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Research article

Thin layer drying behavior of *Ginkgo biloba* L. leaves with respect to Ginkgolide A and Bilobalide content and microbial load

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ABSTRACT

Influence of drying temperature (30–50 °C) and relative humidity (RH: 30–80%) on moisture content, energy requirement and quality of *Ginkgo biloba* leaves with respect to chemical markers namely Ginkgolide A (GA) and Bilobalide (BB), and microbial load of dried materials has been analyzed. Leaves were dried in climate control chamber with varying temperature and relative humidity (RH). Total time required for attaining equilibrium was higher for low temperature at all the RH levels as well as for high RH at all the temperatures. Energy requirement was found to increase at high RH and low temperature. GA and BB concentration increased during drying in comparison to that in fresh material. Microbial load analyzed for dried samples was also found within the limit as prescribed in European Pharmacopeia under the category 3B. 40 °C temperature and 50 % RH with less drying duration was observed as suitable conditions for better recovery of BB and GA content, less microbial load and less energy consumption, during drying of *G. biloba* leaves.

1. Introduction

Natural products based pharmaceutical industries use medicinal plants either in fresh form or in dried state. These medicinal plants are generally grown in far away places, so their transportation to these industries require proper post-harvest processing including drying and storage. Drying helps in reducing the weight of plant material which is helpful for transportation of these materials in bulk [1, 29]. One of the most frequent reasons for the rejection of cultivated medicinal plants by pharmaceutical industries is their microbial load and the lower active constituent concentration that are generally affected by the conditions such as temperature and relative humidity (RH) of the environment where the material is dried and stored [2, 3].

An extensive research has been carried out world-wide on methods used for thermal processing and drying of biological materials [4, 5]. Slow drying may cause harmful changes due to the action of microbial enzymes before the process is completed, while very quick drying hardens the superficial layer of the cells and prevents evaporation of water. The presence of moisture content in plant materials increases the activity of microbial enzymes, which causes degradation of medicinally important compounds. The conventional methods including shade drying [6], and sun drying [7], and artificial methods, such as, freeze drying [8], climate chamber drying [9], oven drying [10], heating [11], and microwave drying [12] are being used for post-harvest processing of medicinal plants.

Ginkgo biloba L (Family- Ginkgoaceae) is a tree and commonly known as Maiden-hair Tree due to resemblance of its leave with that of Adiantum (called maiden hair fern). The tree is native to China, from where it was introduced to other parts of the world eg. Japan, Europe, America, India etc. The tree is cultivated in large scale for its leave and seeds due to increased demand, and a lot of work is also going on in the field of its cultivation practices [13, 14, 15]. Increased usage of *G.biloba* can be understood by observing the trade of its products. Total worldwide sales of ginkgo products in 2012 were US $1.26 billion and it is increasing day by day [16]. Commercially *G. biloba* leave are used in preparation of medicines like Bio-Loba (50 + 50), Enlarge, Ginkgogel, Ginkoriv plus, Multivite gold, Multivite Woman, Viminta Gold, Zyrum etc in the form of extract along with other ingredients. Along with these, it is also used in cosmetic industries in various forms [17].

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The tree parts (leave and seeds) are in demand due to the occurrence of medicinally important compounds like terpene trilactones (TTLs) e.g., diterpene (ginkgolides) and sesquiterpene (bilobalide) and flavonoids mainly flavanol glucosides, biflavanol, proanthocyanidins, alkylphenol etc [18]. Ginkgolide A, B, C, J, K, L and M are potent and selective antagonists of platelet activating factor (PAF) [19, 20] that are useful in prevention and treatment of thrombosis, illness of blood vessels of heart and brain, arhythmia, asthma, bronchitis and allergic reactions [21–23], while the sesquiterpene BB exhibits neuroprotective properties [24]. Being such an important medicinal plant, quality and safety of herbal preparations of *G. biloba* is of great concern. Kressmann et al., 2002 [25] had reported problems in quality of commercially available *G.biloba* based products.

In case of *G.biloba*, Laurain D., 2000 [26] had reported the method of collection of fresh leaves followed by their air drying with in 24 h for better preservation. Fresh leave of *G. biloba* are also reported to be dried in propane or diesel fired drum dryers [27]. and no other reports are available on this aspect. Guan et al. 2013 [28] had studied the effect of sun and shade drying along with baking method on chemical constituents of G.biloba leaves and observed high temperature (80 °C) as an ideal temperature for getting good quantity of active constituents in *G.biloba* leave. No report on microbial loading in *G. biloba* raw and processed material is available, which is an important parameter for deciding the quality of plant material. The present study is designed for optimizing drying conditions (mainly temperature and relative humidity) in a climate controlled chamber, so that loss of medicinally important compounds namely Ginkgolide A (GA) and Bilobalide (BB), present in *G. biloba* leaves, can be minimized along with reduction of microbial load up to the permissible limit as per the standard pharmacopoeia followed.

