Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(6): 96-100
Received: 09-07-2020
Accepted: 06-10-2020

Smiley Gulati
Chemistry and Bioprospecting Division, Forest Research Institute, Indian Council of Forestry Research and Education, Dehradun, Uttarakhand, India

AK Pandey
Chemistry and Bioprospecting Division, Forest Research Institute, Indian Council of Forestry Research and Education, Dehradun, Uttarakhand, India

Amit Gupta
Chemistry and Bioprospecting Division, Forest Research Institute, Indian Council of Forestry Research and Education, Dehradun, Uttarakhand, India

Corresponding Author:
Smiley Gulati
Chemistry and Bioprospecting Division, Forest Research Institute, Indian Council of Forestry Research and Education, Dehradun, Uttarakhand, India

Impact of drying methods on the active phytochemical constituent of *Andrographis paniculata* (Kalmegh)

Smiley Gulati, AK Pandey and Amit Gupta

Abstract
Post-harvest techniques of medicinal plants have direct impact on their quality with respect to colour and their active ingredients. Among the various post-harvest methods, drying is the most common and important method to preserve the quality of herbs. *Andrographis paniculata* (Kalmegh) is one of the most important medicinal plants used in various Ayurvedic formulations for its broad medicinal uses. The therapeutic activity of the herb has been attributed to andrographolide, a bicyclic diterpenoid lactones present in the herb. There are a wide range of methods available for drying the herb but they also possess several disadvantages like economic feasibility, environmental dependency, lower energy efficiency and longer duration. Inadequate drying methods lead to the deterioration of quality rendering the product unsafe for consumption. Therefore, in the present study the impact of drying methods on the colour and andrographolide content of *A. paniculata* herb was analyzed. Kalmegh herb was subjected to different drying treatments viz., sun drying, shade drying, microwave assisted drying, oven drying and hot air drying. Kalmegh samples were analysed for their andrographolide content by HPTLC. The andrographolide content varied from 2.89% to 2.56% with different drying methods. The highest andrographolide content (2.89%) was found in shade dried samples. The results revealed that, the shade drying method is the most suitable method for preserving green colour and andrographolide content in *A. paniculata*.

Keywords: Andrographolide, kalmegh, HPTLC, post-harvest, drying

Introduction
Andrographis paniculata (Burm. f) Nees commonly known as Kalmegh, belongs to family Acanthaceae, is an important medicinal plant used in traditional medicine in China and South Asian countries. This is also called as "King of bitters". It is distributed southwards through Thailand and Peninsular Malaysia to Indonesia and in India it is found in the states of Madhya Pradesh, Chhattisgarh, Orissa, Jharkhand, Maharashtra, Assam, Bihar, West Bengal, Uttar Pradesh, Uttarakhand, Andhra Pradesh, Tamil Nadu, Karnataka and Kerala. *A. paniculata* is an annual, branched, erect, and herbaceous plant which grows throughout the plane lands, hill slopes, waste lands, farms, moist habitat and roadsides. Moist shady places, forests, and wastelands are suitable for its development [1, 2].

Kalmegh has an important place in Indian pharmacopoeia and is being prominently used in 26 ayurvedic formulations [3]. The leaves and aerial parts of the plant are used in Indian traditional medicine for the treatment of fever, malaria, diarrhea and sore throat. Hepatoprotective and antioxidant properties of *A. paniculata* were reported by Trivedi and Rawal [4]. It has immunosuppressive and alexipharine properties and is useful in wounds, ulcers, leprosy, sore throat, tonsillitis, osteodynea, menstrual and post partum haematometra, hypertension etc. [5]. In Thailand, the plant has been recommended for the use in primary health care in cases of sore throat and diarrhoea [6]. Anticancer and immunostimulatory activities are also reported in *A. paniculata*. Kalmegh is one of the ingredients in several polyherbal formulations used as hepatoprotectives in India [7].

The major bioactive constituents of Kalmegh are a group of diterpene lactones (andrographolide). Most of these diterpene lactones are present in the leaves and the stems contain negligible / trace levels. As a result of this, the herbal industry prefers kalmegh having higher content of leaves. The Pharmacopoeia specifies the bare minimum standard of acceptability. The herbal companies prefer to receive Kalmegh (aerial parts) having not less than 40% leaves such that the content of total andrographolide (when measured by HPLC as per USP method) is not less than 1.8% w/w [8]. For Kalmegh leaves (not less than 90% leaves) the industry expects not less than 2.8% w/w total andrographolide which would contain at least 2.5% w/w of pure andrographolide [9].

