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Hari Om Saxena
NWFP Discipline, SFM and Agroforestry Division, Tropical Forest Research Institute, Jabalpur, Madhya Pradesh, India

Ganesh Pawar
NWFP Discipline, SFM and Agroforestry Division, Tropical Forest Research Institute, Jabalpur, Madhya Pradesh, India

Samiksha Parihar
NWFP Discipline, SFM and Agroforestry Division, Tropical Forest Research Institute, Jabalpur, Madhya Pradesh, India

SK Choubey
NWFP Discipline, SFM and Agroforestry Division, Tropical Forest Research Institute, Jabalpur, Madhya Pradesh, India

Pranav Dhar
NWFP Discipline, SFM and Agroforestry Division, Tropical Forest Research Institute, Jabalpur, Madhya Pradesh, India

Correspondence
Hari Om Saxena
NWFP Discipline, SFM and Agroforestry Division, Tropical Forest Research Institute, Jabalpur, Madhya Pradesh, India

Effect of drying methods and storage containers on gallic acid content in fruits of *Terminalia bellerica* L.

Hari Om Saxena, Ganesh Pawar, Samiksha Parihar, SK Choubey and Pranav Dhar

Abstract
The aim of the present study was to see the effect of drying methods and storage containers on gallic acid content in fruits of *Terminalia bellerica* (Baheda). The study showed the sun drying better in comparison to shade and artificial dryings (hot air oven) to maintain quality of fruits in terms of gallic acid content. Besides, low cost HDPE containers, ambient temperature and six month period were found the optimum conditions for storage of *T. bellerica* fruit pulp.

Keywords: *Terminalia bellerica*, fruits, drying, storage, gallic acid

Introduction
The post-harvest management (drying, processing, storage etc.) of medicinal and aromatic plants has great impact on their value chain, because of its direct influence on the quality of medicinal plants/ products in terms of appearance as well as quantity of the active chemical ingredients (Silva and Casali, 2000) [1]. Drying is one of the most important parameter in the post-harvest management of medicinal plants. The quality of drug and consequently the income are significantly influenced by the drying regime (Mahapatra and Nguyen, 2007) [2]. Drying process also contributes to regular supply and facilitates the marketing of medicinal plants, because drying results in reduction of the weight and volume of the plant produce with positive consequences for transport and storage (Calixto, 2000) [3].

Improper drying and storage of medicinal plants and their products lead the early deterioration of their quality and therapeutic efficacy due to rapid hydrolytic decomposition of their bioactive constituents. The extent of such deterioration is influenced ominously by the nature of collection, drying and storage conditions of plant materials (Karlund *et al*., 2014; Kalt *et al*., 1999; Ansari, 2011; Kokate, 2004) [4 – 7]. Inappropriate storage may make the materials susceptible towards the attack of mites, nematode worms, insects/moths, and beetles which destroy herbal drugs during storage (Kamboj, 2012) [8]. As per WHO guidelines, the medicinal plant materials must be stored under specified conditions in order to avoid contamination, deterioration and to enhance long term storage while retaining its medicinal property.

Terminalia bellerica (Gaertn.) Roxb. is one of the highly traded medicinal plant, commonly known as Baheda (family: Combretaceae). It is a medicinal fruit bearing tree found throughout Central Asia. It is distributed throughout the forests of India at an altitude below 10,000 m except in dry and arid regions. The tree is a large deciduous and reaches upto a height of 50 m and a diameter of 3 m with a rounded crown (Kapoor, 1990) [9]. The important part of *T. Bellerica* is the fruit pulp which is utilized by the pharmaceutical industries. Gallic acid (Fig. 1), a strong antioxidant compound, is the main active chemical ingredient of fruit pulp. Estimated annual trade of Baheda fruits is 2000 – 5000 metric tones (Anon, 2019) [10]. Baheda fruit is one of the three fruits utilized in preparation of *Triphala* – a well-established ayurvedic formulation bestowed with a number of pharmacological properties such as appetite stimulation, reduction of hyperacidity, antioxidant, anti-inflammatory, immuno-modulating, antibacterial, antimutagenic, adaptogenic, hypoglycemic, antineoplastic, chemoprotective, and radioprotective effects, and prevention of dental caries (Peterson *et al*., 2017) [11].

