The Application of a New Drying Method on the Quality of Voucher Specimen

Mohd Amril Ramzi Bin Mohd Rahimi[1,*] Furzani Pa'ee[1]

[1]*Department of Technology and Natural Resource, Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (UTHM), 86400 Pagoh,84000 Muar, Johor Darul Takzim, Malaysia.*
[*]*Corresponding author. Email: amrilrahimi@gmail.com*

ABSTRACT
Drying the specimens is one of the crucial steps in plant preservation and the process of making Herbarium voucher specimens. This research was conducted to investigate the effect of the drying method on specimens in the laboratory using the standard method (oven) and drying in the field using a novel method. There are 3 samples used for each drying method and 2 species were chosen which are *Canthium horridum* and *Leptaspis sp.* The novel method is made from recycle items and designed based on Forced-air space heaters. The optimum temperature that is suitable for drying the specimens is also determined. After the drying process is carried out, the herbarium voucher specimens are evaluated for durability from bacteria or fungus growth. Drying the specimen involves removing the moisture or the water in the specimen. Specimens collected are randomly picked at Taman Negara Johor Gunung Ledang (TNJGL). The weight of the specimens is recorded before drying. After drying is completed, the specimens are again weighed to get the final weight. The moisture loss was calculated. The results show that drying in the laboratory using standard method is better. This is because the specimen is well-dried, no fungal growth present, and low fragility. Thus, drying in the laboratory using standard method (oven) is preferred. Some improvements can be made for drying in the field using novel method. In conclusion, it is possible to use the drying method in the field to ensure that the quality of plant specimens is at its best after collection. But some improvements are needed such as in the aspect of uniform drying. This novel method is designed to dry specimen using air and instead of only using heat. In addition, some parts of the specimen are not well-dried using this novel method. Thus, drying in the laboratory using standard method (oven) is recommended.

Keywords*: Moisture loss, Non-Woody plants, Woody plants.*

1. INTRODUCTION

Herbarium voucher specimen is a collection of preserved plants specimens which then used for research and future references. A voucher also defined as demonstrative sample of an expertly identified organism that is deposited and stored at a facility which researchers can retrieve for further examination and research. Herbarium collections have long been used for the research of plant taxonomy, geographic distributions, and stabilizing nomenclature. The specimen could include all available aboveground portions of a representative specimen of a particular plant. It is vital that distinguishing parts of the plant be a part of the herbarium voucher specimen [1]. To provide a source of data regarding changes in plant flowering periods, the specimens collected over many years might be merged with a single baseline season of field observations. To produce high quality of herbarium voucher specimen, the specimen should be well dried. This can avoid the formation of fungus or bacteria growth, which ensure the specimen at the best condition. Herbarium vouchers are crucial as they provide reliability and are the only concrete and verifiable evidence about the taxon which is the focus of the published research. Vouchers specimens are critical for authenticating the identity of a taxonomy. In some situations, the herbarium specimen is the only specimen taken, and it serves as a voucher for ethnobotanical data recorded in field notes; in others, the herbarium

specimen also serves as a voucher for additional specimens such as timber, DNA, or artefacts. The herbarium specimen is the specimen that allows for reliable identification in both circumstances. In the first instance, herbarium vouchers allow following researchers to double-check identifications and revise work in light of new taxonomic notions. [2]. Drying is one of the most important elements in preparing herbarium voucher specimen. There is lack of research done on drying methods of herbarium voucher specimen. Researchers had focused more on how to prepare herbarium voucher specimen generally. Many ways have been used to dry specimen and this drying method is changed and improved from time to time. The drying method may have a negative effect on taxonomic characters such as color if not controlled and monitored. Drying after pressing the specimen is to ensure that all the moisture is removed totally from the plant. Moisture left can lead specimen to rot and would be useless for herbarium and cannot be used for scientific purposes. In this research, the specimens will be dried at laboratory and field to observe the best drying method to dry the specimens. Based on the drying method used, the quality of the herbarium voucher specimen was evaluated.

