the agriculture issues during pandemic situation, moreover the event also has been planned well since last year. After some adjustments, and considering available resources, the conference then held virtually at 25-27 August 2020.

Committee use online meeting platform for two main agendas in this event. First, the parallel session that was held for 3 days in row using website as the media for the researcher to deliver their talk. Each author(s) has 15 minutes to talk about their paper, recorded then uploaded both in Youtube and in the virtual venue (conference website). All the documentation of author(s) talk can be accessed in the conference website (https://icsae.id). Committee set 3 days online meeting, since realizing the time zone different and internet connection differences among the participant(s). Using this strategy, the talks can be enjoyed by the participants during their stay at home, or can be accessed anywhere and anytime. Participants can raise question in every single uploaded talk, by typing their question in the conference website. The system will notify the author(s), then question can be answer it accordingly.

The second program is the General Assembly meeting which held at 27 August 2020. This program was held by online via Zoom meeting, and there were 6 keynote speakers invited and delivering talk about agriculture and its challenge during pandemic situation. Both meeting program, parallel session and general assembly, were organized virtually by committee which stay in Universitas Sebelas Maret Surakarta Indonesia, and in Turkey by the help of Dr. Mithat Direk.

As can be listed, committee receive participation around the globe such as from Europe, Turkey, Egypt, Pakistan, China, India, Indonesia, Japan, Taiwan and etc. Officially, committee receive online registration to participate this virtual conference around 320 participants from around the world. As the commitment to open access all the conference talk, we let the virtual venue open and can be visited online, until now (15 November 2020) the virtual venue has been visited by more than 1,850 viewers from around the world.

Overall, the major obstacles were internet connection stability which vary between participant(s) in his/her region. A condition of can't join the live meeting, breaking up voice during talk, can't see screen share during presentation at the participant(s) side, were associated with the connection. However, according to the participant(s) feedback, ICSAE-7 conference was successfully held by virtually and this was a new experience in participation the international conference.

Lastly, committee would like to thank whole people being at Scientific Board, Managerial Board, authors or participants by supporting their valuable works. Particularly Prof. Ahmad Yunus from Universitas Sebelas Maret Indonesia and his team, for all their contributions on continuity of these conference series since it was held, for the 1st, at 2013 in Surakarta Indonesia, then move around the globe and at 7th ICSAE its back again in Surakarta. I do appreciate all the efforts given to prepare this event. This is the first digital conference of ICSAE series which could be reach wider and bigger participant around the world.

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Peer review declaration

Peer review declaration

All papers published in this volume of IOP Conference Series: Earth and Environmental Science have been peer-reviewed through processes administered by the Editors. Reviews were conducted by expert referees to the professional and scientific standards expected of a proceedings journal published by IOP Publishing.

- Type of peer review: Double-blind with the opportunity to resubmit after revisions
- Conference submission management system: Microsoft's Conference Management Toolkit (Microsoft CMT). The submission url is https://cmt3.research.microsoft.com/User/Login?ReturnUrl=%2FICSAE2021
- Number of submissions received: 224
- Number of submissions sent for review: 198
- Number of submissions accepted: 148
- Acceptance Rate (Number of Submissions Accepted / Number of Submissions Received X 100): 66.07%
- Average number of reviews per paper: 2
- Total number of reviewers involved: 18
- Any additional info on the review process: all papers were checked for its similarity using Turnitin, and 25% similar was set as maximum threshold.
- Contact person for queries:
  Name: Prof. Sri Hartati
  Affiliation: Research and Development Center for Biotechnology and Biodiversity (P3BB) Universitas Sebelas Maret
  Email: tatik_oc@yahoo.com

The morphology and density of pasak bumi (*Eurycoma longifolia*, Jack) leaf trichomes in six natural populations in Indonesia

The morphology and density of pasak bumi (*Eurycoma longifolia*, Jack) leaf trichomes in six natural populations in Indonesia

Zulfahmi[1,4*], Parjanto[2], E Purwanto[2] and A Yunus[2,3]

