STABILITY OF ANDROGRAPHOLIDE IN ANDROGRAPHIS PANICULATA UNDER SELECTED STORAGE CONDITIONS

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ABSTRACT

Fresh Andrographis paniculata was dried by a thin layer drying technique using hot air at a temperature of 55℃ and velocity of 1.0 m/s. The dried sample was then ground and kept in air-tight glass bottles at three selected storage conditions (5±2℃; 25±2℃ with 60% ± 5% RH; 30±2℃ with (60% ± 5%RH) for three months period. The contents of the active compound andrographolide present in the dried Andrographis paniculata at the beginning and at the end of each month during the storage period were determined by using the HPLC. It was found that there was no significant reduction of the active compound andrographolide for all of the selected storage conditions. Storage at ambient conditions (30 ±2℃, 60 ± 5% RH) was also able to maintain andrographolide content in Andrographis paniculata during the three months period. This would enhance the production and supply of Andrographis paniculata as raw material with ease, without requiring the use of cooling equipment for storage purpose.

Keywords: *andrographis paniculata* storage, *andrographolide* content, dried herbs, *andrographolide* stability, herb storage, storage conditions, storage stability.

INTRODUCTION

Andrographis paniculata, a herbal plant belonging to the Acanthaceae family, known as hempedu bumi in Malaysia, can be found in China, India, Indonesia and Sri Lanka, growing normally in the coastal and plain areas. The therapeutic benefit of this herb has been attributed to *andrographolide* and its related diterpenoid compound, i.e. *deoxandrographolide* and *neonandrographolide* (1,2). *Andrographis paniculata* was found to inhibit human breast, liver and prostate cancer cells (3,4), to combat against human immunodeficiency virus (HIV) (5, 6), to overcome liver disorders and acute hepatitis (7, 4) and to combat the common cold and respiratory inflammations (8). In traditional medicine, *Andrographis paniculata* had been used to relief cobra bite (9, 10), to treat diabetes (9, 11,12), dysentery (4), diarrhea (13), dyspepsia (4) and high blood pressure (12). Significant reduction in blood glucose level was observed when hyoglycaemic rats were treated with extract of *Andrographis paniculata* compared to other herbs (14). *Andrographis paniculata* also demonstrated higher anti-malarial effect compared to other plant species studied (15).

Since health benefits of Andrographolide paniculata as mentioned above are many, it would be justifiable to increase its production/supply for utilization in local herbal industry. Knowledge of methods and conditions of storage are very important in order to maintain the quality of a given product after harvest. Temperature and relative humidity are the main parameters affecting quality attributes of fresh agricultural produce during storage (16). Storage of fresh herb at 10^{o}C with packaging was effective compared to ambient conditions (17, 18) studied on the effect of heating, UV irradiation and storage on stability of the anthocyanin-polyphenol co-pigment complex. Generally, most local herbal preparations available in the market are in dry form, either powder or flakes. Thus to ensure constant supply of good quality dried Andrographis paniculata leaves as an ingredient to meet the demand of the herbal products processing industry, appropriate storage conditions for the dried leaves are necessary. The most probable storage conditions that would meet quality preservation of most chemical and bio-based products in dry form for common applications could be considered as follows (19):

i. 5 ± 2°C
ii. 25 ±2°C, 60 ± 5% RH
iii. 30 ±2°C, 60 ± 5% RH

The objective of this study was then to investigate on the appropriate storage conditions for preserving the quality of dried Andrographis paniculata leaves by determining the storage stability of bioactive ingredient *andrographolide* in dried samples of *Andrographis paniculata* under selected conditions for three months storage period. The outcome of the study would then present a valid basis for the provision of appropriate storage conditions to support the effort to mass produce *Andrographis paniculata* in dried form locally as one of the basic raw ingredients/materials to enhance the Malaysian expanding herbal industry.