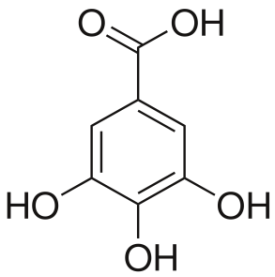


Fig 1: Chemical Structure of Gallic acid

Although, the fruits of *T. bellerica* have commercial trade but information on their drying and storage is still lacking in the literature. In the present article, an attempt has been made to study the effect of different drying methods and storage containers on the gallic acid content in fruit pulp of *T. bellerica* for standardization of proper drying and storage conditions.

Material and Methods

Chemicals and reagents

Gallic acid standard was purchased from Sigma Aldrich, India. Solvents and chemicals used in the experiments were of AR grade.

Collection of Baheda fruits

Mature fruits of Baheda were collected from Chhindwara forest division of Madhya Pradesh in the last week of February month, brought to the laboratory and washed in tap water.

Drying of fruits by different methods

The fruits were dried by three different methods for 48 hrs, (i) shade drying, (ii) sun drying and (iii) artificial drying (hot air oven) at 40ºC. Dried fruits were depulped by manual breaking and the pulp was separated. Pulp received from all drying methods was powdered and evaluated for gallic acid content using HPTLC technique.

Determination of moisture content

Moisture content in sampleswas determined by loss of water in terms of percent w/wusing following formula:

Moisture content = Fresh Weight – Dry weight/ Fresh Weight x 100

Storage of dried fruit pulp in different containers

Dried pulp of Baheda fruits were stored separately in different containers i.e. HDPE bags, Woven sacks, Gunny (jute) bags, Markin cloth, Tin, Glass and Plastic containers in three replicates at room temperature. Unpacked samples kept in open environment were taken as control. Pulp of Baheda fruits were stored in different containers in the month of March and were evaluated for gallic acid content using HPTLC technique at quarterly intervals for one year. The experiments were repeated in the successive year also. Fruit pulp stored in HDPE bags were also kept at 4-5ºC to examine the effect on active chemical ingredient.

Quantification of gallic acid in dried and stored fruit pulp

Quantitative evaluation of gallic acid in dry pulpwas carried out by following the standard method with some modification (Gupta *et al*., 2003) [12].

Preparation of test solution

2.5 gm dried and finely powdered fruit pulp samples were taken in conical flasks containing 50 ml of 2N HCl and heated for 30 minutes over a boiling water bath, cooled and filtered. The filtrate was transferred to a separating funnel and extracted twice with 75 ml (50; 25) of diethyl ether. The pooled diethyl ether layers were washed two times with distilled water, dried over anhydrous sodium sulphate and filtered. The filtrate was evaporated and the concentrated extract was dissolved in 2 ml of methanol for analysis.

Preparation of standard solution

Standard solution of 1 mg/ml of gallic acid was prepared which was further diluted to the stock solution of 0.1 mg/ml concentration.

Mobile phase for TLC

Cyclohexane: Ethyl acetate: Formic acid (4: 6: 1)

Sample application

10 µl of each sample was spotted in triplicate in the form of bands of width 8 mm using a 100 µl CAMAG syringe on 20 x 10 cm aluminum packed TLC plate coated with 0.2 mm layer of silica gel 60F 254 (E. Merck Ltd., Darmstadt, Germany) with the help of Linomat V applicator attached to CAMAG HPTLC system, which was programmed through WinCATS software. Various volumes of the standard solution such as 3, 4, 5, 6 and 7 µl (corresponding to 30, 40, 50, 60 and 70 ng respectively of gallic acid per spot) were applied on TLC plate in the five tracks.

Development of chromatograms

20 ml of mobile phase was used per chromatography run. The linear ascending was carried out in a twin through glass chamber (20 cm x 10 cm) saturated with the mobile phase.

Detection of spots

The developed plate was dried by hot air to evaporate solvents from the plate and kept in photo - documentation chamber and captured the images under UV light at 254 nm. Densitometric scanning was then performed with a CAMAG TLC Scanner 4 equipped with Win CATS software at λ_{max} = 254 nm using deuterium and tungsten light source. The slit dimensions were 6.00 x 0.45 mm. The R_f value, fingerprint data, respective peak areas and spectra were recorded. A calibration curve was prepared by plotting the peak area vs. concentration of gallic acid applied. Amount of gallic acid in the test sample was calculated using the calibration curve of the standard.