2. Materials and methods

2.1. Plant material and chemicals

Plant material (fresh leaves) was collected from the G. B Pant National Institute of Himalayan Environment and Sustainable Development, Kosi-Katarmal Almora (HQ), Uttarakhand (79° 37.405″E; 29° 38.405″N; 1243 amsl), cleaned with distilled water, surface dried and finally stored at 4 °C. Standard compounds GA and BB were purchased from Sigma (India), N–N-dimethyformide (DMF) from SRL, N,O-bistriflouroacetamide: Trimethylchlorosilane (BSTFA:TMCS; 99:1; lot LB92699) from Supelco (India), methanol from Renkam (India), Tryptone yeast extract agar (TYA) and Potato dextrose agar (PDA) from Hi-media (India).

2.2. Drying equipment

Drying was performed in Climate Chamber (Jeio Tech, Korea; model: TH-PE-100) as shown through diagrammatic representation in Figure 1. Dryer was equipped with temperature and relative humidity controllers. A balance (make: Citizen; model: CY510) was placed outside the dryer for measuring the variations in weight of the samples during definite time intervals.

2.3. Controlled drying of raw materials

The stored *G. biloba* leaves were brought to the room temperature conditions and then chopped into small pieces. For drying experiments, 10 g material was dried under different combinations of temperature (30–50 °C) and RH (30–80 %) until constant weight. Variation in weight was monitored at equal time interval of 1 h until reached the equilibrium condition. Drying of samples was carried out in triplicate.

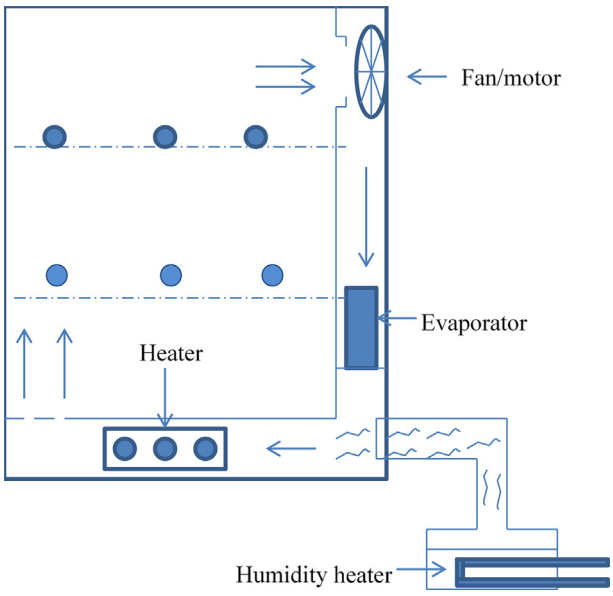

Figure 1. Climate chamber set up used for drying experiments [36].

2.4. Determination of moisture ratio and specific energy requirement during drying

Initial moisture content (M_0) was analyzed for all the samples for each drying experiment. For this, leaf samples were heated at 105°C in an oven (MAC, India), until the constant weight was achieved. Moisture content was calculated on fresh weight basis following Eq. (1).

$$M_0 = \frac{(W_0 - W_t)}{W_0} \times 100 \quad (1)$$

Where W_0 is initial weight of the sample and W_t is dried weight of the sample at time t. Moisture content was also measured at definite time intervals (M_t) and at equilibrium (M_e). Then moisture ratio (M_R) was estimated using Eq. (2) [36]:

$$M_R = \frac{M_t - M_e}{M_0 - M_e} \quad (2)$$

where M_R is the moisture ratio, M_t is the mean moisture content at any time (kg water/kg dry matter), M_0 is the initial moisture content (kg water/kg dry matter), and M_e is the equilibrium moisture content (kg water/kg dry matter).

Total energy consumed in whole drying process and energy required for drying one kilogram of leaves at selected temperature and RH conditions was calculated using Eq. (3) [29–31, 36].

$$E_{kg} = \frac{E_t}{W_0} = \frac{Av\rho_a C_a \Delta T D_t}{W_0} \quad (3)$$

Where, E_t is the total energy in each drying phase (kWh), A is the cross sectional area of the sample holder (m^2), v is the air velocity (m/s), ρ_a is the air density (kg m^{-3}), ΔT is the temperature differences (°C), D_t total time for drying each sample (h) and C_a is the specific heat of air (kWh kg^{-1} $°C^{-1}$), E_{kg} is the required specific energy in kWh/kg and W_0 is the initial weight of material taken for drying study.

Specific heat of air (C_a) used in Eq. (3) was calculated using Eq. (4).

$$C_a = 1.004 + 1.88\omega \quad (4)$$

Where ω is humidity ratio.

