BUKTI SEBAGAI REVIWER INTERNAISONAL BEREPUTASI

1. Reviwer di Journal of Tropical Biodiversity and Biotechnology

- ✓ Judul Artikel: Diversity, Abundance, and Traditional Uses of Asteraceae Species in Mount Bisma, Dieng Plateau, Kejajar, Wonosobo, Central Java.
- ✓ Telah terbit di Journal of Tropical Biodiversity and Biotechnology, 7(1), 66953.
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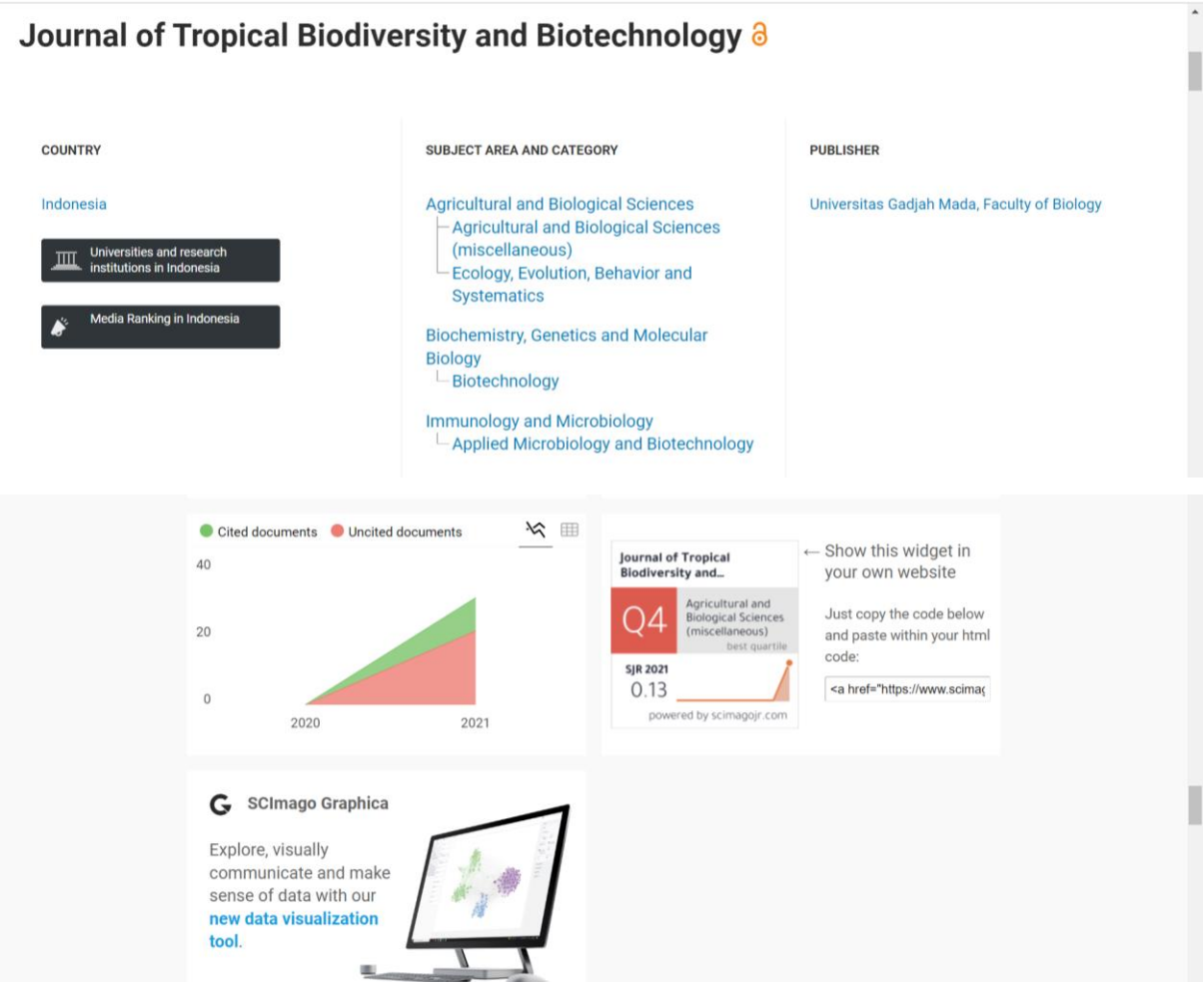

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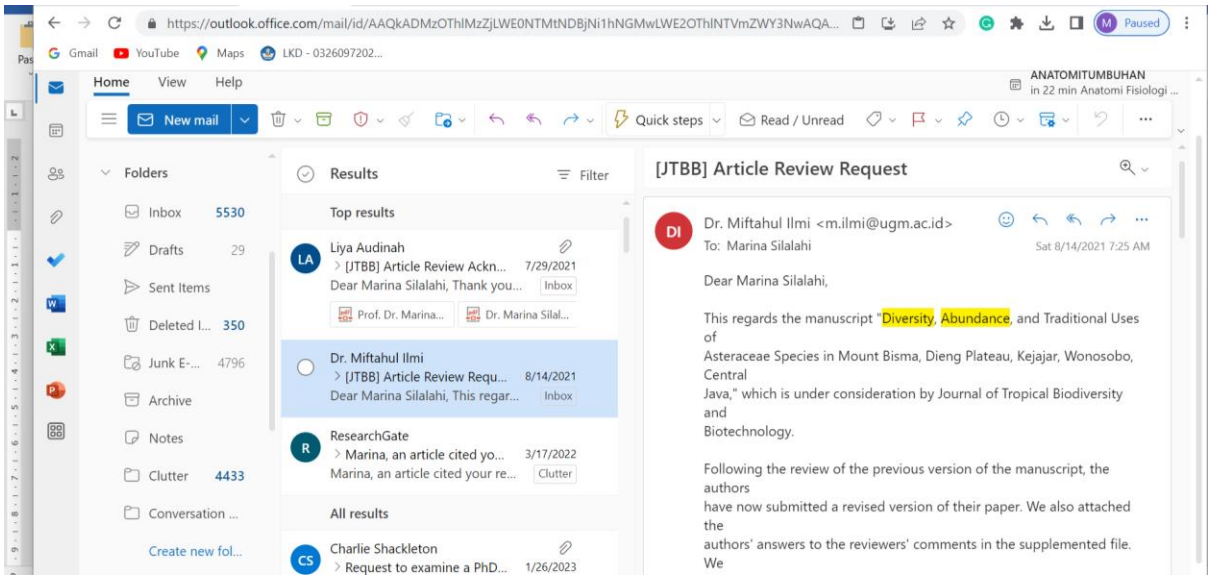

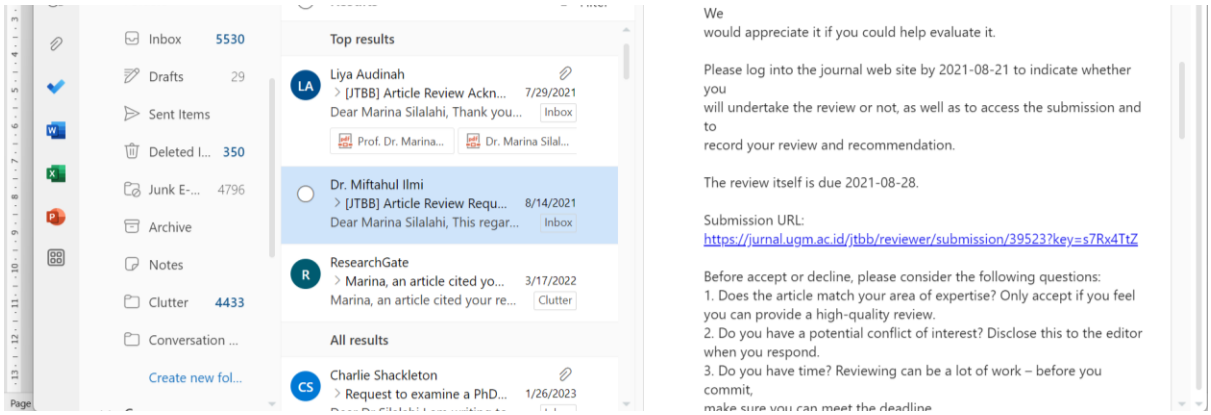

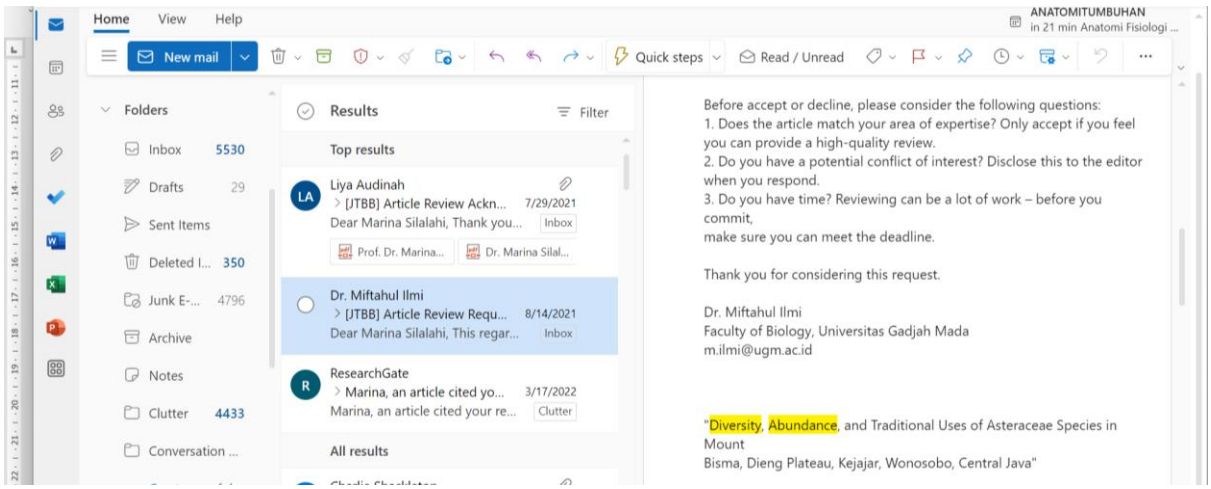

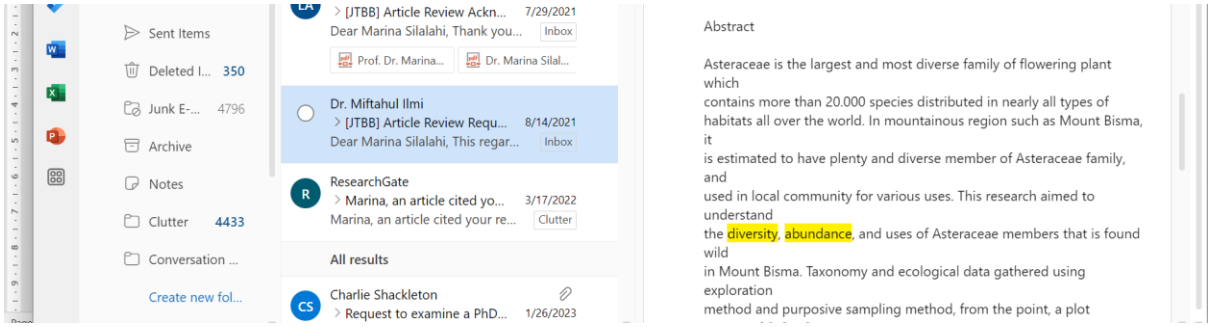
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Asteraceae is the largest and most diverse family of flowering plant which
contains more than 20.000 species distributed in nearly all types of
habitats all over the world. In mountainous region such as Mount Bisma, it
is estimated to have plenty and diverse member of Asteraceae family, and
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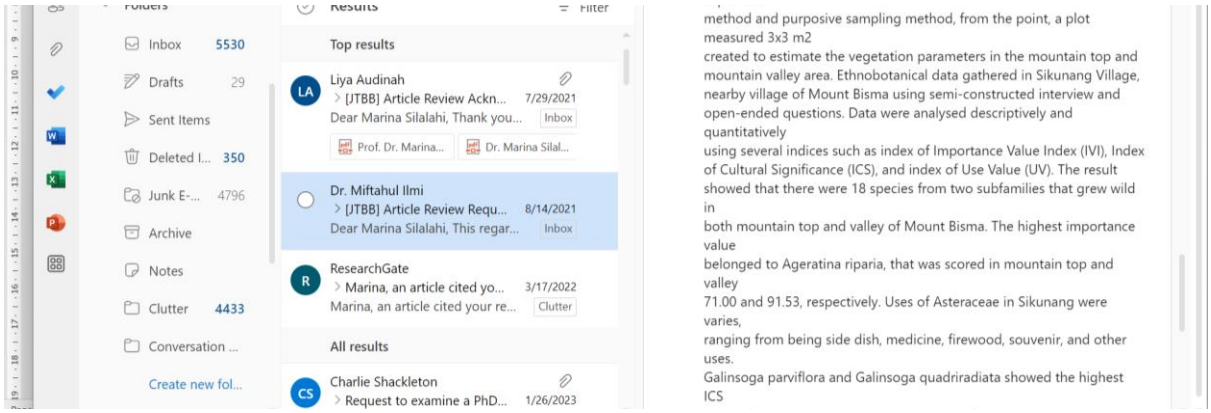
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in
both mountain top and valley of Mount Bisma. The highest importance
value
belonged to Ageratina riparia, that was scored in mountain top and
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Galinsoga parviflora and Galinsoga quadriradiata showed the highest
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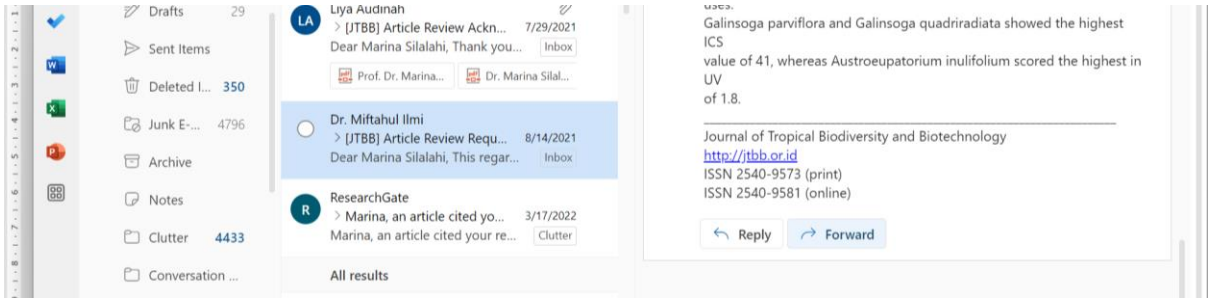
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Galinsoga parviflora and Galinsoga quadriradiata showed the highest
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value of 41, whereas Austroeupatorium inulifolium scored the highest in
UV
of 1.8.
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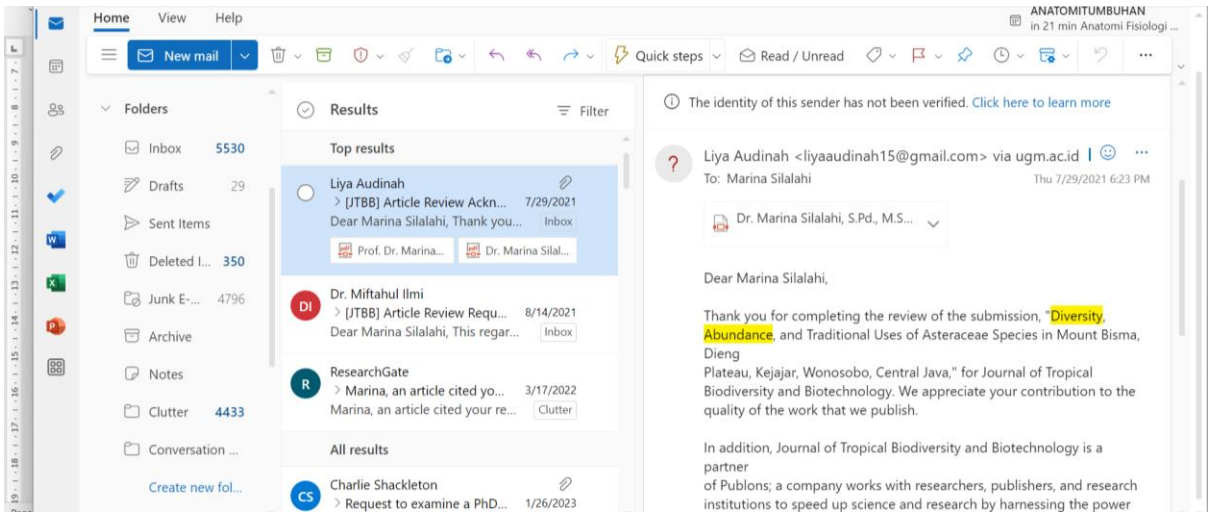
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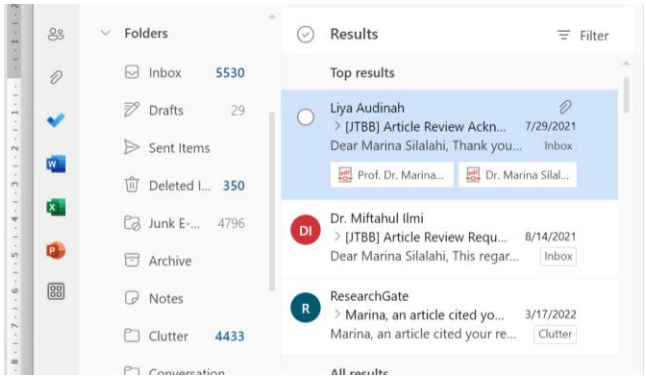

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Journal of Tropical Biodiversity and Biotechnology
http://jtbb.or.id
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ISSN 2540-9581 (online)

CERTIFICATE OF ACKNOWLEDGEMENT

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This is presented to

Dr. Marina Silalahi, S.Pd., M.Si.

for contributing as a reviewer for the Journal of Tropical Biodiversity and Biotechnology in volume 6 issue 3 December 2021

Dr. Miftahul Ilmi

Editor in Chief

Journal of Tropical Biodiversity and Biotechnology

Journal of Tropical Biodiversity and Biotechnology
Volume 07, Issue 01 (2022): jtbb66953
DOI: 10.22146/jtbb.66953

Research Article

Diversity, Abundance, and Traditional Uses of Asteraceae Species in Mount Bisma, Dieng Plateau, Kejajar, Wonosobo, Central Java

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Keywords:
abundance
Asteraceae
Dieng Plateau
ethnobotany
species diversity
Submitted:
23 June 2021
Accepted:
24 September 2021
Published:
09 February 2022
Editor:
Miftahul Ilmi

ABSTRACT
Asteraceae is the largest and most diverse family of flowering plants which contains more than 20,000 species distributed in nearly all types of habitats all over the world. In mountainous regions such as Mount Bisma, it is estimated to have plenty and diverse member of the Asteraceae family, and used in the local community for various uses. This research aimed to understand the diversity, abundance, and uses of Asteraceae members that are found wild in Mount Bisma. Taxonomy and ecological data were gathered using an exploration method and purposive sampling method, from the point, a plot measured 3x3 m^2 was created to estimate the vegetation parameters in the mountain top and mountain valley area. Ethnobotanical data were gathered in Sikunang Village, a nearby village of Mount Bisma using a semi-structured interview and open-ended questions. Data were analysed descriptively and quantitatively using several indices such as Importance Value Index (IVI), Index of Cultural Significance (ICS), and index of Use Value (UV). The result showed that there were 18 species from two subfamilies that grew wild in both mountain top and valley of Mount Bisma. The highest importance value belonged to *Ageratina riparia,* which was scored in the mountain top and valley 71.00 and 91.53, respectively. Uses of Asteraceae in Sikunang were varies, ranging from being a side dish, medicine, firewood, souvenir, and other uses. *Galinsoga parviflora* and *Galinsoga quadriradiata* showed the highest ICS value of 41, whereas *Austroeupatorium inulifolium* scored the highest in UV of 1.8. The study presented high number of Asteraceae diversity and use. Thus, implies that Mount Bisma has vast unexplored biodiversity and locals around Mount Bisma have rich traditional knowledge.

INTRODUCTION
Asteraceae Bercht. & J.Presl (1820); synonym Compositae Giseke (1763), *nom. cons.* is the largest and most diverse family of Angiospermae, composed of ca. 24,000 species from about 1,700 genera that are distributed in nearly all types of habitats all over the world except Antarctica, composing about 10% of recorded diversity of flowering plants (Funk et al. 2009; Tadesse 2014). Member of Asteraceae are undoubtedly widespread due to its highly adaptive characteristics and the specific feature of their flower; a cluster of numerous

tiny flowers (florets) called capitula attractive enough to attract a pollinator, self-pollinating mechanisms of the floret, specific type of fruit called achene that is light enough to be dispersed by wind, supported by the presence of pappus (Bhattacharyya 2016; Purnomo et al. 2016).

A high number of Asteraceae species was considered not proportional with the number of beneficial species from the family. Smaller families, e.g. Poaceae or Fabaceae contain more useful plants than Asteraceae (Funk et al. 2009). In general, members of Asteraceae already used for food (*Lactuca sativa* L., *Helianthus tuberosus* L.), medicine (*Artemisia absinthium* L., *Taraxacum officinale* Wigg.), insecticides (*Tanacetum cinerariifolium* (Trev.) Sch. Bip.), and ornamentals (*Chrysanthemum indicum* L.) (Simpson 2009). A high number of Asteraceae species presents in previous studies are generally acknowledged as a weed. The surrounding community managed to mow the weed because it disturbs the growth of cultivated plants. On the other hand, the surrounding community also finds benefits of Asteraceae species, such as food and medicine. Generally speaking, Asteraceae species are only known to be secondary material as food or medicine, not as the first choice. On the other hand, members of Asteraceae are also recognized as invasive in Indonesia, e.g. *Ageratum conyzoides* L., *Galinsoga parviflora* Cav., *Mikania micrantha* Kunth., and *Sonchus oleraceus* L. (Setyawati et al. 2015) and could affect the survival of native species. Traditional uses of Asteraceae in the local community in unexplored locations need to be researched in order to enrich knowledge on its uses.

Mount Bisma located in Dieng Plateau, Wonosobo, Central Java with a peak reached 2,365 masl, is a volcano, but the volcanic activity is decreased and leaving a wide caldera (Nurpratama et al. 2015). Recently, Mount Bisma is known to be located outside the potential geothermal energy sectors (Harijoko et al. 2016). Mount Bisma was not open for tourist attraction until 2019, thus the mountain has preserved the ecosystem of mountainous region biodiversity. Local communities on Mount Bisma, especially in Sikunang Village, are Javanese, male and female residents mostly work as a farmer, with potato and varieties of vegetables being main crops. Biodiversity and local knowledge of plants in Mount Bisma has not been reported before, especially for the Asteraceae family. Studies on Asteraceae were conducted by Sunarto et al. (2017) in Mount Lawu, Megawati et al. (2017) in Lore Lindu National Park, and Al Farishy & Salamah (2021) in Mount Halimun-Salak. All of the studies conducted before provided general information on Asteraceae diversity in mountainous regions. With the largest number of species, it is highly potential to find any of the beneficial species of Asteraceae. Wonosobo itself offers attractive natural tourism, with edelweiss, *Anaphalis longifolia*, being one of the Asteraceae species that is most attractive for tourists. Local archival research on ethnobotany around Mount Bisma is spoken between generations. There is no known archive of ethnobotany in communities around Mount Bisma. In addition, there is also no known research conducted in the surrounding communities as well. The research aimed to explore

the diversity, abundance, and traditional uses/knowledge about Asteraceae species in Mount Bisma. The result hopefully could present the newest record on the diversity, abundance, and local knowledge of Asteraceae for further research in the future.

MATERIALS AND METHODS

Materials

The materials used in this research were plant specimens collected from two research areas of Mount Bisma; Mountain Top (MT) and Mountain Valley (MV), 300 gr/m^2 acid-free mounting paper sized A3, alcohol 70%, ziplock plastic bag sized 40x30 cm, herbarium envelope, label, document-grade scotch tape, and PVA glue 90%. The tools used in this research were stationary, camera, board, cardboard, voice recorder, and GPS mobile.

Methods

Study area and period

The research was carried out in Mount Bisma, administratively located in Sikunang Village, Kejajar, Wonosobo. The village is located at 7^{0}14'06" S and 109^{0}53'54" E, inhabited by Javanese people, who mainly talk Javanese language with a Banyumasan accent. Mostly, locals are moslem and work as a farmer. Field observation was carried out into two designated areas; Mountain Top (2,365.44 masl) and Mountain Valley (1,599.21 masl). The plot was carried out at two different heights because altitude influences the environment around the plants, limits the growth of certain species and their distribution. Mountain Top following the hiking trails, Mountain Valley started at the intersection between the local's farming area and forest of the valley. Map of the study area presented in Figure 1, red line for MT and blue line for MV. The research was conducted in February 2021 and March 2021.

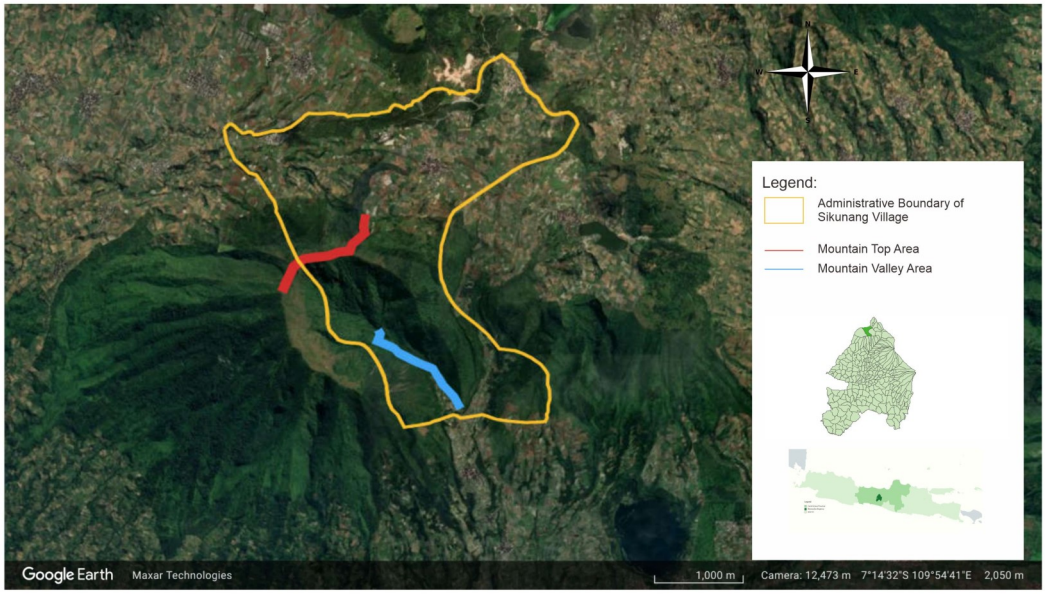

Figure 1. Map of study area showing Sikunang Village administrative boundary (yellow line), Kejajar, Wonosobo, Central Java. Source: Google Earth, 2021.

Field observation and species identification

Field observation aimed to gather the taxonomy and ecological data. The exploration method was used to gather taxonomical data (Rugayah et al. 2004). Purposive sampling was used to find the location that represents Asteraceae diversity following the designated area, then from each point, a 3x3 m^2 plot was made to collect vegetational data, such as a number of species and frequency (Partomihardjo & Rahajoe 2004). A total of 9 plots and 12 plots were made in Mountain Top and Mountain Valley, respectively. Plant samples for each species were taken to make voucher specimens for further identification. The data then were brought to Laboratory of Plant Systematics, Faculty of Biology UGM for voucher specimen construction (Sardiwinata et al. 2008); identification was based on determination key from Flora of Java (Backer & Brink 1965), description and illustration from Mountain Flora of Java (van Steenis 2006).

Interview

The village community survey was used to gather the informants, who are the residents of Sikunang Village, purposive snowball sampling was chosen to found informants with the village chief as a key informant (Silalahi 2016). The respondents are adults (more than 18 years old) from various professions. A total of 15 respondents, mostly by the suggestion of the Village Chief, were interviewed. Fifteen respondents are considered accurate because all of them were chosen by recommendation of a key informant so that all the informants know very well about plants around the village. A semi-structured interview with open-ended questions was performed to gather information from informants (Walujo 2004). The interview was equipped with pictures of Asteraceae species found in Mount Bisma.

Data analysis

Asteraceae species were grouped by taxa, scientific names were rechecked based on Classification of Compositae (Funk et al. 2009) and Global Compositae Database (CWG 2021). The characteristics of invasiveness were determined based on the Guide to Invasive Plant Species in Indonesia (Setyawati et al. 2015). Ecological data in the form of relative density and relative frequency were further analysed by Importance Value Index (IVI) analysis (Barbour et al. 1987). The interview data were analysed to estimate the valuation of the index of cultural significance respective to the categorization of uses (ICS) (Turner 1988), along with the Use Value (UV) analysis (Walujo 2004; Cotton 1996) as general quantification on ethnobotanical data to represent how beneficial a species is for a community.

RESULTS AND DISCUSSION

Asteraceae Diversity

Study of species diversity on Asteraceae family in hiking trails (mountain top) and mountain valley of Mount Bisma was carried out. Based on Funk et al.

(2009), a total of 18 species under 16 genera, eight tribes, and two subfamilies were collected and identified. All species were considered wild. Based on the data obtained, out of the 18 species found in the research location, 17% were recorded only in Mountain Top (MT); 55% were recorded to be present in both locations (MT and MV); and 28% were recorded only in Mountain Valley (MV) (Table 1). The species found only in Mountain Top; *Anaphalis longifolia, Erigeron karvinkianus,* and *Leucanthemum vulgare* were known to inhabit slope ground and unshaded areas. *A. longifolia* was known to be typical to grow in the mountain region of Java (van Steenis 2006), the species also found in Mount Lawu and attract tourism activity (Sunarto et al. 2017).

Table 1. Diversity of Asteraceae in Mount Bisma.

No.	Species	Subfamily	Tribe	Location Found	Specimen Voucher Number
1	*Artemisia vulgaris* L.	Asteroideae	Anthemideae	MV, MT	13/BA/1, 30/BB/10, 64/BB/8, 68/BB/11
2	*Leucanthemum vulgare* Lam.	Asteroideae	Anthemideae	MT	41/BA/3
3	*Dichrocephala integrifolia* (L.f.) Kuntze	Asteroideae	Astereae	MV, MT	02/BA/1, 52/BA/1, 69/BB/11
4	*Erigeron karvinskianus* DC.	Asteroideae	Astereae	MT	14/BA/9, 15/BA/8, 46/BA/7
5	*Erigeron sumatrensis* Retz.	Asteroideae	Astereae	MV, MT	01/BA/1, 47/BA/2, 67/BB/10
6	*Bidens pilosa* L.	Asteroideae	Coreopsideae	MV, MT	04/BA/1, 09/BA/9, 10/BA/1, 12/BA/5, 33/BB/9, 36/BB/11, 42/BA/2, 62/BB/7
7	*Ageratum conyzoides* L.	Asteroideae	Eupatorieae	MV	73/BB/12
8	*Ageratina riparia* (Reg.) R.M.King & H.Rob	Asteroideae	Eupatorieae	MV, MT	05/BA/1, 07/BA/9, 08/BA/9, 16/BA/9, 24/BB/3, 25/BB/1, 26/BB/1, 39/BB/3, 48/BA/1, 53/BA/9, 54/BA/8, 57/BB/1, 58/BB/4, 59/BB/5, 60/BB/3
9	*Austroeupatorium inulifolium* (Kunth.) R.M.King & H.Rob.	Asteroideae	Eupatorieae	MV, MT	03/BA/7, 45/BA/2, 55/BB/1, 56/BB/2, 61/BB/6
10	*Anaphalis longifolia* DC.	Asteroideae	Gnaphalieae	MT	06/BA/5, 18/BA/9, 21/BA/9, 49/BA/13
11	*Gnaphalium purpureum* L.	Asteroideae	Gnaphalieae	MV, MT	37/BB/10, 51/BA/1
12	*Acmella paniculata* (Wall ex. DC.) R.K.Jensen	Asteroideae	Heliantheae	MV	32/BB/10, 63/BB/9
13	*Tithonia diversifolia* (Hemsl.) A.Gray	Asteroideae	Heliantheae	MV	40/BB/12
14	*Galinsoga parviflora* Cav.	Asteroideae	Millerieae	MV, MT	11/BA/1, 27/BB/9, 28/BB/9, 29/BB/10, 31/BB/10, 34/BB/10, 35/BB/11
15	*Galinsoga quadriradiata* Ruiz. & Pav.	Asteroideae	Millerieae	MV	65/BB/11

Table 1. Contd.