Statistical analysis

The data were statistically analyzed. Each experiment was carried out in triplicate and results are expressed as Mean ± SD (n=3). ANOVA was applied to check the results as significant and non – significant.

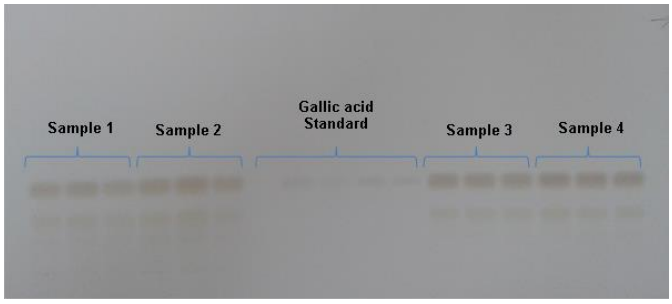
Results and Discussion

Effect of three different drying methods on gallic acid content in pulp of Baheda fruits is given in Table 1. Drying of fruits in shade, sun and hot air oven (40ºC) for 48 hrs reduced the moisture level up to 20.14%, 14.24% and 12.36% respectively. Results showed that the pulp of Baheda fruits dried in sun contained maximum gallic acid (0.216%) content.

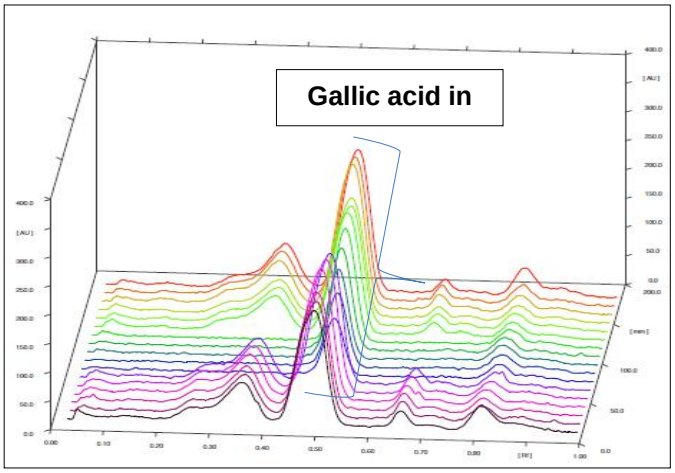
Table 1: Effect of drying on gallic acid content of Baheda fruit pulp

		Shade drying	Sun drying	Hot air drying at 40ºC	SE±	CD at 5 %
Fruits of *T. bellerica*	Time (hrs)	48	48	48		
	Moisture %	20.14	14.24	12.36	0.482	1.699
	Gallic acid %	0.178	0.216	0.212	0.006	0.020

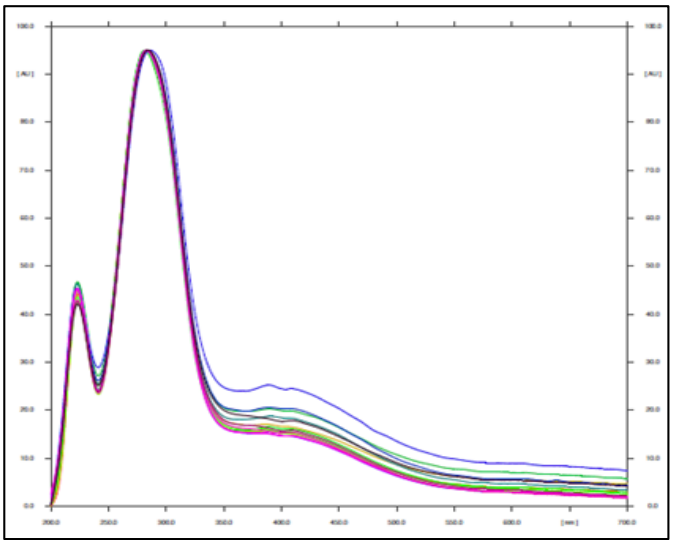
Gallic acid content was also evaluated in dry pulp of Baheda fruits stored in different containers at quarterly intervals. HPTLC plate, 3D HPTLC densitometric scan of plate, spectral pattern of tracks of four samples applied in triplicate and five tracks (different concentrations) of gallic acid standard along with its calibration curve are represented in Fig. 2 (A-D). HPTLC plates for other samples were run in the similar manner and scanned.