2.5. Preparation of extract and their derivatization

Fresh and dried leaves (dried under different temperature and humidity conditions) were powdered and 2 g of it was macerated using 10 mL of methanol (1:5 ratio) in a rotary shaker under ambient condition. Extraction was repeated until the extract become colorless, then the filtrate was dried under ambient conditions. The extracts were then derivatized for gas chromatographic analysis along with GA and BB, separately. For derivatization, dried extracts were initially dissolved in 2 mL DMF. Volume containing 40 mg extract were derivatized using 500 μL BSTFA\TMCS and 120 μL DMF in glass vials. GA and BB standards were also derivatized following the procedures reported by Hasler and Meier [32] and Lang and Wai [33] with some modifications. Salilyzation reaction was carried out in hot air oven at 120 °C for 60 min. For analyzing the accurate peak of GA, spiking of dried extract of sample was carried out by adding known volume and concentration of GA standard before derivatization. Desired peak of GA was identified by observing the increase of area (mV. sec) of the peak after spiking, which was recorded at 16.8 min retention time (RT) while peak of BB was observed at 13.1 min. Calibration curves of GA and BB were plotted by taking standards of different concentrations, and the equation obtained from the curve was utilized for estimation of the amount of GA and BB present in the unknown samples.

2.6. Gas chromatography (GC) analysis

GC analysis was carried out using Chemito GC (Ceres 800 plus) equipped with Flame Ionization Detector (FID). Analysis was done using BP-1 capillary column (30 m × 0.25 mm) in split less mode where the injection volume taken was 1 μL (both for the derivatized samples and standards). The injector and detector temperature were 220 °C and 290 °C, respectively. The flow rate of carrier gas (helium) was maintained at 1 mL/min. The initial oven temperature was kept at 200 °C and increased up to 260 °C at a rate of 6 °C/min and then to 280 °C at the rate of 3 °C/min and held for 10 min with total analysis time of 26.7 min.

2.7. Estimation of microbial load in fresh and dried extracts

Microbial load of the leaf samples was carried out following serial dilution pour plate method using 1 g of samples dried at different conditions. For this, diluted aliquots were poured on the petri plates having Tryptone Yeast (TY), used for bacteria, and Potato Dextrose agar (PDA), used for fungi, and incubated at 25 °C till 1–3 days and 5–7 days for estimating the presence of bacteria and fungi, respectively. All the experiments were carried out in triplicate and microbial load was estimated up to the dilution mentioned in European Pharmacopoeia 5.0 and Indian Pharmacopoeia 2010 with reference to medicinal plants [34,35].

2.8. Statistical analysis

Analysis of variance (ANOVA) was carried out using STATISTICA 8.0 for analyzing the effect of drying conditions on energy requirement, GA and BB. Mean values were considered at 95 % significance level ($p < 0.05$). The graphs were prepared using MS Excel 2016.

3. Results and discussion

3.1. Effect of drying conditions on variation in moisture content of G. biloba leaves

Effect of drying conditions (temperature ranging from 30-50 °C and RH ranging from 30-80 %) on variation of moisture content of *G. biloba*