MATERIALS AND METHODS

Experimental design and statistical analysis

The *Andrographis paniculata* used in this study was obtained fresh from Institute of Bioscience, UPM. The initial moisture content of fresh *Andrographis paniculata* was first determined, after which the *A.paniculata* was dried at 55°C air temperature and 1.0m/s air velocity until its moisture content was less than 7.0% (w.b.). The dried sample was ground and was used to determine the *andrographolide* content. Dried samples were kept and stored in air-tight glass bottles set at selected storage conditions as shown in Table 1. Storage at each condition was replicated three times. The *andrographolide* contents were determined at the start of storage period and consecutively after 1, 2 and 3 months of storage**.** These data on *andrographolide* contents were presented as the mean ± standard deviation**.** The analysis of variance using standard Duncan's test was applied on the results obtained**.** The 5% significant level was used to assess the significant differences among the means of the experimental results

Table 1: Selected storage conditions

Condition	Temperature	Relative Humidity
1	30°C ± 2°C	60% ± 5%
2	25°C ± 2°C	60% ± 5%
3	5°C ± 2°C	-

Andrographolide extraction and determination

Andrographolide was extracted according to the method available in literature (20). The extraction of a*ndrographolide* was done on fresh and dried *Andrographis paniculata* with three replicated samples. The dried sample was ground for determining the *andrographolide* content. The ground *Andrographis paniculata* was percolated with methanol (50ml) and filtered after overnight. The remaining marcs were extracted again twice (over night) in order to completely extract the bioactive compound. The 90% solvent was removed using a rotary evaporator followed by an oven to completely dry the sample.

A high performance liquid chromatography (HP 1100) was used to determine the amount of a*ndrographolide* in *Andrographis paniculata*. The solvent was filtered using a Millipore system and

the *andrographolide* content determination was performed using a Jones Genesis C18 column. A constant flow rate of 0.4 ml/min was used during the content analysis. The composition of the mobile phase was 70% acetonitrile and 30% water. The UV detector wavelength was 230nm and the analysis was run for 15 min.

Freshly prepared samples of standard a*ndrographolide* with four concentrations were injected for the preparation of calibration curve. The test samples were prepared in 1.0 mg/ml for analysis purposes and each test sample was injected three times.

RESULTS AND DISCUSSION

Figure 1, 2 and 3 show *andrographolide* contents of dried samples initially and after keeping for one, two and three months at selected storage conditions, respectively. The analysis of variance with Duncan's test shows that there was no significant difference (α= 0.05) in residual contents of *andrographolide* after three months of storage. Thus the results indicated that there was no significant difference in the quality of dried *Andrographis paniculata* during three months storage, regardless of the storage conditions. As such, the ambient condition (30 ±2 °C, 60±5% RH) is preferable as the appropriate storage condition for dried *Andrographis paniculata* because the a*ndrographolide* is also stable at ambient conditions. Thus, from the economics point of view, no expenses for any special equipment are required to maintain the quality of dried *Andrographis paniculata.*