Kalmegh is one of the commonly traded medicinal plants of India and its demand is more than 1000 MT per annum [10]. The plant is mainly collected from wild sources. However, cultivation has also started in the plains of Uttar Pradesh, Bihar, Madhya Pradesh, Maharashtra, West Bengal, Assam, Orissa, Gujarat, Andhra Pradesh, Tamil Nadu, Kerala and Karnataka [11]. India exports Kalmegh in different farms like dried organic leaves, leaf powder, extract and andrographolide. The aerial part (upper part) of the plant is collected/harvested during October-November when it is in bloom and having maximum number of leaves [12]. The post-harvest (drying) of *A. paniculata* has great importance in the production chain, because of its direct influence on the quality and quantity of the active ingredients in the product [13]. Quality standards need to be followed strictly while exporting *A. paniculata* leaves to any country.

In India, post-harvest loss significantly exists, in rural areas where medicinal plants cultivation/collection takes place. Most of the harvested produce in the fresh form contain 60-90% moisture and therefore, liable to spoilage and deterioration both quantitatively and qualitatively due to development of moulds, contamination by fungus and bacteria, and infestation with a variety of pests. Therefore, proper post harvest management is very crucial stage to maintain the quality of produce. Drying is one of the most antique, fundamental and critical operations in the post-harvest processing of medicinal plants [14]. It is a primary processing technique that removes moisture from the product. The dried product, with reduced weight and volume, facilitates savings in the transportation cost and storage space. The removal of water from the plants hinders possible disintegration of phytochemicals and microbial contamination. In its physical essence, drying is a complex diffusion process, the speed of which is determined by the rate of moisture diffusion from the depth of the dried material into the environment [15]. Several studies have addressed the problems associated with convective drying. In particular, high temperatures can diminish flavour, colour and nutrients in the food. Lowering the process temperature has considerable potential for improving the quality of dried products [16]. Thus, the choice of drying method depends on various factors, such as the type of product, availability of particular dryers and the desired attributes of the desiccated product. The amount of capital costs, and the energy consumed during drying are also important considerations [17]. However, air temperatures between 100^0C and 65^0C appear to be feasible for drying of those medicinal plants that contain glycosides and mucilage, respectively [18]. The European pharmacopoeia recommends maximum final moisture content to be maintained between 8-12% for various medicinal plant species [19]. For instance, the quality of medical dried herbs is defined by the content of bioactive compounds [20]. Drying techniques have been developed that aim to improve quality as well as provide new possibilities to increase the efficiency of the drying process [21].

Generally, harvesters dry Kalmegh in open sun by spreading the herb on ground which often influences the colour and andrographolide content. Harvesters do not have knowledge and access about the proper harvesting techniques. A cost effective, simple and easy to use method is required so that poor collectors can effectively dry their produce without compromising the quality of produce. Under these circumstances, it is appropriate to have a proper drying method which contributes to the quality and sustainability of Kalmegh.

Material and Methods

Plant Material

Fresh aerial part of Kalmegh was collected from the nursery of Forest Research Institute, Dehradun in the month of November. The maximum temperature recorded during the experimental period was 29^0C and minimum of 12^0C, average relative humidity of 80%. Wind speed recorded was 4 km/hr.

Physico- chemical analysis

10gm of powdered herb was weighed and placed it in hot air oven. Temperature was adjusted to 100-105^0C till weight get constant and collected in desiccators and weighed. The loss of weight was regarded as a measure of moisture content and is expressed in percent. The adopted methodology was applied using three replicates for each sample to verify the reproducibility of the experiments.

Processing and drying of Kalmegh herb

Harvested Kalmegh fresh herb was washed thoroughly with running water to remove the dirt and soil. Then the material was subjected to five different drying methods *viz.*, Sun drying, Hot Air drying, Oven drying, Microwave assistant drying and Shade drying.

For sun drying, the fresh material was spread out above the agro shade net (perforated polythene sheet) and dried in open sun with regular upturning. During night hours, samples were wrapped in covers to prevent moisture reabsorption. During this period, the plant samples were weighed at regular intervals. After the attainment of constant weight, the process of sun drying was completed (Fig 1).