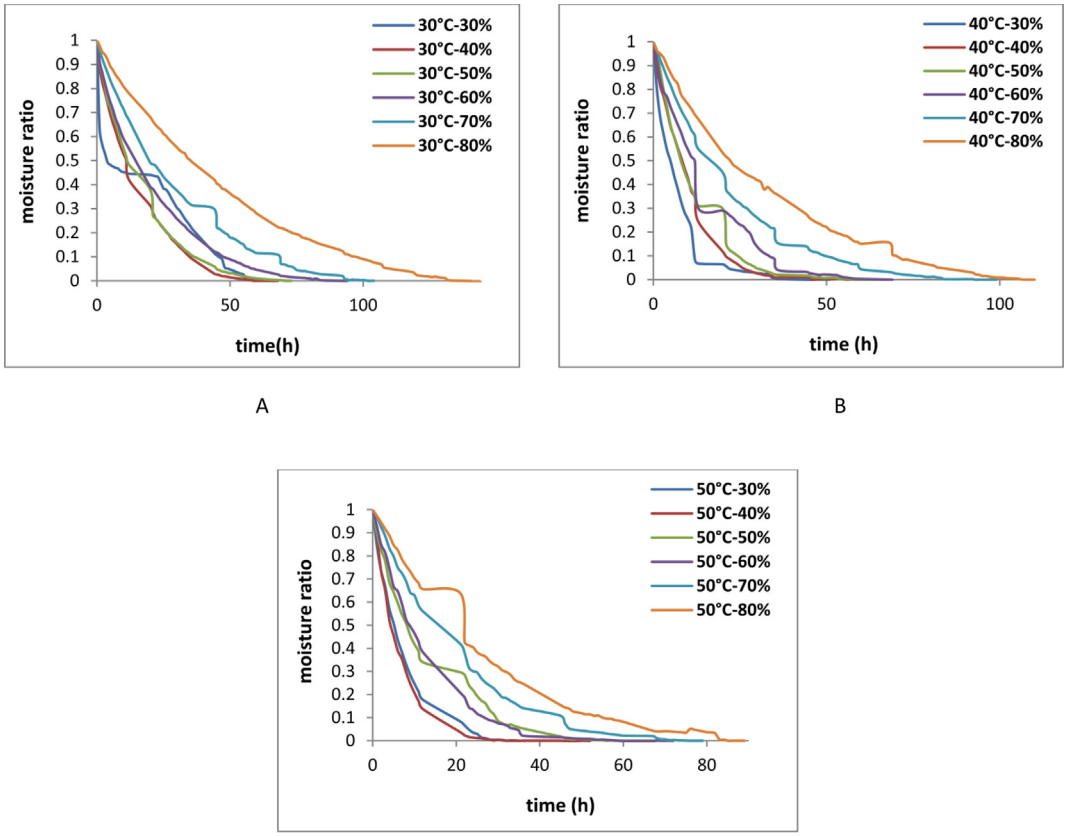

Figure 2. Effect of relative humidity (%) on moisture removal from *G. biloba* leaves during drying at A. 30 °C, B. 40 °C, and C. 50 °C.

Table 1. Effect of drying conditions on concentration of chemical marker compounds (GA and BB) and moisture content variation in *G. biloba* leaves.

Drying Conditions	Specific humidity (g/kg)	BB conc^n (%)	GA conc^n (%)	MC_i (%)	MC_e (%)	MC_f (%)
Fresh		0.15 ± 0.01	2.69 ± 0.03	75.26	-	-
30°C-30%	0.0008	0.06 ± 0.02	2.38 ± 0.85	77.57	21.95	10
30°C-40%	0.0011	0.09 ± 0.03	1.95 ± 0.19	73.49	20.58	16
30°C-50%	0.0013	0.11 ± 0.04	2.20 ± 1.48	75.62	21.53	10
30°C-60%	0.0016	0.03 ± 0.00	1.64 ± 0.17	76.84	22.91	9
30°C-70%	0.0019	0.07 ± 0.05	2.27 ± 0.23	74.87	23.51	12
30°C-80%	0.0021	0.04 ± 0.02	2.41 ± 0.70	77.31	25.46	17
40°C-30%	0.0014	0.04 ± 0.01	2.27 ± 0.80	75.64	14.47	12
40°C-40%	0.0019	0.07 ± 0.01	2.17 ± 0.26	76.73	20.15	12
40°C-50%	0.0023	**0.40 ± 0.03**	**10.75 ± 1.58**	71.68	18.23	20
40°C-60%	0.0028	0.09 ± 0.00	**4.64 ± 0.21**	72.59	18.05	18
40°C-70%	0.0033	0.10 ± 0.01	**4.08 ± 1.39**	76.59	22.72	13
40°C-80%	0.0037	**0.29 ± 0.17**	**5.44 ± 0.42**	78.65	24.72	9
50°C-30%	0.0023	**0.22 ± 0.09**	**2.89 ± 1.39**	74.03	19.82	13
50°C-40%	0.0031	**0.34 ± 0.21**	2.36 ± 0.83	70.44	19.07	11
50°C-50%	0.0039	0.14 ± 0.09	2.58 ± 0.75	72.43	21.39	13
50°C-60%	0.0047	**0.6 ± 0.01**	**3.89 ± 0.20**	74.01	22.38	13
50°C-70%	0.0055	**0.54 ± 0.1**	**3.35 ± 0.62**	68.16	22.88	11
50°C-80%	0.0062	0.02 ± 0.00	0.74 ± 0.41	79.11	30.40	14

BB = bilobalide, GA = ginkgolide, MC_i = initial moisture content, MC_e = equilibrium moisture content, MC_f = final moisture content, °C = Temperature. The values in bold are higher than the same in fresh material.

leaves is presented in Figure 2. Time required to reduce moisture content to equilibrium level was found to be dependent on the drying conditions. Initial moisture contents (MC_i) of the samples, analyzed before starting drying procedure is summarized in Table 1. At every drying condition, initially the moisture content was high, but with time, value of moisture content decreased substantially. Figure 2 shows typical diffusion-controlled behavior under all the drying conditions considered. Similar observations have been reported with respect to *Inula racemosa* [36], bell peppers (*Capsicum annuum* L.) [37], and mint leaves (*Mentha spicata* L.) [38], during drying process. Bonazzi and Dumoulin [3] reported that decrease in moisture content of material under drying causes decrease in the mobility of water molecules in solid matrix. Uniform moisture distribution, observed at low temperature and high humidity, is likely to induce less internal stress and allow the sample to shrink in uniform way until last stage of drying. Under these conditions, drying is likely to require a longer duration.