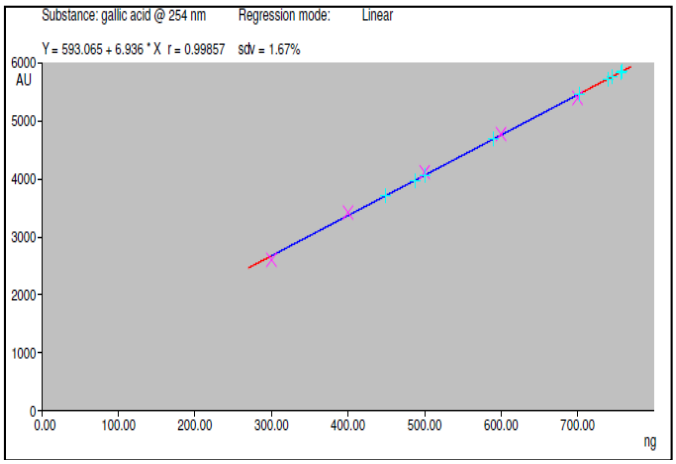
A. HPTLC profiles of test samples of *T. bellerica* fruits



B. HPTLC densitometric 3D image of tracks on HPTLC plate



C. Spectral pattern of tracks on HPTLC plate



D. Calibration curve of gallic acid standard

Fig 2: A B C D Show the HPTLC profiles HPTLC densitometric HPTLC plate and Calibration curve

Gallic acid content evaluated at quarterly intervals in two successive years i.e. 2014 and 2015 in dry pulp stored in different containers is given in Table 2 and 3 respectively which indicated the maximum gallic acid content in dry pulp stored in HDPE bags. The initial concentration of gallic acid in fruit pulp at the time of storage was analyzed as 0.219% and 0.22% in the years 2014 and 2015 respectively.

Table 2: Gallic acid content in dry pulp of *T. bellerica* fruits (year 2014)

Containers	Gallic acid content (%)			
	June 2014	September 2014	December 2014	March 2015
HDPE bags	0.215	0.213	0.178	0.154
Glass	0.189	0.148	0.148	0.128
Plastic	0.182	0.149	0.140	0.125
HDPE bags (4-5° C)	0.160	0.135	0.125	0.120
Steel	0.145	0.129	0.117	0.101
Woven sacks	0.143	0.114	0.104	0.086
Gunny bags	0.140	0.095	0.080	0.073
Tin	0.129	0.072	0.070	0.069
Markin bags	0.066	0.057	0.055	0.029
Open (Control)	0.111	0.059	0.052	0.044
CD at 5 %	0.025	0.014	0.007	0.017
SE±	0.008	0.005	0.002	0.006

Table 3: Gallic acid content in dry pulp of *T. bellerica* fruits (year 2015)

Containers	Gallic acid content (%)			
	June 2015	September 2015	December 2015	March 2016
HDPE bags	0.216	0.214	0.179	0.153
Glass	0.190	0.149	0.148	0.129
Plastic	0.182	0.149	0.141	0.126
HDPE bags (4-5° C)	0.159	0.137	0.126	0.121
Steel	0.146	0.130	0.118	0.102
Woven sacks	0.143	0.115	0.105	0.086
Gunny bags	0.141	0.096	0.081	0.074
Tin	0.130	0.071	0.07	0.070
Markin bags	0.067	0.058	0.054	0.030
Open (Control)	0.111	0.060	0.052	0.045
CD at 5 %	0.025	0.011	0.006	0.016
SE±	0.008	0.004	0.002	0.005

Table 2 and 3 showed the maximum gallic acid content in the pulp of Baheda fruits stored in HDPE bags and minimum content in the pulp stored in Markin bags in both the years

2014 and 2015. It was also observed that the initial gallic acid content was retained upto 2nd quarter in the pulp stored in HDPE bags only while in case of pulp stored in other containers, there was continuously decrease in gallic acid content. The trend of decrease of gallic acid content in different containers for the years 2014 and 2015 can be seen in Fig. 3 and 4 respectively.