In Shade drying, the washed material was sun dried for about two hours to remove excess of water. Shade drying was carried out under ambient temperature. A known weight of fresh Kalmegh herb (10 kg) was used per replicate in each drying methods. The fresh Kalmegh herb was spread on clean agro shade net above 30 cm from the ground level and upturned about two to three times per day. The plant samples were weighed at regular intervals till the time of attainment of constant weight (Fig 2).

Hot Air drying was done in the trays wherein the trays were kept in front of the hot air blower enclosed within the box so that much loss of heat does not occur. Temperature recorded inside the Box was approximately 50^0C and a relative humidity of 78% (Fig 3).

In Oven drying, the plant material was spread uniformly in the hot air oven at temperature 45±2ºC. The samples were weighed at regular intervals and drying was terminated, when the moisture content of the samples attained 8 to 10 per cent (Fig 4).

Microwave drying is based on a unique volumetric heating mode facilitated by electromagnetic radiation at 915 or 2,450 MHz [22]. The sample was placed in microwave for drying in small quantities (Fig 5).

In all the methods of drying, the samples were dried till the moisture content was reduced to <10%. Observations were recorded on drying time and colour of the herb. Further Dried samples were taken for phytochemical analysis.

Extraction and sample preparation for HPTLC analysis

Powdered plant sample (aerial part) [2 gm] in 15ml methanol was sonicated on ultrasonic bath for 30 min, repeated for 4-5 times and filtered. Filtered extract was makeup up to 50 ml with methanol in volumetric flask. The extracted sample was ready for HPTLC analysis.

Equipments
A Camag (Switzerland) HPTLC system equipped with a sample Linomat V, Twin trough Glass Chamber (20 x 10) with SS lid, TLC Scanner III and Wincats an integrated Software 4.02(Switzerland), Sonicator.

Chemicals and Reagents
HPLC grade methanol, toluene, chloroform, acetone, vanillin, sulphuric acid and water were procured from Merck Life Science Private Limited (Mumbai, India). TLC Aluminium pre coated plate with Silica gel 60 F_{254} (20X20 cm^2) used were obtained from E. Merck Ltd. Andrographolide standard 99.8% was procured from Natural Remedies, Bangalore and was used as standard biomarker for determination of andrographolide.

Estimation of Andrographolide
For High-performance thin-layer chromatography (HPTLC) analysis conditions were as follows - application volume- 10 µL, as 5-10 mm bands; developing solvent system-chloroform, acetone and toluene (2:2:1) and spray reagent– a mixture containing 1% vanillin in alcohol and 10% sulphuric acid in alcohol (1:1) and wavelength 223nm [23].

Statistical analysis
The experiment was laid down in Completely Randomized Design with 3 replicates and analysis was done by one way ANOVA model.
$[Y_i = \mu_{i+} \alpha_i + \sum \varepsilon_{ij}]$ where Y_i is the ith observation, μ_i is the General mean effect, α_i is the treatment effect and $\sum_{ij}$ is the error component. The results were considered statistically significant at $P<0.05$ (level of significance = 0.05) When the effect was found to be significant, the post hoc test Tukey's HSD was used to find which group means differed significantly.