2. MATERIALS AND METHODS

2.1 Novel Method

Novel method refers to new method which are made during the research and designed. The purpose to build this novel method is to provide drier for plants specimen after pressing field. The plant specimen is at best when dry as soon as possible after collection. This is also to overcome the problems existed today where the plant specimen is dried after researchers return from the field. Some of the fieldwork might take a week and some up to a month. This causes the specimen starts to rot and lost its morphological characteristics.

The concept for the novel method is to provide a sustainable method that dry specimen using air movement and the principle of this novel method is to extract all the moisture from pressed specimens. This method should be able to dry at least three stacks of specimen included the corrugated paper and the flimsies. It is also should be able to dry most all the plant types, which it needs to have suitable temperature to dry specimen. Some of the disadvantages of drying in the field is that a stove and drier must be carried or built. Thus, this method also was designed to be.

2.2 Plant Collection

The plant specimens are collected from TNJERGL (2°20'31.3" N 102°37'04.4E). The data on the plant specimen has been recorded in a field notebook and the plant specimen are numbered. Data recorded includes local name, common name, scientific name, collectors name, date, location (geographical coordinate) and ecological functions [1]. Specimens were typical and healthy, with at least some fully expanded leaves.

The plants were taken from their typical habitat. The random pick was used to select the plants species. Fleshy-look plants are considered as non-woody plants. For plant parts, a voucher of herbarium specimens was consisting of above-ground structures (leaves, stems, flowers and/or fruits) and below ground structures. Collections of vascular plants were put immediately into a field press because this produces the best-looking specimens. The identifying number is given to each plant specimen. For multiple sheets are pressed as vouchers of that collection, all receive the same number [1]. One of the methods that can be used to collect plant specimen is by using collecting scroll. Field press is rather bulky to carry around and may prove impractical in some cases. Another option available is by using plastic bags. Small plants can be placed singly or two or three together if necessary. Specimen will be taken carefully as risk to damage the specimen is high. The bag will be blown up to add a small amount of moisture and help to cushion the contents [3]. In this research, two types of plants were collected based on physical appearances judging by their moisture content. Duplicates of three were collected for each plant species from TNJERGL. The amount of collection was enough for two sets of herbarium voucher specimen for each species. After the collection, the plants specimen was labeled with one or two letters and numbers.

2.3 Plant Press

In achieving the first objective in this research the specimens were dried using different methods accordingly. A novel drying method was used for the herbarium voucher specimens on field and a standardized drying method in the laboratory (oven). Specimen was pressed immediately after collection. Pressing helps to dry the specimen by removing the moisture as soon as possible the plant is collected. This also helps to flatten the specimen so that later in drying all the surface is dried uniformly. A plant press and straps were required, as well as a small stack of corrugated cardboard sheets cut to the same size as the press and sheets of newspaper.. The plant specimens were cleaned from soil and foreign object. Each specimen was pressed inside an individual folded sheet of newspaper with marked collection label to avoid confusion of specimens. Fragile specimens need to be permanently arranged at first pressing. Some of the big leaves are turned over to fit the press. Excess parts are trimmed off as needed [1]. After pressing plant specimens were done, they were left dried using the methods mentioned in this research which are novel method and standard method (oven).

2.4 Drying Method

In this research, the pressed specimens were dried at field using novel method as soon as specimens are collected and labeled. Before drying the specimen were weighed. The specimens were later checked regularly to avoid over-drying. The preparation area for drying is done in the library at TNJERGL Some of the specimens labeled were pressed after collection and dried at the laboratory using oven. The novel method was created to fulfill these research objectives. After the initial weight of the specimens is obtained, the pressed specimen is dried using this method. During the drying process, the tool was placed close to the plant press. The plant pressed was left dried and the time taken was recorded. After a few minutes, the conditions of the specimens were checked, and the final weight was recorded. Few drying sessions are done before the average moisture content is recorded. The standard method refers to the oven in laboratory which is used to dry the specimen. After the plant specimens are pressed, the plant specimens are dried using the oven. The temperature was fixed at 36℃ and the specimens were left dried for hours. After few drying sessions, the final weight of the specimen was recorded, and the average moisture content is evaluated.