No.	Species	Subfamily	Tribe	Location Found	Specimen Voucher Number
16	*Crassocephalum crepidioides* (Benth.) S. Moore	Asteroideae	Senecioneae	MV, MT	19/BA/1, 22/BA/1, 38/BB/11, 71/BB/11
17	*Sonchus oleraceus* L.	Cichorioideae	Cichorieae	MV, MT	43/BA/3, 44/BA/4, 50/BA/1, 66/BB/10, 70/BB/12
18	*Youngia japonica* (L.) DC.	Cichorioideae	Cichorieae	MV	72/BB/12

Two subfamilies found wild in Mount Bisma were Asteroideae and Cichorioideae. There are informal categories in the Asteroideae subfamily, the Heliantheae allies. From the subfamilies, it was recognized that seven tribes were classified into Asteroideae (four tribes included in Heliantheae allies) and one tribe classified into Cichorioideae. The diversity of Asteraceae species found in Mount Bisma were similar to other research done in mountainous regions of Sulawesi and Mount Lawu (Megawati et al. 2017; Sunarto et al. 2017). Asteroideae leads in the number of species found because Asteroideae is the largest and most diverse subfamily of Asteraceae (Funk et al. 2009). Most species found in the research are considered invasive alien species (IAS), about 13 out of 18 are considered as IAS, four species known to be alien species, and only one species is recognized distributed naturally in Java. The majority of IAS and alien species are originated in New World (Tropical, North, or South America), the rest of them originated from Old World: *C. crepidioides* and *B. pilosa* (Africa); *A. vulgaris, L. vulgare, S. oleraceus* (Europe and Russia Far East); and two species originated in Asia: *D. integrifolia* and *Y. japonica* (Setyawati et al. 2015). The diversity of flower morphology of wild Asteraceae species found in Mount Bisma is presented in Figure 2.

Asteraceae Abundance

The abundance of Asteraceae species found was determined by the value of the Importance Value Index (IVI). The highest IVI in both locations, MT and MV was obtained from species of *Ageratina riparia* with IVI 71.00% and 91.53%, respectively. The result showed that *A. riparia* was dominated the area with the most number of individuals and presented in the most study plot. *A. riparia* covered most of the forest floor in MT and MV. Figure 3 and figure 4 showed the IVI for each species found in MT and MV where the IVI does not distribute equally, two species (*A. riparia* and *Austroeupatorium inulifolium*) have prominent IVI compared to other species; statistically signs that the coverage of both species are considered high, meanwhile, the other species showed low IVI compared to the dominating species. *A. riparia* was recognized to be invasive alien species and dominate the forest floor of several mountainous areas of Java; the IVI scored 69.418% and 81.35% in Mount Lawu and Dieng Plateau (Mount Alang and Mount Klaras), respectively (Setyawati et al. 2015; Sunarto et al. 2017; Abdiyani 2008).

Figure 2. Species of Asteraceae found wild in Mount Bisma. A – H. Asteroideae (without Heliantheae alliance): Gnaphalieae: *Anaphalis longifolia, Gnaphalium purpureum*; Senecioneae: *Crassocephalum crepidioides*; Astereae: *Dichrocephala integrifolia, Erigeron karvinskianus, Erigeron sumatrensis*; Anthemideae: *Leucanthemum vulgare, Artemisia vulgaris.* I – P: Asteroideae (Heliantheae alliance): Coreopsideae: *Bidens pilosa*; Eupatorieae: *Ageratum conyzoides, Ageratina riparia, Austroeupatorium inulifolium*; Millerieae: *Galinsoga parviflora, Galinsoga quadriradiata*; Heliantheae: *Acmella paniculata, Tithonia diversifolia.* Q – R: Cichorioideae: Cichorieae: *Sonchus oleraceus*, *Youngia japonica.* Source: Personal Documentation, 2021.

The diversity and distribution at different altitudes are relatively different. In Mountain Valley, the area with lower altitude, the species richness counted was more than that of Mountain Top (14 species in MV, 12 species in MT). There is one species that is dominant in both locations, *Ageratina riparia*, IVI of MV is higher (91.53%) than in MT (71.00%), so the *A. riparia* is considered more dominant in MV than in MT. Even though had more number species, the dominance of *A. riparia* compared to the other species in MV made MV had lower diversity and distribution, which showed that in MT (higher altitude) it is considered more diverse and the species are distributed more evenly. Asteraceae found in higher altitudes, such as *Anaphalis longifolia* and *Erigeron sumatrensis* are observed to have special adaptation in terms of its morphological character, i.e. adapted to grow well in cliff/ sloping ground; have narrow leaves, drier stem, and strongly attached to the ground. In lower altitudes, the species generally have a wider and thinner leaf, grows perpendicular to the substrate, and wetter stem.

Ageratina riparia was known to be common in Java mountainous region (Purnomo et al. 2016). The invasiveness of *A. riparia* was categorized as high risk, *A. riparia* even had the highest Risk Index in Mount Papandayan. *A. riparia* has an uncommon feature of Asteraceae, that is the species could grow well in shaded areas. Coverage of *A. riparia* was very wide due to the fast-growing characteristics of the plant, even it is considered very fast compared to other invasive species. On the other hand, the species is also a productive

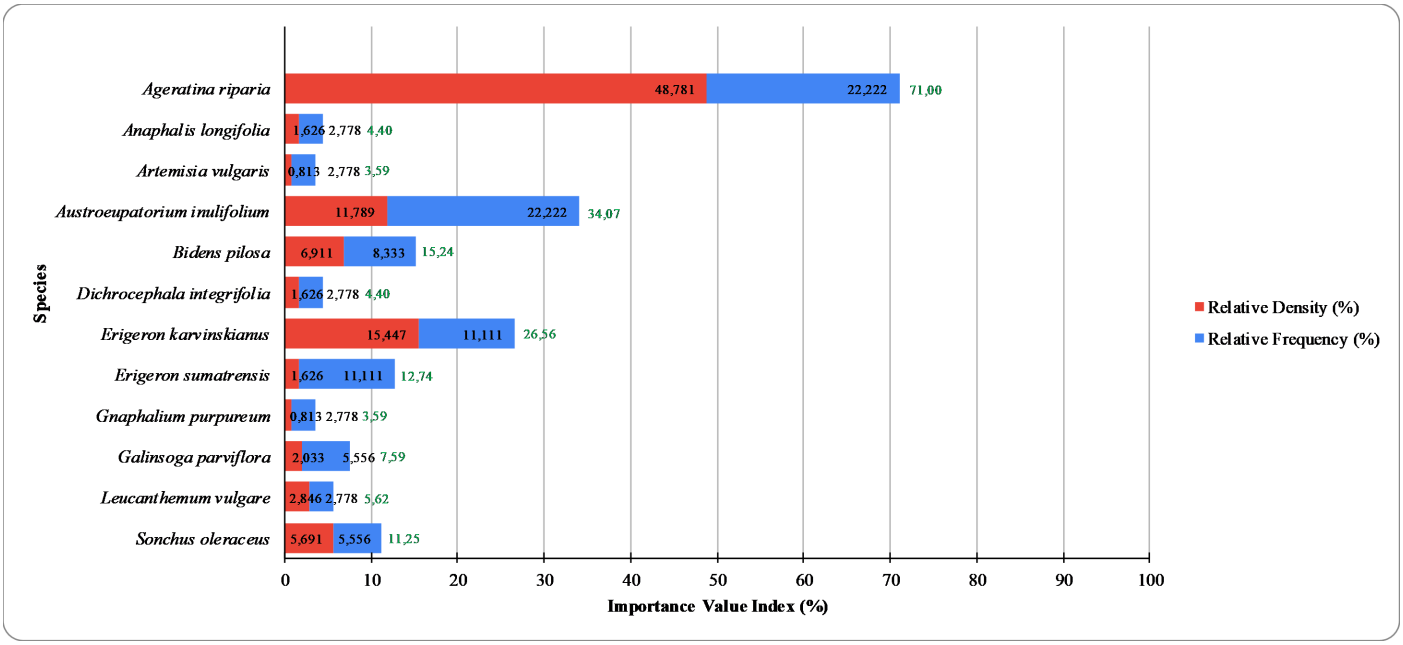

Figure 3. Importance Value Index (IVI) from Mountain Top (MT) Area. Number showed in green symbolized the summation of Relative Density and Relative Frequency (Importance Value Index).

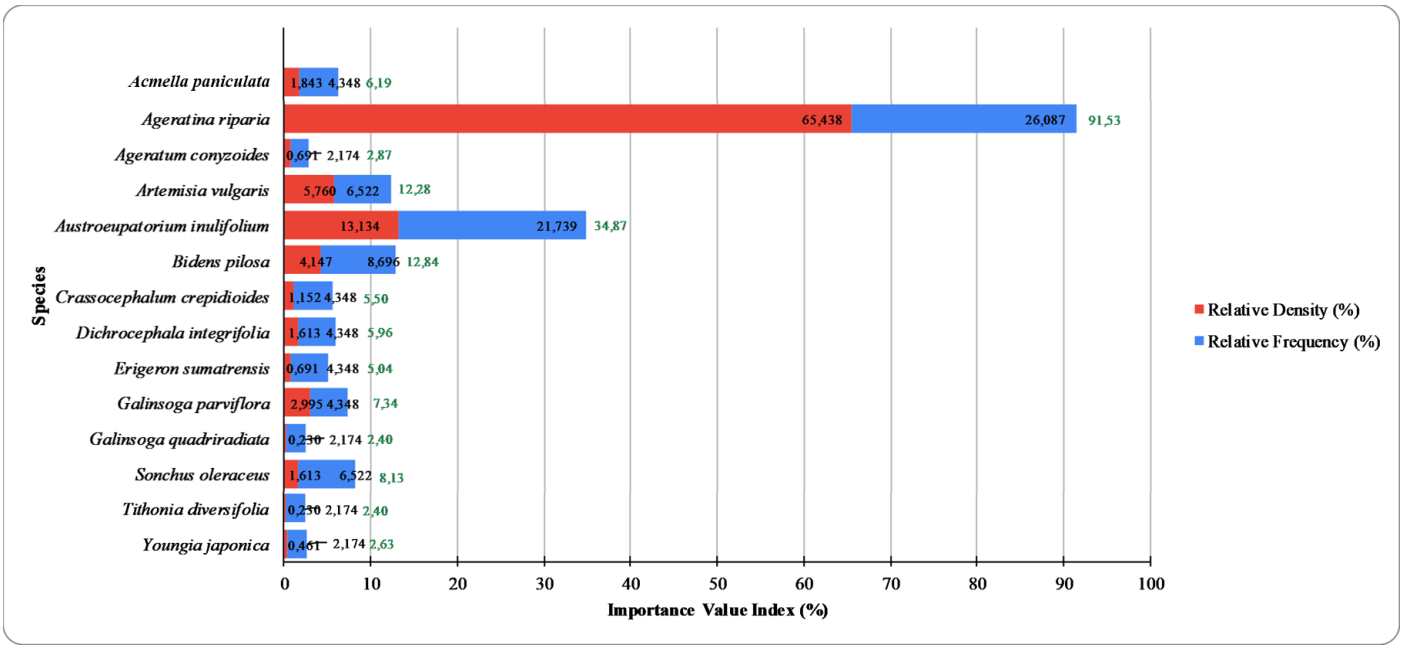

Figure 4. Importance Value Index (IVI) from Mountain Valley (MV) Area. Number showed in green symbolized the summation of Relative Density and Relative Frequency (Importance Value Index).

seeder (Nyuanti et al. 2020). A combination of the characteristics leads the species to invade an area, growing rapidly and dense to dominate the area so that the other species do not have enough space and resources to grow adequately.

Asteraceae Uses

Locals of Sikunang Village, a nearby village of Mount Bisma were interviewed and the result showed that locals had vast knowledge of plant uses in their household; the uses from 17 out of 18 species of Asteraceae found were recognized by the locals. Locals in Sikunang Village perceive the

majority of Asteraceae species found as weed, disturbed their field. Several people also recognized species that grow in the upper part of Mount Bisma, inside the forest, and far from their field. However, locals not only mentioned the species as weeds. Local uses of the plant as told by the people and the valuation of ICS and UV are presented in Table 2.

Locals mentioned that several species could be utilized for consumption, medicine, daily needs, livestock forage, to be sold, etc. Even though locals perceive Asteraceae members in general as weeds, the utilization is a sign that locals have a well-understanding in plant uses. Locals in Sikunang Village maintain vegetation amid their mountainous environment, especially in Asteraceae members, make it possible for them to explore any utilization of plants. Plants mentioned used by locals have an opportunity as bioprospection in the future, because the plants are abundant and the benefits are varied, e.g. *G. parviflora* and *G. quadriradiata* as delicacies, *C. crepidioides* as cosmetics, *L. vulgare* as insecticides. Other bioprospection of Asteraceae members mentioned by locals is *S. oleraceus* (*gembos*) as rabbit forage. Some of the locals mentioned that rabbit which fed using a mixture of grass, rabbit pellets, and *gembos* produced better urine quality than rabbit which fed without mixture of *gembos*, that is highly beneficial as biofertilizer.

The quantification of ICS was based on the quality, intensity, and exclusivity of plant's uses (Turner 1988). The highest score of ICS was obtained by *mondrengan* or *G. parviflora* and *G. quadriradiata* with a score reached 41, that most locals told that the plant could be eaten just raw or cooked. *Sendura* (*A. longifolia*) scored second in ICS (36) because of the valuation of the flower, a rare plant with high demand in tourism attraction. *Gembos* (*S. oleraceus*) scored third highest of ICS (30) because of their utilization as food, high quality rabbit forage, even some medicinal properties. Meanwhile, *malenggo* (*A.inulifolium*) scored fourth highest of ICS (24) because it serves the daily needs of locals as easy-accessed firewood, livestock forage, and economic-importance fragrant flower. On the other hand, UV represents various types of uses known by the locals (Walujo 2004). *Malenggo* (*A. inulifolium*), *mondrengan* (*G. parviflora* and *G. quadriradiata*), *sendura* (*A. longifolia*), and *lengko* (*C. crepidioides*) relatively had high scores compared to other species found in Mount Bisma because various uses are recognized by most locals, meanwhile, the species which had a low score in UV means that the utilization is not as much as the other species or the utilization varies, but only known to the minority of locals (Cotton 1996). As mentioned in the beginning, most species are known as weeds and disturbing cultivated plants, therefore if locals do not utilize the plant for a specific activity, commonly seen plants will be used as livestock forage, e.g. *D. integrifolia* and *E. sumatrensis*. *G. purpureum* is an uncommon plant for locals; locals do not recognize the plant and therefore the utilization is unclear for locals. High UV or ICS value suggests that the species is more beneficial than other species. If the species is beneficial enough to be demanded by more people, the species tend to be cultivated by people, then it will preserve in

Table 2. Local name, traditional uses, ICS, and UV for each species of Asteraceae species found in Mount Bisma.

No.	Species	Local Name	Traditional Uses	ICS*	UV**
1	*Galinsoga parviflora* Cav.	Mondrengan, Jarinten, Jangkungan	Young leaves eaten raw as "*lalapan"* or cooked to be *sayur bobor, sayur bening, oseng,* or *pecel*. Older leaves as rabbit forage.	41	1.53
2	*Galinsoga quadriradiata* Rez. & Pav.				
3	*Anaphalis longifolia* DC.	Sendura	Flower kept as room freshner, dried as ornamental, insect repellent, or sold in nearby tourist attraction.	36	1.40
4	*Sonchus oleraceus* L.	Gembos	Young leaves having medicinal properties to cure hepatitis and other liver diseases, facilitating breast milk. Older leaves used as forage of rabbit.	30	1.27
5	*Austroeupatorium inulifolium* (Kunth.) R.X.King & H.Rob.	Malenggo, Maregol, Sembung, Krenyo, Maitan, Rikowot, Tembulungan, Wedangan	The wood used as firewood; leaves as forage for goat; fragrant flower as room freshner and mixed with sendura to sold in nearby tourist attraction.	24	1.80
6	*Crassocephalum crepidioides* (Benth.) S.Moore	Lengko, Cangklong, Menjangan	Young leaves eaten, having medicinal properties such as skin diseases, ulcer, and face mask; older leaves as forage of rabbit and guinea pig; mix of leaf and root as soporific or cure of vitamin B deficiency; flower as children's toy.	18.5	1.40
7	*Leucanthemum vulgare* Lam.	Jenu, Jenung, Pitrem	Flower used as mosquito repellant, tonic, poultice for sore, or used as ornamentals; root and stem used to strengthen soil, prevent weeds growing, as fish poison or insecticide.	17.5	0.80
8	*Ageratum conyzoides* L.	Bandotan, Rema, Seprah muda, Entut-entutan	Young leaves eaten (cooked, not usual), or used as forage of goat, leaves as poultice for wound or slices; stem latex as substitute of eucalyptus oil, could prevent bleeding.	17	0.80
9	*Erigeron karvinskianus* DC.	Lonte sore, Pitrem gunung, Jenu hitam, Kembang benik, Otot-ototan	Whole part of the plant used to prevent growth of other weeds or used as ornamentals; leaves and flower as poultice for sore; root used as additional materials in tonic.	13.5	0.73
10	*Artemisia vulgaris* L.	Sudamele, Sidamele, Ambril	Leaves used as insecticide and herbicide, root brewed as tonic.	10.5	0.67
11	*Acmella paniculata* (Wall. ex. DC.) R.K. Jensen	Suweng-suwengan, Suket jangkung, Bendotan	Flower in the form of earrings for children's toy; leaves as emergency toothpaste.	7.5	0.40
12	*Youngia japonica* (L.) DC.	Gembos kuning, Kenikir	Leaves as forage or eaten (not usual); flower as mosquito repellant and ornamentals.	5.5	0.71
13	*Ageratina riparia* (Reg.) R.M.King & H.Rob	Lakhar, Suket republik, Repeblik	Leaves used as subtitute of goat forage in dry season; stem and leaves fermented for 15 days as insecticide; root rotted as soil fertilizer.	3	0.53
14	*Bidens pilosa* L.	Kenul, Ketul, Ranjau, Trucukan, Puyengan	Leaves used as poultice/therapy of skin diseases (itch); flower as children's toy.	2.5	0.13
15	*Dichrocephala integrifolia* (L.f.) Kuntze	Rendeng, Semprang, Semprah	Livestock forage.	1.5	0.07
16	*Erigeron sumatrensis* Retz.	Jlantir	Alternative of livestock forage.	1.5	0.20
17	*Tithonia diversifolia* (Hemsl.) A. Gray	-	Flower used as poultice of ulcer.	1.5	0.07
18	*Gnaphalium purpureum* L.	-	- (no known uses)	0	0.00

*ICS: Index of Cultural Significance, **UV: Use Value

the area. In Merapi-Merbabu Slopes, Boyolali there are two Asteraceae species found with relatively low UV; *Tagetes erecta* and *Lactuca sativa* with UV reached 0.1 and 0.07, respectively (Umartani & Nahdi 2021) Other research conducted in Cibodas Biosphere Reserve showed prominent ICS on two Asteraceae species; *Artemisia vulgaris* (ICS: 98.32) and *Bidens pilosa* (ICS: 67.81) (Handayani et al. 2021).

Table 3 showed the known and recorded uses of Asteraceae species found in Mount Bisma by scientific exploration. Traditional knowledge on the uses of each species is found to contribute and enrich the recorded uses of Asteraceae species from studies conducted before. Rich traditional knowledge and scientific exploration should be combined to find the best utilization for the species. On the other hand, in the discussion on Asteraceae diversity, it is known that most species were recognized as alien species. Locals play important role in the population control of alien species by utilizing the plant optimally (Al Farishy & Salamah 2021).

Table 3. Recorded uses of Asteraceae species found in Mount Bisma.

No.	Species	Known Uses
1	*Galinsoga parviflora* Cav.	Young leaves as food, treated as wild vegetables. Contains minerals; older leaves as rabbit forage (Santosa et al. 2020)
2	*Galinsoga quadriradiata* Rez. & Pav.	
3	*Anaphalis longifolia* DC.	Flower sold as souvenir (Utomo & Heddy 2019)
4	*Sonchus oleraceus* L.	Edible (vegetables); treat anemia, liver infection, opium dependency, diurethic, bacteri infection (Jimoh et al. 2011; Setyawati et al. 2015)
5	*Austroeupatorium inulifolium* (Kunth.) R.X.King & H.Rob.	Phytotoxic, cytotoxic, and anti-fungal activity; leaveas as cure for cough and fever, sore, and regulating fertility (Chandrasiri et al. 2015; Quattrochi 2016)
6	*Crassocephalum crepidioides* (Benth.) S.Moore	Young leaves edible (vegetables), cure for gastrointestinal problem, wound or slice, prevent bleeding; rabbit forage (Quattrochi 2016; Dairo & Adanlawo 2007)
7	*Leucanthemum vulgare* Lam.	Ornamentals, mosquito repellent; leaves as food (Clements et al. 2004)
8	*Ageratum conyzoides* L.	Skin disease and wound cure; slices, burnt, ulcer (Syamsuhidayat & Hutapea 1991)
9	*Erigeron karvinskianus* DC.	Ornamentals, skin diseases (Quattrochi 2016; Sharmila et al. 2014)
10	*Artemisia vulgaris* L.	Menstruation cycle, miscarriage, dysentry, nosebleed, intestinal bleeding (Wijayakusuma et al. 1994)
11	*Acmella paniculata* (Wall. ex. DC.) R.K. Jensen	Emergency toothpaste, stomachaches, cure for fever (Quattrochi 2016; Setyawati et al. 2015)
12	*Youngia japonica* (L.) DC.	Consumed as food, larvicidal activity (Rojas-Sandoval 2020; (Liu et al. 2015)
13	*Ageratina riparia* (Reg.) R.M.King & H.Rob	Facilitate urination (Santosa et al. 2017)
14	*Bidens pilosa* L.	Cure for fever, rheumatic, toothache, throat disease, skin diseases, snake bites (Wijayakusuma et al. 1994; (Setyawati et al. 2015)
15	*Dichrocephala integrifolia* (L.f.) Kuntze	Analgesic, antibacterial, antiinflammation, cure for fever (Setyawati et al. 2015; Quattrochi 2016)
16	*Erigeron sumatrensis* Retz.	Wound poultice, headaches, vertigo, TBC, asthma, rheumatic, stomachaches (Silalahi et al. 2019)
17	*Tithonia diversifolia* (Hemsl.) A. Gray	Wound poultice, malaria, diarrhea, ornamentals, fever cure (Silalahi et al. 2019; Setyawati, et al., 2015)
18	*Gnaphalium purpureum* L.	unknown

CONCLUSION

Research in Mount Bisma discovered a total of 18 species of Asteraceae from two subfamilies and eight tribes, with the most abundant species was *Ageratina riparia,* dominating both the area observed due to its invasive nature. Locals of Sikunang Village recognized various uses of Asteraceae plants, such as consumption, livestock forage, medicine, sold in a tourist attraction, etc. *G. parviflora* and *G. quadriradiata*, known as *mondrengan*, scored the highest in ICS, that is 41. Meanwhile, *malenggo* (*A. inulifolium*), scored the highest in UV, which is 1.80. This study presented new information about Asteraceae species in Mount Bisma, implies that there are vast unexplored biodiversities in Mount Bisma and rich traditional knowledge on plant uses in its surrounding community. It needs further research in order to archive and preserve the biodiversity and the traditional knowledge.

AUTHORS CONTRIBUTION

B.K. collected and analysed the data and wrote the manuscript, P designed and supervised the research and revised the manuscript, R.S.K. supervised, revised, and finalized the manuscript.

ACKNOWLEDGMENTS

The author would like to thank Mr. Nur Amin, Chief of Sikunang Village for giving permission to conduct the research in Sikunang Village and local informants of Sikunang Village, Kejajar, Wonosobo for sharing their knowledge on the use of plants by participating in the survey. The manuscript is part of B.K. thesis under supervision of R.S.K.

CONFLICT OF INTEREST

The authors state that there is no conflict of interest in this research.

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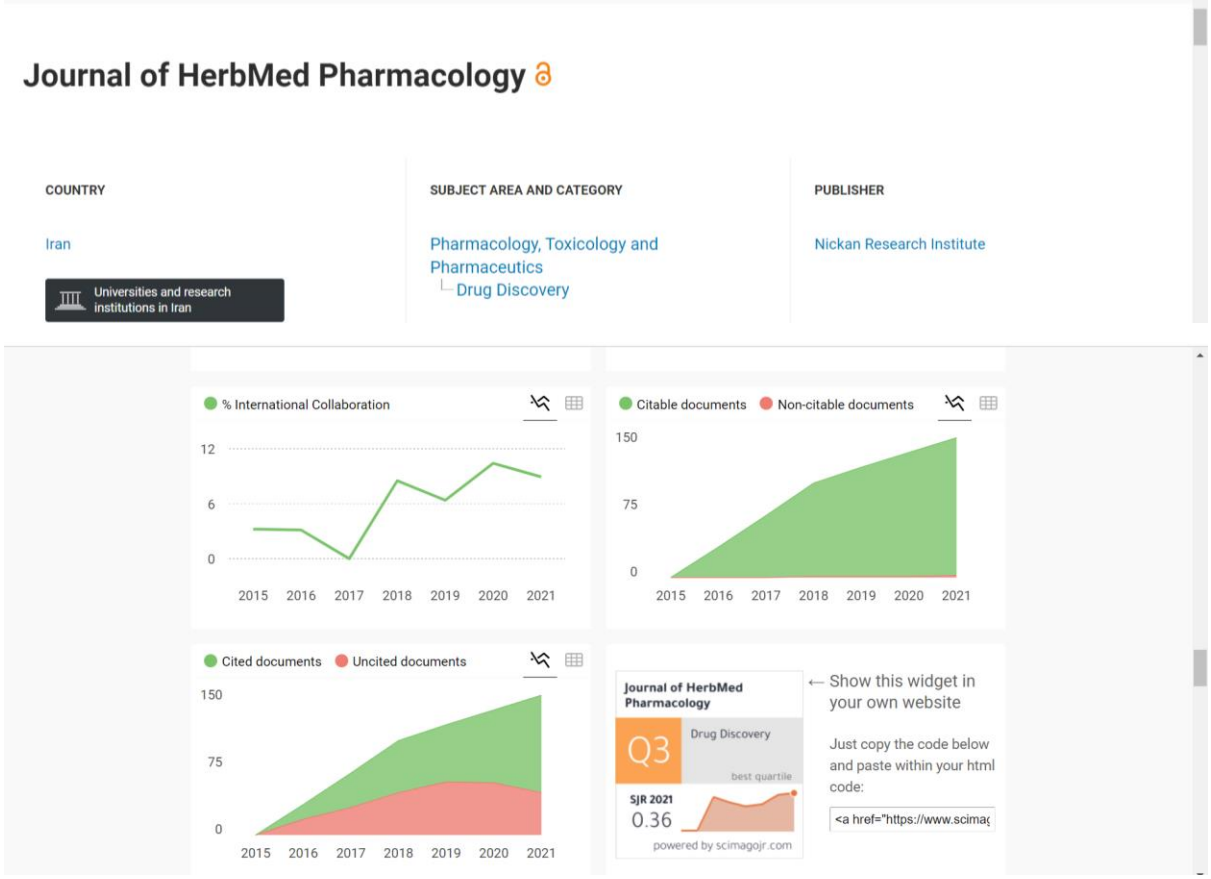

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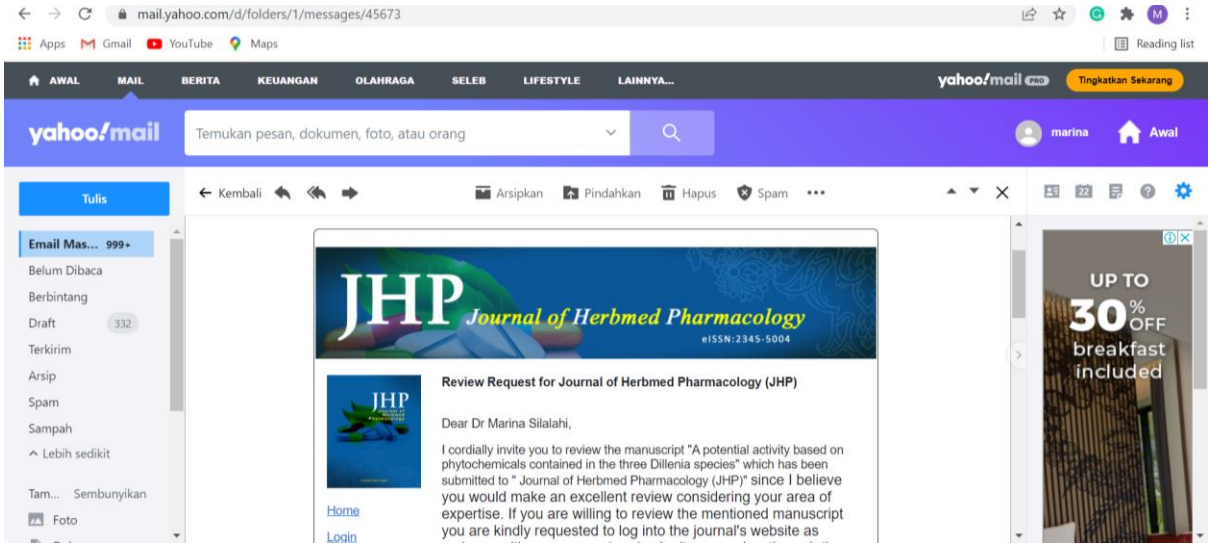

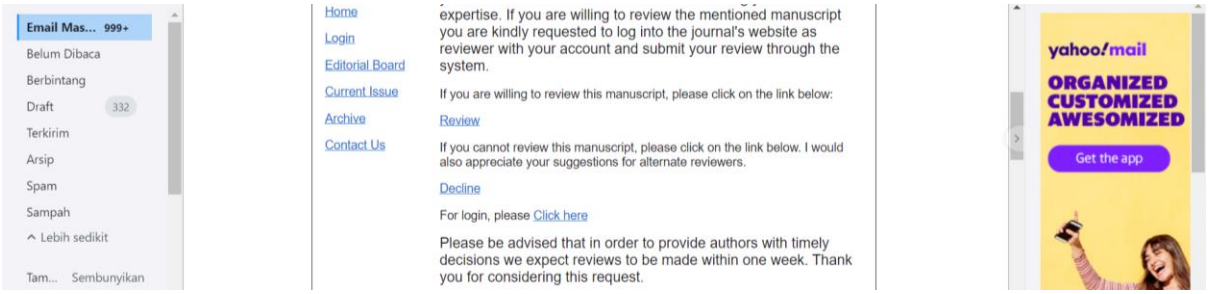
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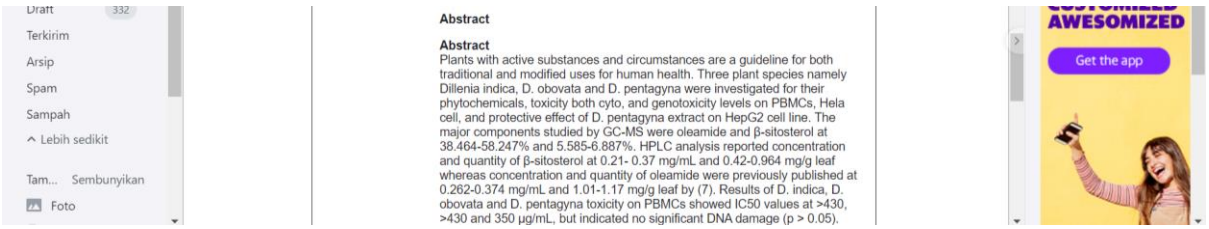
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Abstract
Abstract
Plants with active substances and circumstances are a guideline for both traditional and modified uses for human health. Three plant species namely Dillenia indica, D. obovata and D. pentagyna were investigated for their phytochemicals, toxicity both cyto, and genotoxicity levels on PBMCs, Hela cell, and protective effect of D. pentagyna extract on HepG2 cell line. The major components studied by GC-MS were oleamide and β-sitosterol at 38.464-58.247% and 5.585-6.887%. HPLC analysis reported concentration and quantity of β-sitosterol at 0.21- 0.37 mg/mL and 0.42-0.964 mg/g leaf whereas concentration and quantity of oleamide were previously published at 0.262-0.374 mg/mL and 1.01-1.17 mg/g leaf by (7). Results of D. indica, D. obovata and D. pentagyna toxicity on PBMCs showed IC50 values at >430, >430 and 350 µg/mL, but indicated no significant DNA damage (p > 0.05).
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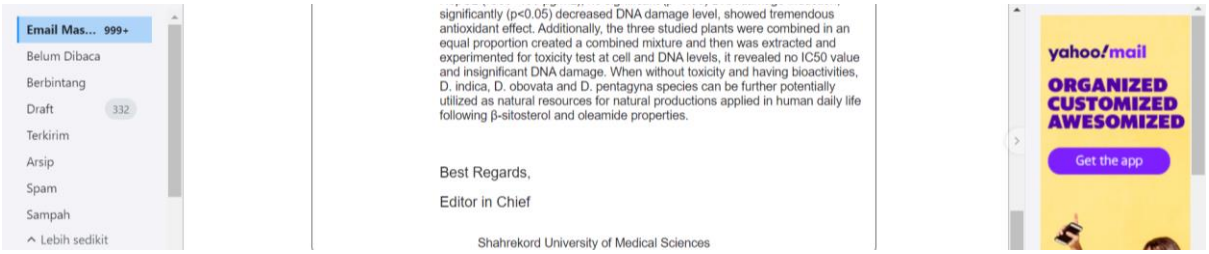
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significantly (p<0.05) decreased DNA damage level, showed tremendous antioxidant effect. Additionally, the three studied plants were combined in an equal proportion created a combined mixture and then was extracted and experimented for toxicity test at cell and DNA levels, it revealed no IC50 value and insignificant DNA damage. When without toxicity and having bioactivities, D. indica, D. obovata and D. pentagyna species can be further potentially utilized as natural resources for natural productions applied in human daily life following β-sitosterol and oleamide properties.
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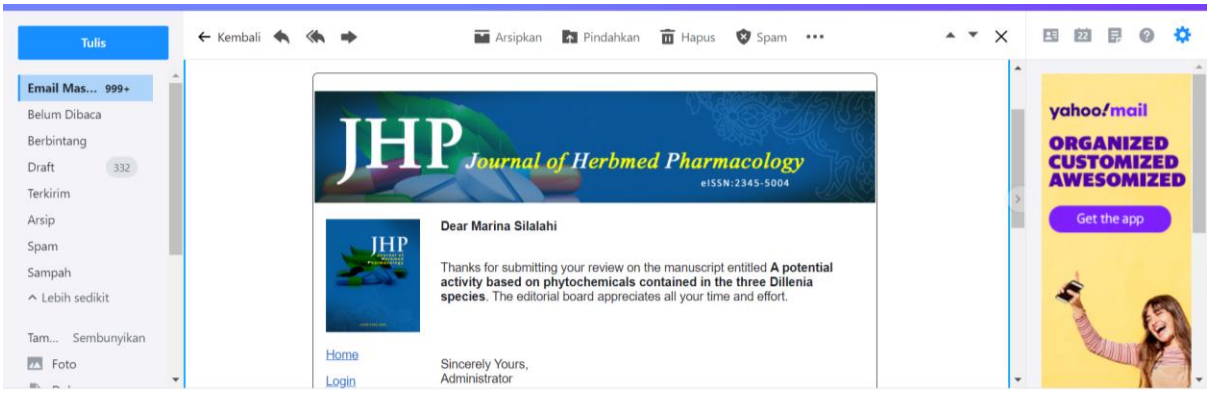
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Journal of Herbmed Pharmacology
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Dear Marina Silalahi
Thanks for submitting your review on the manuscript entitled A potential activity based on phytochemicals contained in the three Dillenia species. The editorial board appreciates all your time and effort.
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J Herbmed Pharmacol. 2022; 11(3): 339-347.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2022.39