3.2. Effect of temperature and RH on energy requirement

Total and specific energy required for drying of *G. biloba* leaves was calculated for each experiment using Eq. (2) (Table 2). Total energy required during drying was maximum (61.23 kWh) at 40 °C temperature with 80 % RH, while the minimum (21.29 kWh) was consumed when drying temperature was 50 °C with 30 % RH. Similarly, maximum specific energy (2041.15 kWh/kg) was consumed at drying temperature of 40 °C with RH of 80 %, while

Table 2. Energy requirement during complete drying of *G. biloba* leaves at different drying conditions.

Drying conditions	E_t (kWh)	E_{kg} (kWh/kg_{fwt})	E_{kg}(kWh/kg_{water})	E_{kg}(kWh/kg_{dwt})
30°C-30%	29.17 ± 0.00	972.40 ± 0.00	4044.19 ± 6.43E-13	10468.47 ± 0
30°C-40%	25.58 ± 0.00	852.75 ± 0.00	3553.14 ± 0	9136.63 ± 0
30°C-50%	31.42 ± 0.00	1047.28 ± 0.00	4396.21 ± 0	11011.07 ± 0
30°C-60%	33.07 ± 1.05	1102.17 ± 34.91	4692.30 ± 148.63	11195.86 ± 354.62
30°C-70%	43.24 ± 2.99	1441.35 ± 99.75	6303.27 ± 436.22	13770.84 ± 952.99
30°C-80%	61.20 ± 2.11	2039.90 ± 70.36	9124.8 ± 314.72	18582.09 ± 640.91
40°C-30%	24.14 ± 1.93	804.64 ± 64.37	3047.86 ± 243.82	11605.31 ± 928.43
40°C-40%	32.06 ± 1.84	1068.62 ± 61.41	4347.90 ± 249.86	12205.03 ± 701.38
40°C-50%	30.52 ± 0.70	1017.18 ± 23.21	4283.86 ± 97.75	10607.88 ± 242.07
40°C-60%	35.93 ± 2.03	1197.51 ± 67.83	5005.84 ± 283.55	12724.41 ± 720.75
40°C-70%	52.15 ± 1.77	1738.42 ± 59.01	7415.06 ± 251.70	17579.53 ± 596.73
40°C-80%	61.23 ± 0.19	2041.15 ± 6.44	8930.67 ± 28.17	19480.79 ± 61.46
50°C-30%	21.29 ± 0.47	709.64 ± 15.60	2907.05 ± 63.89	7953.67 ± 174.81
50°C-40%	21.29 ± 1.53	709.71 ± 51.14	2919.30 ± 210.36	7866.29 ± 566.84
50°C-50%	36.97 ± 2.76	1232.37 ± 91.96	5246.62 ± 391.49	12518.45 ± 934.11
50°C-60%	49.38 ± 3.04	1645.92 ± 101.41	7077.55 ± 436.05	16332.22 ± 1006.25
50°C-70%	50.79 ± 0.62	1692.90 ± 20.64	7644.78 ± 93.20	15130.14 ± 184.47
50°C-80%	54.77 ± 3.91	1825.70 ± 130.32	8896.20 ± 635.02	14250.89 ± 1017.25

E_t = total energy (kWh), E_{kg} (kWh/kg_{fwt}) = specific energy per kg fresh wt of sample, E_{kg} (kWh/kg_{dwt}) = specific energy per kg dry wt of sample, E_{kg} (kWh/kg_{water}) = specific energy per kg water removed from the sample, °C = Temperature.

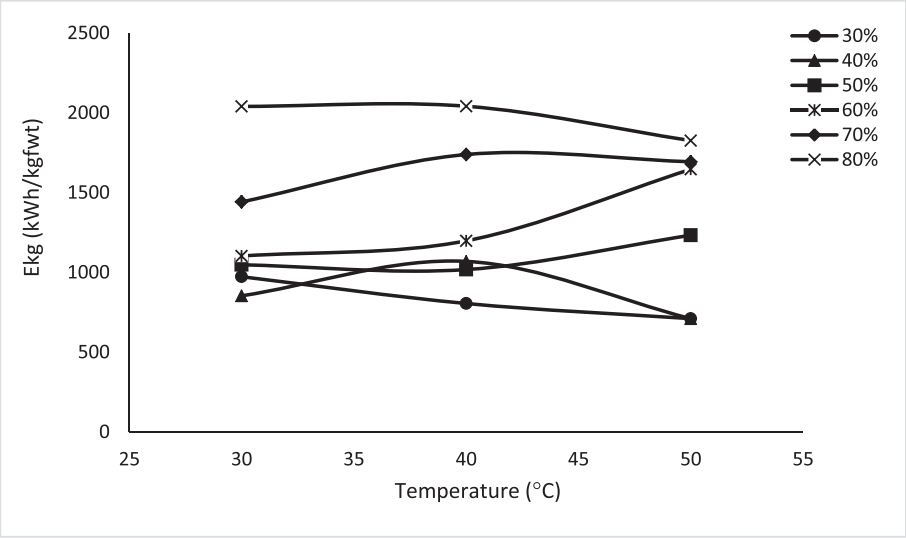

Figure 3. Effect of relative humidity (%) and temperature (°C) on specific energy consumption (kWh/kgfwt) during drying.