2.5 Quality Assessment

Data gathering and analysis are used in quality assessment to indicate the degree of adherence to established standards and criteria. If the quality is deemed to be unacceptable as a result of this process, efforts are made to determine why. Remedial activities are taken as a result, and the quality is re-evaluated after a sufficient time period. [4]. In this research, there were two things observed. Firstly, the moisture content of the specimen and secondly the fungal growth. The quality assessment was done a week after all specimens were dried at field and laboratory, respectively. The specimens are removed from the plant press carefully so that the specimens are not damaged. This quality assessment needed to be done to know the condition of the specimens after undergo drying process from both methods which are using novel method at field and using standard method (oven) at laboratory.

2.5.1 Moisture Content Percentage

The weight of a sample was measured and recorded. The sample was then placed in an oven to dry, and the dry weight was calculated. The amount of water is calculated by subtracting the dry weight from the initial weight, dividing by the initial weight, and multiplying by 100 to get the percentage. The Equation (1) [1] is used, where *w1* is the initial weight of specimen (g), and *w2* is the final weight of specimen (g).

$$\frac{w1-w2}{w1} x100 \quad (1)$$

The moisture content percentage of the specimen should be less than 40%. Dry has no real scientific meaning and has never been well defined. The term dry is often defined as the point at which weight loss ends. The moisture content percentage should be less than 40% so that the moisture was totally extracted. It was avoided to dry less than 20% as this will cause over-drying. This assessment was also repeated for the specimens dried at field using novel method

2.5.2 Observation of Fungal Growth

The specimen was observed for its durability. This has been done by observation of fungal growth on the specimens. There was no fungal growth such as mold and mildew on the specimen f the specimen is well dried. The specimen was placed under sufficient light and was observed thoroughly. The plant part that needed more attention is leaves. The leaves need to be checked upside down carefully. Plant specimen fragility was also observed. If the specimen is breaking down after a small pressure it shows that the plant is over-dried.

2.6 Data Analysis

Solid For Data Analysis, the data was obtained from the results based on the drying methods used. Both methods, which are drying at field using novel method and drying at laboratory by using standard method were compared. The temperature used was observed to know the suitability for drying specimens. Next, the time taken for the tools to dry the specimens were recorded to determine methods that can provide efficient time to dry specimen. The shorter the time taken to dry the specimen, the more efficient the drying method. This is applicable if the specimens are well-dried. From the weight, the moisture content can be recorded. Thus, the effect of the drying method on the herbarium voucher specimen can be determined

3. RESULTS AND DISCUSSION

3.1 Plant specimens collected

Plants from the Rubiaceae and Poaceae families were collected. Leaves facing one other with stipules or in whorls, unbroken leaf edges, and leaflike appendages at the base of the leafstalks characterise members of the family. In tropical species, the leaves are normally big and evergreen, deciduous in temperate species, and needle-like or scalelike in desert species. The plants may produce a single blossom or a cluster of tiny flowers. Berries, drupes, capsules, and schizocarps are examples of fruits (dry fruits that split into segments of a single seed). The scientific name of this species is *Canthium horridum.*

Secondly, the plant specimen collected is from family *Poaceae*. *Poaceae,* formerly called *Gramineae*, grass family of monocotyledonous flowering plants, a division of the order *Poales*. The *Poaceae* are the world's single most important source of food. Leaves 5-20 x 2-5 cm, ovate-elliptic, elliptic or elliptic-lanceolate, acuminate, narrowed at base, pseudo-petiolate, surfaces with projections of false reticulate veins. Sheaths compressed, keeled, bases brownish to black. Ligules obscure. Panicles very lax, 5-20 cm long, 10-30 cm wide, loosely speculate: peduncles hairy. Utricles pyriform or obovate, 4-8 mm long, pores open at the top enclosing the ovary. Palea linear slightly exserted. Lodicules truncate. The scientific name of this species is *Leptaspis urceolata.*

3.2 Effect of drying method to the specimen and optimum temperature.

For drying specimens at field using novel method, the result can be seen in Table 3.1. The average moisture content for *Canthium sp* is 23.64 % while for Non-Woody Plant average moisture content for the *Leptaspis sp.* is 23.78%.

Table 3.1. The moisture content of drying specimen using novel method.