Journal of Herbmed Pharmacology

Screening of phytochemicals, toxicities, and activities of three *Dillenia* species

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ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 11 October 2021
Accepted: 2 April 2022

Keywords:
β-Sitosterol
Comet assay
Oleamide
Toxicity

ABSTRACT

Introduction: Plants containing β-sitosterol and oleamide are important for various diseases. So, *Dillenia indica*, *D. obovata*, and *D. pentagyna* were investigated for phytochemicals, cytotoxicity and genotoxicity levels on peripheral blood mononuclear cells (PBMCs) and Hela cells. The protective effect of *D. pentagyna* extract on a HepG2 cell line was also investigated.
Methods: Gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) were used for phytochemical analysis. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction (MTT) and comet assays were performed for toxicity testing and protective effects against DNA oxidative damage.
Results: The major components were oleamide and β-sitosterol at 38.464-58.247% and 5.585-6.887% with concentration and quantity of β-sitosterol at 0.2-0.37 mg/mL and 0.42-0.964 mg/g leaf. The *D. indica*, *D. obovata*, and *D. pentagyna* toxicities on PBMCs showed IC_{50} values at >430, >430, and 350 µg/mL respectively, with no significant DNA damage ($P > 0.05$) compared to the negative control group. All plant extracts showed toxic activity on Hela cell with IC_{50} values at <0.43 µg/mL and induced significant DNA damage ($P < 0.05$) compared to the negative control group. Conversely, the activity of the *D. pentagyna* extract indicated low cytotoxic activity against HepG2 (IC_{50}>430 µg/mL), no significant ($P > 0.05$) DNA damage induction, significantly ($P < 0.05$) decreased DNA damage level, and tremendous antioxidant effect. Additionally, a combined mixture of all plants in an equal proportion revealed no IC_{50} value and insignificant DNA damage.
Conclusion: All the studied species contained oleamide and β-sitosterol, with toxicity on Hela cells without toxicity on PBMC. The *D. pentagyna* species showed high antioxidant effects and no toxicity on HepG2.

Implication for health policy/practice/research/medical education:
This research emphasizes phytochemicals concentrated on oleamide and β-sitosterol, toxicities, and activities of *D. indica, D. obovata*, and *D. pentagyna* species. This information can support further uses in human health.
Please cite this paper as: Siripiyasing P, Silawong K, Thooptianrat T, Sudmoon R, Babayan N, Khondkaryan L, et al. Screening of phytochemicals, toxicities, and activities of three *Dillenia* species. J Herbmed Pharmacol. 2022;11(3):339-347. doi: 10.34172/jhp.2022.39.

Introduction

Plants, plant-derived extracts, and phytochemicals isolated from plants have long been used worldwide in the treatments of many diseases. For example, the alkaloid morphine, a strong painkiller, is used to treat severe pain after an operation, serious injury, and pain from cancer and heart attacks; the anti-tussive agent codeine; and the anti-spasmodic alkaloid papaverine isolated from poppy (*Papaver somniferum*) (1). Cannabidiol (CBD), tetrahydrocannabinol (THC), and

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other phytocannabinoids and non-phytocannabinoid chemicals, such as terpenes and flavonoids have myriad pharmacological properties (2-5). Hence, phytochemicals are natural resources, which lead to many beneficial uses with more sophisticated and modern preparation methods. Plants are important sources of novel pharmacologically active compounds, with 50% of the approved drugs being derived directly or indirectly from plants. Despite the current preoccupation with synthetic chemistry as a vehicle to discover and manufacture drugs, the contribution of plants to disease treatment and prevention is still enormous (6). Also, the two substances β-sitosterol and oleamide are important in human body function. Oleamide is a protective agent against scopolamine-induced memory loss and has been suggested to be useful as a preventive agent against Alzheimer's disease, insomnia, loss of appetite, inflammation, prevention and treatment of atherosclerosis, thrombosis, arthritis, and cancer (7-11). β-sitosterol is as important to health as oleamide; it possesses various biological actions such as anxiolytic and sedative effects, analgesic, immunomodulatory, antimicrobial, anticancer, anti-inflammatory, lipid-lowering effect, hepatoprotective, protective effect against non-alcoholic fatty liver disease and respiratory diseases, wound healing effect, antioxidant and anti-diabetic activities (12).

There are still many important plants and substances that have not yet been studied and mentioned. The more plant species we know and study, the more benefits we find for humans. Given this, the two substances β-sitosterol and oleamide in *D. indica*, *D. obovata* and *D. pentagyna* species were examined; the cytotoxicity and genotoxicity, as well as DNA protective activity, were also evaluated, aimed at plant compounds and usage.

Materials and Methods

Plant materials

The mature leaves of three *Dillenia* species, *D. indica*, *D. obovata* and *D. pentagyna* were collected. The plants were identified by a proficient botanist, prof. Dr. Aunrat Chaveerach, the specimens were kept at the Department of Biology, Faculty of Science, Khon Kaen University, Thailand, collector no. A. Chaveerach 930, 931, and 934. Then the leaves were further processed following the below steps.

Methods

Preparation of chemical extracts

To prepare the extracts, the leaf samples were rinsed with water and air-dried. A 20 g of sample was then ground into a powder, mixed with 125 mL of hexane (analytical grade) and filtered through a filter paper at room temperature. Next, 80 mL of the filtrate was obtained and stored at -20°C until use. The highest extract concentration was obtained at 4300 µg/mL. This concentration was used as a stock and 10-fold serial dilutions were performed to prepare a total of four testing concentrations, 430, 43, 4.3, and 0.43 µg/mL for cytotoxicity and genotoxicity assays.

The three study plants were combined in an equal proportion as a combined mixture, which was then extracted to obtain a concentration of 4,300 µg/mL. Then the four testing concentrations, 430, 43, 4.3, and 0.43 µg/mL were made and used for cytotoxicity and genotoxicity assays.

Gas chromatography-mass spectrometry (GC-MS) analysis and component identification

The GC-MS analysis of the crude extracts was performed using an Agilent Technologies GC 6890 N/5973 inert mass spectrometer fused with a capillary column (30.0 m × 250 µm × 0.25 µm). Helium gas was used as the carrier at a constant flow rate of 1 mL/min. The injection and mass-transferred line temperature was set at 280°C. The oven temperature was programmed for 70°C to 120°C at 3°C/min, then held isothermally for 2 minutes, and raised to 270°C at 5°C/min. A 1 µL aliquot of the crude extract was injected in split mode. The relative percentage of the crude constituents was expressed as a percentage using peak area normalization. Component identification was determined by comparing the obtained mass spectra with the reference compounds in the Wiley 7N.1 library.

Cell culture

In this study human peripheral blood mononuclear cells (PBMCs), human cervical carcinoma (HeLa) and human hepatoma (HepG2) cell lines were used. PBMCs were obtained from 24 mL blood sample collected from healthy human from the Central Blood Bank at Srinagarind hospital, (Khon Kaen University) into heparinized blood collection tubes using sterile techniques. PBMCs were isolated using Ficoll-Paque density gradient technique (Ficoll-Paque Plus, GE Healthcare). The cells were collected by centrifugation and resuspended in phosphate-buffered saline (PBS). The cells were cultured in growth medium RPMI (Sigma Aldrich, Germany) supplemented with 10% fetal bovine serum (HyClone, UK), 2 mM L-glutamine (Sigma Aldrich, Germany), 100 IU/mL penicillin (Sigma Aldrich, Germany), and 100 µg/mL streptomycin (Sigma Aldrich, Germany) at 37°C and 5% CO_2. The human HeLa and HepG2 cell lines were routinely maintained in the growth medium DMEM (Sigma Aldrich, Germany) supplemented with 10% fetal bovine serum (HyClone, UK), 2 mM L-glutamine (Sigma Aldrich, Germany), 100 IU/mL penicillin (Sigma Aldrich, Germany), and 100 µg/mL streptomycin (Sigma Aldrich, Germany) at 37°C and 5% CO_2.

Estimation of cytotoxicity

The cytotoxicity of plant extracts in PBMCs was assessed using trypan blue exclusion test (13). The cell suspension

was prepared at a concentration of viable cells at 4-6 × 10^5 cells/mL. The cell suspension was divided into 1.5 mL microtubes (500 µL/tube) and incubated with the plant extract (50 µL/tube) at 37°C for 24 hours. The cells were treated with different concentrations of the plant extract diluted with dimethyl sulfoxide (DMSO): 4,300, 430, 43, and 4.3 µg/mL. The untreated cells (negative control) were incubated in a culture medium only. The DMSO treated samples were used as vehicle control. Cells incubated with 100 µM of H_2O_2 for 15 minutes were used as a positive control. The cytotoxicity of plant extracts in HeLa and HepG2 cell lines was analyzed using MTT colorimetric cell viability assay (14). The cells were seeded at the density of 0.2-0.25 × 10^6 cell/mL into 96-well plates (Greiner, Germany) and incubated for 24 hours at 37°C and 5% CO_2. Afterwards, plant extracts were added to the cell cultures at concentrations ranging from 4.3 µg/mL to 430 µg/mL. Untreated cells were used as a negative control, while DMSO treated cells served as solvent control. After further incubation for 24 hours, the MTT assay was performed. The absorbance was measured using an ELISA plate reader (Human Reader HS, Germany) at a wavelength of 570 nm. Cell viability was expressed as a percentage of the negative control. Doses inducing 50% inhibition of cell viability (the IC_{50} value) were calculated using GraphPad Prism 5.01 (GraphPad Software, USA). Each experiment was performed in triplicate.

Genotoxicity assay

The alkaline single-cell gel electrophoresis (comet assay) was performed to evaluate DNA damages induced by plant extracts according to the method described by (15) with slight modifications. Briefly, after incubation with the extracts as described above, cell pellets were obtained by centrifugation and then resuspended in PBS. A 100 µL of 0.5% low melting point agarose (LMA) was mixed with40 µL of the cell suspension. The mixture was dropped onto slides that were precoated with 1% normal melting point agarose. Next, cover slips (22 mm × 50 mm) were placed on top of the slides and stored at 4°C. After 5-10 minutes, the cover slips were removed, and slides were submerged in a lysis solution (8 M NaCl, 0.6 M EDTA pH 8, 0.2 M Tris, 0.1% Triton X-100) for at least 1 hour. Slides were then soaked in electrophoresis buffer (6 mM EDTA pH 10, 0.75 M NaOH) for 40 minutes. Next, electrophoresis was performed for 25 minutes at 26 volts and 300 milliamps at 4°C. After electrophoresis, the slides were immediately neutralized with 0.4 M Tris buffer (pH 7.5) for 5 minutes. The neutralization process was repeated three times for 5 minutes each. The slides were then stained with 1 µg/mL ethidium bromide (60 µL per slide) overnight at 4°C in the dark. Comets were observed at 360x magnification with a fluorescence microscope Zeiss III RS (Germany) equipped with 560 nm excitation filter, 590 nm barrier filter and a CCD video camera PCO (Germany). At least 150 cells (50 cells for each of triplicate slides) were examined for each experimental point. Image analysis software Komet 4 (Perceptive instruments, UK) was used to analyze the content of DNA in the tail (by the relative tail fluorescence intensity in percent to the untreated control) and olive tail moment (OTM), representing the product of a tail length and the percentage of DNA content in the tail of comets.

Protective effect of plant extracts towards DNA oxidative damage in HepG2 cell line

Antioxidant activity of non-toxic plant extract, *D. pentagyna* against hydrogen peroxide-induced DNA damage was evaluated using comet assay in HepG2 cell line. The plant extract at the concentration of 430 µg/mL was added to the cultures of HepG2 cell line 24 hours after seeding. Experiments were performed in two experimental protocols: (I) co-treatment of cells with hydrogen peroxide and plant extract, which tested the ability of the plant extracts to directly scavenge ROS and (II) 24 hours pre-treatment with plant extract followed by co-treatment (pre+co-treatment) which, in addition to direct scavenging activity, allowed accumulation of plant extracts in the cell and induction of enzymatic and non-enzymatic cellular antioxidants and detoxifying enzymes. The untreated cells were used as a negative control (NC), cell cultures treated only with H_2O_2 (50 µM of H_2O_2 for 5 minutes) were used as a positive control (PC). The comet assay was performed as described above.

Statistical analysis

Data analysis was performed with GraphPad Prism 5.01 (GraphPad Software, USA). All experiments were repeated at least three times. At least triplicate cultures were scored for an experimental point. All values were expressed as means ± SE. Data were analyzed by non-parametric Mann-Whitney U test. $P < 0.05$ was considered as the statistically significant value.

Results

The phytochemical study on three *Dillenia* species, *D. indica*, *D. obovata,* and *D. pentagyna* by GC-MS indicated that oleamide was found as the most abundant constituent with 38-58% from the hexane leaf extracts. Additionally, β-sitosterol was also found at 6.887%, 5.679%, and 5.585%, respectively. The GC-MS chromatogram is shown in Figure 1, and the phytochemical contents are presented in Table 1. The HPLC analysis following chromatograms (Figure 2) reported that they contained β-sitosterol concentrations of 0.21 mg/mL, 0.25 mg/mL and 0.37 mg/mL, and quantities of 0.42 mg/g, 0.55 mg/g, and 0.964 mg/g dried leaf material, respectively (Table 2).

The results of dose dependent effect of plant extracts on the viability of PBMC, HeLa and HepG2 cells are shown in Figure 3, and the calculated IC_{50} values are presented in Table 3. The three *Dillenia* extracts showed slight or

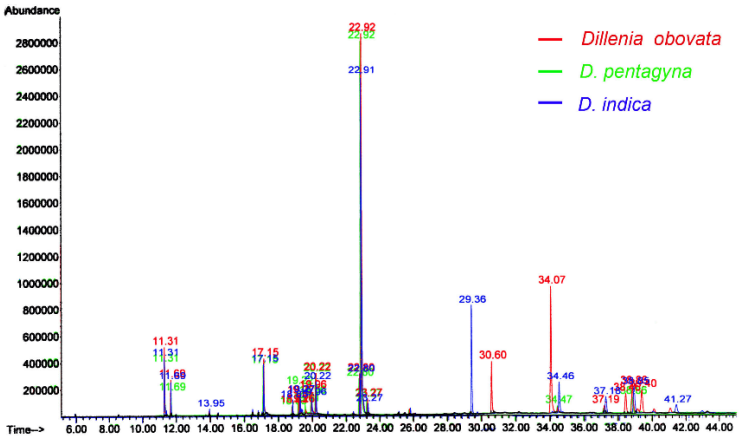

Figure 1. Chromatograms of hexane crude extracts from the leaves of *Dillenia indica*, *D. obovate*, and *D. pentagyna*.

no cytotoxic effect on PBMCs. In the case of *D. indica* and *D. obovata* extracts treatments, the cell viability at the highest tested concentration of 430 µg/mL was 77-79% demonstrating a non-cytotoxic profile for PBMC cells. For the *D. pentagyna* extract, the cell viability at the treatment concentration of 430 µg/mL was 48.10±7.02% with an estimated IC_{50} value of 350 µg/mL. The PBMCs viability incubated with 100 µM H_2O_2 (positive control) was 39.60 ± 7.09%.

For the genotoxicity assessment in terms of induction of DNA strand breaks in PBMCs, the extract concentrations of 430 µg/mL, 430 µg/mL, and 350 µg/mL were tested for *D. indica*, *D. obovata* and *D. pentagyna*, respectively, using comet assay (Figure 4). The three *Dillenia* extracts were shown to be non-genotoxic since the level of DNA damage was statistically insignificant compared to the negative control (Table 4). The dose-dependent cytotoxic effects of three species on HeLa and HepG2 cells are revealed in Figure 3a and 3b.

However, HeLa cells demonstrated higher sensitivity

Table 1. Percentage of chemical constituents in relative content by GC-MS of the three *Dillenia* species

Common name	Chemical formula	Relative content (%)		
		D. indica	*D. obovata*	*D. pentagyna*
Oleamide	$C_{18}H_{35}NO$	42.21	38.46	58.25
Squalene	$C_{30}H_{50}$	11.29	-	-
β-Sitosterol	$C_{29}H_{50}O$	6.89	5.68	5.59
BHT quinone methide	$C_{15}H_{22}O$	5.43	4.50	-
Vitamin E	$C_{29}H_{50}O2$	5.30	-	-
Palmitic acid or myristic acid or tridecanoic acid	$C_{16}H_{32}O_2$	5.00	4.11	5.55
Stigmasterol	$C_{29}H_{48}O$	3.90	1.38	-
Palmitamide	$C_{16}H_{33}NO$	3.21	3.27	5.00
Cycloartenol	$C_{30}H_{50}O$	2.34	-	-
n-Hentriacontane	$C_{19}H_{40}$	0.49	17.13	-
1,5-Dimethyl-6-(1,5-dimethylhexyl)	$C_{28}H_{46}O_2$	-	5.68	-
Taraxerol	$C_{30}H_{50}O$	-	5.03	-
D-Friedoolean-14-en-3-one	$C_{30}H_{48}O$	-	3.65	-
n-Heptadecane	$C_{17}H_{36}$	-	3.39	-
Stearic acid	$C_{18}H_{36}O_2$	-	2.31	2.80
Butylated hydroxytoluene (BHT)	$C_{15}H_{24}O$	-	1.81	-
Phytol	$C_{20}H_{40}O$	-	1.24	3.37
2,6-Di-tert-butylbenzoquinone	$C_{15}H_{22}O$	-	-	5.73
Linolenic alcohol or α-linolenic acid	$C_{18}H_{32}O$	-	-	2.02
2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	-	-	1.87
Unknown		13.95	2.35	9.82

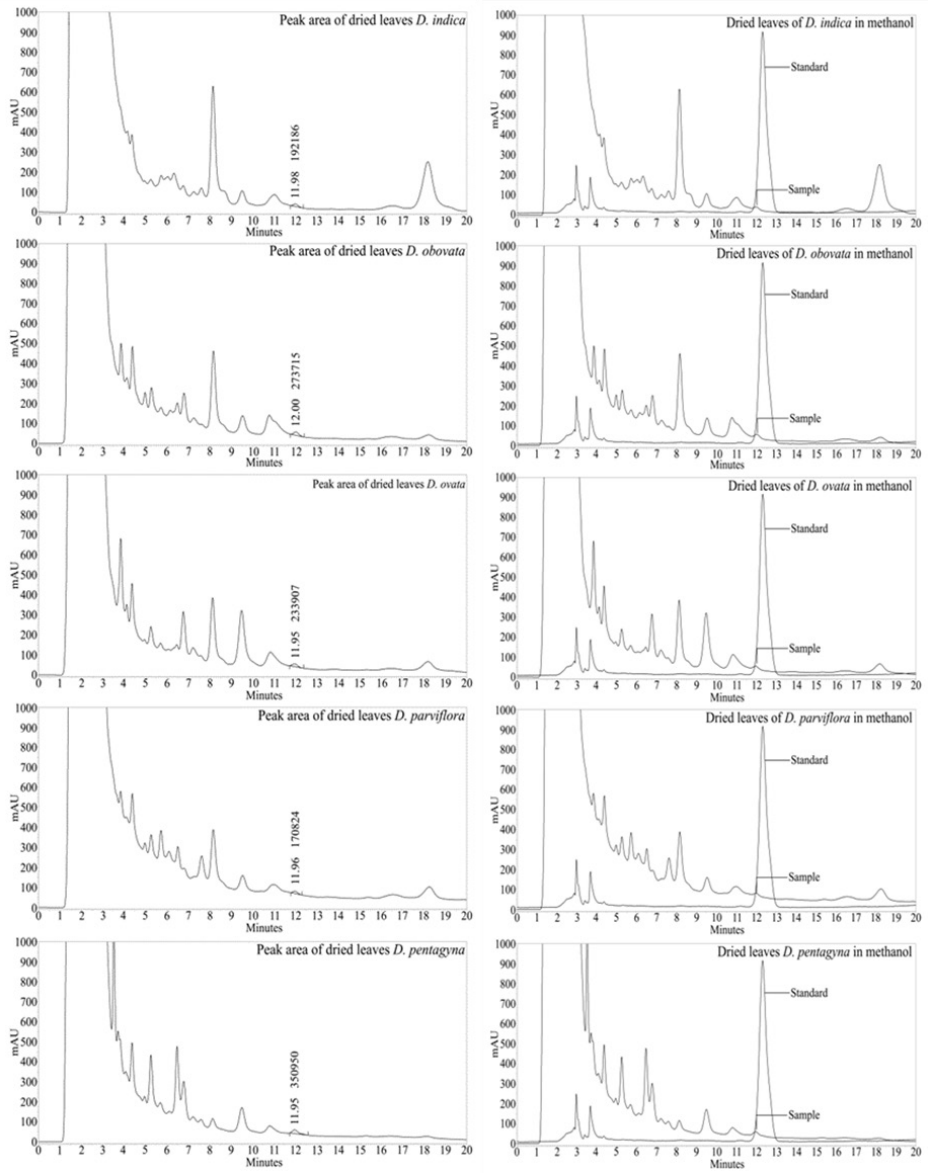

Figure 2. Chromatograms showing peaks and peak areas of β-sitosterol in *Dillenia indica, D. obovata,* and *D. pentagyna.*

compared to HepG2 cells, since at the lowest tested concentration (0.43 µg/mL) the viability of HeLa cells was estimated to be 23.7 ± 2.3%, 34.2 ± 1.8%, and 42.5 ± 3.4% for *D. pentagyna, D. indica,* and *D. obovata,* respectively. For the positive control, 100 µM H_2O_2, the HeLa cells' viability was 2.68 ± 1.15%. The final concentration used to analyze the induction of DNA damages in HeLa cells after treatment with *D. indica*, *D. obovata,* and *D. pentagyna* extracts, was 0.43 µg/mL. The statistically significant increase in the level of DNA damage was observed with *D. indica* and *D. obovata* extracts treatment; however, it was notably lower compared to the positive control (Table 4). The extract from *D. pentagyna* was shown to be non-genotoxic against HeLa cells. As mentioned, the dose-dependent cytotoxic effect was observed in case of HepG2 cells after treatment with plant extracts (Figure 3b). The IC_{50} values were possible to estimate for *D. indica* and *D. obovata* species, whereas *D. pentagyna* was shown to be non-cytotoxic at the highest tested concentration (430 µg/mL) (Table 3). The slight genotoxic potential in HepG2 cells was revealed for the *D. obovata* extracts, whereas *D. indica* and *D. pentagyna* demonstrated non-genotoxic potential (Table 4, Figure 4).

Table 2. Concentration and amount of β-sitosterol measured by HPLC in the three methanol *Dillenia* species extracts

Plant	Concentration (mg/mL)	Amount (mg/g sample)
Dillenia indica	0.21	0.42
D. obvata	0.29	0.59
D. pentagyna	0.37	0.96

The PBMC cells treated with a combined mixture

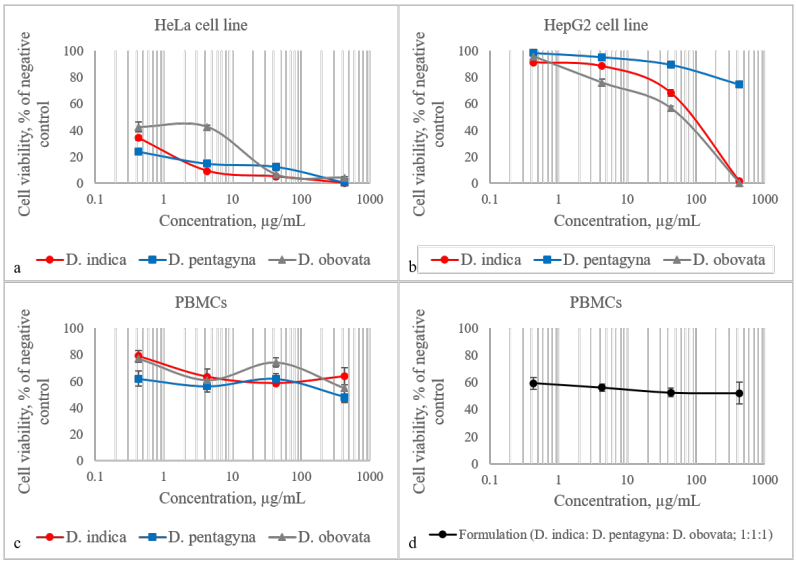

Figure 3. Cytotoxic potential of hexane leaf extracts of *Dillenia indica, D. pentagyna,* and *D. obovata* species in HeLa (a), HepG2 (b), and PBMC (c). The cytotoxicity of formulation in PBMCs (d).

showed no sign of dose-dependent decrease of cell viability (Figure 3d). The cell viability percentages were 52.19 ± 14.01% (430 µg/mL), 52.59 ± 5.57% (43 µg/mL), 56.18 ± 4.36% (4.3 µg/mL), and 59.36 ± 7.37% (0.43 µg/mL). The cell viability did not drastically differ between low and high concentrations. Because of no IC_{50} value, the highest concentration (430 µg/mL) of the combined mixture was selected for the comet assay. PBMCs did not show significant sign of DNA damage, the Olive tail moment value was 0.01 ± 0.002, while in the case of negative control it was 0.15 ± 0.01. The results of protective and antioxidant activity against hydrogen peroxide-induced DNA damage of non-toxic plant extract of *D. pentagyna* in HepG2 cells are presented in Table 5. It was shown that the extent of DNA damage significantly increased in hydrogen peroxide treated cultures (OTM: 13.2 ± 0.96) as compared to the negative control (OTM: 5.9 ± 0.65; $P < 0.05$). Both pre-treatment and co-treatment of cell

Table 3. The IC_50 values of hexane leaf extracts of *Dillenia indica, D. pentagyna,* and *D. obovata* species estimated for HeLa, HepG2, and PBMC cells

Plant species	IC50 values, µg/mL		
	HeLa cells	HepG2 cells	PBMCs
D. indica	<0.43	139±2.6	>430
D. pentagyna	<0.43	>430	350
D. obovata	<0.43	81±2.1	>430
Combined mixture (*D. indica: D. pentagyna: D. obovata;* 1:1:1)	-	-	>430

Table 4. The level of DNA damage evaluated by comet assay based on Olive tail moment after treatment with hexane leaf extracts of *D. indica, D. pentagyna,* and *D. obovata* species

	NC	PC	*D. indica*	*D. obovata*	*D. pentagyna*	Combined mixture
HeLa cells						
Concentration	-	100 µM	0.43 µg/mL	0.43 µg/mL	0.43 µg/mL	-
OTM	0.02±0.006	26.07±2.00*	0.08±0.005*	0.81±0.05*	0.06±0.01ns	-
HepG2 cells						
Concentration	-	50 µM	139 µg/mL	81 µg/mL	430 µg/mL	-
OTM	5.2±0.89	15.6±1.4*	7.4±1.82 ns	8.2±2.12*	4.2±0.07 ns	-
PBMCs						
Concentration	-	100 µM	430 µg/mL	430 µg/mL	350 µg/mL	430 µg/mL
OTM	0.15±0.01	9.69±1.26*	0.22±0.04ns	0.09±0.01 ns	0.14±0.01 ns	0.01±0.002 ns

OTM, olive tail moment; NC, negative control; PC, positive control (H2O2); PBMCs, peripheral blood mononuclear cells.
* $P < 0.05$ compared to negative control; ns – $P > 0.05$ compared to negative control

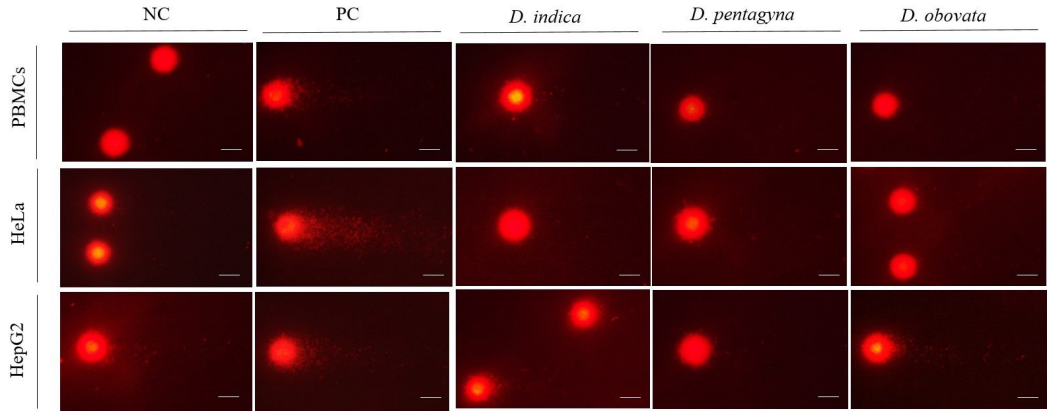

Figure 4. The comet assay performed in PBMCs, HeLa, and HepG2 cells treated with hexane leaf extracts of *D. indica, D. pentagyna*, and *D. obovata* species at IC50, 430 µg/mL or 0.43 µg/mL (if IC_{50} value was higher or lower of the tested concentrations) concentrations. NC: negative control; PC: positive control; The scale bar is 10 µM.

cultures by the extract resulted in a significant ($P < 0.05$) DNA repair (OTM: 3.6 ± 0.27 and 7.3 ± 0.34 compared to the positive control hydrogen peroxide treated cells). However, pre-treatment of cultures with the plant extracts was more efficient in reducing DNA damage levels than co-treatment.