the minimum (709.64 kWh/kg) was consumed when drying temperature was 50 °C with 30 % RH (Figure 3). Total energy requirement has shown significant variation with RH at constant temperature ($p < 0.05$) while temperature did not show significant effect on energy consumption at constant RH ($p < 0.05$). In general, energy requirement was found to increase at high RH and low temperature. Water absorption capacity decreases as the RH increases, thereby needing a major quantity of air mass flow for increasing evaporation rate during drying. Therefore, more energy will be required to heat the air with increased RH, which leads to increase water absorption capacity due to absorption of moisture [39].

3.3. Effect of drying conditions on GA and BB concentration of dried G. biloba leaves

Concentration of GA and BB in *G. biloba* leaves, dried under different conditions as well as in fresh state, is shown in Table 1 and GC chromatograms of leaves dried at 40 °C temperature under different RH conditions are shown in Figure 4 A-F. GA and BB

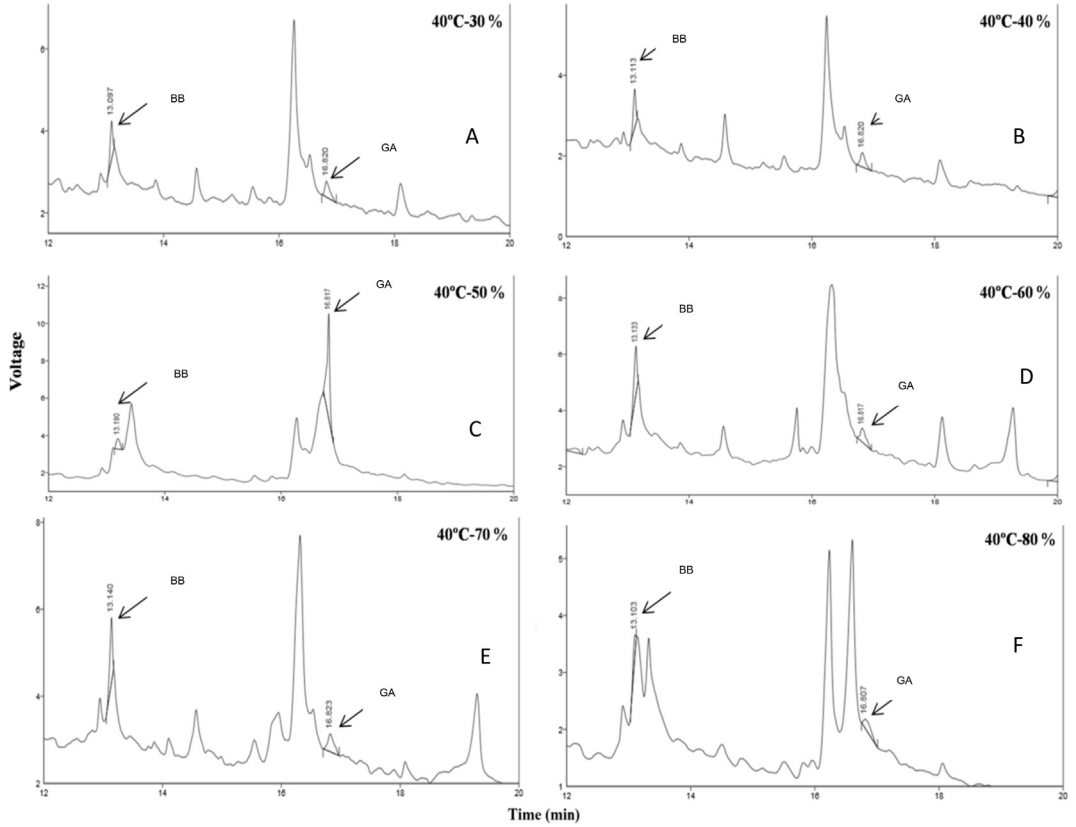

Figure 4. GC chromatograms of G. biloba dried leaves under different combinations of temperature (°C) and relative humidity (%) showing concentration of Ginkgolide A (Rt = 16.8 min) and Bilobalide (Rt = 13.1 min) (Rt= Retention time): A.40°C-30%; B. 40°C-40%; C. 40°C-50%; D. 40°C-60%; E. 40°C-70%; F. 40°C-80%.