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Abstract. *Eurycoma longifolia* Jack is one of the important medicinal plants in Indonesia and is used in many traditional and modern medicines. Information on trichome characteristics of *E. longifolia* and its relationship with environmental factors is limited. The purpose of this study was to investigate the morphology and density of the leaf trichomes of *E. longifolia*, to observe its correlation with geographic and climate factors of population, and to identify trichome characters that can discriminate among populations. The morphology and density of the leaf trichome of six populations of *E. longifolia* were investigated using a light microscope. Analysis of variance, correlation analysis, principal component analysis (PCA), and clustering of the population were performed. The results of this study detected two types of glandular trichome in the leaf of *E. longifolia*, namely peltate and capitate. Analysis of variance showed a significantly different density of trichome among populations. Variation of trichome density among populations may be caused by different micro-environmental of population and genetic factors of plant individuals. We confirmed that geographic and climate factors of the population have significant positive/negative correlations with types and density of trichomes. The PCA analysis exhibited trichome density could be used as a distinguishing characteristic among populations.

1. Introduction

Pasak bumi (*Eurycoma longifolia*, Jack) is one of the medicinal plants that have economically potential to be developed in Indonesia. They are grown and distributed in the primary forest of the Sumatra and Borneo islands [1–3]. *E. longifolia* is shrubs or small trees with a tall of up to 10 m. This plant possessed many chemical compounds that can be used as anti-inflammatory, antioxidant, antibacterial, anticancer, anti-plasmodial, and increasing the testosterone hormone [4–6]. Now, the

extract of this plant has been traded in herbal medicinal shape as tea and coffee, as well as the capsule that produced the pharmaceutical industry.

Trichomes are uni or multi-celled pustule, which originate from the epidermal cells. They varied in morphological characters, locations, ability to secrete, and type of secretion [7]. In general, trichome (hairs) can be classified into two main groups, namely non-glandular and glandular trichomes. These trichomes have many roles in each plant species, and they can be found in several organs such as the leaf, petal, bark, etc. Leaf trichomes have two functions in the plant i.e. structural defense against herbivores and protection against environmental factors such as strong UV radiation, solar radiation, drought, high temperature, and freezing [8, 9].

Currently, many researchers used trichomes as a tool for taxonomic identification of plants, not only in the intra-generic classification level [10, 11] but also at the species and sub-species level [12, 13]. This is closely related to the character of trichomes that are relatively stable, relatively diverse, easy in the preparation for study, and commonly found in several plant families [14].

Information about morphology and density of trichomes of the E.longifolia species as well as comparison variation of the trichome density between plant populations in different environmental conditions are not available. Description of the morphology of trichomes and their relationships to environmental factors is particularly important to be known since trichome structures produce some of the active compounds [15], and the therapeutic efficacy of the plants might be also affected by environmental factors. Therefore, we conducted the study on trichomes morphology and density present on the leaves of *E. longifolia*. The purpose of this study was to determine the morphology and density of trichomes in the leaves of *E. longifolia,* to observe its relationship with geographic and climate factors of population, and to identify trichome characters that can discriminate among population studies.

2. Materials and methods

2.1. Plant samples

The samples of *E. longifolia* were collected from six populations, one population from West Borneo, two populations from Riau islands, and three populations from Riau province. The climatic and geographic data of each population were shown in Table 1. Five mature plants are taken from each population, which are selected randomly and the minimum distance among plants is 20 m. Each plant was taken one leaf expanded fully, and health. The leaves were then made into a herbarium and sent to the laboratory.

Table 1. Research sites characteristic of *E. longifolia*.

Population name	Research sites status	Longitude	Latitude	Altitude (m.a.s.l)	Temperature mean annually (^oC)	Precipitation mean annually (mm $year^{-1}$)
Pokomo, Riau Province	Protected forest	100°56’31” E	0°15’40” N	170	27.50[a]	225.17[a]
Tahura, Riau Province	Forest park	101°25’23” E	0°41’8” N	70	27.50[a]	208.60[a]
Mandor, West Borneo Province	Forest nature reserve	109°20’5” E	0°18’40” N	50	26.80[b]	319.10[b]
Lingga-1, Riau Archipelago	Natural forest	104°40’25” E	0° 10’39”S	90	27.20[c]	236.50[c]
Lingga-2, Riau Archipelago	Protected forest	104°35’12” E	0°12’66”S	160	27.20[c]	236.50[c]
Sentajo, Riau Province	Protected forest	101°30’22” E	0°28’42”S	106	27.50[a]	279.66[a]