Discussion

The research results showed that the three *Dillenia* species, including *D. indica*, *D. obovata,* and *D. pentagyna* are new potential resources for improving human health to use given the high levels of oleamide and β-sitosterol they contain. Aside from the three studied *Dillenia* species, *D. indica*, *D. obovata,* and *D. pentagyna*, nine *Dillenia* species found in Thailand have been studied for their oleamide contents (16), but β-sitosterol was not studied in this plant group. This study is the first to show the two important substances found together in these three *Dillenia* species, which can be used in various diseases given the substances' physiological properties. Beta-sitosterol in the form of plant, substance, or plant extract can be used in supplements, cosmetics, medical settings, modern herbal products, etc. The physiological actions in the human body include anti-inflammatory, chemopreventive, hypocholesterolemic, wound healing, antioxidant, and anti-diabetic effects. It is also used for benign prostatic hyperplasia and prostatic cancer treatment (17-21). The plants or extracts can be used in natural product creation such as hair supplements for hair growth improvement and hair loss reduction, given the better absorption and improved activity for the treatment of alopecia in shampoos and hair serums (22-24). In this regard *Polygonum multiform* and saw palmetto (*Serenoa repens*) have been used in hair care products (23). In addition to the β-sitosterol substance contained in the *Dillenia* species, there were high quantities of oleamide percentage-wise as elucidated by the GC-MS method. However, HPLC was used for the amount and concentration measurements and showed oleamide at 1.01 mg/g plant sample and 0.326 mg/mL, 1.12 mg/g plant sample and 0.374 mg/mL, and 1.17 mg/g plant sample and 0.262 mg/mL in *D. indica*, *D. obovata,* and *D. pentagyna*, respectively (7). The role of oleamide in human body is very important, such as stress-reducing, memory improvement used for Alzheimer's, inducing deep sleep, improving appetite, and as an anti-inflammatory without toxicity (7,8,9,16).

When plants contain vital phytochemicals, the combined mixture needs to be examined in terms of toxicity, both cytotoxicity and genotoxicity. The mixture showed non-toxicity on PBMCs and on the cell level because the mixture had greater proportions of the non-toxic *D. indica* and *D. obovata* than the proportion of the more toxic *D. pentagyna*. However, even though there was an IC_{50} value of 350 µg/mL for *D. pentagyna*, this predicted an LD_{50} of 934.100 mg/kg, which categorized it as a WHO Class II (50-2,000 mg/kg body weight, oral), and Class

Table 5. The level of induced oxidative DNA damage in human hepatoma HepG2 cell line after treatment with *D. pentagyna* hexane leaf extract

Plant extract	Pre-treatment		Co-treatment	
	%DNA in tail	OTM	%DNA in tail	OTM
Negative control (un-treated cells)	13.3 ± 1.3	5.9 ± 0.65	13.3 ± 1.3	5.9 ± 0.65
Positive control (PC)	29.8 ± 1.84	13.2 ± 0.96	29.8 ± 1.84	13.2 ± 0.96
Dillenia pentagyna	6.74 ± 0.81#*	3.6 ± 0.27#*	16.5 ± 0.67*	7.3 ± 0.34*

#$P < 0.05$ in comparison with negative control; * $P < 0.05$ in comparison with the positive control; PC: cells treated with 50 µM H_2O_2 for 5 min. OTM: Olive tail moment.

Ib (50-200 mg/kg body weight, dermal) moderately to highly hazardous toxic chemical. In order to meet this classification, a 50 kg body weight person would have to consume a 2500-100 000 mg dose or apply on the skin a 2500 -10 000 mg dose. In terms of humans, who are quite different from rats both in body size and genetics, it is very difficult for people to consume or apply to the skin such a high dose corresponding to going over the threshold for no toxicity in our DNA results.

The study of protective effects of *D. pentagyna* extract showed that pre-treatment of the cultures with the extract was more efficient in reducing DNA damage levels than co-treatment. An especially remarkable protective effect was found in the case of cells pre-treated with *D. pentagyna*, as DNA damage level significantly decreased even in comparison with the level of background DNA damage in the negative control ($P<0.05$). Therefore, stronger protection was obtained via pre-treatment than in co-treated cells for the tested extract, indicating that in addition to direct scavenging of ROS, the accumulation of plant extract in the cell increased the induction of enzymatic and non-enzymatic cellular antioxidants and detoxifying enzymes, thus leading to the elevation of cellular antioxidative defenses. Here the tremendous antioxidant effect on the cellular level, based on biological response (DNA-damage) was shown. It is assumed that phenolics and flavonoids appear to be major contributors to the antioxidant potential of those extracts. However, the major constituents of *D. pentagyna* extract, oleamide and β-sitosterol, and their role in cell antioxidant defenses cannot be ignored. Indeed, oleamide is a ligand of thioredoxin reductase enzyme known to catalyze the reduction of thioredoxin, a component of the cellular defense system against oxidative damage (25).

The experiment of the combined mixture further assures that the utilization of these three plants for health promotion and disease treatment is good, non-toxic, and is likely to be highly effective based on the properties of the two active ingredients.

Conclusion

All of the experimental aspects of this study after phytochemical measurements, including toxicity testing in PBMCs, HeLa, and HepG2 cell lines plus antioxidant activity, were done to ensure that they will be able to be fully utilized for human health. Given this, *D. indica*, *D. obovata,* and *D. pentagyna* may be used in traditional and modified forms according to modern scientific measurements that are presently available. They can be potentially used as natural resources with several advantages stemming from the β-sitosterol and oleamide compounds that the plants contained.

Authors' contributions

Conceptualization: AC and NB. Data curation: PS, KS, and TT. Formal analysis: PS, TiT. Formal analysis: AC and NB. Investigation: RS and TaT. Methodology: PS, TiT, LK, LA. Project administration: RS. Supervision: AC. Manuscript writing, review & editing: AC, RS, and NB.

Conflict of interests

The funders had no role in the design of the study, collection, analyses, interpretation of the data, writing of the manuscript, or in the decision to publish the results.

Ethical considerations

Ethical issues including text plagiarism, data fabrication, double publication, falsification and etc. have been carefully observed by the authors. All the experiments were performed in accordance with relevant guidelines and regulations.

Funding/Support

RS and AC were financially supported for the research by Research and Graduate Studies, Khon Kaen University (Grant number is RS and AC, 2021).

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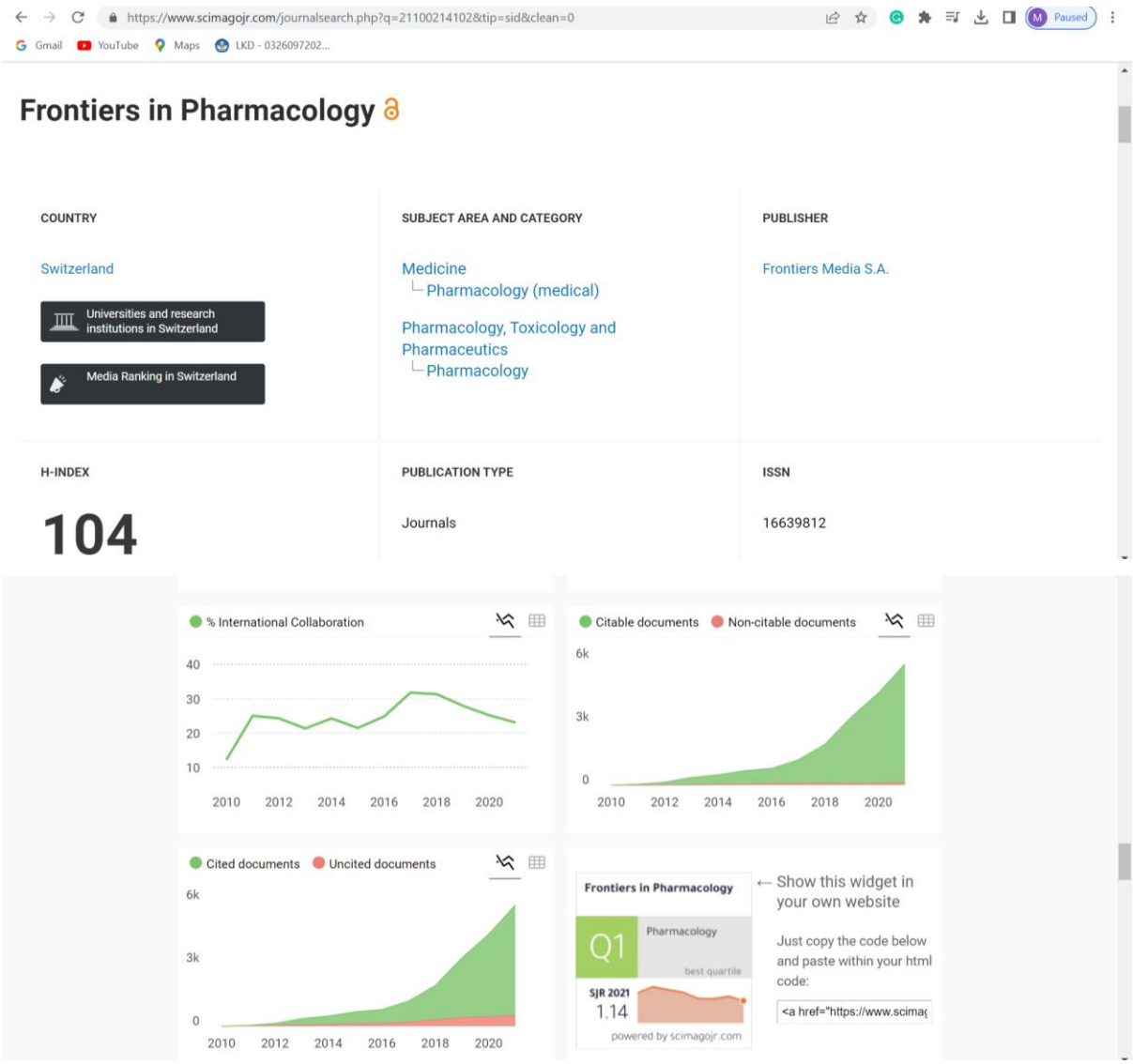

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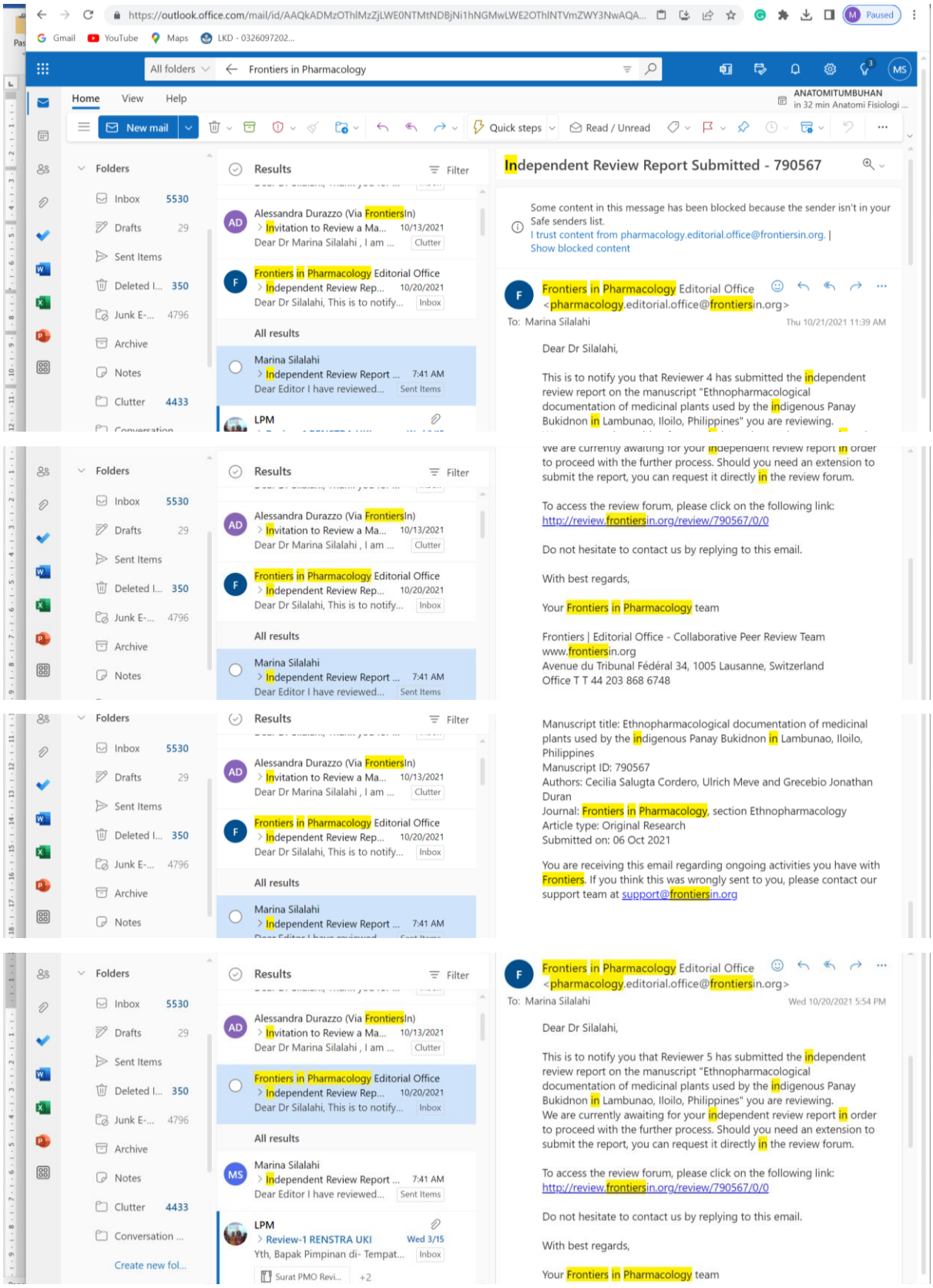

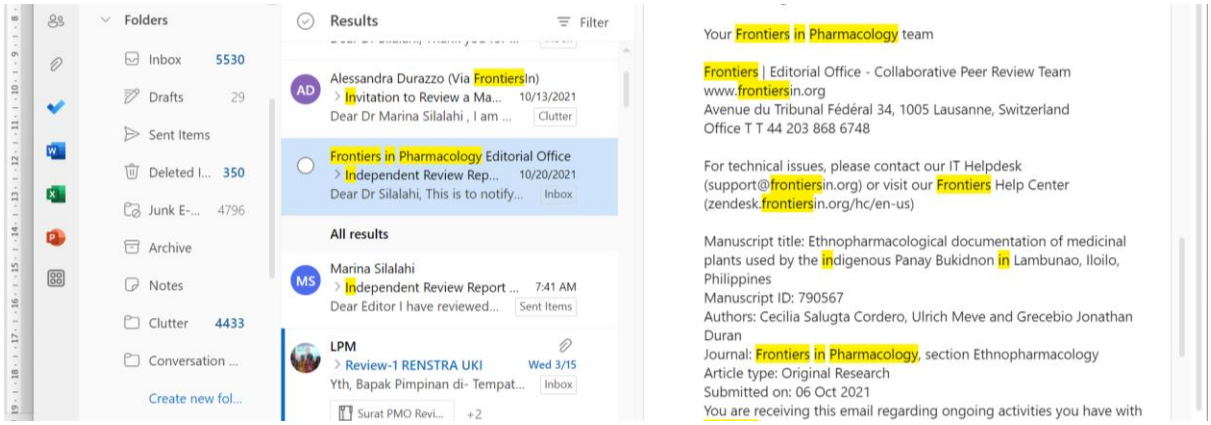
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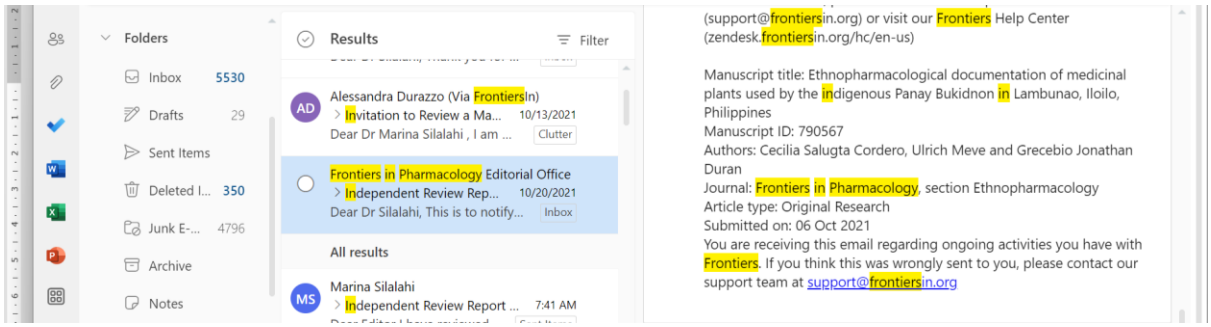
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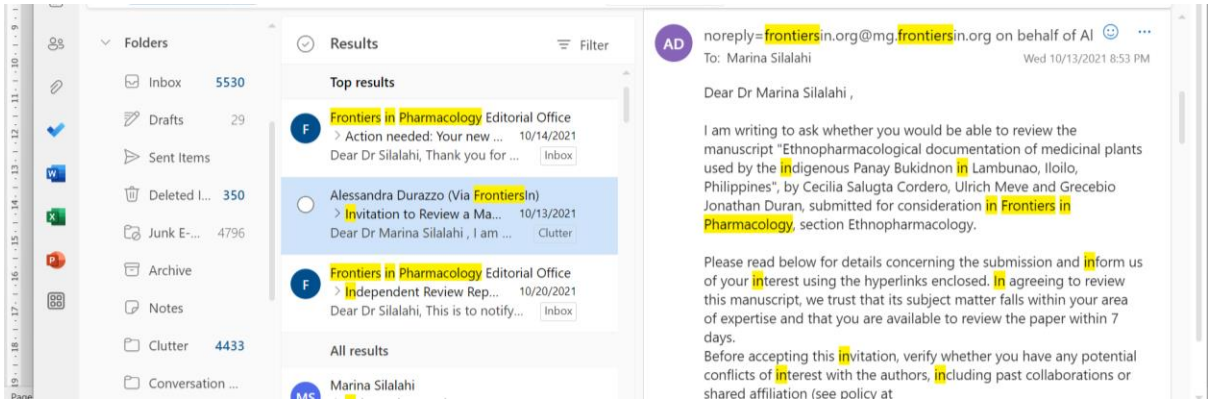
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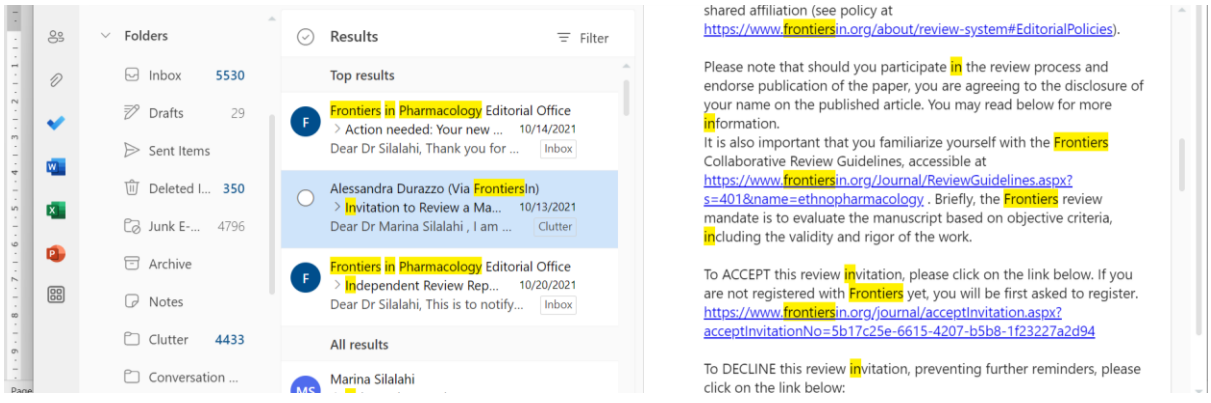
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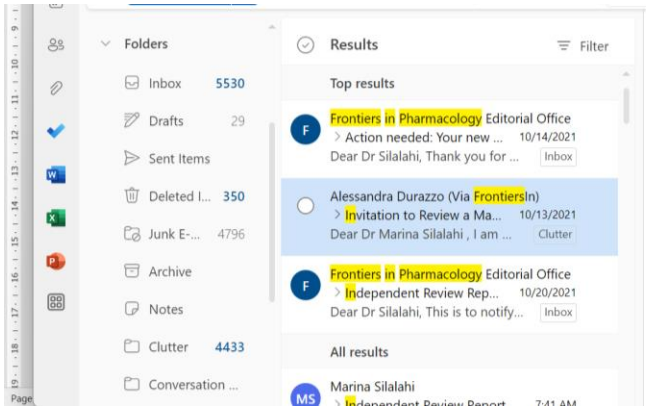

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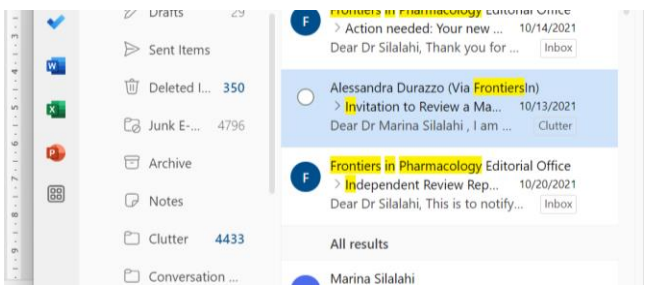

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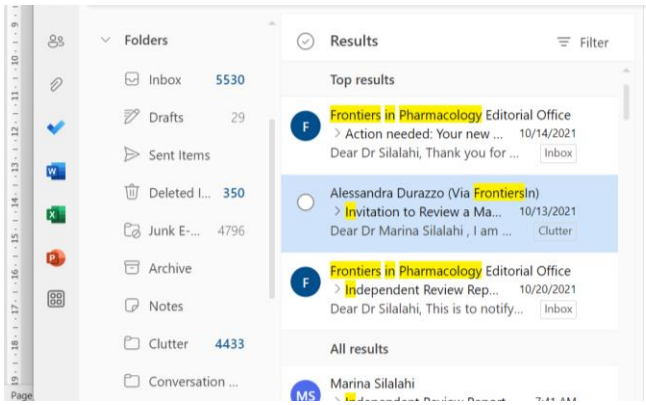
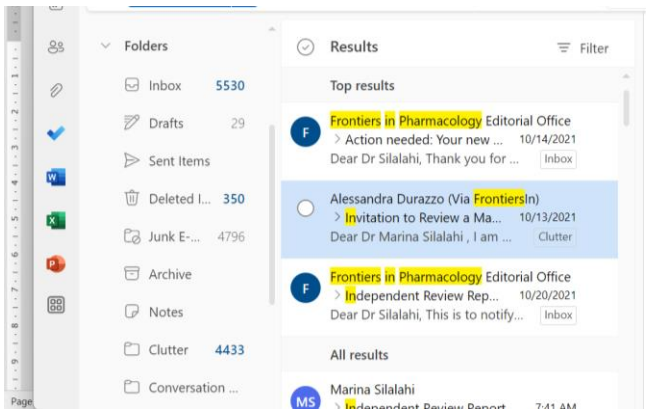


Abstract: The Panay Bukidnon is a group of indigenous peoples living in the interior highlands of Panay Island, in Western Visayas, Philippines. Little is known about their ethnopharmacological knowledge due to limited written records and no recent research has been conducted on the medicinal plants they used in ethnomedicine. This study aims to document the medicinal plants used by the indigenous Panay Bukidnon in Lambunao, Iloilo, Panay Island. A total of 131 medicinal plant species distributed in 121 genera and 57 families were used to address 91 diseases in 16 different use or disease categories. The family Fabaceae was best represented with 13 species, followed by Lamiaceae with nine species, and Poaceae with eight species. The leaf was the most frequently used plant part and decoction was the most preferred form of preparation. To evaluate the plant importance: use value (UV), relative frequency citation (RFC), informant consensus factor (ICF), and fidelity level (FL) were used. Curcuma longa L. had the highest UV (0.79), Artemisia vulgaris L. had the highest RFC value (0.57), and Annona muricata L. had the highest RI value (0.88). Diseases and symptoms or signs involving the respiratory system and injury, poisoning and certain other consequences of external causes recorded the highest ICF value (0.80).



consequences of external causes recorded the highest ICF value (0.80). Chromolaena odorata (L.) R.M.King & H.Rob. had the highest FL value (100%) used for cuts and wounds. The results of this study serve as a medium for preserving cultural heritage, ethnopharmacological bases for drug research and discovery, and preserving biological diversity.

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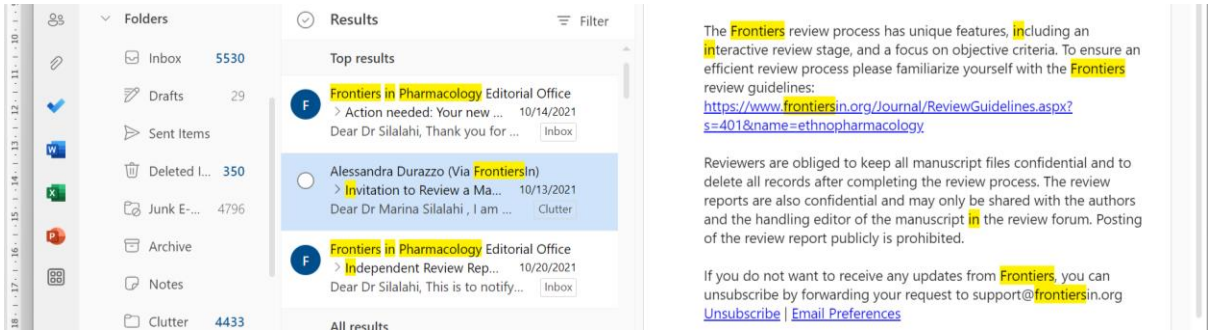
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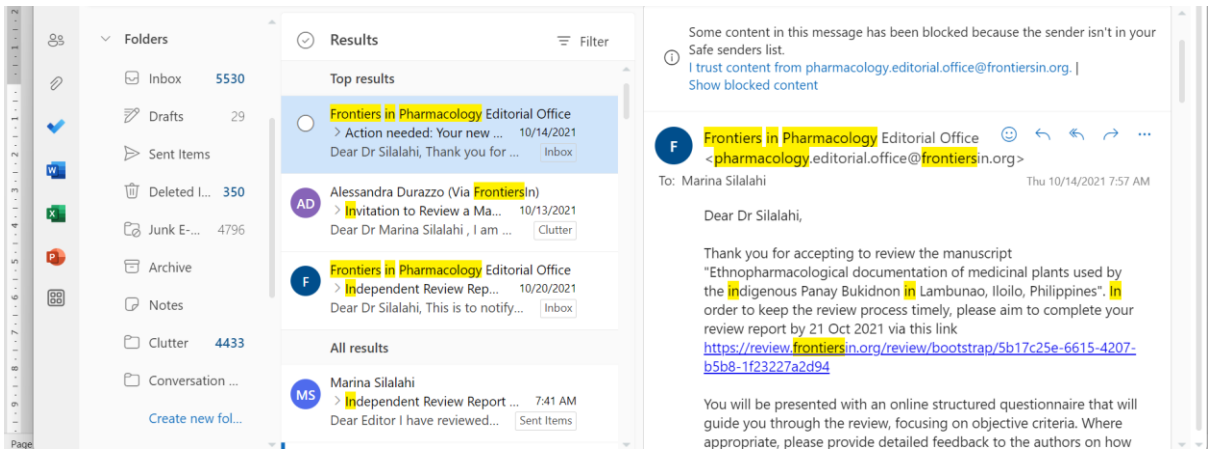
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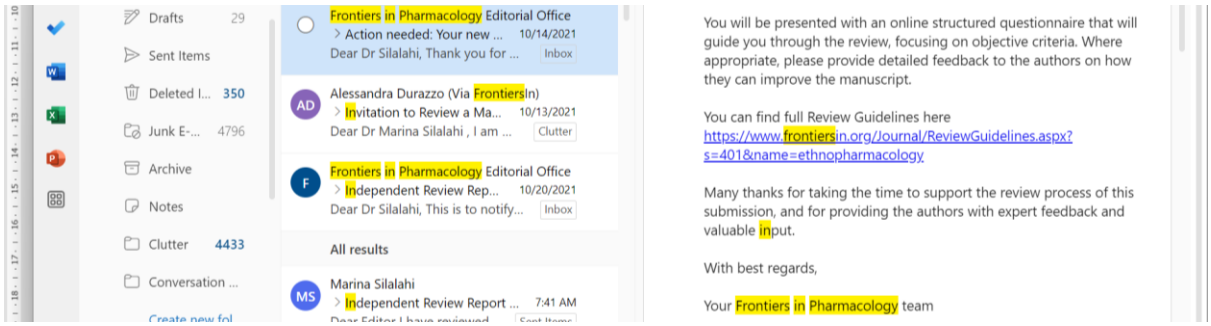
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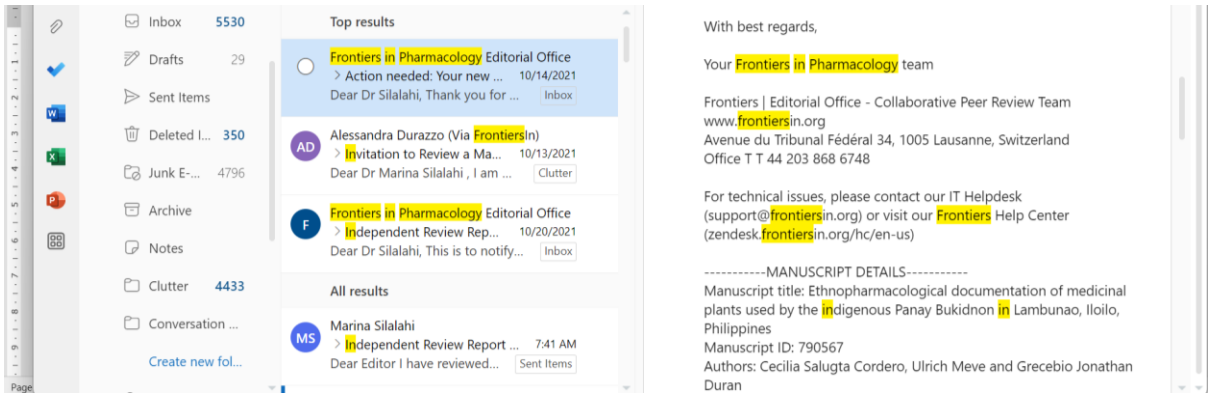
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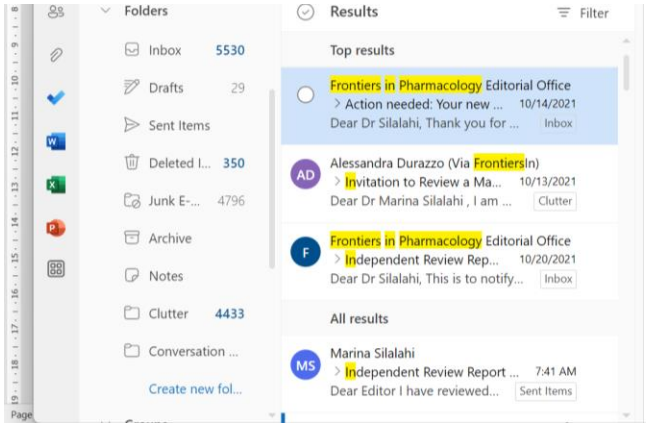

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Manuscript ID: 790567
Authors: Cecilia Salugta Cordero, Ulrich Meve and Grecebio Jonathan Duran
Journal: Frontiers in Pharmacology, section Ethnopharmacology
Article type: Original Research
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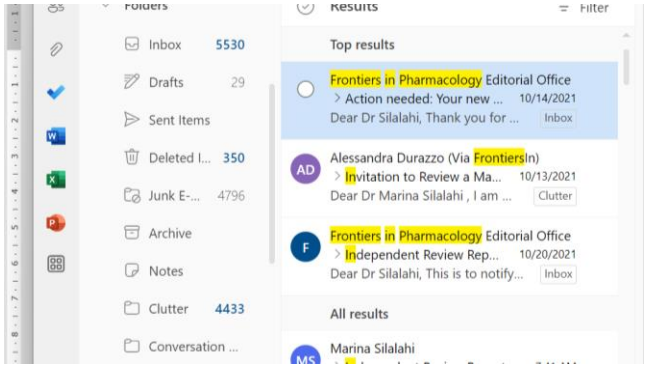

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published: 10 January 2022
doi: 10.3389/fphar.2021.790567

Ethnobotanical Documentation of Medicinal Plants Used by the Indigenous *Panay Bukidnon* in Lambunao, Iloilo, Philippines

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Specialty section:
This article was submitted to Ethnopharmacology, a section of the journal Frontiers in Pharmacology

Received: 06 October 2021
Accepted: 18 November 2021
Published: 10 January 2022

Citation:
Cordero CS, Meve U and Alejandro GJD (2022) Ethnobotanical Documentation of Medicinal Plants Used by the Indigenous Panay Bukidnon in Lambunao, Iloilo, Philippines. Front. Pharmacol. 12:790567. doi: 10.3389/fphar.2021.790567

The *Panay Bukidnon* is a group of indigenous peoples living in the interior highlands of Panay Island in Western Visayas, Philippines. Little is known about their ethnobotanical knowledge due to limited written records, and no recent research has been conducted on the medicinal plants they used in ethnomedicine. This study aims to document the medicinal plants used by the indigenous *Panay Bukidnon* in Lambunao, Iloilo, Panay Island. Semi-structured interviews were conducted with 75 key informants from June 2020 to September 2021 to determine the therapeutic use of medicinal plants in traditional medicine. A total of 131 medicinal plant species distributed in 121 genera and 57 families were used to address 91 diseases in 16 different uses or disease categories. The family Fabaceae was best represented with 13 species, followed by Lamiaceae with nine species and Poaceae with eight species. The leaf was the most frequently used plant part and decoction was the most preferred form of preparation. To evaluate the plant importance, use value (UV), relative frequency citation (RFC), relative important index (RI), informant consensus factor (ICF), and fidelity level (FL) were used. *Curcuma longa* L. had the highest UV (0.79), *Artemisia vulgaris* L. had the highest RFC value (0.57), and *Annona muricata* L. had the highest RI value (0.88). Diseases and symptoms or signs involving the respiratory system and injury, poisoning, and certain other consequences of external causes recorded the highest ICF value (0.80). *Blumea balsamifera* (L.) DC. and *Chromolaena odorata* (L.) R.M. King & H. Rob were the most relevant and agreed species for the former and latter disease categories, respectively. *C. odorata* had the highest FL value (100%) and was the most preferred medicinal plant used for cuts and wounds. The results of this study serve as a medium for preserving cultural heritage, ethnopharmacological bases for further drug research and discovery, and preserving biological diversity.