Result and Discussion
In the present investigation, the moisture content of fresh samples varied from 85% to 94% while average moisture content was 91.16%. After shade drying, the moisture content varied from 5% - 9% while average moisture content was 7%. Method of drying had a marked influence on colour and andrographolide content of the herb. The andrographolide content in (percent w/w on dry weight basis) in Kalmegh samples dried by different dying methods (Sun drying, Shade drying, Oven drying, Hot air drying and Microwave drying) are presented in Table 1. The andrographolide content varied from 2.56% to 2.89% with respect to different drying methods. Sun drying and oven drying ($45^0C \pm 1^0C$) took about 4 days and 2 days respectively. Whereas, microwave drying was the quickest method followed by hot air drying which took only 20 minutes and 12 hours respectively to complete drying process to reach the desired moisture content. The drying rate was found to be the lowest in shade drying (8 days). It could be reasoned that in shade drying, due to the less temperature in the month of November which was not enough to remove the moisture quickly. In addition, the open sun drying and microwave drying caused more severe damage on the morphological appearance (colour) of Kalmegh samples which resulted in the reduction of andrographolide content.
While drying the herb under shade took more time than other methods but retained the green colour of the herb which is also a criterion to ascertain the quality. Compared to sun drying, the retention of green colour of the herb, which is necessary for fetching premium prices in the market, was found to be high in the sample dried under ambient temperature (Shade drying). The poor colour of the sun dried samples was due to thermal degradation of chlorophyll pigment under high temperatures. Similar results were reported by Padmapriya and Rajamani (2016) [24]. Maximum andrographolide content was also found in the samples dried by shade drying method followed by hot air drying and lowest in microwave drying. The present findings are in accordance with the results obtained by Padampriya and Rajamani (2016) in *G. sylvestre* leaves with highest gymnemic acid content in the shade dried sample under ambient temperature [24]. As regards to stevioside, the major glycoside found in fresh leaves of stevia, all the drying methods resulted in substantial reduction except shade drying [25]. However, Ambrose and Naik (2013) reported that mechanical drying of senna leaves at 45 ^0C was superior compared to shade drying and showed higher sennoside content (2.69%), while shade dried sample contain a lower sennoside content of 2.2% [26]. The results revealed that, the shade drying method was found to be most suitable method for preserving the green colour and andrographolide content in *A. paniculata*. According to its p value it is considered statistically significant ($P<0.05$). Similar results were reported by Alara [27] in *Vernonia amygdalina* leaves and Lim and Murtijaya [28] in *Phyllanthus amarus*.

Table 1: Andrographolide content (w/w) on dry weight basis from *Andrographis paniculate*

	Sun Drying	Shade Drying	Oven Drying	Air Drying	Microwave Drying	F-Ratio	P-value	S/NS
Mean percent of andrographolide (W/W)	2.62^{bd}	2.89^{a}	2.64^{b}	2.76^{c}	2.55^{d}	64.98	<0.05	S
SEM	0.03	0.01	0.01	0.02	0.01			

* Means with same superscripts are not significantly different from each other

Fig 1: Sun Drying

Fig 2: Shade Drying

Fig 3: Air Drying

Fig 4: Oven Drying

Fig 5: Microwave Drying

Conclusion

Post-harvest management including drying is a primary processing method which can be performed by the collectors at their place is the need of the hour for production of cost-effective and quality raw material with high andrographolide and less microbial contamination to harness the national and international demands. The colour and andrographolide content was significantly influenced by different drying methods. Shade drying of Kalmegh was found superior method of drying as it maintained green colour and highest andrographolide content compared to other drying methods. Thus, shade drying would be preferable method in preserving the quality of *A. paniculata*.

Acknowledgement

The authors are grateful to the Director, Forest Research Institute, Dehradun for providing facilities. Authors are thankful to Dr. Raman Nautiyal for his assistance in statistical analysis. National Medicinal Plants Board, New Delhi is acknowledged for providing financial assistance to carry out the research work.