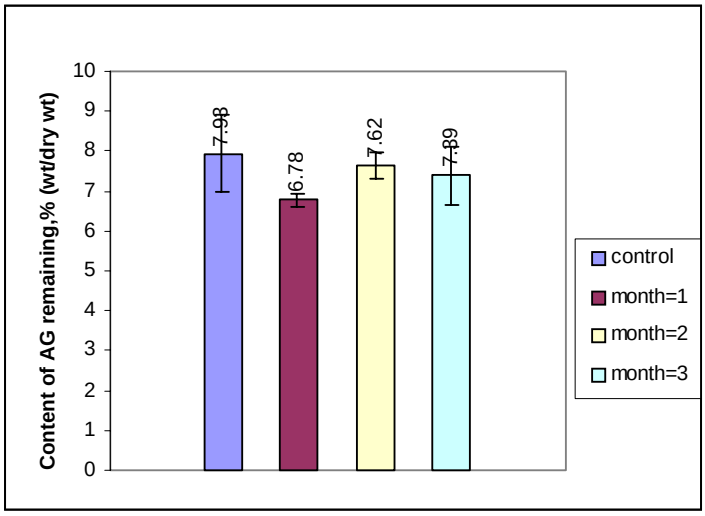

Figure 1: Content of andrographolide for storage condition 5 ±2 ℃

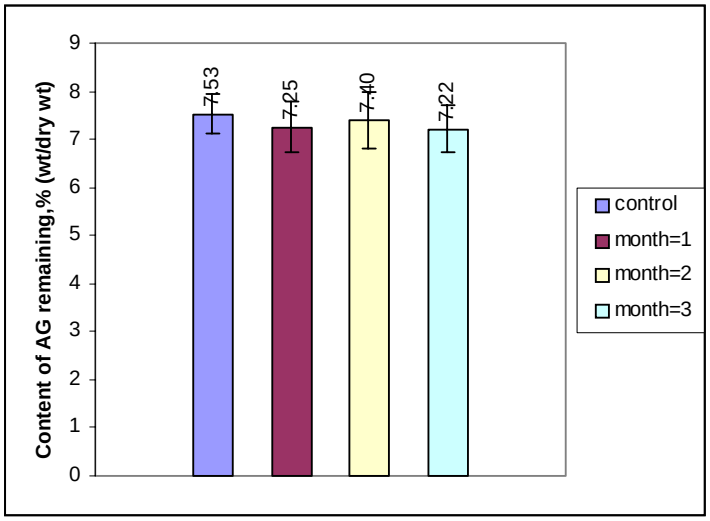

Figure 2: Content of andrographolide for storage condition 25 ±2℃, 60 ±5% RH

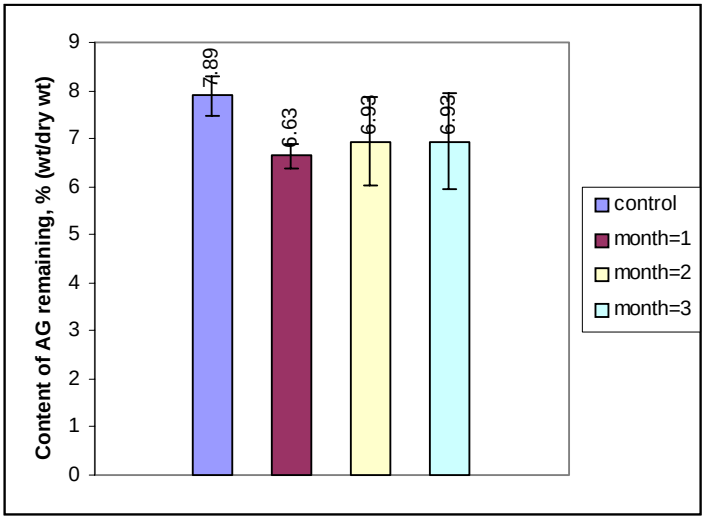

Figure 3: Content of andrographolide for storage condition 30 ±2°C, 60 ±5% RH

CONCLUSIONS

Since the result of this study indicated that the local ambient condition did not cause significant reduction of *andrographolide* content of dried *Andrographis paniculata* for the storage duration of three months, the preferable storage condition would then be the ambient condition (30±2°C, 60±5%RH). There is no necessity for provision of cool environment; thus no expenditure for any special equipment is required for preserving the quality of dried *Andrographis paniculata.* This would enhance its production as raw material supply for the local herbal industry as well as for export purposes.

REFERENCES

[1] Choudhury, B.R., Haque S.J. and Poddar, M.K. "*In vivo* and *in vitro* effects of kalmegh (*Andrographis paniculata*) extract and andrographolide on hepatic microsomal drug metabolising enzymes", *Planta Med.* **53**: 135–140.

[2] Rajani, M., Shrivastava N.and Ravishankara, M.N. "A rapid method for isolation of andrographolide from *Andrographis paniculata* Nees (Kalmegh)", *Pharmaceut. Biol.* **38**: 204–209

[3] Anon. (2008) *Andrographis* In-depth Review. http://www.altcancer.com/andcan.htm. AlphaOmegaLab.com, Guayaquil. Equador. (accessed: 5 November 2008).