Keywords: ethnobotany, ethnomedicine, Panay Bukidnon, Panay Island, Philippines

INTRODUCTION

About 370–500 million indigenous peoples live in 90 countries worldwide, making up 5% of the global population. They represent 5,000 distinct and diverse cultures, but they also account for 15% of the extremely poor and deprived communities from social services and economic resources (United Nations Development Programme, 2019).

In the Philippines, there are 110 ethnolinguistic groups with more than 14 million people spread across the archipelago, with the highest population in Mindanao (63%), followed by Luzon (32%) and Visayas (3%) (National Commission on Indigenous

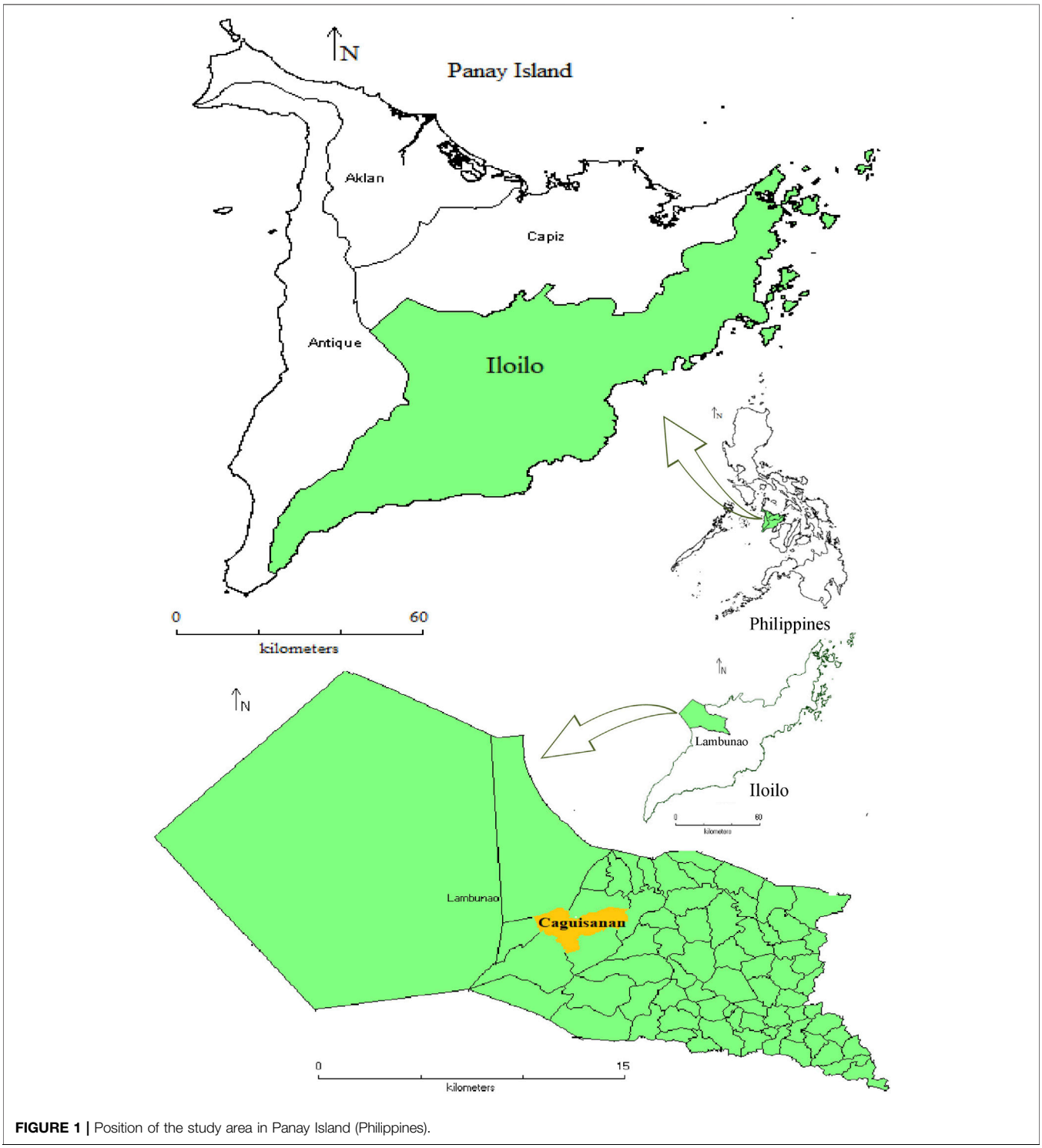

FIGURE 1 | Position of the study area in Panay Island (Philippines).

Peoples, 2011), who occupy around 13 million hectares (ha) (45%) of the national land territory (National Economic Development Agency, 2017). The *Bukidnon* is the major indigenous group in the Central and Western Visayas in terms of population size, followed by the *Ati/Ata* (Negritoes). In Panay Island in Western Visayas, the *Bukidnon* population is about 112,000. The province of Iloilo is one of the four provinces in Panay Island, together with Aklan, Antique, and Capiz. It has the highest *Bukidnon* population with 62,245 individuals (National Commission on Indigenous Peoples, 2019). *Bukidnon,* which literally translates to the "mountain people," were once coastal dwellers, but due to the piratical raids from Mindanao and political suppressions during the reign of the Spanish government, they moved to the hinterlands of the island (Magos, 2004). This was depicted in their epic tradition of chanting called *Sugidanon* (Magos, 1999). To distinguish the *Bukidnon* in Panay from the other *Bukidnon* groups in Mindanao, Negros, and other neighboring islands, the "*Panay*" is added (Gowey, 2016). Other authors used *Mundo* (Beyer, 1917), *Monteses* "mountaineer" (Ealdama, 1938), *Sulod* or *Sulodnon* "enclosed by the mountains" (Jocano, 1968; German, 2010), *Tumandok* "native of the place" (Talledo, 2004), and *Bukidnon* (Smith, 1915; Magos, 1999) to designate the *Panay Bukidnon* people.

The *Panay Bukidnon* primarily utilized the forest resources, rivers, and streams for their food and livelihood. They also engaged in slash and burn farming and building boats to transport their goods to the lowlands. In the 1970s, when logging activities were prohibited by the Philippine government, they shifted to other means of living, including farming various crops (Magos, 1999). Their social organization is relatively similar to the lowlanders. Their community membership pattern is composed of the *baylan/babaylan, mirku* (herb doctor), *parangkutun* (advisor), and the *husay* (arbiter). The *baylan* is considered the most important status in society and regarded with high respect. The *baylan* is the one who communicates with the spiritual world, interprets dreams, and handles religious performances. He or she may also administer herb medicine to the sick and practice folk medicine and physical therapy. Their language is *Kiniray-a*, a dialect that is similar to Ilonggo/Hiligaynon. Today the *Panay Bukidnon* settled in the interior "barangays" (villages) of at least 24 municipalities of the four provinces of Panay (Provincial Planning Development Office, 2018; National Commission on Indigenous Peoples, 2020) and most of their communities are located in the mountainous areas of the Central Panay Mountain Range.

Iloilo province is situated in the southeastern part of Panay Island in Western Visayas. It is geographically located at the center of the archipelago, and it is known as the "Heart of the Philippines." Its excellent port facility and strategic location made the province the center of trade during the 1890s when the sugar industry was booming and it was once given the title of "Queen City of the South." It is also known for the "*Dinagyang Festival,*" one of the most spectacular religious and cultural celebrations in the country in honor of Senior Sto. Nino (Child Jesus) (Province of Iloilo, 2015). The province has a total land area of 491,940 ha, 24% of which is classified as forestland, while 76% is classified as alienable and disposable land (Department of Environment and Natural Resources, 2019).

Several ethnobotanical surveys in Panay Island have been conducted on the *Ati* (Negritoes) indigenous groups (Madulid et al., 1989; Ong and Kim, 2015; Cordero et al., 2020; Cordero and Alejandro, 2021), but there is no study focused exhaustively on the medicinal plants used by the *Panay Bukidnon* in ethnomedicine. Nevertheless, several plants were listed with medicinal purposes in the anthropological case studies documented in the interior barangays of Tapaz, Capiz in Central Panay in 1945–1959 (Jocano, 1968). Given the absence of recent research about the ethnobotanical knowledge of *Panay Bukidnon*, it is therefore urgent to document this indigenous knowledge before it is forgotten. The documentation of traditional knowledge will serve as a medium for preserving cultural heritage, ethnopharmacological bases of drug research and discovery, and preserving biological diversity. Thus, this study is the first attempt to extensively survey the ethnobotanical knowledge in one of the indigenous *Panay Bukidnon* communities in the province of Iloilo in Panay Island, Western Visayas, Philippines.

MATERIALS AND METHODS

Study Area and Permits

The town of Lambunao is a first-class municipality in the third district of Iloilo province, with a population of 81,236 individuals as of May 2020 (Philippine Statistics Authority, 2021). It is the largest municipality in the province in terms of land area (40, 709 ha), about 26.12% of which are forestlands, and the rest are alienable and disposable land. It is bounded by the municipalities of Calinog in the North, Duenas and Pototan in the East, Janiuay and Badiangan in the South, and Janiuay and Valderrama, Antique, in the west (**Figure 1**). It is a mountainous municipality and has the highest elevation (194 m a.s.l.) in the province. The climate of the area has two pronounced seasons: dry from the months of November to April and wet for the rest of the year. Seven out of its 73 barangays are inhabited by the *Panay Bukidnon* people. Brgy. Caguisanan, which lies between 11°05′37.6″N and 11°04′42.3″N latitude and 122°24′26.6″E 122°26′51.6″E longitude, has a land area of about 5.20 km^2. According to the recent survey, it is one of the seven indigenous *Panay Bukidnon* barangays with a population of 1,842 in 394 households. The main source of livelihood in the barangay is farming of various crops such as rice, banana, corn, and other vegetables. Some of the younger generations are professionals working in various private and government sectors and some are working as overseas Filipino workers abroad. The landscape of the study site is dominated by hills and mountains with scattered rice terraces, grasslands, and patch forests.

Certification Precondition was acquired from the National Commission on Indigenous Peoples (NCIP)-Region VI/VII and the researchers have satisfactorily complied with the requirements for securing the Indigenous Knowledge and Systems Practices (IKSPs) and Customary Laws (CLs). It was issued in compliance with Section 59 of the Republic Act No.

TABLE 1 | Demographic profile of the *Panay Bukidnon* informants in Lambunao, Iloilo, Philippines.

Social group	Variables	No. of informants (75)	Percentage (%)
Sex	Male	31	41
	Female	44	59
Age	24–39	9	12
	40–55	29	39
	56–70	25	33
	71–89	12	16
Civil Status	Married	71	95
	Single	2	3
	Widowed	2	3
Education	No formal education	5	7
	Elementary	37	49
	Secondary	16	21
	Tertiary	17	23
Occupation	Farmer	19	25
	Housewife	29	39
	Self-employed	11	15
	Employed	5	7
	Barangay officials	7	9
	Albularyo (herb doctor)	2	3
	Paltera (midwife)	1	1
	IP leader	1	1

8371 "The Indigenous Peoples Rights Act (IPRA) of 1997." Several meetings were conducted: Pre-FPIC (Free and Prior Informed Consent) Conference; Disclosure Conference with the Indigenous Peoples (IP) Community and Presentation of Application; Community Decision Meeting; Memorandum of Agreement Preparation and Signing; and Output Validation Meeting. A Wildlife Gratuitous Permit was issued by the Department of Natural Resources Region (DENR) VI before conducting the study.

Data Collection

Fieldworks and interviews were conducted from June 2020 to September 2021. Interviews were carried out using semi-structured questionnaires, ethically reviewed, and approved (**Supplementary Material S1**). A purposive sampling technique was used, and the principal key informants were determined during the community decision meeting in the presence of the NCIP officers, barangay officials, IP leader, and council of elders. The informants were composed of the tribal leader, council of elders, herb doctors (*mirku/surhano/albularyo*), midwife (*paltera*), and other members of the community who have indigenous knowledge of using medicinal plants in treating and addressing health problems and conditions. A total of 75 informants, 31 males and 44 females, aged between 24 and 89 years old, were interviewed at their own convenience in their community during the study. Questions regarding personal information and the medicinal plants they used when they experienced any health-related problems were asked during the surveys. The information about the demographic profile of the participants, such as age, gender, civil status, educational attainment, and occupation, is shown in **Table 1**. The plant part used, mode of preparation, and administration were also recorded during the interviews. A focus group discussion was conducted with the 10 members of the council of elders to verify the acquired data among the informants during the output validation meeting. The meeting was facilitated by the NCIP officers, IP leader, and Brgy. Captain.

Plant Collection and Identification

Collecting medicinal plant samples was carried out with the help of the informants, if available in their immediate surroundings or their home gardens right after the interview. Some field collections were assisted by the informants who have the knowledge of the location of some plants that were not available in the home gardens. Plants were also photographed for documentation purposes. Voucher specimens were prepared using three to five branches with preferably reproductive parts (flowers and fruits), inserted in newspapers, and positioned in a way that best represents the plant in the wild. The plants were poisoned with a generous amount of denatured alcohol in polyethylene bags. Poisoned specimens were then transferred to a new newspaper and placed in a presser. Pressed and dried plant specimens were then mounted on herbarium sheets with proper documentation labels. Voucher specimens were accessioned and deposited in the Herbarium of the Northwestern University Luzon (HNUL). Identification of the collected medicinal plants was made using different online databases such as Co's Digital Flora of the Philippines, (https://www.philippineplants.org/), Phytoimages (http://www.phytoimages.siu.edu), Stuartxchange (http://www.stuartxchange.org/), and Plants of the World Online (http://plantsoftheworldonline.org/), then verified by Mr. Danilo Tandang, a botanist at the Philippine National Museum Herbarium and Mr. Michael Calaramo of the Herbarium of Northwestern University Luzon (HNUL). For the validation of the family and scientific names, Tropicos (Tropicos, 2021), World Flora Online (World Flora Online, 2021), and International Plant Names Index (International Plant Names Index, 2021) were used. To identify the geographical distribution and endemicity of the medicinal plants, Co's Digital Flora of the Philippines (Pelser et al., 2011) and Plants of the World Online (Plants of the World Online, 2021) were used.

Data Analyses

There were five values used to quantify the plant importance: use value (UV), relative frequency of citation (RFC), relative importance index (RI), informant consensus factor (ICF), and fidelity level (FL). The UV was calculated to determine the relative importance of the medicinal plant species using the following formula: $UVs = Ui/N$, where U_i is the number of use reports cited or mentioned by each informant for a particular species and N is the total number of informants (Phillips and Gentry, 1993). A use report was considered every time an informant cited or mentioned a plant being used for any medical condition or purpose. RFC was used to determine the culturally important medicinal plants using the following formula: $RFCs = FCs/N$, where FC_s is the number of informants who cited or mentioned a plant species (frequency citation) and N is the total number of informants who

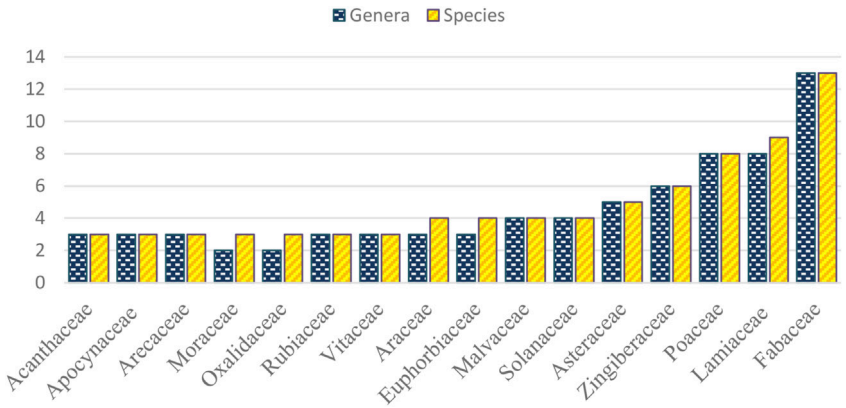

FIGURE 2 | Family of medicinal plants with a high number of genera and species.

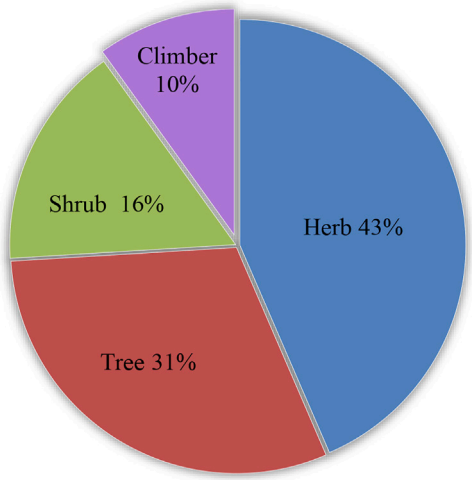

FIGURE 3 | Medicinal plant growth habit.

participated in the study. The values range from 0 to 1, 1 being the highest and indicating that all informants cited or mentioned a particular plant species (Tardío and Pardo-De-Santayana, 2008). RI was used to assess the relative importance of medicinal plants by use category using the following formula: $RIs = [RFCs(max) + RNUs(max)]/2$, where $RFC_{s(max)}$ ($RFC_{s(max)} = FC_s/FC_{max}$) is the relative frequency of citation of the species and is obtained by dividing the frequency citation of informant/s for a particular species (FC_s) by the number of informants citation of the species that has the maximum or highest frequency citation (FC_{max}). $RNU_{s(max)}$, ($RN_{s(max)} = NU_s/NU_{max}$) is the relative number of the use categories and is obtained by dividing the number of use categories of a particular species (NU_S) by the number of use categories of the species with the highest use categories (NU_{max}). Values closest to 1 indicate that the medicinal plants are most frequently cited as useful in different use categories (Tardío and Pardo-De-Santayana, 2008). ICF was used to evaluate the consensus or homogeneity of the ethnobotanical information from the participating informants using the following formula: $ICF = (Nur - Nt)/(Nur - 1)$, where N_{ur} is the number of use reports for each disease category and N_t is the number of species used in that category (Heinrich et al., 1998). FL was used to assess the percentage of the most preferred medicinal plant for a particular disease category using the following formula: $FL = (Np/N)\,x100$, where N_p is the number of informants who cited or mentioned the use of a medicinal plant for a particular disease category and N is the total number of informants who cited that plant for any other use or purpose (Friedman et al., 1986). A high value indicates that a medicinal plant has the highest use report and the most preferred species within a particular disease category. There were 16 different use or disease categories adapted and modified from the ICD-11 International Classification of Diseases 11th Revision of the World Health Organization (World Health Organization, 2021), which is used in this ethnopharmacological documentation.

RESULTS

Medicinal Plants Characteristics

The present study documented a total of 131 medicinal plant species distributed in 121 genera and 57 families. The family Fabaceae was best represented with 13 species, followed by Lamiaceae with nine species and Poaceae with eight species (**Figure 2**). Fabaceae are used to treat 28 diseases in 13 different use or disease categories, Lamiaceae in 24 diseases in ten disease categories, and Poaceae in 21 diseases in 12 disease categories.

The medicinal plants recorded possess different growth forms such as herbs (43%), trees (31%), shrubs (16%), and climbers (10%) (**Figure 3**). The plants were collected within the vicinity of the barangay mostly cultivated in the informant's home gardens or backyards that serve as ornamentals and vegetables and used for medicinal purposes; some were cultivated as crops in the farmland; some were grown on the riverbanks and forest; others

TABLE 2 | Top ten medicinal plants with the highest UV, RFC, and RI values.

Rank	Medicinal plants	UV	Medicinal plants	RFC	Medicinal plants	RI
1	*Curcuma longa*	0.79	*Artemisia vulgaris*	0.57	*Annona muricata*	0.88
2	*Blumea balsamifera*	0.64	*Curcuma longa*	0.47	*Curcuma longa*	0.87
3	*Artemisia vulgaris*	0.59	*Blumea balsamifera*	0.44	*B lumea balsamifera*	0.80
4	*Annona muricata*	0.56	*Zingiber officinale*	0.43	*Zingiber officinale*	0.78
5	*Jatropha curcas*	0.55	*Psidium guajava*	0.40	*Artemisia vulgaris*	0.77
6	*Psidium guajava* L	0.55	*Pseuderanthemum carruthersii*	0.40	*Jatropha curcas*	0.72
7	*Justicia gendarussa*	0.52	*Musa balbisiana* cv	0.40	*Moringa oleifera*	0.72
8	*Pseuderanthemum carruthersii*	0.52	*Plectranthus scutellarioides*	0.40	*Plectranthus scutellarioides*	0.62
9	*Musa balbisiana* cv	0.52	*Annona muricata*	0.39	*Psidium guajava*	0.62
10	*Plectranthus scutellarioides/Zingiber offinale*	0.49	*Justicia gendarussa*	0.37	*Carica papaya/Justicia gendarussa*	0.60

do grow as weeds pervasively around the community. Of all the 131 medicinal plants listed, 91 species were collected as cultivated plants and 40 species were collected in the wild. Out of 127 plants identified up to the species level, 78 species are not native (introduced, naturalized, cultivated) in the Philippines and 49 species are native. Three species (*Areca catechu* L., *Musa textilis* Née, and *Mussaenda philippica* A. Rich.) of the native plants are considered endemic and their occurrence is widespread in the country. Information about the plant growth habit, collection sites, and geographical distribution and endemicity are found in = **Supplementary Table S1**.

The medicinal plant details are summarized in **Supplementary Table S2**. The scientific, local, and family names are also listed along with the plant part used, disease or purpose, quantity, mode of preparation, the form of administration, adverse or side effects, use value, relative frequency citation, and relative importance index.

Plant Part Used and Mode of Preparation and Administration

Sixteen different medicinal plant parts were used to address 94 diseases and health-related conditions documented in this study. The most frequently used plant parts for the preparation of the remedy were leaf (51%), followed by bark (8%), fruit (6%), and rhizome (6%) (**Figure 4**). Root, stem, whole plant, flower, seed, bulb, shoot, sap, and aerial root were also used but less frequently. The least utilized plant parts were tuber, petiole, and tendril. There were ten different ways to prepare the medicinal plants and the most common forms were decoction (35%), followed by crushing or pounding (23%) and direct application (20%) (**Figure 5**). Eat/chew/drink, heat/roast, soak in water, and grate/slice were also practiced. The least forms of preparation were cooking, processing into vinegar or oil, and burning for smoke or ash. The plant parts used and the mode of preparation of the medicinal plants depend on the ailments to be addressed and to whom they will be administered. Occasionally, some of the preparations include animal parts and products such as blood, egg, beeswax (*kabulay*), slaked lime (*apog*), minerals like salt, and chemicals like kerosene but in minute amounts. Sugar or breastmilk was also added to reduce or mask the bitterness of plant extracts to be taken orally by infants and children.

More than half of the medicinal plant preparations (52%) recorded were administered externally or topically by applying plant parts directly on the body, rubbing plant extracts, bathing, and burning for smoke and ash. The rest were taken orally (48%) by drinking decoction, eating, chewing, drinking extracts or liquids and used as a mouthwash.

Quantity and Dosage

The quantity of the medicinal plants used is influenced by the guided cultural and religious beliefs of the *Panay Bukidnon* and should be prepared or administered in odd numbers (3, 5, or 7). For example, in treating headaches, three different medicinal plants such as *Pseuderanthemum carruthersii* (Seem.) Guillaumin (3, 5, or 7 leaves depending on the leaf size), *Curcuma longa* L. (7 thinly sliced rhizomes), and *Zingiber officinale* Roscoe (7 thinly sliced rhizomes) were applied on the forehead. The frequency of the administration was dependent on the disease to be treated. For decoction, it was often administered by drinking a full glass to be taken twice or thrice a day or as a replacement for water intake. The detailed quantity and frequency of administration of medicinal plants are shown in **Supplementary Table S2**.

Use Value and Relative Frequency of Citation

The use value was used to evaluate the relative importance of the medicinal plants: high values indicate high use report, while relative frequency citation determined the usefulness of the plant by high FC or being mentioned by all the informants.

The top three medicinal plants with the highest use value were *Curcuma longa* L. (0.79), *Blumea balsamifera* (L.) DC. (0.64), and *Artemisia vulgaris* L. (0.59) (**Table 2**). *C. longa* is used to treat 13 diseases in nine disease categories and recorded a high use report in suppressing fever, headache, and *sinda*. It is usually prepared with two or four medicinal plants. The preparation and mode of administration for headache and fever were the same and with few modifications for *sinda*. *C. longa* is also used for muscle pain, stomachache, bloated stomach, tooth decay, typhus, typhoid fever, memory loss, cancer, cuts/wounds, and tetanus. It is cultivated in the informant's home gardens for medicinal purposes.

B. balsamifera was used to treat nine conditions or purposes under eight different disease categories and is widely known to cure cough, used in postpartum care and recovery, and relieved headache. It is also used for muscle pain, bloated

TABLE 3 | Use or disease category, reported disease or uses, ICF, and FL of the most cited species.

Use or disease- category	Reported diseases or uses under each category*	No. of used taxa	Use report	ICF	Most cited species for each category	Fidelity level (%)	Use or purpose of the most cited taxa
Infectious and parasitic diseases	Amoebiasis (4), athletes foot (1), boil (11), chicken pox (2), dengue (2), helminthiases (2), measles (3), mumps (6), oral thrush (1), shingles (3), ringworm (2), tetanus (2) Pityriasis versicolor (2), tuberculosis (3), typhus (2), typhoid fever (11), wart (2)	59	153	0.62	*Carica papaya*	60	Typhoid fever
Neoplasms	Cancer (12)	12	27	0.58	*Annona muricata*	31	Cancer
Endocrine, nutritional and metabolic diseases	Diabetes (5), goiter (1), high uric acid (1)	7	8	0.14	*Morinda citrifolia*	15	Diabetes
Diseases and symptoms or signs involving the nervous system	Headache (35), migraine (1), dizziness (8), cramps/spasm (4)	48	178	0.73	*Pseuderanthemum carruthersii*	93	Headache
Diseases of the ear or mastoid process, visual system, and symptoms or signs involving speech or voice	Hearing impairment (2), conjunctivitis (1), hoarseness (2)	5	9	0.50	*Moringa oleifera*	15	Conjunctivitis
Diseases of the circulatory system and blood or blood-forming organs	Anemia (6), hypertension (11)	17	28	0.41	*Annona muricata*	24	Hypertension
Diseases and symptoms or signs involving the respiratory system	Cough (24), pneumonia (1), rhinorrhea (2), itchy throat (1), chest pain (1), tonsilitis (1)	30	148	0.80	*Blumea balsamifera*	91	Cough
Diseases and symptoms or signs involving the digestive system or abdomen	Abdominal pain (2), angular cheilitis (1), bleeding gums (1), bloated stomach/ gas pain (17), blood in feces (1), constipation (2), diarrhea (17), gastric ulcer (1), halitosis (1), indigestion (1) intestinal cleansing (1), nausea (1), stomachache (39), teething syndrome (1), tooth decay (11) vomiting blood (7)	103	252	0.59	*Chrysophyllum cainito*	67	Diarrhea
Diseases and symptom or signs involving the skin	Dandruff (1), eczema (1), lump (5), Pityriasis rosea (2), rashes (2), skin lesion (5)	16	35	0.56	*Psidium guajava*	20	Skin lesion
Diseases and symptoms or signs of the musculoskeletal system or connective tissue	Lower back pain (2), limb pain (3), muscle pain (8), muscle swelling (2), rheumatoid arthritis (8)	22	51	0.58	*Artemisia vulgaris*	26	Muscle pain
Diseases of the genitourinary system	Induce period/menstruation (1), kidney stones (7), kidney trouble (13), swelling of male genitalia (2), urinary tract infection (22)	45	92	0.52	*Annona muricata*	55	UTI
Pregnancy, childbirth and the puerperium	Breast engorgement (3), birth control (4), induce labor (2), lactation support (1), postpartum bleeding (2), postpartum care and recovery (22)	34	118	0.72	*Bambusa spinosa*	93	Postpartum care and recovery
Injury, poisoning and certain other consequences of external causes	Animal bite (2), bruise (1), burn (1), caterpillar dermatitis (1), circumcision (1), cuts and wounds (21), fracture (7), splinter (2)	36	176	0.80	*Chromolaena odorata*	100	Cuts and wounds
General symptoms and signs	Chill (2), fever (19), malaise (1)	21	59	0.66	*Curcuma longa*	43	Fever
Mental or behavioral symptoms, signs or clinical findings	Memory loss	1	1		*Curcuma longa*	2	Memory loss
Other cultural uses	*Doklong* (14), *hiwit* (2), *inaswang* (5), *kolebra* (3), *sinda* (10)	34	89	0.63	*Curcuma longa*	46	*Sinda*

**Number inside the parenthesis denotes the number of species in each reported disease or use.*

stomach/gas pain, goiter, urinary tract infection (UTI), vomiting blood, and *inaswang*. It was collected growing in the farmland, but some informants also cultivated it in their backyards.

A. vulgaris was used to treat seven ailments in six disease categories and is the best-known therapy for cough, fever, headache, and body pains. It is also used for the remedy of chest pain, fracture, and hearing problems. It is grown in the

TABLE 4 | Medicinal plants used to strengthen immunity against infection.

Medicinal plants	Preparation and administration
Capsicum annuum	Eat one fruit before dinner
Vitex trifolia	Boil handful of leaves and drink decoction like water
Euphorbia hirta	Boil handful of whole plant and drink decoction as water intake
Musa balbisiana cv	Eat ripe fruits three times a day
Peperomia pellucida	Soak a handful of whole plants in warm water then drink like water
Curcuma longa	Pound a handful of rhizomes then rub extract on the body three times a day
Zingiber officinale	Boil rhizome and drink one glass of decoction three times a day
Areca catechu, *Piper betle*, and *Nicotiana tabacum*	Wrap a pinch of *A. catechu* seeds in a *P. betle* leaf daubed with slaked lime (*apog*) and plug cut dried leaf of *N. tabacum* then chew (chewing betle quid)

home gardens as medicinal plants for future use. Seven medicinal plants were recorded with only one use report and FC for each species: *Cheilocostus speciosus* (J.Koenig) C.D.Specht, *Luffa aegyptiaca* Mill., *Syzygium cumini* (L.) Skeels, *Lygodium circinnatum* (Burm. f.) Sw., *Solanum melongena* L., *Nauclea orientalis* (L.) L., and *Leea guineensis* G. Don, which garnered the lowest value for UV (0.01) and RFC (0.01).

Medicinal plants with the highest RFC were *Artemisia vulgaris* (0.57), followed by *Curcuma longa* (0.47) and *Blumea balsamifera* (0.44) (**Table 2**). Out of the 75 informants who participated in the survey, *A. vulgaris* had the highest informant citation or FC.