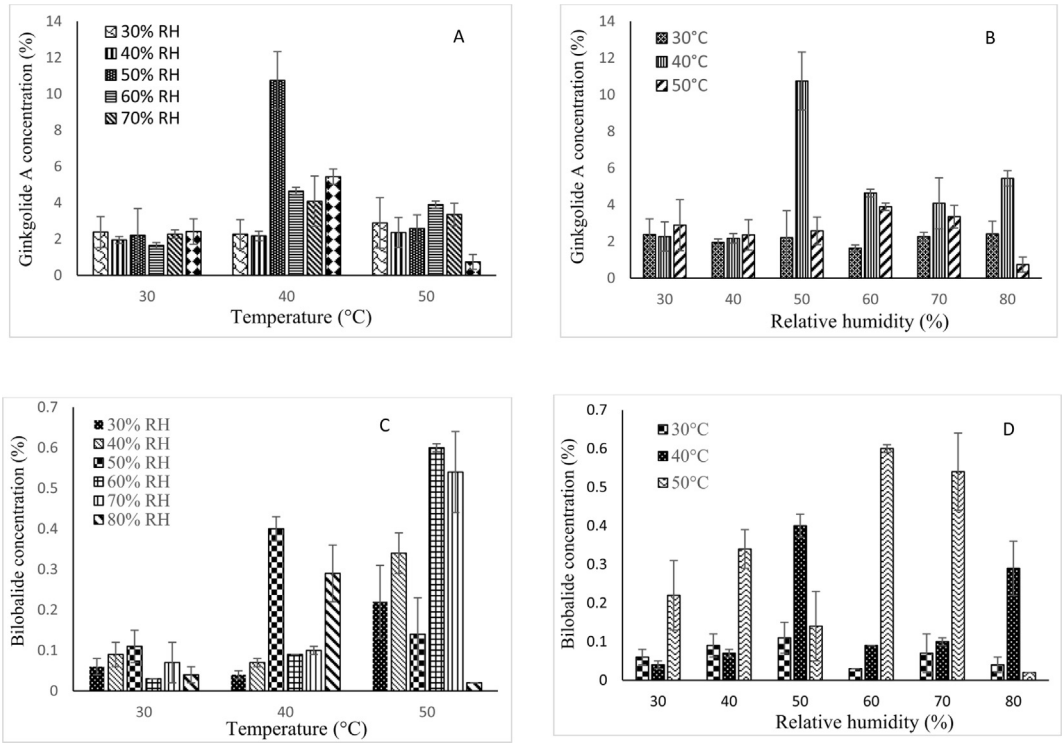

Figure 5. Variation of Ginkgolide A with varying A. relative humidity (%), and B. temperature (°C); variation of Bilobalide with varying C. relative humidity (%), and D. temperature (°C).

concentration varied significantly with the varying RH and temperature ($p < 0.05$). At 30 °C, concentration of GA and BB was found to be lower ranging from 1.64 ± 0.17 % to 2.41 ± 0.70 % and 0.02 ± 0.00 % to 0.11 ± 0.04 %, respectively. At 40 °C, GA concentration varied from 2.17 ± 0.26% to 10.75 ± 1.58 % while BB concentration varied from 0.04 ± 0.01 % to 0.40 ± 0.03 %. GA concentration at 50 °C varied from 0.74 ± 0.41 % to 3.89 ± 0.20 % while concentration of BB ranged from 0.02 ± 0.00 % to 0.6 ± 0.01 %. Standard error was on higher side which might be due to improper derivatization of one of the replicates.

Volatile chemicals might evaporate during drying due to heating of the product and removal of water [40]. However, in the present study, the release of volatile compounds, GA and BB, from the dried leaves was on lower side. In some of the drying conditions, the concentration of these volatiles was also observed to increase at equilibrium. This might be due to the hardening of material's surface or due to different types of bonding like covalent bonding, hydrogen bonding, steric entrapment or sorption on protein or lipid [41]. Results obtained from these experiments showed the response on the similar line. At the drying conditions 40°C-50%, 40°C-80%, 50°C-30%, 50°C-40%, 50°C-70% and 50°C-80% (temperatures and RH combinations) BB concentration was found at the higher end in dried *G. biloba* leaves in comparison to the fresh ones. Similarly, at 40°C-50%, 40°C-60%, 40°C-70%, 40°C-80%, 50°C-60% and 50°C-70% drying conditions, the concentration of GA was found higher in dried *G. biloba* leaves in comparison to the fresh material (Table 1 and Figure. 5 A-D). Similar observation has been reported from *I. racemosa* rhizomes [36] where increase in concentration of active constituents (alantolactone and isoalantolactone) was observed during drying and in *Artemisia annua* leaves [42]where increase in artemisinin content in leaves was 43% in plants dried in oven and shade, and 94% in sun-dried plants. In case of *A. annua* leaves, this increase was observed due to rapid bioconversion of dihydroartemisinic acid (DHAA) to artemisinin (ART) during drying conditions. Therefore, an increase in GA and BB concentration, in the present study, might be attributed to the increase in rate of bioconversion of precursor of these compounds that needs to be further examined.