References

1. Niranjan A, Tewari SK, Lehri A. Biological activities of Kalmegh (*Andrographis paniculata* Nees). Indian Journal of Natural Product and Resources 2010;1:125- 135.
2. Mishra SK, Sangwan NS, Sangwan RS. Phcog Rev. Plant Review *Andrographis paniculata* (Kalmegh): A Review. Pharmacognosy Reviews 2007;1(2):283-298.
3. Kumar RA, Sridevi K, Kumar NV, Nanduri S, Rajagopal S. Anticancer and immunostimulatory compounds from *Andrographis paniculata*. Journal of Ethnopharmacology 2004;92:291-295.
4. Trivedi NP, Rawal UM. Hepatoprotective and antioxidant property of *Andrographis paniculata* (Nees) in BHC induced liver damage in mice. Indian Journal of Experimental Biology 2001;39:41-46.
5. Puri A, Saxena R, Saxena RP, Saxena KC, Srivastava V, Tandon JS. Immunostimulant agents from *Andrographis paniculata*. Journal of Natural products 1993;56(7):995-999.
6. Prathanturarug S, Soonthornchareonnon N, Chuakul W, Saralamp P. Variation in growth and diterpene lactones among field-cultivated *Andrographis paniculata*. Journal of Natural Medicines 2007;61(2):159-163.
7. Ram VJ. Herbal preparations as a source of hepatoprotective agents. Drug News and perspective 2001;14(6):353-363.
8. Srivastava A, Misra H, Verma RK, Gupta MM. Chemical fingerprinting of *Andrographis paniculata* using HPLC, HPTLC and densitometry. Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques 2004;15(5):280-285.
9. Jain DC, Gupta MM, Saxena S, Kumar S. LC analysis of hepatoprotective diterpenoids from *Andrographis paniculata*. Journal of pharmaceutical and biomedical analysis 2000;22(4):705-709.
10. Goraya GS, Ved DK. Medicinal Plants in India: An Assessment of their Demand and Supply. National Medicinal Plants Board, Ministry of AYUSH, Government of India, New Delhi and Indian Council of Forestry Research & Education, Dehradun 2017.
11. Pandey AK, Mandal AK. Cultivation of Andrographis paniculata, Journal of Tropical Medicinal Plants 2008; 9(1):178-182.
12. Pandey AK, Mandal AK. Variation in morphological characteristics and andrographolide content in *Andrographis paniculata* (Burm. f.) Nees of central India. Iranica Journal of Energy and Environment 2010; 1(2):165-169.
13. Pandey AK, Savita. Harvesting, Post Harvest Processing and Value Addition of Medicinal plants. The Pharma Innovation Journal 2017;6(12):229-235.
14. George SDS, Cenkowski S. Modelling of thin-layer drying on an inert sphere. Drying Technology 2009;27(6):770-781.
15. Sultanova SA, Safarov JE, Raxmanova TT. Study of temperature profile in drying process. Chemical Technology, Control and Management 2020;2020(3):29-34.
16. Raghavan GV, Rennie TJ, Sunjka PS, Orsat V, Phaphuangwittayakul W, Terdtoon P. Overview of new techniques for drying biological materials with emphasis on energy aspects. Brazilian Journal of Chemical Engineering 2005;22(2):195-201.
17. Achariyaviriya S, Soponronnarit S, Terdyothin A. Mathematical model development and simulation of heat pump fruit dryer. Drying Technology 2000;18(1-2):479-91.

18. Müller JO, Heindl AL. Drying of medicinal plants. Frontis 2006, 237-252.
19. Anonymous. European Pharmacopoeia 2005.
20. Ebadi MT, Azizi M, Sefidkon F, Ahmadi N. Influence of different drying methods on drying period, essential oil content and composition of *Lippia citriodora Kunth*. Journal of Applied Research on Medicinal and Aromatic Plants 2015;2(4):182-187.
21. Thamkaew G, Sjöholm I, Galindo FG. A review of drying methods for improving the quality of dried herbs. Critical Reviews in Food Science and Nutrition 2020; 18:1-24.
22. Feng H, Yin Y, Tang J. Microwave drying of food and agricultural materials: basics and heat and mass transfer modeling. Food Engineering Reviews 2012;4(2):89-106.
23. Pandey AK, Smiley Gulati, Amit Gupta, Tripathi YC. Variation in andrographolide content among different accessions of *Andrographis paniculata*. The Pharma Innovations Journal 2019;8(4):140-144.
24. Padmapriya S, Rajamani K. Standardization of post-harvest technology for *Gymnema sylvestre* and *Plectranthus forskohlii* (Wild) Briq (Syn: Coleus forskohlii). Journal of Medicinal and Aromatic Plants Sciences 2016;4(4):11-17.
25. Periche A, Castelló ML, Heredia A, Escriche I. Influence of drying method on steviol glycosides and antioxidants in *Stevia rebaudiana* leaves. Food Chemistry 2015; 172:1-6.
26. Ambrose DCP, Naik R. Mechanical Drying of Senna leaves (*Cassia angustifolia*). Current Agriculture Research Journal 2013;1(1):65-68.
27. Alara OR, Abdurahman NH, Mudalip SK, Olalere OA. Mathematical modeling of thin layer drying using open sun and shade of *Vernonia amygdalina* leaves. Agriculture and Natural Resources 2018;52(1):53-58.
28. Lim YY, Murtijaya J. Antioxidant properties of *Phyllanthus amarus* extracts as affected by different drying methods. LWT-Food Science and Technology 2007;40(9):1664-1669.