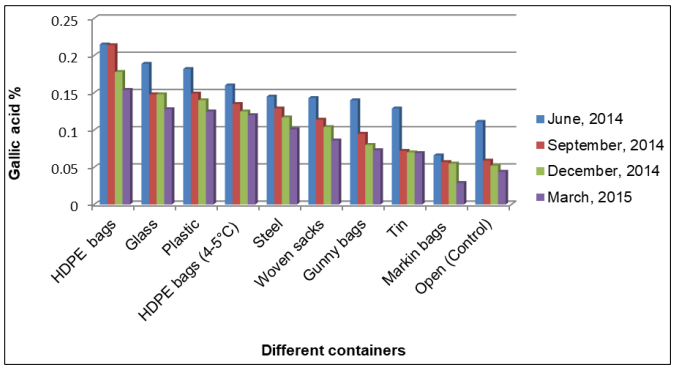


Fig 3: Gallic acid content in dry fruit pulp stored in different containers (year 2014)

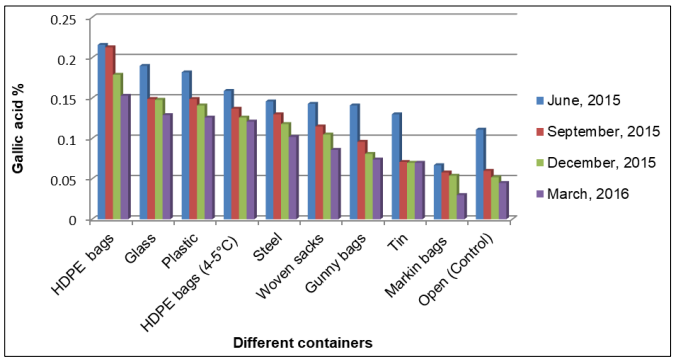


Fig 4: Gallic acid content in dry fruit pulp stored in different containers (year 2015)

HDPE bags were found the best containers to keep the fruit pulp for six months. The trend of decrease of gallic acid content in fruit pulp stored in HDPE bags in different quarters in both the years can be seen in Table 4 and Fig. 5.

Analysis of variance indicated significant ($P< 0.05$) variation in gallic acid content of fruit pulp stored in different containers (except HDPE bags) within the quarters as well as between the quarters in both the year 2014 and 2015. In HDPE bags, the variation was non-significant ($P>0.05$) between the initial two quarters in both the years while it was significant ($P< 0.05$) between last two quarters.

Table 4: Gallic acid content in dry pulp of *T. bellerica* stored in HDPE bags

Quarters	Gallic acid content (%)	SE±
March 2014	0.215	0.004
June 2014	0.213	0.002
September 2014	0.178	0.002
December 2014	0.154	0.003
March 2015	0.216	0.003
June 2015	0.214	0.001
September 2015	0.179	0.002
December 2015	0.154	0.003
CD at 5 %	0.008	

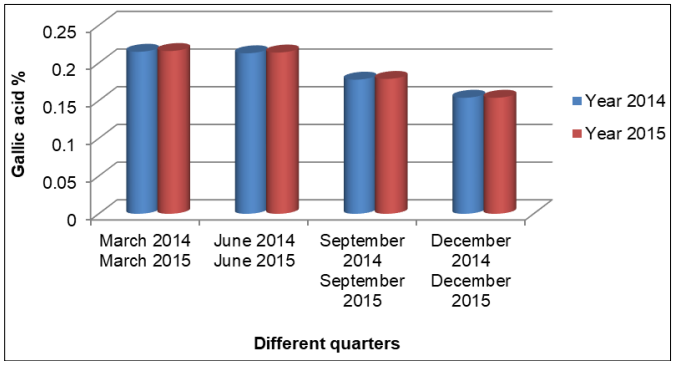


Fig 5: Gallic acid content in dry fruit pulpstored in HDPE bags in the years 2014 and 2015

Raw herbs collected from field are contaminated with soil and other unwanted materials. They undergo processing (cleaning and drying) before storage and for utilization in formulations. Drying is the simplest method of processing (primary processing) crude drugs, making them possible to store for reasonable period (Ulhas, 2015) [13].