Data sources: a[16], b[17], c[18]

2.2. Procedure for trichome observation

One dry leaflet of *E. longifolia* was taken from each sample leave for trichome observation. The method of collecting of trichome used the nail polish. Lightly paint a section of the leaflet middle veins with clear fingernail polish. Allow the fingernail polish for minutes to completely dry. Place the clear tape on the dried nail polish. Press the tape onto the leaflet gently and firmly, and then peel the tape from the leaf and place the tape sticky side down of the microscope slide. The tape contains the trichome is observed under a light microscope Nikon Eclipse 50i (Nikon, Japan) with 400x magnifications. The images are captured using Camera Nikon DS-Fi1 and were analyzed using NIS-Element software. Move the slide to find the trichomes in the other area. The trichome was observed on the abaxial and adaxial leaflet surface. For measurement, each leaflet and side was captured six images, and a total of 30 images for each side per population. The different types and distribution of trichome are described and then compared. The general trichome terminology follows [19] and [14]. Trichome density was calculated by dividing the number of trichome by the view field area [20].

2.3. Statistical analyses

The mean and standard deviation of the observed trichome number was calculated. Analysis of variance (ANOVA) was used to assess the trichome differences among populations and the parson correlation coefficient was also used to exhibit a significant correlation between different hair types. The multivariate analyses such as principal component analysis (PCA) and the dendrogram of UPGMA (Unweighted Pair Group Method with Arithmetic Mean) were performed. The used software for statistical analysis was SAS ver.9. [21] and NTSYS ver. 2.01 [22].

3. Results and discussion

3.1. Trichome morphology

In general, two types of glandular trichomes were observed on the abaxial and adaxial of the leaflet of *E. longifolia*; namely peltate and capitate. Both trichomes were different in size, structure, and mode of secretion. Peltate trichome was constituted by a cell at the base, a very short stalk cell, and a large secretory head forming the central and peripheral cells (Figure 1). The head of this trichome displayed a typical spherical shape due to cuticle expansion during the accumulation of essential oil in the sub-cuticular space. This peltate trichome is also observed in Lamiaceae family [14], and *Salvia argentea* [23].

Capitate trichome was constituted by one basal cell, a stalk of variable length, and uni-or bi-cellular head, in which the head size is smaller than that of peltate trichomes (Figure 2–4). In this study, capitate trichomes can be distinguished into three types based on the morphology and dimension of the stalk, namely capitate trichome of Type 1, capitate trichome of Type II, and capitate trichome of Type III. Capitate trichome of Type I had a bicellular rounded to oval head, a body with a unicellular short stalk, and a unicellular basis (Figure 2).

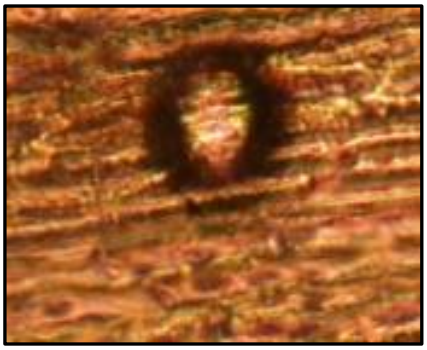

Figure 1. Peltate trichome.

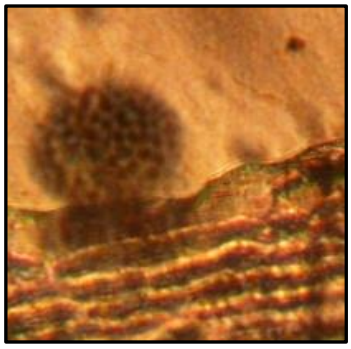

Figure 2. Capitate trichome of Type-I.

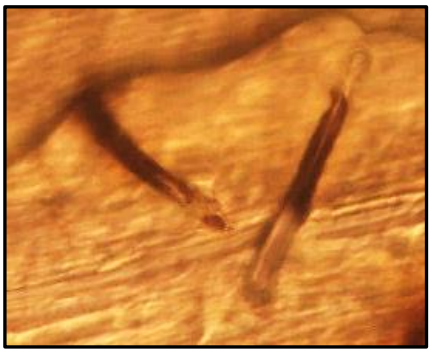

Figure 3. Capitate trichome of Type-II.