Relative Importance Index

The RI was used to assess the relative importance of the medicinal plants by use or disease categories. A high value indicates that a particular medicinal plant species is most frequently cited as useful with a high number of use categories or having multiple uses. The top three plants with the highest RI values were *Annona muricata* L. (0.88), *C. longa* (0.87), and *B. balsamifera* (0.80) (**Table 2**). *A. muricata* is used in 11 different use or disease categories: diseases of the genitourinary system; neoplasms; diseases of the circulatory system and blood or blood-forming organs; endocrine, nutritional and metabolic diseases; diseases and symptoms or signs involving the respiratory system; diseases and symptoms or signs involving the nervous system; diseases and symptoms or signs involving the digestive system or abdomen; infectious and parasitic diseases; diseases and symptoms or signs involving the skin; diseases and symptoms or signs of the musculoskeletal system or connective tissue; and other cultural uses. It is used to address 13 diseases or purposes and recorded the high use report in treating UTI, cancer, and hypertension by drinking the leaf decoction or soaking the young leaves in warm water and drink or by just eating a medium sliced fruit three times a day. *A. muricata* is also used for the remedy of high uric acid, pneumonia, dizziness, intestinal cleansing, kidney trouble, itchy throat, amoebiasis, lump, arthritis, and *doklong*. *C. longa* was used in nine categories: injury, poisoning and certain other consequences of external causes; neoplasms; diseases and symptoms or signs involving the nervous system; general symptoms and signs; diseases and symptoms or signs involving the digestive system or abdomen; infectious and parasitic diseases; diseases and symptoms or signs of the musculoskeletal system or connective tissue; mental or behavioral symptoms, signs, or clinical findings; other cultural uses. *B. balsamifera* is used in eight disease categories: diseases and symptoms or signs involving the digestive system or abdomen; endocrine, nutritional, and metabolic diseases; diseases of the genitourinary system; diseases and symptoms or signs of the musculoskeletal system or connective tissue; diseases and symptoms or signs involving the nervous system; diseases and symptoms or signs involving the respiratory system; pregnancy, childbirth, and the puerperium; other cultural uses. The top ten medicinal plants with the highest use value, relative frequency citation, and relative importance index are shown in **Table 2**.

Informant Consensus Factor

There were 91 diseases and purposes in 16 different use or disease categories recorded in this study (**Table 3**). ICF was used to evaluate the consensus in the medicinal plant information among the informants. High ICF values indicate one or few medicinal plants mentioned by a high number of informants within a particular disease category, and low values indicate that more species are being used and the informants differ in their preference on which plant to use. The highest ICF value (0.80) was in the diseases and symptoms or signs involving the respiratory system and in injury, poisoning, and certain other consequences of external causes. The diseases and symptoms or signs involving the respiratory system were cough, pneumonia, rhinorrhea, itchy throat, chest pain, and tonsillitis. *B. balsamifera* had the highest use report within the category and was frequently used plant for treating cough by consuming the young leaves or by drinking leaf or root decoction or by rubbing leaf extract on the head of the afflicted ones. A high proportion of informants mentioned and agreed upon the use of *B. balsamifera* in treating cough within the category. Injuries, poisoning, and certain other consequences of external causes recorded, such as animal bite, bruise, burn, caterpillar dermatitis, circumcision, cuts and wounds, fracture, and splinter, were the reported purposes or medical uses. *Chromolaena odorata* (L.) R.M.King & H. Rob. had the highest use report within the category and was the most preferred medicinal plant used to treat cuts/wounds by applying crushed leaves on the affected area. The next highest value was in the diseases and symptoms or signs involving the nervous system (0.73) with headache, migraine, and dizziness as the reported medical condition. *Pseuderanthemum carruthersii* had the highest use report and was widely used for treating headaches by applying leaves on the forehead alone or with *C. longa* and *Z. officinale*. The lowest ICF value was recorded in mental disorder (0.00) with memory loss

TABLE 5 | Comparative presence-absence matrix of the medicinal plants used by the *Panay Bukidnon* with other ethnobotanical studies.

Scientific name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	New medicinal plant uses/purpose
Andrographis paniculata (Burm.f.) Nees	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	1	0	0	1	1	
Justicia gendarussa Burm.f.*	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	1	0	0	1	1	1	0	1	
Pseuderanthemum carruthersii (Seem.) Guillaumin	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	
Acorus calamus L.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	
Amaranthus viridis L.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Anemia
Alternanthera sessilis (L.) R. Br. ex DC.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Anemia, vomiting blood
Allium sativum L.	1	1	0	1	1	0	1	0	1	0	0	0	1	1	1	1	0	0	0	0	1	0	1	
Allium fistulosum L.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Teething syndrome
Mangifera indica L.	1	1	0	1	1	1	1	0	1	1	0	0	1	1	0	1	1	0	0	0	1	0	1	Stomachache, cough, kidney trouble,
Spondias pinnata (L.f.) Kurz	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	
Annona muricata L.	0	1	1	0	1	0	1	0	1	0	0	1	1	1	1	1	1	0	0	1	1	1	1	Pneumonia, lumps
Annona squamosa L.	0	0	1	1	1	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	1	Postpartum care and recovery
Centella asiatica (L.) Urb	1	0	1	0	0	1	1	1	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1	
Alstonia scholaris (L.) R.Br.*	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	1	
Catharanthus roseus (L.) G.Don	0	0	1	0	0	0	1	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	1	
Tabernaemontana pandacaqui Poir.*	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	1	
Alocasia macrorrhizos (L.) G.Don *Alocasia* cultivar	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	
Colocasia esculenta (L.) Schott	0	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	Breast engorgement
Homalomena philippinensis Engl	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	
Schefflera elliptica (Blume) Harms	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Areca catechu L.	1	0	1	1	1	0	1	0	1	1	0	0	0	0	0	1	1	0	0	1	0	0	1	
Cocos nucifera L.	0	1	1	1	1	1	1	0	1	0	0	1	1	0	0	1	0	0	0	1	0	0	1	
Corypha utan Lam.	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	
Cordyline fruticosa (L.) A.Chev.*	1	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	
Aloe vera (L.) Burm.f.	0	0	0	0	0	1	1	0	1	0	0	0	1	1	1	1	0	0	0	0	0	0	1	
Artemisia vulgaris L.	1	1	0	0	1	1	1	0	1	0	0	1	1	0	1	1	1	0	1	1	1	1	1	
Bidens pilosa L.	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	Toothache
Blumea balsamifera (L.) DC.*	1	0	1	1	1	1	1	0	1	0	0	1	0	0	0	1	1	1	1	1	1	1	1	
Chromolaena odorata (L.) R.M.King and H.Rob	0	1	0	0	1	1	1	0	1	1	0	0	1	1	0	1	1	1	1	1	1	0	1	
Elephantopus tomentosus L.*	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	1	0	0	1	
Impatiens balsamina L.	0	0	1	1	0	1	1	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	1	
Basella alba L.	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	Mumps
Bixa orellana L.	0	0	1	0	1	1	1	0	1	0	0	0	1	0	1	0	0	0	0	0	1	0	1	Bloated stomach/gas pain
Cordia dichotoma G.Forst.	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	1	1	0	0	1	
Brassica rapa L.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Kidney problem, anemia
Ananas comosus (L.) Merr.	1	0	0	1	1	0	1	0	1	0	0	1	1	0	0	1	1	0	0	1	0	0	1	Bleeding gums
Carica papaya L.	1	1	1	1	1	1	1	0	1	0	0	0	1	1	1	1	1	0	1	1	1	0	1	
Ipomoea batatas (L.) Lam	0	0	1	1	1	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	Breast engorgement
Decalobanthus peltatus (L.) A.R.Simões and Staples*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	
Cheilocostus speciosus (J.Koenig) C.D.Specht	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	1	
Kalanchoe pinnata (Lam.) Pers.*	1	1	1	1	1	1	1	0	1	0	0	0	1	1	0	1	1	0	1	0	1	1	1	
Cucurbita maxima Duchesne	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Fever, swelling of female genitalia

(Continued on following page)

TABLE 5 | (*Continued*) Comparative presence-absence matrix of the medicinal plants used by the *Panay Bukidnon* with other ethnobotanical studies.

Scientific name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	New medicinal plant uses/purpose
Luffa aegyptiaca Mill	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
Momordica charantia L	1	0	0	1	0	0	1	0	1	1	0	1	1	0	1	1	0	0	0	1	1	0	1	
Cyperus mindorensis (Steud.) Huygh	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
Dioscorea esculenta (Lour.) Burkill	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Mumps, ringworm
Euphorbia hirta L.*	1	1	1	0	0	1	1	0	0	1	0	0	0	1	1	1	1	1	1	1	1	0	1	UTI, angular cheilitis
Euphorbia tirucalli L.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Tooth decay
Jatropha curcas L.	1	1	1	1	1	1	1	0	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	Tetanus
Manihot esculenta Crantz	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	Fracture, gas pain, lower back pain
Caesalpinia sappan L.*	0	1	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	1	1	1	1	Typhoid fever
Cajanus cajan (L.) Huth	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	
Clitoria ternatea L.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Cancer, hypertension
Desmodium triflorum (L.) DC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	
Gliricidia sepium (Jacq.) Kunth ex Walp	0	0	1	1	1	0	1	0	0	0	0	1	1	1	0	1	1	0	0	1	0	0	1	
Indigofera tinctoria L	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	
Leucaena leucocephala (Lam.) de Wit	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	1	
Mimosa pudica L.*	0	0	1	1	1	1	1	0	1	1	0	1	1	1	0	0	1	1	1	1	0	1	1	
Phaseolus lunatus L.	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	
Pithecellobium dulce (Roxb.) Benth.	0	0	1	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	
Senna alata (L.) Roxb.*	1	0	0	1	0	0	1	1	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	
Tamarindus indica L.	0	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0	0	1	1	0	0	1	
Vigna unguiculata (L.) Walp	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Bloated stomach/gas pain, gas pain
Cratoxylum sumatranum (Jack) Blume*	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	
Eleutherine palmifolia (L.) Merr.	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	Rashes, headache
Clerodendrum quadriloculare (Blanco) Merr.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	
Gmelina arborea Roxb. ex Sm.	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	1	1	0	0	1	1	0	1	
Hyptis capitata Jacq.	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	1	Helminthiasis
Mentha arvensis L.	1	0	1	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	1	
Orthosiphon aristatus (Blume) Miq.	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	Cancer
Plectranthus amboinicus (Lour.) Spreng.	0	1	0	0	1	0	1	0	0	0	0	1	0	0	0	1	1	0	1	1	1	0	1	
Plectranthus scutellarioides (L.) R.Br.	0	1	0	1	0	0	1	0	1	0	0	0	1	1	0	1	1	0	1	1	0	1	1	
Tectona grandis L.f.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	Cuts, wounds, rheumatoid arthritis
Vitex trifolia L.*	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	
Persea americana Mill	1	1	0	1	1	1	1	0	1	1	0	1	1	1	0	1	0	0	1	0	1	1	1	
Barringtonia asiatica (L.) Kurz	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Lygodium circinnatum (Burm. f.) Sw.*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Headache
Lagerstroemia speciosa (L.) Pers.	1	0	0	1	1	0	1	0	1	0	0	1	1	1	0	0	1	0	1	1	1	0	1	Cancer
Abelmoschus esculentus (L.) Moench	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	UTI, cancer, hypertension
Corchorus olitorius L.	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Birth control
Hibiscus acetosella Welw. ex Hiern*	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	Cuts, wounds, boils, anemia, hypertension, diabetes, rhinorrhea, cough
Urena lobata L.*	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	1	
Sandoricum koetjape (Burm.f.) Merr.	0	0	1	0	1	0	1	0	1	1	0	0	1	1	1	1	1	0	1	0	1	0	1	Kidney trouble
Swietenia mahagoni (L.) Jacq	0	1	1	0	1	0	1	0	0	0	0	1	1	0	1	1	1	0	0	0	0	0	1	
Tinospora crispa (L.) Hook. f. and Thomson*	0	1	0	0	0	0	1	0	0	0	0	1	0	0	1	1	1	0	0	1	1	0	1	
Artocarpus heterophyllus Lam	0	1	1	0	1	1	1	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	1	
Ficus benjamina L. HNUL 0021383	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	1	0	0	1	1	0	0	1	Postpartum care and recovery, stomachache
Ficus septica Burm.f.	0	0	0	1	0	0	1	1	0	0	0	1	0	1	0	1	1	0	0	1	1	0	1	

(Continued on following page)

TABLE 5 | (*Continued*) Comparative presence-absence matrix of the medicinal plants used by the *Panay Bukidnon* with other ethnobotanical studies.

Scientific name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	New medicinal plant uses/purpose
Moringa oleifera Lam.	0	1	1	1	1	1	1	0	1	0	0	1	1	0	0	1	0	0	1	1	1	1	1	
Muntingia calabura L.	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	1	0	1	
Musa balbisiana cv. Colla	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	
Musa textilis Née	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	
Musa x *paradisiaca* L.	0	1	0	1	1	1	1	0	1	1	0	0	1	0	1	1	0	0	0	1	1	0	1	
Psidium guajava L.	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	
Syzygium cumini (L.) Skeels	0	0	1	0	1	0	1	0	1	1	0	0	1	1	0	1	0	0	0	1	0	0	1	
Averrhoa bilimbi L.	0	0	1	1	1	0	1	0	1	0	0	1	1	0	1	1	0	0	1	0	0	0	1	Kidney trouble
Averrhoa carambola L.	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Kidney trouble, postpartum care and recovery
Oxalis triangularis A.St.-Hil	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	Cuts/wounds
Peperomia pellucida (L.) Kunth	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0	1	1	1	
Piper betle L	1	0	1	1	0	0	1	0	1	1	0	0	0	0	0	1	0	0	1	1	0	0	1	Mumps
Antidesma bunius (L.) Spreng	0	0	1	1	1	0	1	0	1	0	0	0	1	0	1	0	0	0	0	1	0	0	1	
Bambusa spinosa Roxb	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	1	Cancer
Chrysopogon aciculatus (Retz.) Trin	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Stomachache, tooth decay, wart
Cymbopogon citratus (DC.) Stapf	1	1	0	0	0	1	0	0	1	1	0	1	1	1	0	1	0	0	0	1	1	1	1	Halitosis
Eleusine indica (L.) Gaertn.*	0	1	0	0	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	1	1	1	1	
Imperata cylindrica (L.) Raeusch	1	1	0	1	1	1	1	0	1	1	0	0	1	1	1	1	1	1	1	1	0	1	1	Cancer
Oryza sativa L	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	1	Postpartum care and recovery
Saccharum officinarum L.	0	0	1	1	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	Hoarseness
Zea mays L.	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	
Chrysophyllum cainito L.	0	1	1	1	0	1	1	0	1	1	0	1	1	1	1	1	0	0	0	1	0	1	1	
Capsicum annuum L.	1	1	1	1	1	1	1	0	1	0	0	0	0	1	1	1	1	0	1	0	1	0	1	
Solanum lycopersicum L.	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	
Nicotiana tabacum L.	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	
Solanum melongena L.	0	0	0	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	
Nauclea orientalis (L.) L.	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	
Morinda citrifolia L.	0	0	1	1	1	0	1	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	
Mussaenda philippica A.Rich	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	1	
Citrus maxima (Burm.) Merr.	0	0	0	0	1	0	1	0	0	0	0	1	1	1	1	1	0	0	0	1	1	0	1	Kidney trouble, chicken pox
Citrus microcarpa Bunge	1	1	1	0	0	1	1	0	1	0	0	1	0	1	1	1	0	0	0	1	1	0	1	
Pipturus arborescens (Link) C.B. Rob.*	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	Breast engorgement
Stachytarpheta jamaicensis (L.)*	0	1	0	1	1	1	0	1	0	1	0	0	0	0	1	1	1	1	1	1	1	0	1	
Cissus sp.																								
Leea guineensis G. Don	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	
Tetrastigma sp. Planch.																								
Alpinia galanga (L.) Willd	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	
Amomum sp.																								
Curcuma longa L.	0	1	0	1	0	1	1	0	0	1	0	0	0	1	0	0	1	0	1	0	1	1	1	Typhus, memory loss, tetanus
Etlingera philippinensis (Ridl.) R.M.Sm.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	Typhoid fever, tuberculosis, headache
Kaempferia galanga L.	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	1	0	1	1	1	
Zingiber officinale Roscoe	1	1	0	1	1	1	1	0	1	0	0	1	1	1	1	1	0	0	0	1	1	1	1	Tetanus

as the

reported condition and *Curcuma longa* was used for the treatment.

Fidelity Level

The FL was used to determine the relative importance of a medicinal plant within each category. Medicinal plants with the highest FL values were *Chromolaena odorata* (100%), *Bambusa spinosa* Roxb. (93%), and *Pseuderanthemum carruthersii* (93%) (**Table 3**). *C. odorata* is exclusively used to treat cuts and wounds and can be seen growing invasively along the paths and roadsides in the community. *B. spinosa* recorded the highest use report for postpartum care and recovery under the pregnancy, childbirth, and puerperium category. It is preferably used and highly suggested by many informants for postpartum care and recovery therapy. Decoction of at least three up to ten different medicinal plants was used for drinking (1–2 glasses), body steaming, and bathing to be performed nine days after a mother gave birth. *B. spinosa* has also been used to treat cancer, UTI, and kidney stones but with only one citation recorded for each ailment *P. carruthersii* has the highest use report and is the most preferred medicinal plant for relieving headaches under the diseases and symptoms or signs involving the nervous system. The lowest FL value was recorded for *C. longa* in treating memory loss under the mental disorder category with only one informant mentioned for its curative effect.

Comparing Different Indices

Table 2 shows the top 10 medicinal plants with the highest UV, RFC, and RI values. High UV and RFC values indicate the high number of use reports and frequency citations (FC) from the informants, while high RI values consider the multiplicity of uses or the high number of uses in different disease categories. This implies that medicinal plants with high UV, RFC, and RI values are the most important and valued medicinal plants in the community. There are a few considerable differences in species ranking yielded by the three indices set out in **Table 2**. The ranks of the first three species (*C. longa, Blumea balsamifera,* and *Artemisia vulgaris*) are nearly the same in all indices except in RI where *Annona muricata* had the highest value but ranked 4th in UV and only 9th in RFC. *C. longa, B. balsamifera,* and *A. vulgaris* had the highest use reports and FC from the informants and only next to *A. muricata* in terms of multiple uses in different disease categories. *A. muricata* had the highest number of uses or purposes in different disease categories (11 disease categories); however, its use reports and FC are not quite as high as those of the first three species mentioned above. *A. muricata* is the most frequently used medicinal plant in a wide range of diseases (13 diseases). Another noticeable difference is the inclusion of *Moringa oleifera Lam.* and *Carica papaya* L. in the RI index, which are not shown in the top ten species with high UV and RFC values. *M. oleifera* ranks 12th in UV and 13th in RFC, while *C. papaya* ranks 17th in UV and 14th in RFC (values are not shown in **Table 2** but available in **Supplementary Table S2**). Their UV and RFC values are not quite as high, but they

1-Balangcod and Balangcod, 2011.
2-Oliowa et al., 2012.
3-Tantiado, 2012.
4-Abe and Ohtani, 2013.
5-Ragragio et al., 2013.
6-Gruyal et al., 2014.
7-Ong and Kim, 2014.
8-Raterta et al., 2014.
9-Balangcod and Balangcod, 2015.
10-Pizon et al., 2016.
11-Odchimar et al., 2017.
12-Baddu and Ouano, 2018.
13-Tantengco et al., 2018.
14-Agapin, 2019.
15-Pablo, 2019.
16-Cordero et al., 2020.
17-Dapar et al., 2020.
18-Paraguison et al., 2020.
19-Belgica et al., 2021.
20-Cordero and Alejandro, 2021.
21-Nuñeza et al., 2021.
22-Montero and Geducos, 2021.
23-PTKD (Philippine Traditional Knowledge Digital Library on Health).
**Medicinal plants recorded in Panay Bukidnon communities in the Province of Capiz (Jocano, 1968).*
Plants in bold are the newly recorded species with medicinal values.
Values in bold indicate the absence of medicinal value of the newly recorded species compared with the other existing ethnobotanical studies in the Philippines.

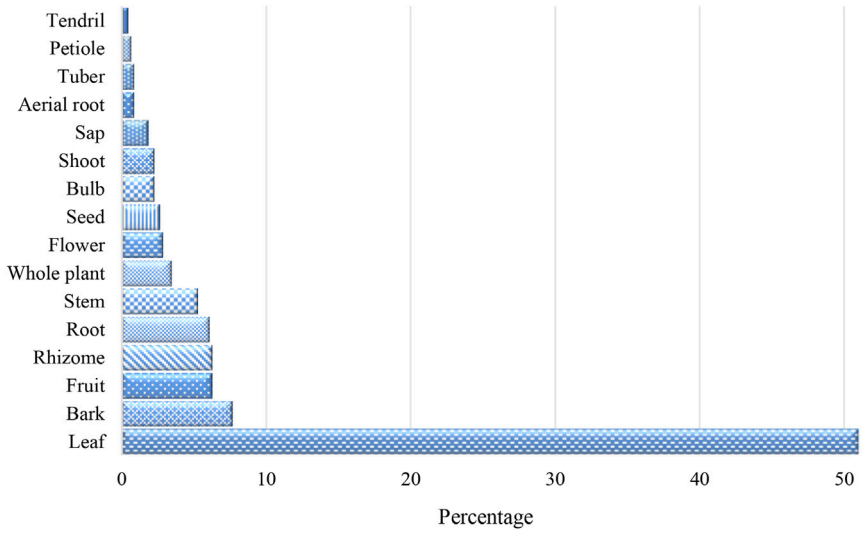

FIGURE 4 | Medicinal plant parts used.

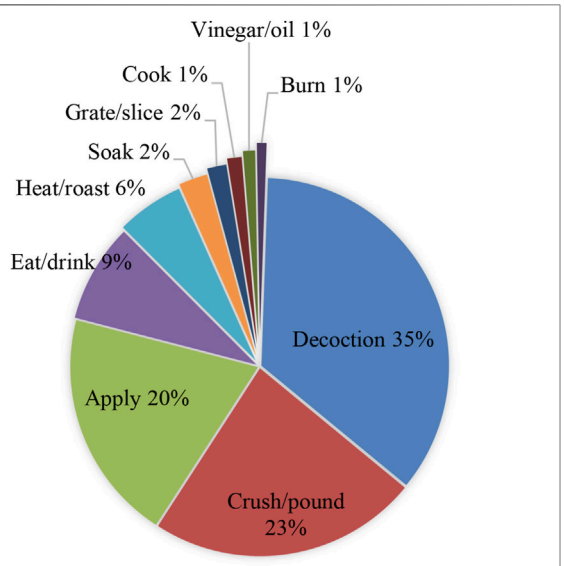

FIGURE 5 | Medicinal plant preparation.

have multiple uses in different disease categories. Low UV, RFC, and RI values indicate a low number of use reports, FC, and have one or few uses in disease categories. For example, *Andrographis paniculata* (Burm.f.) has low UV (0.04), RFC (0.03), and RI (0.07) values, indicates low use report (2) and FC (2 informants), and is only used in one disease category (diseases and symptoms or signs involving the digestive system or abdomen). This implies that *A. paniculata* is a less important and less preferred medicinal plant species in the *Panay Bukidnon* community. On the other hand, **Table 3** shows the use or disease categories and ICF and FL values. High ICF values are considered the most culturally relevant medicinal plants and the agreement of its use within a disease category in the community, while FL highlights the most preferred species for a particular disease. Medicinal plants with the high ICF values were *Blumea balsamifera* (0.80), *Chromolaena odorata* (0.80), and *Pseuderanthemum carruthersii* (0.73) and these species are highly agreed upon by most informants for the therapy of diseases in their respective disease categories. Medicinal plants with high FL values were *C. odorata* (100%), *P. carruthersii* (93%), and *Bambusa spinosa* (93%) and these are the most preferred species to a particular disease in each disease category. Though *B. spinosa* is not included in the top medicinal plants with high UV, RFC, and RI values, its curative effect for postpartum care and recovery is preferred by a high proportion of informants. Medicinal plants with high UV, RFC, RI, ICF, and FL values are the most culturally important, relevant, preferred, and agreed on species in the *Panay Bukidnon* communities.

Cultural Important Medicinal Plants

Indigenous peoples are strongly tied with their spiritual beliefs and practices. Interestingly, up to date, the *Panay Bukidnon* still believe that some of the illnesses and diseases are caused by spirits, supernatural beings, and sorcery. Some diseases were mentioned that were caused by *aswang* (witch), *hiwit* (sorcery), *sinda* (charm of spirits) and some health conditions like *doklong* and *kolebra* with more complicated and sometimes unexplained symptoms. *Sinda* is a condition with symptoms like dizziness and fever caused by spirits or supernatural beings, while *kolebra* has symptoms like chills, stomachache, nausea, shortness of breath, and paleness. *Doklong* is somewhat similar to "relapse" and sometimes accompanied by other symptoms like headache, muscle pain, and weakness. For *inaswang*, five medicinal plants were recorded for the therapy and a cultivar of *Alocasia* is frequently used by applying the heated leaf to the stomach area. For the treatment of *hiwit*, a species of *Amomum* is used by rubbing the stem extract on the body or by crushing the

stem with the inner bark of *Pipturus arborescens* (Link) C.B. Rob. and taking the extract orally. *Curcuma longa* is the most used medicinal plant used to cure *sinda* by rubbing the rhizome's extract on the head or applying the sliced rhizome along with other plants on the forehead. *Jatropha curcas* L. is frequently used as a remedy for *kolebra* by drinking the extract of the inner bark and for *doklong*, drinking the leaf decoction of *Citrus maxima* (Burm.) Merr. alone or with other medicinal plants is the most preferred.

Medicinal Plants Used to Strengthen Immunity Against Infection and for Potential COVID-19 Therapy

With the current situation of the novel coronavirus disease (COVID-19) in the country and the resurgences of the infection waves, communities in the far-flung areas tend to explore different medical plants as an alternative for potential therapy while waiting for the vaccine. There were ten medicinal plants mentioned by the council of elders that they used to boost their immunity against COVID-19 infection (**Table 4**). If someone is suspected of having a COVID-19 infection or exhibits symptoms related to COVID-19, they use the available medicinal plants (**Table 2**) available to alleviate their condition. Medicinal plants such as *Curcuma longa, Zingiber officinale, Capsicum annuum* L., and *Peperomia pellucida* (L.) Kunth are traditionally used by the *Panay Bukidnon* to treat fever, headache, cough, and body pains which were also the symptoms of COVID-19 and influenza. They also believed that chewing betel quid which is composed of *Areca catechu* L., *Piper betle* L., *Nicotiana tabacum* L., and slaked lime (*apog*) can help them fight the infection and help them feel substantially better.

Comparative Review of the Medicinal Plants With Other Ethnobotanical Studies

The anthropological study conducted in the *Panay Bukidnon* communities in the Province of Capiz in the 1950s recorded a total of 54 medicinal plant species and 21 of which are cited in this current ethnobotanical study. An additional 109 taxa were documented to the medicinal flora used by the *Panay Bukidnon* in Panay Island from this present study.

To identify the new medicinal plants and plant use, a comprehensive comparison was performed with 22 ethnobotanical studies published from 2011 up to the present and with one online database. Only the scientific names and their synonyms were used for the comparative review; local names were not considered because they are arbitrary within different cultures and dialects. Of the 127 medicinal plants identified up to the species level, three species (*Eleutherine palmifolia* (L.) Merr., *Hibiscus acetosella* Welw. ex Hiern, and *Oxalis triangularis* A.St.-Hil.) show some novel medicinal uses that were not documented in other existing ethnobotanical studies conducted in the country. These medicinal plant species are not native to the Philippines. *E. palmifolia* is used for the therapy of rashes and headaches. Its red bulb is the preferred plant part for the treatment. It is usually used as an ornamental plant grown in the home gardens and community center. *H. acetosella* is used to cure cuts/wounds, boils, anemia, hypertension, diabetes, rhinorrhea, and cough. Filipinos also used this medicinal plant as a vegetable and normally use it to "sour" the dishes. With its deep red-purple foliage, it is also served as an ornamental plant. *O. triangularis* is used to treat cuts/wounds by the *Panay Bukidnon* and serves as a hanging ornamental plant for its striking deep maroon trifoliate leaves. Forty-seven medicinal plants were recorded to have an additional therapeutic use or purpose not mentioned in the other previous ethnobotanical studies, while 80 species have the same medicinal values as mentioned in the existing literature. Some of the additional uses or purposes of the medicinal plants that are rarely listed in other studies are angular cheilitis, breast engorgement, and promoting teething in toddlers. The detailed information about the comparative review of the medicinal plants and the additional plant uses is shown in **Table 5**.

DISCUSSION

The documentation of 131 medicinal plant species used in the indigenous health care practices showed the extensive usage of *Panay Bukidnon* ethnobotanical knowledge and indicative importance for their rich cultural heritage. The families of Fabaceae, Lamiaceae, and Poaceae were represented with a high number of medicinal plant species. Fabaceae as the most preferred medicinal plant family used by the *Panay Bukidnon* is parallel to the other folkloric studies conducted in Western Visayas (Madulid et al., 1989; Tantiado 2012; Ong and Kim, 2014; Cordero and Alejandro, 2021) and other indigenous communities in the country (Ragragio et al., 2013; Obico and Ragrario, 2014; Tangtengco et al., 2018; Pablo, 2019). Fabaceae is highly used by the *Panay Bukidnon* to treat infectious and parasitic diseases and diseases and symptoms or signs involving the digestive system or abdomen. The family constitutes phytochemicals that have antibacterial, antifungal, antioxidant, and insecticidal activities (Wanda et al., 2015).

The use of leaves as the most preferred medicinal plant part to address medical conditions is comparable to other ethnobotanical surveys conducted throughout the archipelago (Balangcod and Balangcod, 2011; Olowa et al., 2012; Abe and Ohtani, 2013; Gruyal et al., 2014; Ong and Kim, 2014; Raterta et al., 2014; Balangcod and Balangcod, 2015; Pizon et al., 2016; Odchimar et al., 2017; Baddu and Ouano, 2018; Tantengco et al., 2018; Agapin, 2019; Pablo, 2019; Cordero et al., 2020; Dapar et al., 2020; Belgica et al., 2021; Cordero and Alejandro, 2021; Madjos and Ramos, 2021; Montero and Geducos, 2021; Nuñeza et al., 2021). As a tropical country, leaves are always available for most plant species at all seasons and are readily accessible in case of emergencies. The collection of leaves is more sustainable than gathering other plant parts such as barks and roots that can cause damaging effects and even mortality to a plant if harvested in large quantities. Leaves contain the highest secondary metabolites with an antimicrobial effect (Chanda and Kaneria, 2011),

antioxidant property, antibiotic activity, and antidiabetic potential compared with other plant parts (Jain et al., 2019).

Decoction is the most common form of preparation and preferably to be taken orally and occasionally used for body steaming, bathing, and washing. It is also an evident form of preparation in other indigenous communities in the country (Balangcod and Balangcod, 2015; Pizon et al., 2016; Odchimar et al., 2017; Baddu and Ouano, 2018; Tantengco et al., 2018; Cordero et al., 2020; Cordero and Alejandro, 2021; Madjos and Ramos, 2021; Nuñeza et al., 2021). Decoction is done with the use of one medicinal plant species or in a combination of two or more. The *Panay Bukidnon* are culturally used to combine three, five, or seven (colloquially known as *pito-pito*) different medicinal plants for higher efficacy. Each plant constitutes phytochemical compounds and is sometimes present in small quantities and inadequate to achieve desirable therapeutic effects. To yield better results and effectiveness, the combination of different medicinal plants demonstrates the synergistic effects. Some bioactive chemicals work significantly when combined with other plants rather than used singly (Parasuraman et al., 2014).

Curcuma longa recorded the highest use value and is used as therapy for headache, fever, body pain, stomachache, bloated stomach, tooth decay, typhus, typhoid fever, anti-tetanus, memory loss, cancer, and *sinda*. The rhizome's extract is usually used for the treatment. It is also used by other indigenous groups in the country for diarrhea, abdominal pain, flatulence, arthritis, and hypertension by the *Higaonon* tribe in Iligan City (Olowa et al., 2012); arthritis, cough, and cuts and wounds by the *Ivatan* tribe in Batan Island (Abe and Ohtani, 2013); fever, burn, dizziness, and abdominal pain by the *Ati* tribe in Guimaras Island (Ong and Kim, 2014); arthritis by the *Subanen* tribe in Zamboanga del Sur (Pizon et al., 2016); flatulence, headache, numbness, rheumatism, stomachache, and vomiting by the *Aetas* tribe in Bataan (Pablo, 2019); cancer by the *Manobo* tribe in Bukidnon (Pucot et al., 2019); skin eruptions and gastric pain by the *Ati* tribe in Aklan (Cordero et al., 2020); ten different diseases by the *Manobo* tribe in Agusan del Sur (Dapar et al., 2020); myoma, hepatitis, relapse, sore eyes, and stye by the eight ethnolinguistic groups in Zamboanga Peninsula (Madjos and Ramos, 2021); bruise and boils by the *Mamanwa* tribe in Surigao del Norte and Agusan del Norte (Nuñeza et al., 2021). In India, the use of *C. longa* dates back to 4,000 years ago not only as a culinary spice but also for religious and medicinal importance. It contains bioactive compounds that have antioxidant, antimutagenic, antimicrobial, antimutagenic, antimicrobial, antifungal, anticancer, and other countless medicinal uses (Prasad and Aggarwal, 2011).