The superiority of sun drying in comparison to other methods is supported by the fact that in winter season due to low temperature, the shade-drying of raw materials with high moisture content is slow and inefficient. Such conditions promote mould growth which results in deterioration of raw materials (Singh, 2008) [14]. Fresh fruits of Baheda have higher moisture content which needs high energy to bring down the moisture level early along with to avoid the loss of bioactive compounds. In present investigation, hot air drying reduced the moisture level more in comparison to sun drying within the same time frame of 48 hrs but it also reduced the gallic acid content. Tanko *et al*., (2005) [15] expressed that in natural drying, exposure to sun and/or the desiccating effect of air currents promotes the removal of water from the materials. Downs and Compton (1955) [16] reported that natural air-drying is easy to control and seldom damages the crop. Natural drying is a popular method of medicinal plant drying, especially in areas where maturity and harvesting of the plants coincides with the beginning of the dry season, and their phytochemicals are not photosensitive. Cinnamon (*Cinnamonum cassia* Prel.) bark is usually sun-dried after harvesting in July and August when the quality of the bark is high (Cai *et al*., 2004) [17]. However, Downs and Compton (1955) [16] reported that the reliance on favorable weather conditions limits the use of natural drying. Jambor and Czosnowska (2002) [18] reported that the selection of a better drying method can produce a sufficiently shelf stable product without major losses in the active chemical ingredients and herb value.He further emphasized that there are numerous methods for drying of medicinal plant materials but adoption of improper method may cause major losses of their medicinal, culinary, visual, and nutraceutical properties, negatively affecting the product value.

Apart from drying, storage is also essential to maintain the product with the physical and chemical characteristics closer to those found in the fresh plant, and failures in drying and storage influence the quality of the final product (Cristiane *et al*., 2018) [19] both in term of microbial infestation and active chemical ingredients. Gallic acid has been reported to be the main active chemical constituent present in *T. bellerica* fruits.

The present investigation revealed that fruit pulp stored in

HDPE bags contained maximum gallic acid content followed by glass containers while minimum gallic acid content was recorded in the fruit pulp stored in markin bags. The reason for minimum content in markin bags may be due to its more moisture absorbing capacity which leads the deterioration of chemical ingredients in stored materials. The study is also supported by Sinh (1999) [20] who reported packing wise biodeterioration of phytoconstituents that was found high in paper and cloth packings. He also reported the minimum microbial load in polythene packing because polythene did not permit the growth of micro-organisms and found much more effective in controlling the loss of the phytoconstituents. Masand *et al*., (2014) [21] reported that there are no guidelines for proper storage of herbal raw materials till date. It is only the proper handling, packaging and storage of herbs which can preserve the safety, efficacy and quality of herbs. Thillaivanan and Samraj (2014) [22] reported that the physical factors such as air (oxygen), humidity, light, and temperature can bring about deterioration of plant materials directly or indirectly. Saxena *et al*., (2018) [23] in a similar study on Vaividang (*Embelia tsjeriam –cottam*) fruits reported the sun drying and HDPE bags better choice in comparison to other drying methods and storage containers.

Conclusion

The post harvesting process of medicinal plants has great importance in maintaining their chemical, medicinal, culinary, visual, nutraceutical and ultimately the product value. Drying and storage are among the essential postharvest processes which play a major role in maintaining the quality of plant materials equal to the fresh one and failures in these steps influence the eminence of the final product. The selection of the correct drying method and storage container increases the value of the plant produce to get economic benefits. In the present study, it appeared to be feasible to dry the fresh fruits of Baheda in sun initially for 48 hrs before decorticating them to get the pulp for medicinal use. Besides, the low cost moisture proof HDPE bags were found the best materials for storing the fruit pulp upto six months with minimum quality loss in terms of gallic acid content. Markin bags are the least choice for storage purpose because these absorb moisture rapidly which affect biochemicals/quality of materials.