Figure 4. Capitate trichome of Type-III.

Capitate trichome of Type II constituted by a unicellular rounded head and body with a short neck cell, a bicellular long stalk, a large unicellular pedestal, and a 6–8 celled basis (Figure 3). Capitate trichome of Type III constituted by one cell head, long-stalked (4–7 cells), and one cell basal. This type matches the description and typology of [13], in which the capitate trichome of type III corresponds to their type 14A (Figure 4). This type is commonly found form in *E. longifolia*. Peltate and capitate trichomes found on the leaflet of *E. longifolia* showed similar characteristics to the glandular trichome reported for the genus *Leucas* [14]. The presence of peltate and capitate trichome appears to be a common characteristic for a large number of medicinal plant species [24].

3.2. Variation of the trichome morphology and density between populations

The results of the analysis of variance showed that the number of trichomes peltate, capitate-type I, and capitate-type II were significant differences between populations in the abaxial leaf surface, while the numbers of capitate-type I, capitate-type II, and capitate-Type III trichomes were significant differences in adaxial leaf surface (Table 2). On the abaxial surface, the highest number of peltate trichome was observed in the West Borneo population, and the lowest value was observed in the Tahura population. The highest number of capitate-1, capitate-2, and capitate3 trichomes were observed in the Lingga-1, Sentajo, and Lingga-2 populations, respectively. On the adaxial surface, the highest number of peltate trichome was observed in the Pokomo population, followed by the Lingga-1 and West Borneo populations, whereas the rest populations were not found of peltate trichome. The highest numbers of capitate-1, capitate-2, and capitate-3 trichomes were observed in the Lingga-2, Sentajo, and Pokomo populations, respectively, while the lowest value was observed in the Sentajo, Lingga-1, and Sentajo populations.

The differences in the number of each trichome between populations are closely related to the environmental conditions of the population and the genetic differences between individuals within the population. Some environmental factors that influenced appearing of trichome were water availability, soil properties, herbivore abundance [8] whereas genes involved in trichome production were *GLABROUS1* (*GL1*), *GLABROUS2* (*GL2*), *GLABROUS3* (*GL3*), *ENHANCER OF GLABRA 3 (EGL3), TRANSPARENT TESTA GLABRA 1 (TTG1) and TRYPTYCHON (TRY)* [25,26]. Furthermore, [25] stated that *GLABROUS1* (*GL1*) gene is the most promising candidate for trichome variation in the Arabidopsis plant.

The density of *E. longifolia* trichomes on the adaxial leaf surface was higher than the abaxial leaf surface. The average value of trichome density on the adaxial and abaxial leaf surfaces was 415.34 mm^{-2} and 63.39 mm^{-2}, respectively, in which was 6.55 times higher in the adaxial than abaxial leaflet surface. The results of the analysis of variance (ANOVA) showed that the density of trichomes in the abaxial and adaxial leaf surfaces showed a highly significant difference ($P < 0.0001$). The high density of glandular trichomes on the upper surface compared to the lower surface is an important strategy of the plant to deal with high light intensity striking the plant leaves the surface, increasing sunlight reflection, reduce water loss in leaf surface, and the role as the chemical defense against biotic

agents [27, 28]. The higher density of trichomes in adaxial leaf surface compared to the abaxial leaf surface was also reported by [29] in *Hyptis villosa* Pohl ex Benth, [28] in the species of Stachytarphyta, and [12] in *Lipia graveolens* H.B.K., but many researchers also reported that trichome density was higher on the abaxial in some plants [15, 30].

Table 2. Mean value of each trichome in the population studies.