Artemisia vulgaris has the highest relative frequency citation. It is a cosmopolitan weed and is available nearly everywhere. It thus does not surprise that it is commonly used for cough, fever, headache, body pains, chest pain, fracture, and hearing problems. Other ethnobotanical surveys mentioned its efficacy against cough and scabies by the *Kalanguya* tribe in Ifugao (Balangcod and Balangcod, 2011); stomachache (Olowa et al., 2012); sore eyes, ear infection, and cough by the *Ayta* tribes in Pampanga (Ragragio et al., 2013); cough with phlegm, fever, abdominal pain, body pains, and headache (Ong and Kim, 2014); colds by the *Talaandig* tribe in Bukidnon (Odchimar et al., 2017); dysmenorrhea by the *Y'Apayaos* in Cagayan arthritis (Baddu and Ouano, 2018); fever, sore throat, colds, cough, and phlegm *Ayta* in Bataan (Tantengco et al., 2018); fever, headache, dizziness, stomachache, bloated stomach, and cough (Cordero et al., 2020); 11 different folkloric uses (Madjos and Ramos, 2021); cough and gas pain (Cordero and Alejandro, 2021); fever, cough, and cough with phlegm (Nuñeza et al., 2021). In medieval times, it was known as the "mother of herbs" due to its beneficial effects. Studies have been conducted worldwide for its antioxidant, bronchodilatory, hepatoprotective analgesic, antihypertensive, estrogenic, cytotoxic, antifungal and antibacterial, anti-inflammatory, anti-allergenic, antimalarial, and anthelmintic activities (Ekiert et al., 2020). *Artemisia,* per se, is an extremely important plant genus, pharmacologically as well as economically. *A. annua* L. makes the most important example, famous for its many pharmacologically active substances but especially for Artemisin (Tu, 2011), an effective remedy against malaria.

Annona muricata, an important, widely grown fruit tree, has the highest relative importance index value (0.88) and is used to treat 13 diseases in 11 different use or disease categories. It recorded the high use report for treating UTI, cancer, and hypertension by drinking the leaf decoction or eating just the ripe fruit. It is also used by the *Panay Bukidnon* for high uric level, pneumonia, dizziness, intestinal cleansing, kidney trouble, *doklong*, itchy throat, amoebiasis, lump, and arthritis. In traditional medicine across the country, it is also used for the treatment of diarrhea (Olowa et al., 2012); dermatological diseases (Tantiado, 2012); fever, insect repellent, headache, and stomachache (Ragragio et al., 2013); tetanus (Pizon et al., 2016); gastrointestinal cleansing and tumors (Odchimar et al., 2017); fever and arthritis (Baddu and Ouano, 2018); stomachache and dizziness (Tantengco et al., 2018); diabetes, high blood, stomachache, UTI, and vertigo (Pablo, 2019); cancer (Agapin, 2019); 12 different diseases (Dapar et al., 2020); kidney problems, urinary tract infection, goiter, and anthelmintic (Cordero et al., 2020); at least 16 medical problems (Madjos and Ramos, 2021); cuts and wounds, stomach ulcer, intestinal cleansing, UTI, cough, and cancer (Cordero et al., 2020); cancer (Montero and Geducos, 2021); cough, wound, asthma, and UTI (Nuñeza et al., 2021); cancer, stomach acidity, hypertension, and cough (Belgica et al., 2021). Phytochemical constituents investigated on *A. muricata* exhibited antiarthritic, anticancer, anticonvulsant, antidiabetic, anti-inflammatory, antioxidant, antihypertensive, antiparasitic, antiplasmodial, cytotoxic, gastroprotective, and wound healing activity (Moghadamtousi et al., 2015; Coria-Téllez et al., 2018).

The highest ICF value is in the diseases of the respiratory system category and *Blumea balsamifera* is the most frequently used medicinal plant to treat cough. A high number of informants agreed on the effectiveness of *B. balsamifera* against the diseases on the respiratory system, particularly for treating cough in the community. However, this therapeutic claim must be seriously considered for further pharmacological investigations to determine its efficacy. *B. balsamifera* is one of the ten medicinal plants endorsed by the Philippine Department of Health (DOH) as part of basic healthcare and clinically proven

to have diuretic and antiurolithiasis properties. It is manufactured in the country for national distribution and marketing by the National Drug Formulary (World Health Organization, 1998). It also contains compounds (monoterpenes, diterpenes, sesquiterpenes) that have antitumor, antioxidant, antimicrobial and anti-inflammation, antiplasmodial, antityrosinase, wound healing, anti-obesity, disease and insect resistance, and hepatoprotective effects and radical scavenging activities (Pang et al., 2014).

The medicinal plant with the highest FL was the *Chromolaena odorata* under the injury, poisoning, and certain other consequences of external causes category. All informants who cited *C. odorata* preferred to use it as first aid for cuts and wounds. This suggests that *C. odorata* might contain valuable bioactive compounds with pharmacological effects for cuts and wounds that must be proven scientifically. Several ethnobotanical studies also recorded the use of *C. odorata* for cuts and wounds in the country (Olowa et al., 2012; Ong and Kim, 2014; Pizon et al., 2016; Odchimar et al., 2017; Tantengco et al., 2018; Cordero et al., 2020; Dapar et al., 2020; Belgica et al., 2021; Cordero and Alejandro 2021; Madjos and Ramos, 2021). The leaves of *C. odorata* are rich in flavonoids and have the highest concentration of allelochemicals. They have antimalarial, anti-inflammatory, antibacterial, analgesic, antipyretic, antioxidant, anticancer, and wound healing properties (Vijayaraghavan et al., 2017).

Traditional medical practices in the indigenous groups in the Philippines are generally influenced by their cultural, spiritual, and religious beliefs of supernatural beings. *Curcuma longa* is the most preferred medicinal plant administered by the *Panay Bukidnon* to a sick person with conditions caused by unseen beings. In Hindu worship rights, *C. longa* has been used for offerings and magic (Velayudhan et al., 2012).

Plant-based compounds have been in constant use since ancient times for any emerging disease. There were several bioactive compounds extracted from medicinal plants with promising antiviral properties against the novel coronavirus (COVID-19) (Adhikari et al., 2020). In Thailand, 60 medicinal plant species were used to treat mild symptoms of COVID-19 (Phumthum et al., 2021). In Nepal, there were also 60 medicinal plants used (Khadka et al., 2021) and 23 plants in Morroco (El Alami et al., 2020) for potential COVID-19 therapy and *Zingiber officinale* is one of the common species used. In Bangladesh, phytochemicals extracted from *Calotropis gigantea* exhibited positive inhibitory effects against the COVID-19 virus (Dutta et al., 2021), as well as the alkaloids and terpenoids isolated from plants of African origin (Gyebi et al., 2021). *Curcumin* from *C. longa* also showed promising effects against the virus (Adhikari et al., 2020).

In the Philippines, the Department of Science and Technology (DOST) has been conducted clinical trials and explored the therapeutic effects of the virgin coconut oil (VCO), *Euphorbia hirta* (tawa-tawa), and *Vitex trifolia* (lagundi) for their potential efficacy against COVID-19 infection (Arayata, 2021). In the recent updates published in the Global Media Arts (GMA) news articles, clinical trials for *V. trifolia* and *E. hirta* have been proven to decrease mild-to-moderate symptoms of COVID-19. Mild-to-moderate symptoms of 172 random COVID-19 patients disappeared within 3–5 days after taking a 1,950 mg capsule of *E. hirta* thrice a day for ten days as a food supplement. *V. trifolia* also showed a positive result in decreasing mild symptoms of COVID-19. Community trials of VCO as an adjuvant for mild symptoms of COVID-19 patients showed a positive result in decreasing the virus count by 60–90%. A clinical trial of VCO on mild and severe symptoms of COVID-19 conducted in Philippine General Hospital is still ongoing (Global Media Arts News, 2021a; Global Media Arts News, 2021b).

V. trifolia, *C. longa, Z. officinale, Capsicum annuum, E. hirta,* and *Peperomia pellucida* were used by the *Panay Bukidnon* as an alternative medicine to strengthen their immunity and they have claimed that these species can alleviate the symptoms of the COVID-19 infection. They used these plants traditionally to treat fever, headache, cough, and body pains, which were also the common indications of COVID-19. Further pharmacological research and investigations are highly suggested for these medicinal plants to explore their potential uses and therapeutic effects against COVID-19 infection especially for *Zingiber officinale, Capsicum annuum*, and *Peperomia pellucida* species. The *Panay Bukidnon* also believed that chewing betel quid could give them the strength to fight the virus. Chewing betel quid has been a customary practice of Filipinos since the pre-Spanish colonial period throughout the Philippines. It is part of the social undertakings and ceremonies and is believed to increase stamina, good health, and longevity (Valdes, 2004). In India, the practice of chewing betel dates back to around 75 AD and it is known for centuries for its therapeutic properties (Toprani and Patel 2013). A review was conducted on the synergistic prophylaxis effects of *Piper betle* and gold ash can hypothetically limit and manage the COVID-19 infection (Sharma and Malik, 2020).

For the comparative review performed on the medicinal plants with other ethnobotanical studies conducted in the country, three species (*Eleutherine palmifolia*, *Hibiscus acetosella*, and *Oxalis triangularis*) showed no record of medicinal value in the previous studies. *E. palmifolia* is used by the *Dayaks* in Indonesia to treat a variety of diseases such as diabetes, cancer, hypertension, stroke, and sexual disorders and as a galactagogue. Bioactive compounds from this species contain various pharmacological activities such as antibacterial, anti-inflammatory, anticancer, and antidiabetic (Kamarudin et al., 2021). *H. acetosella* is used as therapy for anemia in Southern Uganda (Ssegawa and Kasenene, 2007) and its phenolic compounds exhibit antioxidant and antibacterial properties (Lyu et al., 2020). Limited literature is available for *O. triangularis,* but its medicinal uses include remedies for fever, UTI, mouth sores, cuts, rashes, and skin infections (Arakelyan and Arakelyan, 2020). The comparison of the medicinal plants and their uses was performed with five ethnobotanical studies that were previously conducted in the rural and urban communities and villages (Tantiado, 2012; Gruyal et al., 2014; Agapin, 2019; Belgica et al., 2021; Montero and Geducos, 2021), 17 studies conducted in the IP communities (Balangcod and Balangcod, 2011; Olowa et al., 2012; Abe and Ohtani, 2013; Ragragio et al., 2013; Ong and Kim, 2014; Raterta et al., 2014; Balangcod and Balangcod, 2015; Pizon et al., 2016; Odchimar et al., 2017; Baddu and Ouano, 2018; Tantengco et al., 2018; Pablo, 2019; Cordero et al., 2020; Dapar et al., 2020; Paraguison et al., 2020; Cordero and Alejandro, 2021; Nuñeza

et al., 2021) all over the country, and one online database: the Philippine Traditional Knowledge Digital Library on Health (Philippine Traditional Knowledge Digital Library on Health, 2021). The PTKDL is an electronic library that documented 16,690 enumerations of medicinal plant preparations and 66 healing rituals and practices mentioned by 509 traditional healers in 43 different research sites in the country (World Health Organization, 2019). The database (https://www.tkdlph.com/) recorded about 1,200 medicinal plants used by the local and indigenous communities from different ethnobotanical studies, lexicographic and linguistic texts, and current researches conducted in selected indigenous communities nationwide.

CONCLUSION

The ethnobotanical use of many different plant species is an important predominating practice in the Philippines. It is an integral part of Filipino custom and tradition and has been culturally accepted for ages. The results of this ethnobotanical documentation of 131 medicinal plants used in addressing 91 diseases across 16 different disease categories portray the strong dependence of the *Panay Bukidnon* in the medicinal flora in their area. This could be attributed to the great distance of the study site to the town and the health centers or well-functioning hospitals. The most culturally relevant and important species recorded in this study in terms of UV, RFC, RI, ICF, and FL are *Curcuma longa*, *Blumea balsamifera*, *Artemisia vulgaris, Annona muricata,* and *Chromolaena odorata,* respectively. The efficacy and effectivity of the therapeutic claims of these species must be further pharmacologically investigated and validated. These species have been used for centuries by many people worldwide and have proven to cure a myriad of diseases. The comparative study of the medicinal plants with other ethnobotanical studies revealed some novel and additional therapeutic uses that are valuable to the immense body of traditional knowledge and practices in the country. The traditional knowledge and practices from indigenous peoples add more treatment opportunities for potential therapy of pre-existing and novel diseases. The indigenous knowledge on the medicinal plants used by the *Panay Bukidnon* is passed from one generation to the other mostly in oral forms with the influence of their religious and cultural beliefs. Furthermore, it is urgent to document the indigenous knowledge before it is forgotten because of environmental and social challenges such as species extinction, climate change, acculturation, modernization, availability and accessibility of prescribed medicines, and lack of interest of the younger generations. The results of this study also serve as a medium for preserving cultural heritage, ethnopharmacological bases for further drug research and discovery, and preserving biological diversity. The ethnobotanical study on the *Panay Bukidnon* communities in Panay Island is limited by the expensive and lengthy process of acquiring government permits and by the fact that some communities are infested by leftists (New People's Army) that could risk the safety of researchers and there are no access roads in the upland areas. Lastly, it is strongly recommended to conduct further comprehensive surveys on other *Panay Bukidnon* communities in other provinces of the Panay Island and to conduct pharmacological studies and investigations on the important medicinal plants, especially the ones that have high ICF and FL values for potential drug development and formulation.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Graduate School–Ethics Review Committee, University of Santo Tomas. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CSC processed and acquired the necessary government permits, conducted the field works, and drafted the manuscript. All authors designed the study, GJDA and UM supervised, reviewed, and made the final revision of the manuscript.

FUNDING

The research had partial funding from the scholarship of the first author from the Commission on Higher Education K-12 Transition Program (CHED K-12) and the Digital Cooperation Fellowship from the Alexander von Humboldt Foundation awarded to GJDA and UM.

ACKNOWLEDGMENTS

The authors are forever grateful to the *Panay Bukidnon* IP leader, the council of elders, members, and Brgy. officials of Brgy. Caguisanan, Lamunao, Iloilo; DENR Region VI for the Wildlife Gratuitous Permit; NCIP Iloilo-Guimaras Service Center officers for facilitating the community meetings; NCIP Region VI/VII for the issuance of the Certification Precondition. Heartfelt gratitude is also given to the Ethics Review Committee of the University of Santo Tomas Graduate School, Danilo Tandang of the Philippine

National Museum, and Michael Calaramo of the Herbarium of the Northwestern University Luzon. CSC would like to thank CHED K-12 for her scholarship. GJDA and UM acknowledge the Alexander von Humboldt Foundation for the Digital Cooperation Fellowship.

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SUPPLEMENTARY MATERIAL

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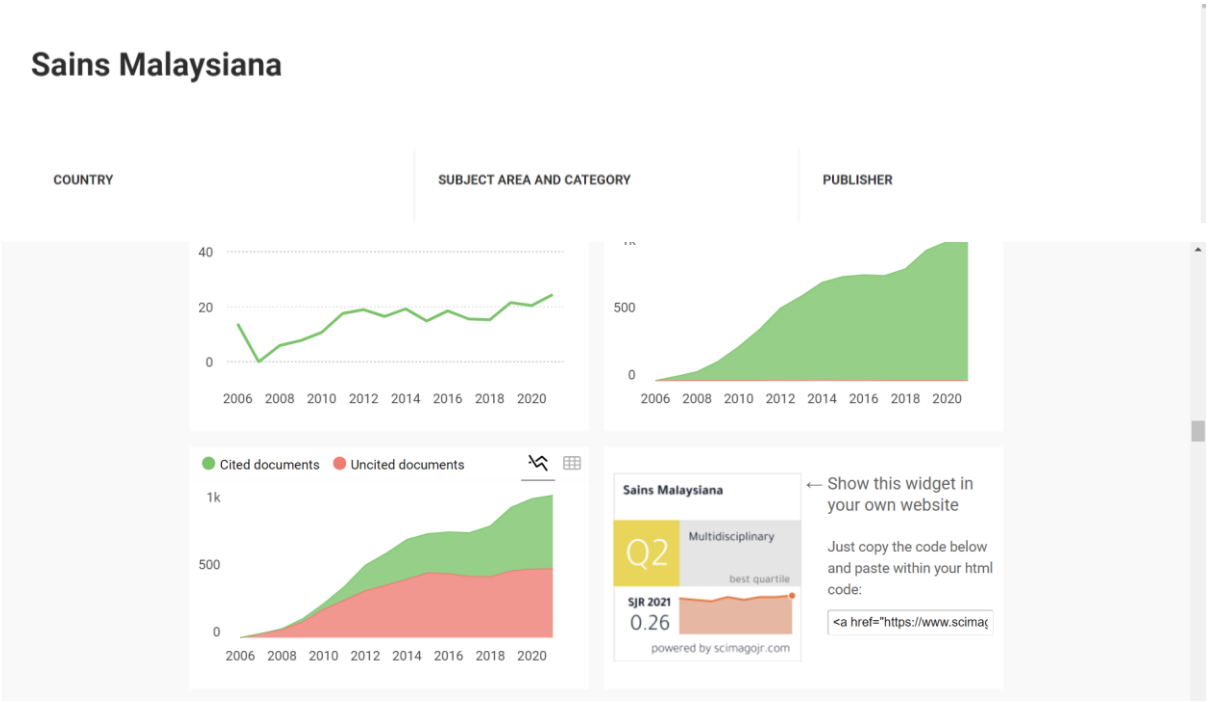

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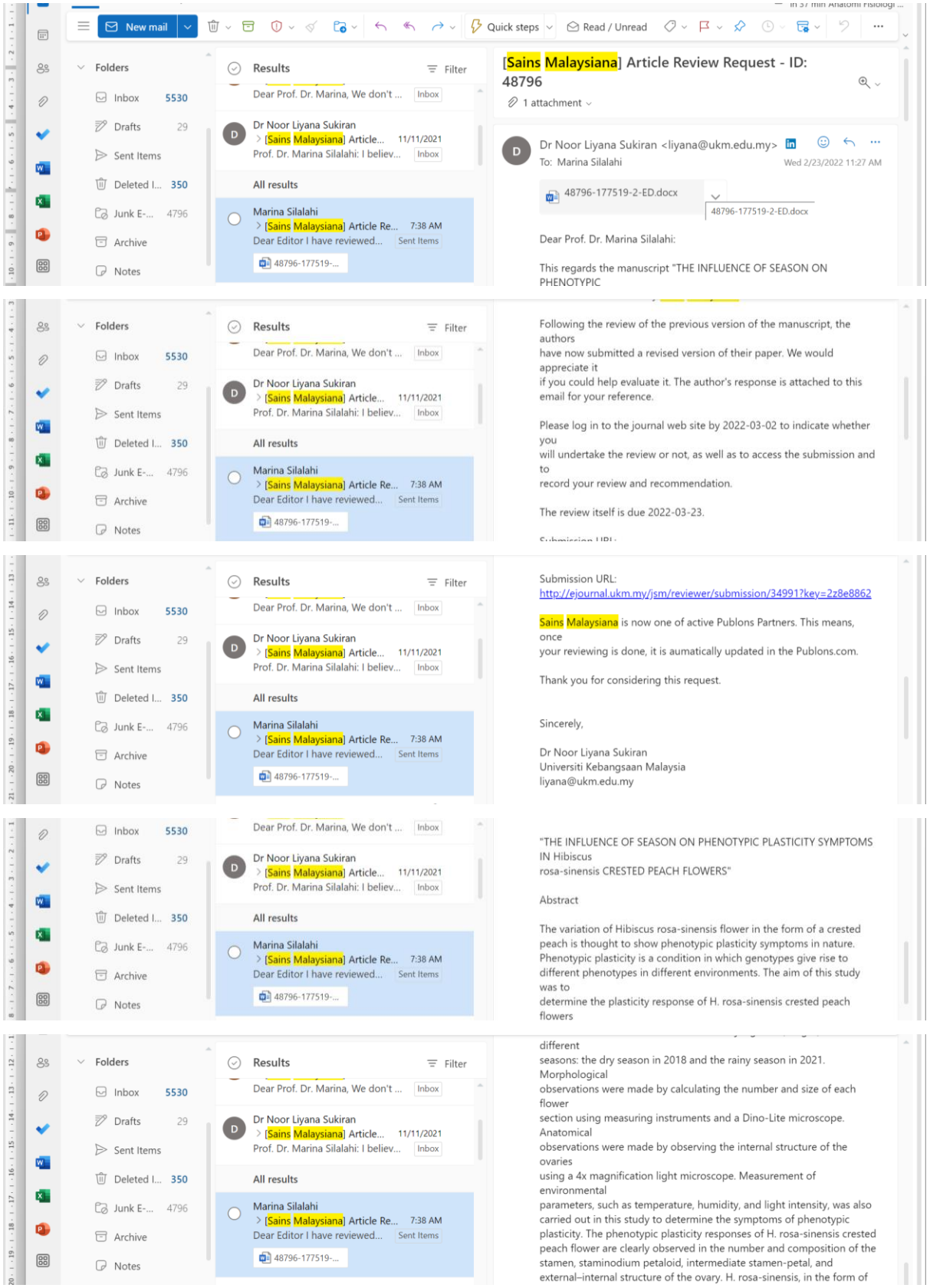
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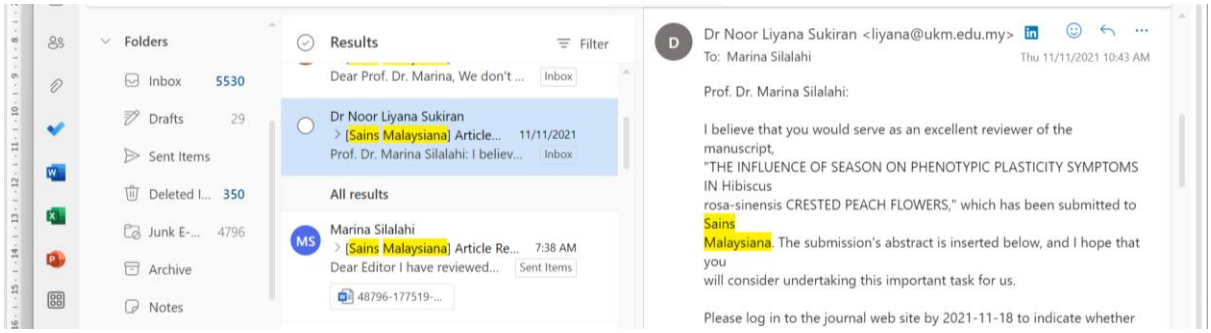
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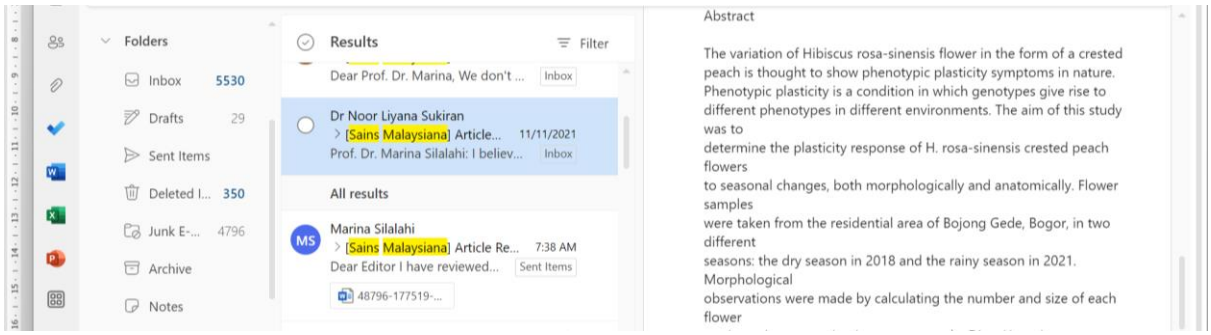
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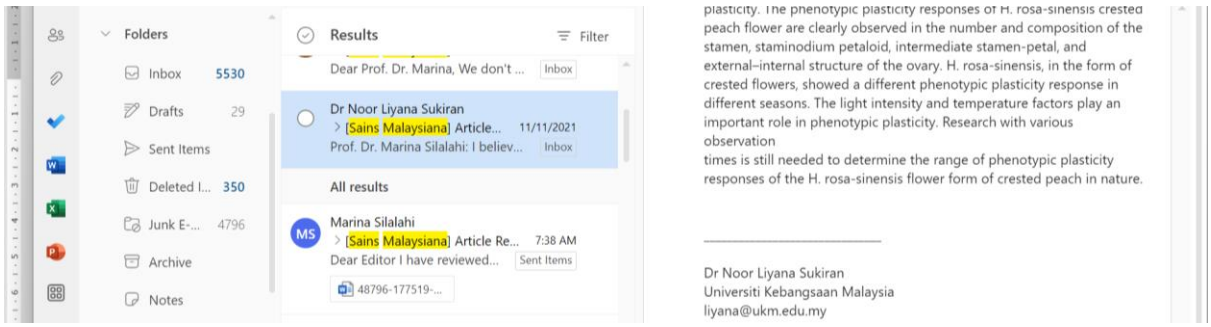
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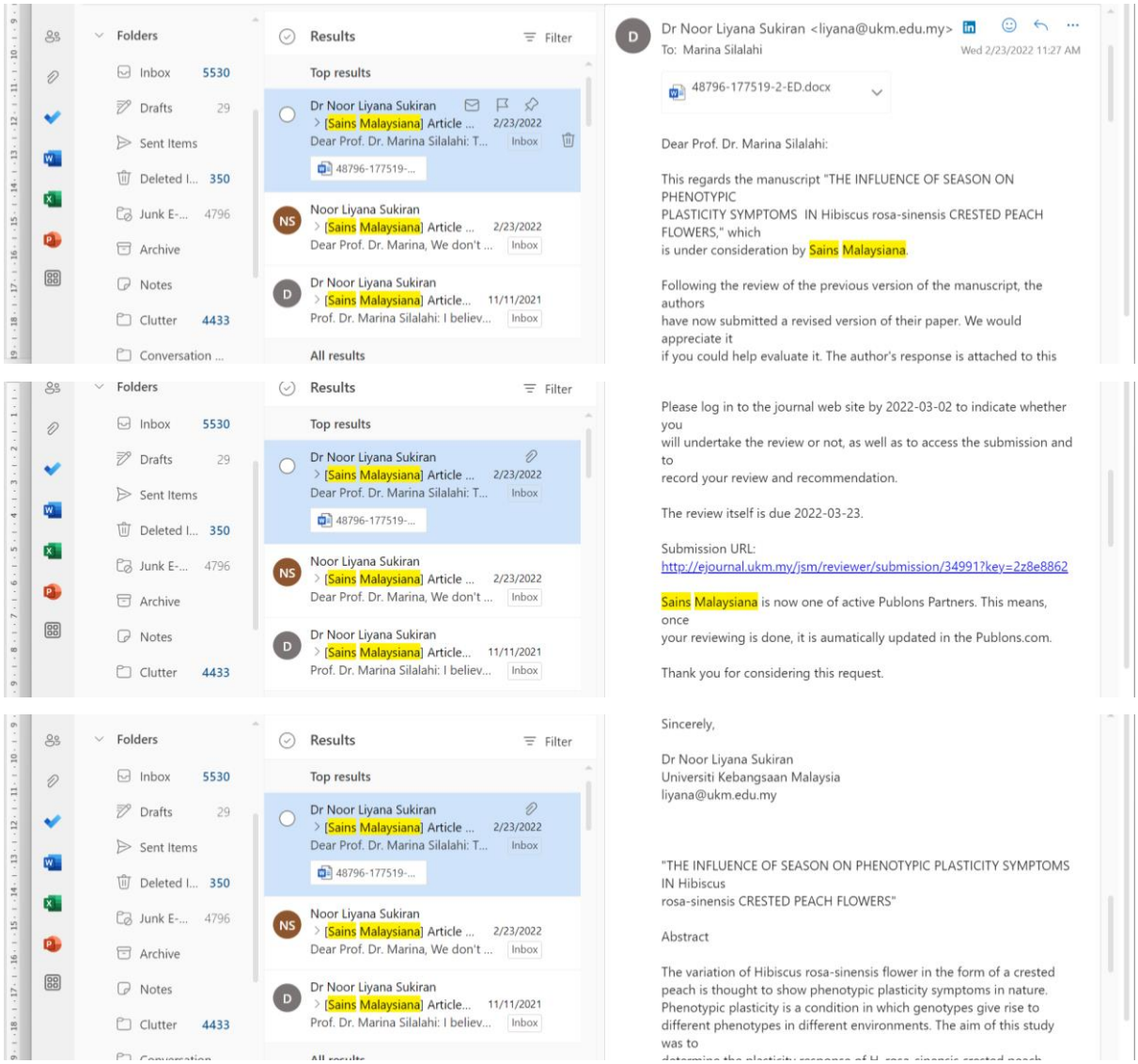
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The Influence of Season on Phenotypic Plasticity Symptoms in *Hibiscus rosa-sinensis* Crested Peach Flowers

(Pengaruh Musim terhadap Gejala Keplastikan Fenotip pada Bunga *Crest* Pic *Hibiscus rosa-sinensis*)

SAIFUDIN & ANDI SALAMAH*

Cellular and Molecular Mechanisms in Biological System (CEMBIOS) Research Group, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok 16404, West Java, Indonesia

Received: 15 June 2021/Accepted: 14 April 2022

ABSTRACT

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Keywords: Crested; flower; *Hibiscus rosa-sinensis*; phenotypic; plasticity

ABSTRAK

Variasi bunga *Hibiscus rosa-sinensis* berbentuk *crest* pic dianggap menunjukkan gejala keplastikan fenotip secara semula jadi. Keplastikan fenotip ialah keadaan apabila genotip menimbulkan fenotip yang berbeza dalam persekitaran yang berbeza. Matlamat kajian ini adalah untuk menentukan tindak balas keplastikan bunga *crest* pic *H. rosa-sinensis* terhadap perubahan musim secara morfologi dan anatomi. Sampel bunga diambil daripada kawasan perumahan Bojong Gede, Bogor, dalam dua musim berbeza: musim kering pada tahun 2018 dan musim hujan pada tahun 2021. Pemerhatian morfologi dilakukan dengan mengira bilangan dan saiz setiap bahagian bunga menggunakan alat pengukur dan Mikroskop Dino-Lite. Pemerhatian anatomi dibuat dengan memerhati struktur dalaman ovari menggunakan mikroskop cahaya pembesaran 4×. Pengukuran parameter persekitaran, seperti suhu, kelembapan dan keamatan cahaya, juga dijalankan dalam kajian ini untuk menentukan gejala keplastikan fenotip. Tindak balas keplastikan fenotip bunga *crest* pic *H. rosa-sinensis* diperhatikan dengan jelas dalam bilangan dan komposisi stamen, staminodium petaloid, stamen-kelopak perantaraan dan struktur luar-dalaman ovari. *H. rosa-sinensis*, dalam bentuk *crest*, menunjukkan tindak balas keplastikan fenotip yang berbeza pada musim yang berbeza. Faktor keamatan cahaya dan suhu memainkan peranan penting dalam keplastikan fenotip. Penyelidikan dengan pelbagai masa pemerhatian masih diperlukan untuk menentukan julat tindak balas keplastikan fenotip bagi bentuk bunga *H. rosa-sinensis crest* pic dalam persekitaran semula jadi.

Kata kunci: Bunga; *Crest*; fenotip; *Hibiscus rosa-sinensis*; keplastikan

Introduction

The *H. rosa-sinensis* flowers mainly have two shapes that are single and double. There is also a crested, a transitional shape between single and double flowers (Beers & Howie 1990). The difference between the forms of *H. rosa-sinensis* flowers is the presence of the additional organ resembling a petal called staminodium petaloid and stamen-petal intermediate. Those two additional petals were not found in a single flower, while in double and crested flowers, the additional petals varied in size, number and composition (Saifudin & Salamah 2019). The form of the *Hibiscus* flower used in our research samples is crested. According to Salamah et al. (2017), crested peach flowers are categorized into double type I, with the number of staminodium petaloid ranging from 7 to 28 and intermediate stamen-petal totaling 1-21 (Figure 1).