Type of plants	*Canthium* sp.	*Leptaspis* sp.
Time (hrs)	1 hr 30 mins	
Temperature(°C)	45	45
Average Moisture Content (%)	23.64	23.78

For drying specimens at laboratory using standard method oven, the result can be seen in Table 3.2. The average moisture content for *Canthium sp* is 23.43 % while for Non-Woody Plant average moisture content for the *Leptaspis sp.* is 14.70 %.

Based on the results, using standard method (oven) provides good drying. Although the novel method took less time but both specimens are uniformly heated and dried when using the oven method. The specimen decreases in size as it dries, and it will shrivel unless the press is regularly tightened during drying (Plain J, 1968). Most of the plant specimen parts have the same color. But using novel method, both plant specimen is not uniformly heated and dry. The outermost part of the specimens is drier than the middle part. This shows that standard method can provide a better drying session as it can provide uniform heating. A specimen will become brittle and discoloured if exposed to a high temperature for an extended period of time. If the drying duration is too short or the temperature is too low, the specimens will remain damp to the touch and mildew may develop.

Table 3.2. The moisture content of drying specimen using novel method (oven)

Type of plants	*Canthium* sp.	*Leptaspis* sp.
Time (hrs)	21 hrs 37 mins	
Temperature(°C)	36	36
Average Moisture Content (%)	23.43	14.70

The time taken for drying using standard method is longer because this method involves only heat. While the novel method, it involves heat and air movement. Thus, the water or moisture in the specimen can dry faster. The temperature used in drying specimen at field using novel method is 45℃ while the temperature of used in drying specimen at laboratory using standard method is 36℃. Using 45℃ can provide shorter time to dry specimen. but the specimen might damage and become brittle. Thus, temperature at 36℃ can be used to dry the specimen with less supervision.

Table 3.3. The fungal growth and brittleness test for specimen dried at field using standard method (oven)

Type of plants		*Canthium* sp.			*Leptaspis* sp.		
Specimen number		W1	W2	W3	NW1	NW2	NW3
Fungal Growth	Mold	x	x	x	x	x	x
	Mildew	x	x	x	x	x	x
Brittleness		Medium	Medium	Medium	Low	Low	Low

3.3 Quality of Voucher Specimen

For quality assessment Observation is done thoroughly through the specimen which includes the leaf skeleton parts and the back part of the leaf to identify any mold or mildew growth. Pressure is applied

to the specimen to test the brittleness. There was no fungal growth on both types of specimen, *Canthium sp.* and *Leptaspis sp.* after a week from the drying session using novel method and standard method (oven). This is because the plant specimens were dried after the collection. But two weeks after the drying session, there were some growths spotted. On the leaves of *Canthium sp.* there were some molds grew. This cause the plant specimen to be damaged and changed the morphological characteristics of this species. Mold growth and mycotoxin production are influenced by environmental factors.

Water activity (aw), temperature, pH, and the environment can all have a significant impact on growth and mycotoxin production patterns. [5]. In this case, the plant specimen was damaged because of incomplete drying which cause present of water activity and influenced the mold growth. The possible common molds are *Acremonium* and *Fusarium. Acremonium* is a very polyphyletic fungus genus that includes fungi that are distantly related. [6]. Mold grows on wet surfaces and becomes powdery. It comes in a variety of colours, including white, pink, orange, and grey.

The imperfect fungal genus *Fusarium,* with perfect states *in Calonerctria Gibberella*, *Microneciriella*, and *Nectria***,** is one of the plant pathogenic groups of major importance for the plant breeder. *Fusarium* looks like a colony with texture ranging from flat to wooly or cottony. The color of the colonies can be white, tan, salmon, cinnamon, yellow, red-violet, pink, or purple.

The brittleness of the specimen using each method shows a difference. Specimen dried using novel method has higher brittleness compared to specimen dried using standard method. The temperature of the novel method during the drying session causes some parts of the specimen over dry and increase its brittleness.