Position	Trichome type	Population					
		Tahura	Sentajo	Pokomo	Lingga-1	Lingga-2	West Borneo
Abaxial	Peltate*	0.067^{b}	0.267^{b}	0.100^{b}	0.467^{ab}	0.233^{b}	0.833^{a}
	Capitate-Type 1**	0.633^{ab}	0.267^{bc}	0.000^{c}	1.033^{a}	0.200^{c}	0.400^{bc}
	Capitate-Type II**	0.433^{bc}	2.967^{a}	0.500^{bc}	0.733^{bc}	0.133^{c}	1.000^{b}
	Capitate-Type III	0.767^{ab}	0.467^{b}	0.900^{ab}	0.767^{ab}	1.267^{a}	0.667^{b}
Adaxial	Peltate	0.000	0.000	0.167	0.067	0.000	0.067
	Capitate-Type 1**	3.667^{bc}	1.100^{d}	4.80^{ab}	6.167^{a}	7.067^{a}	1.900^{cd}
	Capitate-Type II**	2.833^{b}	14.667^{a}	1.533^{b}	0.567^{b}	1.033^{b}	2.100^{b}
	Capitate-Type III**	13.100^{a}	4.767^{c}	8.367^{b}	7.633^{bc}	6.767^{bc}	7.833^{b}

Note: * and ** showed significant difference at P< 0.05, and P < 0.01, respectively. The same letters in the same line are no significant differences.

The results of the analysis of variance (ANOVA) showed that the density of trichomes in the abaxial and adaxial leaf surfaces was a highly significant difference among populations (P <0.0001). The highest value of trichome density on the adaxial leaf surface is observed in the Sentajo population of 517.21 mm^{-2}, whereas the lowest value of trichome density is observed in the population of West Borneo of 358.52 mm^{-2}. The result of the Duncan tested showed that Sentajo and Tahura populations were significantly different from other populations. The average value of trichome density on the abaxial surface was ranged from 37.78–99.92 mm^{-2}, which the highest of the density of trichome was observed in the Sentajo population and the lowest value of the trichome density was observed in the population of Pokomo. The difference in trichome density between populations may be caused by genetic differences among individuals within the population and micro-environment conditions of each population. These results are the same as those reported by [11] in *Salvia nemorosa* L.

Table 3. Density of trichome between populations and both leaf surfaces of *E. longifolia*.

Position	Populations						Mean
	Tahura	Sentajo	Pokomo	Lingga-1	Lingga-2	West Borneo	
Abaxial***	47.86^{cd}	99.92^{a}	37.78^{d}	75.57^{ab}	46.18^{d}	73.05^{bc}	63.39^{b}
Adaxial***	493.70^{a}	517.21^{a}	376.99^{b}	363.56^{b}	382.03^{b}	358.52^{b}	415.34^{a}

Note: *** showed significant differences level at P<0.0001 among populations according to the result of anova.

3.3. Correlation between trichome morphology and density with site environmental factors

The results of correlation analysis among trichomes types and density with climatic and geographic factors were exhibited in Table 4. Peltate trichome in the abaxial leaflet surface was positive and significantly correlated to mean annual precipitation and longitude and was negative significantly correlated with mean annual temperature and altitude. Capitate trichome of type 1 in the abaxial leaflet surface was negative significantly correlated to altitude, whereas capitate trichome of type I in the adaxial leaflet surface was a positive significant correlation with altitude, and was a negative significant correlation with mean annual precipitation. Capitate trichome of type II in abaxial leaflet surface was negative significantly correlated to altitude and positive significant correlation with mean annual precipitation, whereas capitate trichome of type I in the adaxial leaflet surface was positive significantly correlated with mean annual precipitation, mean annual temperature, and was negative significantly correlated to longitude and latitude. Capitate trichome of type III in the abaxial leaflet surface was not significantly correlated to climatic and geographic factors of the population studies, whereas capitate trichome of type III in the adaxial leaflet surface was a positive significant correlation with latitude and was negative significantly correlated with mean annual precipitation.

Trichome density in the abaxial leaflet surface was negative significantly correlated to altitude and latitude and was positive significantly correlated to mean annual precipitation, whereas trichome density in the adaxial leaflet surface was a positive significant correlation with mean annual temperature and was negative significantly correlated to longitude. Our results were a line with those reported by [31] in which trichome density in the abaxial leaflet showed a negative significant relationship with altitude. [27] also reported a correlation of trichome density with mean monthly precipitation. In addition, [32] and [33] found that the soil type of plant habitat has an intense influence on the trichome of *Ziziphora tenuior* L and *Acinos graveolens* (M.B.) Link, respectively.