[4] Basak, A., Cooper, S., Roberge, A.G., Banik, U.K., Chretien, M., & Seidah, N.G. (1999). Inhibition of proprotein convertases-1,-7 and furin by diterpines of Andrographis paniculata and their succinoyl esters. Biochemical Society **338**: 107-113.

[5] Calabrese, C., Berman, S.H. and Babish, J.G. "A phase I trial of andrographolide in HIV positive patients and normal volunteers", *Phytother. Res.* **14**: 333-338.

[6] Chang, R.S., Lu, D., Chen, G.Q., Pan, Q.C., Zhao, Z.L., and Smith, K.M. (1991). Dehydroandrographolide succinic acid monoester as an inhibitor against the human immunodeficiency virus. Society for Experimental Biology and Medicine **197**: 59-66.

[7] Puri, A., Saxena, R., Saxena, R.P., and Saxena, K.C. (1993) Immunostimulant agents from *Andrographis paniculata*. Natural Products **56**(7): 995-999

[8] Cheung, H.Y., Cheng, C.S., and Kong, C.K. (2001) Determination of bioactive diterpenoids from *Andrographis paniculata* by micellar electrokinetic chromatography. Chromatography **930**: 171-176.

[9] Md Salleh, S. (1991) The use of *Andrographis paniculata* extract for treatment of diabetes mellitus (Kegunaan ekstrak hempedu bumi (*Andrographis paniculata*) untuk rawatan diabetes mellitus) Bachelor of Science Project Report, Department of Biology, UPM, Malaysia

[10] Selvanayagam, Z.E., Gnavavendhan, S.G., Chandrasekkharan, P., Balakrishna, K., Rao, B.R. (1994) A review on pharmacological and clinical studies. Fitoterapia **65**(2): 99-111.

[11] Norhaida, A.H. (1994) The effect of different fractions of *Andrographis paniculata* extract on blood glucose level in diabetic rats (Kesan berbagai pecahan ekstrak *Andrographis paniculata* ke atas paras glukosa darah tikus diabetic) Bachelor of Science Project Report, Department of Biology, UPM, Malaysia Malaysia.

[12] Zaridah, M.Z., Idid, S.Z., Wan Omar, A., and Khozirah, S., (2001) In vitro antifilarial effects of three plant species against adult worms of subperiodic Brugia malayi. Ethno-Pharmacology **78**: 79-84.

[13] Huang, K.C. (1993) *The pharmacology of Chinese herbs.* Boca Raton. CRC Press.

[14] Rafidah, H., Azimahtol, H.L.P. and Nallapan, M (2004) Screening for antihyperglycaemic activity in several local herbs of Malaysia. Ethnopharmacology **95**(2-3): 205-208.

[15] Nik Najib, N.A.R., Furuta, T., Kojima, S,. Takane, K. and Mustafa, A.M. (1999). Antimalarial activity of extracts of Malaysian medicinal plants. Ethnopharmacology **64**(3): 249-254

[16] Paull, R.E. (1999) Effect of temperature and humidity on fresh commodity quality. Postharvest Biology and Technology **15**(3): 263-277

[17] Sankat, C.K. and Maharaj,V (1996) Shelf life of green herb Eryngium foetidum L. stored under refrigerated condition. Postharvest Biology and Technology **7**(1-2): 109-118.

[18] Bakawska, A and Kuchaska, A.Z (2003) The effect of heating, UV irradiation and storage on stability of the anthocyanin-polyphenol copigment complex. Food Chemistry **18**(3): 349-355

[19] Anon (2003) Stability Testing of New Drug Substances and Products. ICH Harmonised Tripartite Guideline, ICH Expert Working Group. February.

[20] Jain, D.C., Gupta, M.M., Saxena, S., and Kumar, S. (2000) Lc analysis of hepatoptrotective diterpenoids from Andrographis paniculata. Pharmaceutical and Biomedical Analysis **22**: 705-709.