3.4. Effect of drying conditions on microbial load on G. biloba leaves

Drying controls, the shelf life of the material by suppressing the growth of microorganisms, by reducing the rate of chemical reactions and by inhibiting enzymatic deterioration [43]. Therefore, in the present study, the microbial load was determined for the fresh as well as dried *G. biloba* leaves having highest concentration of GA and BB content (Table 3), which was found high at 30°C- 30 % RH, 40°C-50 % RH, and 50°C-60 % RH. Microbial load was estimated in terms of colony forming units (cfu/g sample) that is used to estimate the number of viable bacterial or fungal cells in a sample. The bacterial colonies, in fresh leaves, were counted up to10^9 dilution. During drying process this showed a decreasing trend: colonies up to 10^6 (30°C-30 %), up to 10^3 (40°C-50 %) and up to 10^2 (50° C-60 %) dilutions. Fungal colonies, that were observed up to 10^6 dilution in fresh samples, were restricted to 10^4 (30°C-30%), 10^3 (40°C-50%) and 10^2 (50°C-60%) in dried conditions (Table 3).

The microbial load also decreased with the decreasing moisture content of the samples, indicating that high moisture content favours the growth of microorganisms. Therefore, 30°C-30% condition was found to be the best suited conditions for the growth of both bacteria and fungi (molds) with the ability to grow in the mesophilic range of temperature. Different Pharmacopeia are available which explains the limit of microbial loads in medicinal plants used for different medicinal purposes. According to European Pharmacopeia, category 3B is relevant for using medicinal plants for preparations for oral administration containing 'raw materials of natural origin', where the total count of aerobic bacteria in herbal raw materials should not exceed 10000 (10^4) per g or per mL. Similarly, total count of fungi should not exceed 100 (10^2) in 1g or 1mL and *E. coli* should be totally absent [34]. Indian pharmacopeia described the limit of microbial count ranging from 10^7 to 10^3 for total aerobic

Table 3. Microbial load on fresh and dried leaf samples of *G. biloba*.

Dilution factor	Category under European pharmacopeia	Type of microbes	Colony forming units (cfu/g) ±SE			
			Fresh sample	Dry sample		
				30°C-30%	40°C-50%	50°C-60%
10^2	3 B (Aerobic bacteria: 10^4) (Molds: 10^2)	Aerobic bacteria	48.33 ± 2.8	26.66 ± 2.5	8.33 ± 2.0	2.33 ± 0.5
		Molds	9.66 ± 2.3	7.33 ± 1.15	1.0 ± 1.0	0.66 ± 0.5
10^3		Aerobic bacteria	46.66 ± 7.6	17.66 ± 1.5	3.66 ± 1.5	-
		Molds	8.66 ± 1.5	5.0 ± 1.0	0.33 ± 0.5	-
10^4		Aerobic bacteria	32.3 ± 4.04	11.66 ± 3.5	-	-
		Molds	2.66 ± 2.0	3.33 ± 1.5	-	-
10^5		Aerobic bacteria	22 ± 2.0	9.0 ± 1.0	-	-
		Molds	1.33 ± 0.5	-	-	-
10^6		Aerobic bacteria	10.66 ± 1.5	2.66 ± 2.0	-	-
		Molds	0.66 ± 0.5	-	-	-
10^7		Aerobic bacteria	6.55 ± 1.5	-	-	-
		Molds	-	-	-	-
10^8		Aerobic bacteria	2.66 ± 1.15	-	-	-
		Molds	-	-	-	-
10^9		Aerobic bacteria	1.0 ± 1.0	-	-	-
		Molds	-	-	-	-

SE = Standard Error (among the replicates), - = No growth.

count and 10^5 to 10^2 for total fungal count based upon the usage [35]. Results of microbial load, analyzed for different samples, clearly exhibited the reduction in microbial load after drying under different conditions (Table 3). Verification of data with the European and Indian Pharmacopeia indicated that the fresh material will not be acceptable due to the exceeding microbial load, while the dried materials will be acceptable due to the permissible limit of microbial load.

4. Conclusion

The present study has shown the effect of drying temperature and RH on moisture diffusion from *G. biloba* leaves, energy requirement, and quality in terms of GA and BB concentration and microbial load in *G. biloba* leaves dried under different controlled drying conditions. To the best of our knowledge, this is the first report on the evaluation of the effect of drying conditions on GA and BB content of *G. biloba* leaves and the microbial load. These findings are likely to be applicable in commercial production of GA and BB and long-term preservation of the plant material. Higher concentration of both the GA and BB at 40 °C temperature and 50 % RH with less drying duration will influence the economy of drying process.

Declarations

Author contribution statement

Vasudha Agnihotri: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Priyanka Adhikari: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Neha Pandey, Priyanka Sati: Performed the experiments.

Anita Pandey: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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