The results of correlation analysis also explained that high trichome density in the adaxial leaflet surface is closely related to temperature. Adaxial leaflet surface receives higher temperatures than abaxial leaflet surface, and plants will present higher trichomes density as a defense mechanism against excessive radiation and higher temperature. [34] explained that the secretion of chemical compounds from trichomes acts as a protector of plant photosynthetic tissue. The secretions reflect excess solar radiation and also help dissipate absorbed heat. As a result, leaf temperatures are maintained at near-optimal levels for photosynthesis and carbon fixation.

Table 4. The correlation coefficients among trichomes types and density with climatic and geographic factors of the population studies.

Position	Variable	Environmental factors				
		Longitude	Latitude	Altitude	MAT*	MAP**
Abaxial	Peltate	0.2421 **(0.0011)**	-0.0238 (0.7517)	-0.1534 (**0.0397)**	-0.2395 (**0.0012)**	0.2179 (**0.0033)**
	Capitate - Type 1	0.0693 (0.3552)	0.0167 (0.8235)	-0.1948 (**0.0088)**	-0.0619 (0.4089)	-0.0404 (0.5908)
	Capitate - Type II	-0.0974 (0.1932)	-0.3297 (**<.0001)**	-0.13 (0.0819)	0.1215 (0.1043)	0.3141 (**<.0001)**
	Capitate -Type III	0.0085 (0.909)	0.0093 (0.902)	0.1445 (0.053)	-0.0181 (0.8093)	-0.1094 (0.1436)
	Trichome Density	0.0822 (0.2725	-0.241 (**0.0011)**	-0.1941 (**0.009)**	-0.0644 (0.3905)	0.2592 (**0.0004)**
Adaxial	Peltate	-0.0054 (0.9418)	0.0419 (0.5764)	0.056 (0.4237)	-0.0032 (0.9657)	-0.0094 (0.9007)
	Capitate - Type 1	-0.0244 (0.7454)	-0.033 (0.6604)	0.236 (**0.0014)**	0.0068 (0.9277)	-0.2773 (**0.0002)**
	Capitate - Type II	-0.2677 (**0.0003)**	-0.3787 (**<.0001)**	-0.0652 (0.3844)	0.2989 (**<.0001)**	0.2619 (**0.0004)**
	Capitate -Type III	-0.0757 (0.3124)	0.3834 (**<.0001)**	-0.1339 (0.0732)	0.0763 (0.3086)	-0.2287 (**0.0020)**
	Trichome Density	-0.3279 (**<.0001)**	-0.0219 (0.7705)	-0.0954 (0.2027)	0.3527 (**<.0001)**	-0.0755 (0.3140)

Note: * mean annual temperature, ** mean annual precipitation.

3.4. Principal component analysis and dendrogram

To know the contribution of each variable in separating of population, the principal component analysis was conducted. Two principal components (PC1 and PC2) explained 99.79% of the total variation among populations (Table 5). PC1 explained 91.01% of the total variation in which the highest contribution was trichome density in adaxial while PC2 explained 8.79% which the variable responsible for distinguishing along the PC2 was trichome density in abaxial. This result indicates that the variable of trichome density could be used for separating the population of the species studies.

The scatter plot of the first two components from the principal component analysis can separate the population of *E. longifolia* based on trichome morphology and density (Figure 5). The first component was clustered Sentajo and Tahura population into the first group, and rest populations were clustered into second groups (axis x). The second component was separated the West Borneo, Lingga-1, and Sentajo populations with Tahura, Lingga-2, and Pokomo populations (axis y). This scatters plot of

PC1 and PC2 considered acceptable for discriminating of the *E. longifolia* population due to explained 99.78% of the total variation. The similar result was reported in *Eurycoma apiculata*, A.W Benn (PC1–PC2= 90%) [35]; and *Eurycoma longifolia* Jack (PC1–PC2= 80.16%) [3].

Table 5. Principal component analysis for trichome character of *E. longifolia*.