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References

1. Silva F, Casali VWD. Aromatic plants medicinais: Postoperative essenciais colheita and oils. Viçosa: Art and Livros, 2000, 135.
2. Mahapatra A, Nguyen CN. Drying of medicinal plants. Acta horticulturae. 2007; 756 (756):47-54.
3. Calixto JB. Efficacy, safety, quality control, market and regulatory guidelines for herbal medicines (phytotherapeutic agents). Brazilian Journal of Medical and Biological Research. 2000; 33:179-189.
4. Karlund A, Moor A, Sandell M, Karjalainen RO. The Impact of harvesting, storage and processing factors on health-promoting phytochemicals in berries and fruits. Processes. 2014; 2:596-624.
5. Kalt W, Forney FC, Martin A, Prior RL. Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. Journal of Agricultural and Food Chemistry. 1999; 47:4638-4644.
6. Ansari SH. Essentials of Pharmacognosy. Birla publications Pvt ltd, 2011, 10-16.
7. Kokate CK, Gokhale SB. Pharmacognosy. Nirali prakashan, Delhi, 2004.
8. Kamboj A. Analytical Evaluation of Herbal Drugs. In: Vallisuta O (Ed) Drug Discovery Research in Pharmacognosy. 1st ed. Rijeka, Croatia: Tech Publication, 2012.
9. Kapoor LD. Handbook of Ayurvedic Medicinal Plants. CRC Press Boca Raton, USA, 1990, 321.
10. Anonymous. https://www.nmpb.nic.in/medicinal_list, 2019.
11. Peterson CT, Denniston K, Chopra D. Therapeutic uses of *Triphala* in ayurvedic medicine. Journal of Alternative and Complementary Medicine. 2017; 23(8):607-614.
12. Gupta AK, Tandon N, Sharma M. Quality Standards of Indian Medicinal Plants. An ICMR Publication, New Delhi. 2003; 1:198-209.
13. Ulhas AS. Storage, packaging and labeling concept with perspective of raw herbs and their formulation. Unique Journal of Ayurvedic and Herbal Medicines. 2015; 03(04):42-46.
14. Singh N. Processing techniques of NWFPs of Chhattisgarh TBOs – *Madhuca latifolia*, *Shorea obusta*, *Schleichera oleosa*, *Pongamia pinnata* and *Buchanania lanzan*. A project report submitted to TFRI, Jabalpur, 2008.
15. Tanko H, Carrier DJ, Duan L, Clausen E. Pre - and post-harvest processing of medicinal plants. Plant Genetic Resources. 2005; 3(2):1-11.
16. Downs S, Compton E. Crop drying with heated air. Agricultural Engineering. 1955; 36:90-92.
17. Cai T, Chen F, Qi J. Dehydrated oriental mushrooms, leafy vegetables and food preparation herbs and condiments. In: Hui YH, Ghazala S, Graham DH, Murrell KD and Nip WK (Eds) Handbook of Vegetable Preservation and Processing. Marcel Dekker, New York, 2004, 373-393.
18. Jambor J, Czosnowska E. Herbal medicines from fresh plants. Post Fitoter. 2002; 8(1-2): 2-5.
19. Cristiane FL, Evandro d CM, Sérgio MLD. Influence of storage conditions on quality attributes of medicinal plants. Biomedical Journal of Scientific & Technical Research. 2018; 4(4):4093-4095.
20. Sinh M. Effect of gamma radiation and different packings used for storage of the drug bahera fruit (*Terminalia bellerica* Roxb.) on microflora population (fungi, pathogenic bacterial sp. & Spc) and on the phytoconstituents (protein and tannin). Ancient Science of Life. 1999; 18(3&4):218-227.
21. Masand S, Madan S, Balian SK. Modern concept of storage and packaging of raw herbs used in Ayurveda. International Journal of Research in Ayurveda and Pharmacy 2014; 5(2):242-245.
22. Thillaivanan S, Samraj K. Challenges, Constraints and Opportunities in Herbal Medicines – A Review. International Journal of Herbal Medicine. 2014; 2(1):21-24.
23. Saxena HO, Deshmukh AK, Pawar G, Choubey SK. Standardization of drying and storage conditions of vaividang (*Embelia tsjeriam –cottam*) fruits in terms of embelin content. Indian Journal of Tropical Biodiversity. 2018; 26(2):197-204.