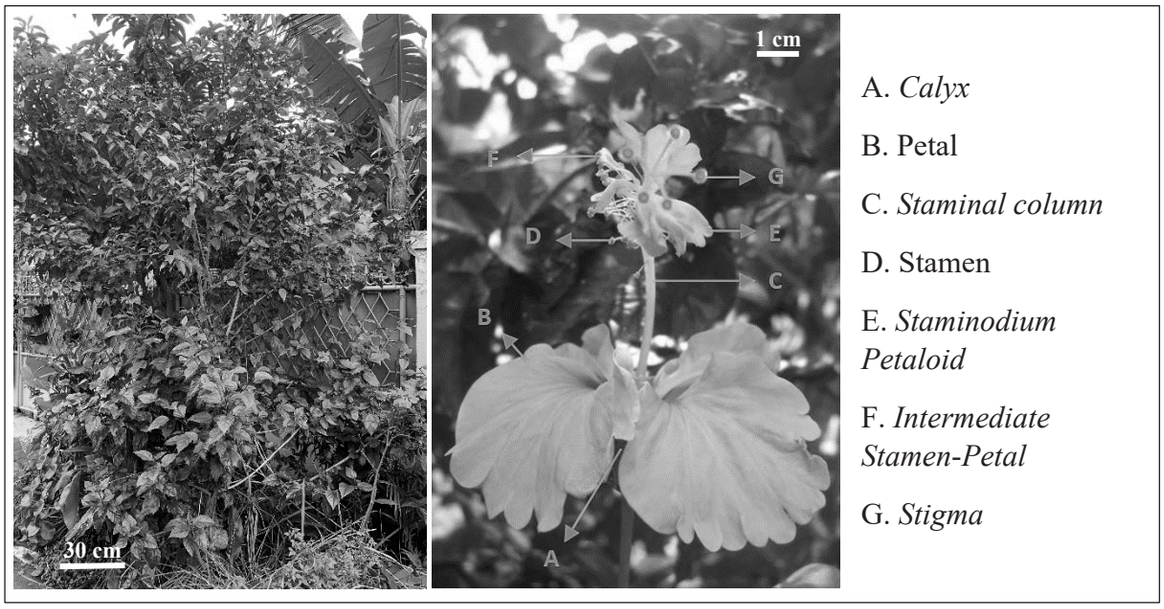

FIGURE 1. Crested peach form of *H. rosa-sinensis* flower

Hibiscus rosa-sinensis, a crested peach flower form, is a shrub 2-4 m in height (Figure 1). It has simple ovate-shaped leaves with a glossy dark green color. Leaf tips of *H. rosa-sinensis* are tapered (acuminatus) with sawed (serratus) or ringed (crenatus) leaf margins. The composition of the leaves of *H. rosa-sinensis* is alternate, where leaves are arranged alternately in a spiral pattern around the twigs. Each branch's node has only one leaf (Gilman 1999) with flowers in the leaf axils (flos lateralis or flos axillaris) (Tjitrosoepomo 2000).

Variations in the shape, color and size of *H. rosa-sinensis* flowers can occur in one individual plant. Individuals who usually produce crested peach flowers can produce single peach, single red and double peach flowers (Prihatiningsih 2011). Saifudin and Salamah (2019) have proven that the *H. rosa-sinensis* flower, in the form of crested peach, has morphological and anatomical variations, especially in the additional organs of flowers and ovaries. This variation is thought to be closely related to the phenomenon of phenotypic plasticity in nature, which is a form of plant adaptation to environmental changes (Gao et al. 2018). Phenotypic plasticity occurs when a genotype gives rise to different phenotypes in different environments (Turcotte & Levine 2016).

Research on phenotypic plasticity in plants is generally carried out on vegetative organs, mainly in leaves. Epidermal thickness, palisade, number of stomata and chlorophyll content were the parameters observed for plasticity responses (Gratani 2014). Research on phenotypic plasticity in flowers has been conducted by Arnold et al. (2019), proving that extreme temperature changes affect a plant's flowering time in the population. In addition, seasonal changes affect

the size and colour of flowers in *Moricandia arvensis,* which determines the number of pollinators (Gómez et al. 2020). Meanwhile, Sobral et al. (2021) proved that the presence of herbivores affects *Raphanus sativus* flower colour plasticity.

There are also studies on changes in flower structure in *Hibiscus rosa-sinensis* due to environmental factors (Rostina 2017; Saifudin 2019; Saifudin & Salamah 2021). However, these studies are generally viewed from the molecular aspect related to the symptoms of homeosis. Based on our previous study (Saifudin & Salamah 2019), homeosis is considered one of the symptoms of phenotypic plasticity in *Hibiscus rosa-sinensis* flowers. This study aimed to determine the plasticity response of *H. rosa-sinensis* crested peach flowers to environmental changes, both morphologically and anatomically. So far, no report has discussed this matter, so the implementation of this research is important. Understanding the symptoms of phenotypic plasticity in nature is essential to anticipate environmental changes, especially related to global warming. This research is also needed as a basis for further research, especially from the genetic and molecular aspects.

Materials and Methods

Study species, site, and period

Research on phenotypic plasticity in *Hibiscus rosa-sinensis* crested peach flowers was carried out in residential area of Bojong Gede, Bogor, Western Indonesia. The location determination was based on a field survey which showed that the phenomenon of phenotypic plasticity of *H. rosa-sinensis* in Bojong Gede tends to be high. Previous studies have shown a high variety of crested flowers both morphologically and anatomically (Saifudin & Salamah 2021, 2019).

The residential area of Bojong Gede, Bogor, is located at coordinates 06°28.072' S and 106°48.667' E with an altitude of 135 above mean sea level (AMSL). According to data from the Meteorological, Climatological, and Geophysical Agency (BMKG), the Bojong Gede area during September 2018 had low rainfall or was in the dry season, while in February 2021, it had high rainfall or was in the rainy season (Figure 2).

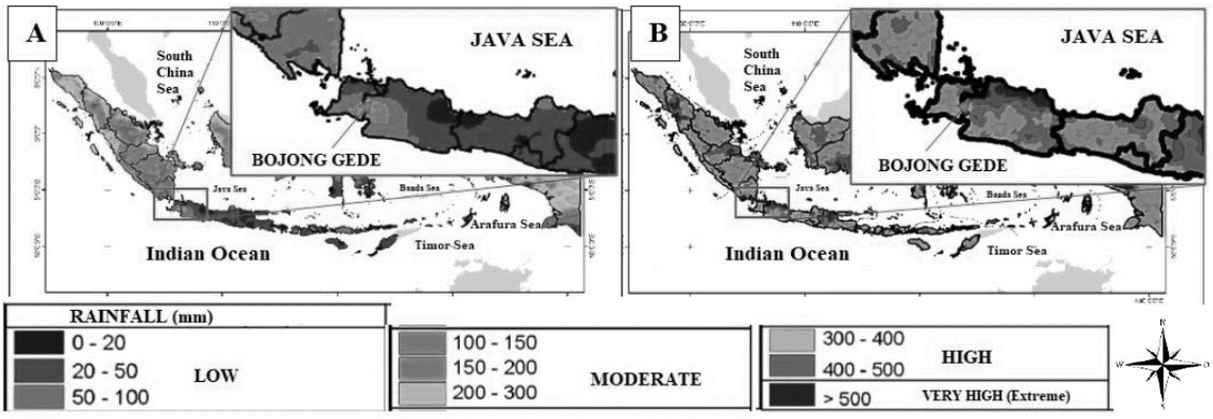

FIGURE 2. Rainfall in the Bojong Gede area in September 2018 (A) and February 2021 (B) (Source: BMKG, 2021)

A total of 200 samples of *H. rosa-sinensis* anthesis flower in the form of crested peach were taken from the residential area of Bojong Gede, Bogor, with different sampling times: September 2018 (100 flowers) and February 2021 (100 flowers). The flower sampling in 2018 represents phenotype plasticity data in the dry season period, while the flower sampling in 2021 represents phenotype plasticity data in the rainy season period.

The flower samples were taken every day from one plant during September 2018 and February 2021 until they reached the desired number. Generally, the number of flowers ranges from 1 to 28 anthesis flowers from different branches per day. Flowers with defects due to disease or insects were not used as samples to prevent data from being biased. Not all samples of flowers obtained have a representative appearance. There is a possibility of

human error that makes the sample damaged. Thus, only representative samples were used as 100 flower samples in each observation period in this study.

OBSERVATION AND DATA COLLECTION

Observation and data collection were carried out qualitatively and quantitatively. Qualitative data was collected by morphological and anatomical observation with the same sample sources. The qualitative data includes the process of flower development before anthesis (pre-anthesis), the shape of the flower during anthesis, the phenotype of additional organs of the flower, and the external–internal structures of the ovaries. Morphological observations of the shape of the flower, the additional organs and the external structure of the ovary were carried out by direct observation or by using a Dino-Lite microscope, while anatomical observations of the internal structure of the flower ovaries, both at the pre-anthesis and anthesis stages, were carried out using a 4× magnification light microscope.

Quantitative data was collected by counting the number of stamens and additional organs of flowers. The calculation of the number of epicalyx, calyx, corolla, and stigma was not carried out because, based on preliminary studies, there were no significant differences in each part of the flower between individual flowers. Quantitative data collection was also done by measuring the length of the petiole and staminal column. Measurement of the length of the staminal column is carried out by measuring the closest distance between the position of the petal growth and the first appearance of the stamen or additional organ.

Quantitative data collection was also carried out on environmental parameters, such as temperature, air humidity, and light intensity. Measurement of temperature and humidity was carried out using a Thermo hygrometer, while light intensity measurement was carried out using a 3 in 1 multipurpose soil meter. Measurements of environmental parameters were carried out three times a day: morning (09:00-10:00, Western Indonesia Time [WIB]), afternoon (12:00-13:00, WIB) and evening (15:00-16:00, WIB). Subsequent observation data is presented in the form of figures and tables.

DATA ANALYSIS

Qualitative data is presented in the form of pictures, while quantitative data is presented in tabular form. The data obtained was then tested by chi-square and analyzed descriptively. The chi-square test was carried out to compare the observed data for 2021 with the observations in 2018. Descriptive analysis of phenotypic plasticity symptoms begins by comparing the variation in crested flowers in the two observation periods to determine which flower organs have the most potential to show a plasticity response. The plasticity response can be determined based on changes in the shape (morphogenesis) of flower organs and the number and composition of each part of the flower. Based on the results of morphological and anatomical observations, as well as the measurement of environmental parameter data equipped with literature studies, a reaction norm curve can be designed to describe the level of phenotypic plasticity in crested flowers and the factors that influence it.

RESULTS AND DISCUSSION

ENVIRONMENTAL PARAMETERS

The results of measurements of environmental parameters at different periods, September 2018 and February 2021, are shown in Table 1. Chi-square tests on the parameters of temperature, air humidity and light intensity show a difference between the observed data for September 2018 and February 2021 (df = 2, $\alpha = 0{,}05$,

TABLE 1. Average values of environmental parameters at Bojong Gede Residence in 2018 and 2021

Observation time	Average values of environmental parameters								
	Temperature (℃)			Air humidity (%)			Light intensity (Lux)		
	Morning	Afternoon	Evening	Morning	Afternoon	Evening	Morning	Afternoon	Evening
2018	25.5	32.5	29.3	75	46	54	2532	3004	2098
2021	29.3	31.3	30.2	80.2	73.8	74.3	1858	1983	1285

X2 > 5,9915). Measurements of temperature, air humidity, and light intensity show that the Bojong Gede area in September 2018 has a warmer or drier climate than in February 2021. During the observation period in 2021, no significant changes in each environmental parameter at each observation time (morning–afternoon–evening) were observed compared with the observation period in 2018, which shows significant change. This condition is thought to make an important contribution to each symptom of the plasticity of the *H. rosa-sinensis* phenotype of crested peach flowers. Environmental factors affect the number of flowers formed on *H. rosa-sinensis*. It is known that in the 2018 observation period, the number of flowers produced ranged from 0-23 with an average appearance of 15 flowers per day, while in the 2021 observation period, it ranged from 0-28 with an average appearance of 17 flowers per day.

PHENOTYPIC PLASTICITY OF STAMEN

The average number of stamens formed in the single peach flower, which appears in the upper third of the staminal column is 38-54, while the number of stamens formed in the crested peach flower was 0-50. These observations indicated a reduction in the number of stamens in crested peach flowers compared with the number of stamens in single peach flowers. The reduced number of stamens in crested peach flowers is due to the transdifferentiation of the stamens into new, petal-like structures called intermediate stamen-petal (ISP) and staminodium petaloid (SP).

Transdifferentiation is the ability of an adult cell to transform directly into another functional adult cell (McManus et al. 1998). Transdifferentiation can occur without going through the cell division phase (Shoji et al. 1996) and without any change in the shape or size of the cells (Krishnamurthy et al. 2015). Transdifferentiation is part of the morphogenesis process in plants and can occur both *in vitro* and *in vivo* (Almeida et al. 2015). The transdifferentiation process that leads to morphogenesis is thought to make an important contribution to the phenotypic plasticity symptoms of the crested peach form of *H. rosa-sinensis* flowers in nature.

The formation of a petal-like sheet on the stamen, starting from the area around the anthers and then widening to follow the formation of sheets throughout the filament (Figure 3). The new structure resembles petals but still contains a part of the stamen, which is then known as the intermediate stamen-petal (ISP). The process of transdifferentiation of stamen to form sheets is thought not to stop with the formation of ISP only but continues to develop until no more stamen components are found. The new structure resembles a petal that no longer contains a stamen component, known as the staminodium petaloid (SP).

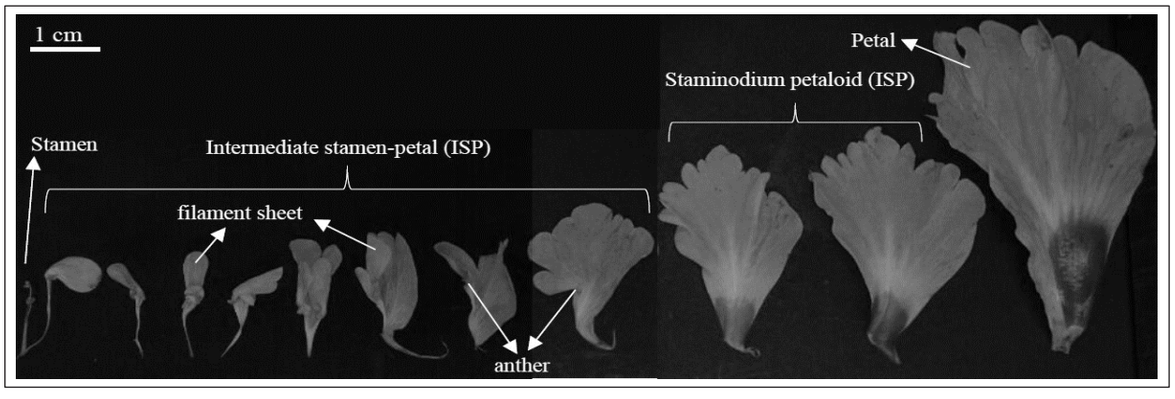

FIGURE 3. Stages of stamen morphogenesis suspected of forming ISP and SP

Based on the results of these morphological observations, it is suspected that the formation of ISP and SP is a manifestation of phenotypic plasticity symptoms in the stamen. The assumption that ISP and SP are new structures as a result of the stamen transdifferentiation process is strengthened by the composition of the number of stamens, ISP, and SP, which, when accumulated, have the same number range as the total number of stamens in single peach flowers (Table 2). Chi-square test on the number and composition of ISP, SP and stamen shows a difference between the observed data for September 2018 and February 2021 (df

= 2, α = 0.05, X2 > 5.9915). The phenotypic plasticity symptoms of the stamen forming ISP and SP in the two observation periods showed that the emergence of ISP in the observation period in 2021 is more prominent than in the observation period in 2018. This condition is inversely proportional to the range of SP that are more numerous in 2018 than in 2021 (Table 3).

TABLE 2. Data for the calculation of crested and single forms of flower perianths

		Single peach flowers								Crested peach flower							
Sample Number		15	16	19	33	50	51	52	65	9	24	39	40	66	67	68	70
Number of flower parts	SP	0	0	0	0	0	0	0	0	25	1	11	20	10	22	15	8
	ISP	0	0	0	0	0	0	0	0	5	4	24	9	24	6	17	24
	Stamen	45	43	38	54	46	43	44	40	0	50	6	6	13	7	22	8
Total		45	43	38	54	46	43	44	40	30	55	41	35	47	35	54	40

TABLE 3. Average number of ISP and SP in 2018 and 2021

Observation Period	Amount range			Average number		
	ISP	SP	Stamen	ISP	SP	Stamen
2018	1-19	6-33	0-28	8.5	23,8	4.65
2021	2-24	0-30	0-50	11,2	17,3	10,2

ISP: intermediate stamen-petal; SP: staminodium petaloid

Based on the entire observation samples, the accumulated number of ISP, SP, and stamen in crested peach flowers was in the range of 30-55. This is not much different from the range of the number of stamens in single peach flowers (38-54) observed in this study and is also almost similar to that observed by Salamah et al. (2017), that is 39-54. The similarity in the range numbers of stamens in single peach flower, as well as the total number of ISP, SP, and stamen in crested peach flower, could lead to the conclusion that both ISP and SP are derived from the stamen. This result supports Prihatiningsih's (2011) assumption, which states the ISP and SP are included as stamen modification because they appear to replace stamens in a single crested flower. However, regarding whether ISP and SP originate from stamen, molecular observations still have to be done.

The results of stamen number observation show that the range of stamens in 2021 is higher than in 2018. Therefore, it can be concluded that the stamen is more triggered to perform phenotypic plasticity into ISP or SP in the dry season, which is suggested that the dry season will more easily trigger plasticity symptoms in *H. rosa-sinensis* crested peach flower by forming more strand-like organs. According to Gritani (2014), climate change and other environmental pressures will trigger plants to show heterogeneity, and phenotypic plasticity is a plant's effort to overcome these environmental pressures. Baranov et al. (2019) added that the level of phenotype plasticity is closely related to climatic conditions, particularly changes in light intensity and temperature.

Environmental stress that triggers the phenotypic plasticity of crested flowers can be seen in temperature, air humidity and light intensity data (Table 1). It is known that in 2018 observations, there was a significant temperature change, especially from morning to noon. If in 2021, the observed temperature increase was only 2 ℃, then in 2018, the increase would reach 7 ℃. This condition also applies to the parameters of air humidity and light intensity. The humidity measurement results show that from morning to noon, there is a 6.4% decrease in 2021 and 29% in 2018. Meanwhile, from morning to noon, the light intensity measurement results show an increase of 125 Lux in 2021 and 472 Lux in 2018. Changes in temperature, humidity, and light intensity

from morning to afternoon are thought to be factors that influence the phenotypic plasticity process of crested peach flowers in nature.

Changes in temperature, humidity, and light intensity are thought to be closely related to the various physiological activities of plants that tend to be actively carried out in the morning before noon, such as photosynthesis, hormone biosynthesis and transportation of nutrients to every part of the plant body, including flowers. According to Trivellini et al. (2011), photosynthetic carbohydrates play an essential role in plant metabolism. Apart from being a source of energy, carbohydrates play a role in the osmoregulatory function and act as signal molecules involved in important processes of plant life, one of which is flower death.

PHENOTYPIC PLASTICITY OF OVARY

The symptom of phenotypic plasticity found in the outer structure of the ovary is the twisted outer wall of the ovary. The depth and direction of the twisting pattern are thought to influence the presence or absence of the changes in the internal structure of the ovaries. Although generally showing a twisted ovary pattern, an ovary with a non-twisting outward appearance, which resembles the morphology of a single-flowered ovary, is still found. In addition, there is also a symptom of phenotypic plasticity in the form of the character of the ovary wall forming a sheet (Figure 4).

Table 4 shows that the ovarian wall of flower samples observed in the period 2018 differs from 2021. This data is confirmed by the results of the chi-square test, which shows that the null hypothesis is rejected, which means there are differences in ovarian wall morphology between the observation periods of 2018 and 2021 (df = 2, $\alpha = 0.05$, $X2 > 5.9915$). Most of the ovaries from the period of 2018 showed a twisted pattern in the wall, while most ovaries of the 2021 samples showed a non-twisted wall pattern. Another striking difference is that the modification of the walls to form sheets was only found in the 2018 observation period, while the ovarian wall pattern without twisting was only found in the 2021 observation period.

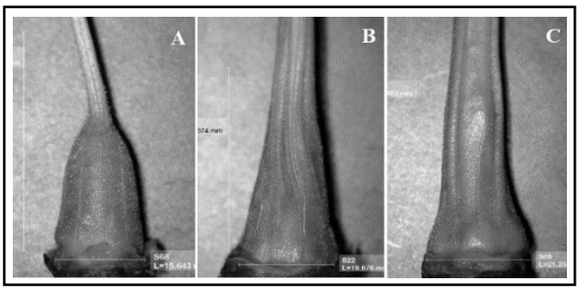

FIGURE 4. Ovarian wall morphological variations of crested peach flowers: without twisting (A), twisting (B), and forming sheet (C)

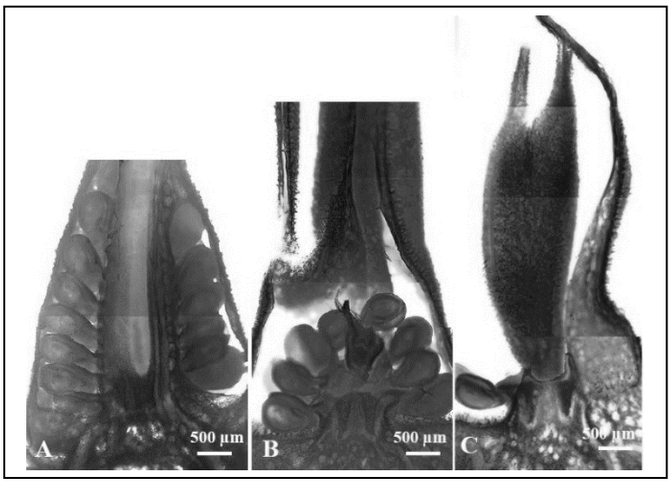

FIGURE 5. Three types of internal structure of the ovary of a crested flower: resembles a single flower (A), with changes or reduction (B), and form a new structure resembling pistil (C)

TABLE 4. Morphological data of the outer wall of the ovary of the crested peach flower

Observation period	Ovarian wall morphology		
	Not twisted	Twisted	Forming sheets
2018	0	87	13
2021	75	25	0

The twisting pattern and modification of the ovary wall to form a sheet are thought to be part of the phenotypic plasticity symptoms of the crested peach *H. rosa-sinensis* flower. Based on anatomical observations of flower development, it is known that the twisting pattern in the ovary wall has occurred since the bud, to be precise, in the third stage of pre-anthesis (Saifudin & Salamah 2019). This finding shows that the response of plants to environmental changes that lead to phenotypic plasticity symptoms started since the beginning of the flower formation process.

Meanwhile, based on the results of anatomical observations done previously (Saifudin & Salamah 2019), it is known that the internal structure of the ovary of the crested peach flower can display three symptoms of phenotypic plasticity: 1) a character such as a single flower ovary whose internal structure does not change, 2) a character that indicates a change in the internal structure of the ovary, either in the form of an arrangement of ovules that appear random or is experiencing reduction, 3) a character indicating the formation of a new structure resembling a pistil (Figure 5). Most of the samples in the observation period in 2018 showed changes in the internal structures of the ovaries, while the samples in the observation period in 2021 mostly showed the structure of the ovaries that did not change (resembles single flower ovaries) (Table 5).

TABLE 5. Comparison of the number of samples from crested flower ovary anatomy observations

Observation period	The internal structure of the ovaries		
	does not change (resembles a single flower)	undergoes a change or reduction	forms a new structure resembling a pistil
2018	0	59	41
2021	75	25	0

Chi-square test on the internal structure of the ovaries showed a difference between the observed data for September 2018 and February 2021 ($df = 2$, $\alpha = 0.05$, $X2 > 5.9915$). In the form of an internal ovarian structure, the symptoms of phenotypic plasticity forming a new structure resembling a pistil found in 2018 were not found in 2021. Ovaries, whose internal structure resembles a single flower ovary, were not found in 2018 observations but became the dominant character found in 2021 observations.

The ovary, whose internal structure forms a new structure resembling the pistil, and the ovary, whose internal structure is reduced and undergoes changes, both have a twisting pattern that is not much different. Thus, it can be assumed that the depth of the twist pattern is not associated with the formation of new structures resembling the pistil. The formation of a new pistil-like structure is reinforced by the presence of an internal ovule-like structure. In one sample, the pistil-like structure can even give rise to another new structure that also resembles the pistil-form inside the pistil (Saifudin & Salamah 2019).

Saifudin and Salamah (2021) made morphological observations and categorized crested peach flowers into three main types based on the length of the staminal column. The first one is a single-like crested flower, which resembled single flowers and had a staminal column length of more than 4 cm. The second and third ones were double-like crested flowers (crested flowers that resemble double flowers, with staminal columns less than 1 cm) and crested intermediate-like flowers (crested flowers that have the appearance of single-like and double-like flowers that have staminal columns less than 1 cm and less than 4 cm).

When the results of morphological observations in 2018 and 2021 were compared, the frequency of appearance of single flowers in one individual *H. rosa-sinensis* crested form was higher than double flowers. Even double flowers were not found in the two observation periods of 2018 and 2021. Different conditions were observed in single flowers: in the 2018 period, only one single crested flower occurred, while in 2021, there were 15. The single flowers tend to be found more frequently in the rainy season (2021), and this reinforces the assumption of a range of phenotypic plasticity levels in crested flowers.

Symptoms of phenotypic plasticity in the ovaries of crested peach blossoms can be observed in the external and internal structures of the ovaries. The outer structure of the ovary showed a twisting pattern of the outer wall, while the internal ovary showed a change in the number of ovules or in the formation of a new structure that resembles a pistil. Most samples in the observation period in 2018 showed a change in the internal structure of the ovaries, while the samples in the observation period in 2021 mostly showed the structure of the ovaries that did not change (resembling single-flowered ovaries). Based on these conditions, in general, it can be concluded that the phenotypic plasticity that encourages the formation of new structures in crested peach blossoms is triggered, especially in the dry season, where the light intensity tends to be high, which increases the temperature and decreases the humidity around the plant. The drastic change in the three environmental parameters is thought to be an important factor in the appearance of phenotypic plasticity symptoms. According to Callaway et al. (2003), in addition to competition factors and the presence of herbivores, variations in the abiotic environment that triggers plant stress are one of the factors that increase the plasticity expression of plant phenotypes.

The observation results on 41 ovaries samples from the 2018 period showed the formation of a new structure resembling a pistil, indicating as if there had been a phenomenon of a new flower formation in the flower, and so on, which showed the pistil flower phenomenon that leads to the growth of multiple flowers. Therefore, it can be assumed that the peak symptom of phenotypic plasticity in *H. rosa-sinensis* crested peach flower, which is depressed by environmental pressure, will cause double flowers. This assumption is further strengthened by the results of morphological observations in the dry season or the observation period in 2018, where the size and number of each part of the crested peach flower are more similar to the double flower of *H. rosa-sinensis*.

NORM OF REACTION

So far, studies related to flower variation in *H. rosa-sinensis* species are still limited to its external appearance. Beers and Howie (1990), for example, categorized *H. rosa-sinensis* flowers into two main forms, namely, single and double forms, and one transitional form in the form of crested flowers. Through their study, Salamah et al. (2018) further developed this categorization and divided the form of flowers into four types: Single, double type I, double type II and double type III. The crested flower itself is included in the double type I category.

Based on the results of this study, it is assumed that the range of symptoms of the phenotypic plasticity of the crested peach form of *H. rosa-sinensis* is between single and double flowers. The wetter (rainy season) an environment is, the more the character of the crested flower is thought to be like a single flower, while the drier (summer) an environment is, the more the character it has is thought to be like double flowers. Therefore, the crested flower is evidence of the plasticity process of gene expression of a hybrid towards the phenotype of its parent, both single and double parents, which are the natural formation of *Hibiscus* flowers in nature. Environmental factors are the main factor determining the plasticity of the gene expression so that if a norm of reaction curve is made, it will look like the curve in Figure 6.

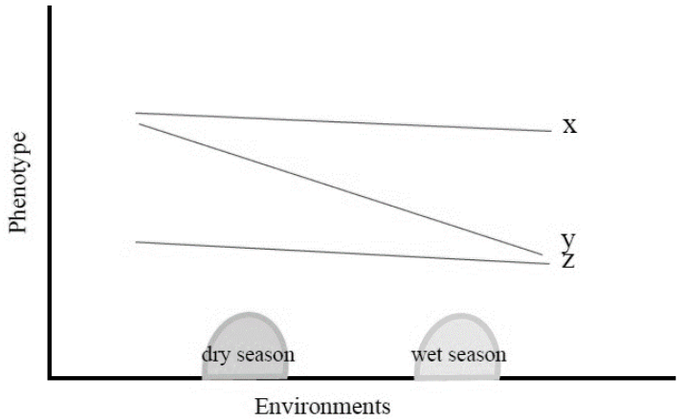

FIGURE 6. Illustration of norm reaction of double-flower (x), crested peach–flower (y), and single-flower (z) genotypes

Based on this curve, the norm of reaction lines in the genotype of single and double flowers appears a little non-horizontal and parallel (line x, z), which means that although environmental factors influence the plasticity of the phenotype, the gene factor determines the phenotypic character more. Meanwhile, the norm of the reaction line on the crested flower genotype is not horizontal at all and will intersect with the norm of the reaction lines of double and single flowers (line y), which indicates that environmental factors have more influence on genotype to bring up the phenotypic characters of crested flowers. Under certain conditions, environmental factors can even trigger the formation of a crested flower phenotype that resembles single and double flowers. The double flower is a wild type of *H. rosa-sinensis* in nature, so it is possible that under extreme environmental conditions (observation of the dry season in 2018), the phenotypic character of the crested flower is more likely to bring out the double flower character.

When referring to environmental factors, single, double, and crested flowers can co-exist. That is, genetic factors remain an important factor in determining the character of crested forms in nature. If we look at the fact that the twisting pattern has been formed since the early stages of preanthesis, then the 'cell fate' in forming the new structure has been specified since the beginning of flower development. According to Schiefelbein (1994), the determination of cell fate will trigger cell differentiation towards morphogenesis through genetic control that is influenced by the environment.

In this study, the crested flower showed no plasticity in forming a double flower as a whole. Regarding this finding, several assumptions were made. First, Indonesia's climate is still below the maximum temperature tolerance limit for *H. rosa-sinensis* crested peach flower to survive without having to make maximum plasticity into a new form. Additionally, crested flowers need a short time to flower (ephemeral flower) so that the plasticity process of crested flowers forming a double flower as a whole becomes difficult. In other words, it takes longer with more extreme temperatures and light intensities to change the shape of a crested flower into a double flower.

According to Trivellini et al. (2011), *H. rosa-sinensis* has a bloom time of 12 hours to die. The short flowering time is thought to be the reason the process of transdifferentiation and morphogenesis of the stamen into a petal-like structure is imperfect so that only intermediate structures appear in nature, in this case, the ISP and SP. If various external and internal factors make it possible for the crested form of *H. rosa-sinensis* to plasticize, the morphogenesis process sequence should occur from stamen to ISP, then ISP to SP, and then SP to a petal structure, thus forming double flowers. In other words, crested flowers are authentic evidence of phenotypic plasticity events that are still or have been taking place in nature. In contrast, single flowers are the only variety in *H. rosa-sinensis* where most of the individual flowers are unable to complete the plasticity journey.

The composition of the additional organs in the double-like type, where the number of SP is far greater than the number of SPI and stamen, also supports the 'journey' of single peach flowers to double flowers as a form of phenotypic plasticity. Even in some samples, the stamen was no longer found. In addition, in double-like flowers, morphologically, a staminal column is found, which gets shorter in size and begins to flatten into sheets, as if to form a double flower-like appearance. If the flowering time is extended, the SP size may continue to widen until it looks more like a petal and adds to the appearance of a double flower.

It is suspected that there are still many symptoms of phenotypic plasticity in the crested form of *H. rosa-sinensis,* which are influenced by various factors other than abiotic environmental factors, particularly those related to the competition or presence of neighboring plants, as well as the presence of herbivores. These factors are connected to affect the level of expression of plant genes towards their plasticity character.

Conclusions

Hibiscus rosa-sinensis in the form of crested flowers showed a different phenotypic plasticity response in different seasons. The light intensity and temperature factors play an important role in the phenotypic plasticity norm reaction. Light intensity and high temperature in summer will direct the phenotypic plasticity of the crested flower to the double flower shape. It is suspected that this is related to the dominance of double parent genes, which are more resistant to summer than single flower parents. The imperfection of the crested flower to form a single flower is thought to be due to the Indonesian climate, which is still below the tolerance limit for crested flower life and the short inflorescence time.

Acknowledgements

This work is supported by Hibah PUTI 2020 and funded by DRPM Universitas Indonesia with contract number NKB-4557/UN2.RST/HKP.05.00/2020 for Andi Salamah.

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