Position	Variables	PC1	PC2	PC3	PC4
	Peltate	0.00	0.01	0.03	-0.05
	Capitate - Type 1	0.00	0.01	0.11	0.07
Abaxial	Capitate - Type II	0.01	0.03	-0.11	-0.08
	Capitate - Type III	0.00	-0.01	-0.02	0.07
	Trichome Density	0.13	**0.98**	0.15	0.05
	Peltate	0.00	0.00	-0.01	-0.01
	Capitate - Type 1	-0.02	-0.05	0.03	0.91
Adaxial	Capitate - Type II	0.06	0.11	-0.72	-0.23
	Capitate -Type III	0.00	-0.09	0.66	-0.32
	Trichome Density	**0.99**	-0.13	0.02	0.03
	Eigenvalue	5095.47	491.90	8.12	3.39
	Proportion	91.01	8.79	0.15	0.06
	Cumulative (%)	91.01	99.79	99.94	100.00

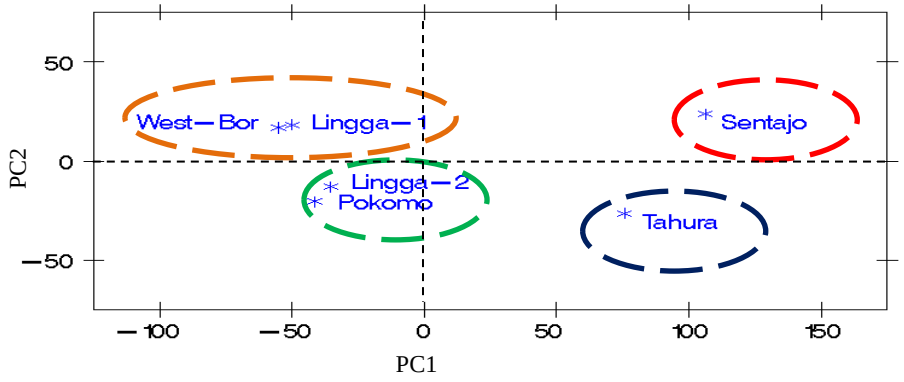

Figure 5. Scatter plot of PCA of E. longifolia populations.

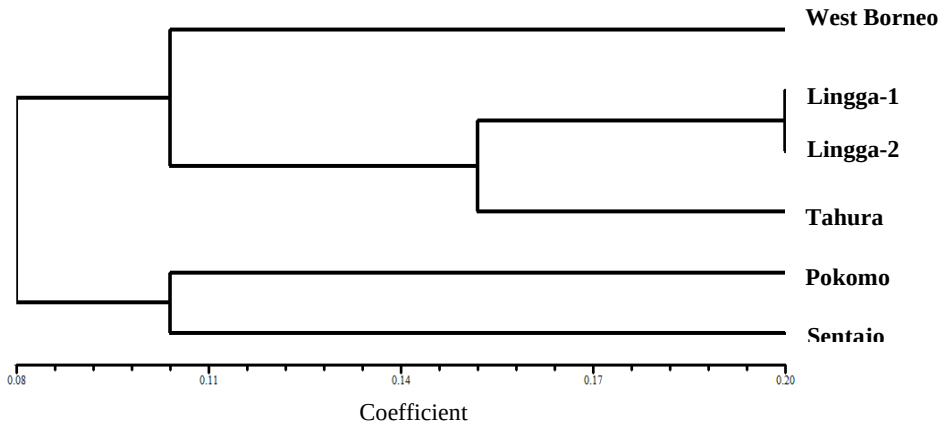

Figure 6. UPGMA dendrogram of *E. longifolia* based on similarity coefficient of trichome character.

The UPGMA dendrogram of *E. longifolia* based on the similarity coefficient of micromorphology characters among populations is exhibited in Figure 6. The UPGMA dendrogram of *E. longifolia* slightly different from the scatter plot of PCA projection. At the coefficient similarity among populations was 0.14, the UPGMA dendrogram of *E. longifolia* divided populations studied into four groups, the first and second groups were Sentajo and Pokomo populations, respectively, the third groups consisted of Lingga-1, Lingga-2, and Tahura populations, and the fourth groups was West Borneo population.

4. Conclusion

Two types of glandular trichomes were detected in leaflet E. longifolia, namely peltate and capitate trichomes. Trichome density in the adaxial leaflet surface is higher than in the abaxial leaf surface and varies between populations. Trichome density in the abaxial was a positive correlation with mean annual precipitation whereas trichome density in the adaxial was a positive correlation with mean annual temperature. The variable of Trichome density can be used for separating the population of the species studies.

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