Table 3.4. The fungal growth and brittleness test for specimen dried at field using novel method

Type of plants		*Canthium* sp.			*Leptaspis* sp.		
Specimen number		W1	W2	W3	NW1	NW2	NW3
Fungal Growth	Mold	x	x	x	x	x	x
	Mildew	x	x	x	x	x	x
Brittleness		High	High	High	Medium	Medium	Medium

Table 3.5. Comparison on both drying methods in the research

	Standard Method (Oven)	Novel Method
Temperature	36	45
Time Taken	Longer	Shorter
Uniform heating	Yes	No
Fungal Growth after two weeks	No	Yes
Brittleness	Specimen less brittle	Specimen more brittle
Suitability for plants specimen	Suitable for both plant specimens	Suitable for both plant specimens

3.4 Quality of Voucher Specimen

The standard method provides suitable temperature for drying specimen, which is 36°C as 45°C can cause over-drying if not monitored. There are differences between drying specimen at field using novel method and drying specimen at laboratory using standard method (oven). Novel method can be used to dry specimen in short time with sufficient monitor. Next, the standard method provides uniform heating compared to novel method and this is preferable to ensure specimen quality. The standard method shows no fungal growth after two weeks and provides specimen that has low brittleness. Last but not least, both methods can be used to dry specimen.

In conclusion, both woody and non-woody plants are suitable for both methods which are drying using drying at field using novel method and standard method (oven). But to dry specimens in fields novel method is preferred and needs to be monitored regularly to avoid over-drying. The herbarium voucher specimen dried from standard method was well-dried and has good quality in terms of fragility and fungal growth. Thus, the first objective of this research is achieved. This research also shows that the suitable temperature for drying specimen is 36°C and with this the second objective of this research is achieved.

This research shows the durability of the specimen from the effect of both drying methods. The woody plants specimen dried from both drying methods had a low fragility in quality and no fungal growth after preservation. The non-woody plants specimen also has low fragility and no fungal growth after preservation. With these, the last objective for this research is achieved. This also indicates that both types of plant specimen can be dried using both methods.

ACKNOWLEDGMENT

First and foremost, praises to Almighty Allah for His showers of blessings, this research is completed and giving me strength and ability to understand, learn and complete this report.

I acknowledged Universiti Tun Hussein Onn Malaysia for providing necessary funding, facility, and assistance. Special thanks to Taman Negara Johor Endau Rompin Gunung Ledang (TNJERGL) for giving me permission and assistance to finish the research.

My deepest and sincere gratitude to my supervisor, Dr Furzani Binti Pa'ee for continuous support and valuable advises for my research, for her patience, motivation, and immense knowledge. With all the advises, guidance and suggestions all the time helped me in research and this thesis writing.

Finally, I am enormously grateful to my family for their acceptance and patience during this research is carried out. I would also like to give my gratitude to my friends for their support, sense of humor, sacrifices and tolerant to make sure this thesis is completed.

REFERENCES

[1] J. Hildreth, E. Hrabeta-Robinson, W. Applequist, J. Betz, & J. Miller, Standard operating procedure for the collection and preparation of voucher plant specimens for use in the nutraceutical industry, Analytical and Bioanalytical Chemistry, vol. 389(1), 2007, pp. 13–17. DOI: 10.1007/s00216-007- 1405-x

[2] M. Nesbitt, Use of herbarium specimens in ethnobotany. In: Curating Biocultural Collections, eds J. Salick, K. Konchar, and M. Nesbitt, Royal Botanic Gardens, Kew: Kew Publishing., 2014, pp. 313– 328.

[3] J.M. Bowles, Plant Identification for the Ministry of Natural Resources in 1982, Guide to plant collection and identification, 2004.

[4] J. Mainz, E.M. Hammershøy, A.M. Worning, S. Juul, Kvalitetsvurdering, kvalitetssikring og kvalitetsforbedring i sundhedsvaesenet. Begreber og terminology, Quality assessment, quality assurance and quality improvement in health care, Concepts and terminology, Ugeskr Laeger, vol. 154(17), 1992, pp. 1151-6. Danish.

[5] M.D. Northolt, L.B. Bullerman, Prevention of Mold Growth and Toxin Production through Control of Environmental Conditions, J Food Prot., vol. 45 (6), 182, pp. 519–526. DOI: https://doi.org/10.4315/0362-028X- 45.6.519

[6] A.E. Glenn, C.W. Bacon, R. Price, & R.T. Hanlin, Molecular phylogeny of Acremonium and its taxonomic implications, Mycologia, vol. 88 (3), 1996, pp. 369- 383. DOI: 10.1080/00275514.1996.12026664