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Proximate composition of *Ginkgo biloba* L. leaves, from Indian Himalayan Region, with reference to their importance in natural medicine

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Abstract: Plant nutrients, from the medicinal plants in particular, play important role in the treatment of a range of diseases. *Ginkgo biloba* L. belongs to family Ginkgoaceae, a medicinal tree, that has been recognized for its potential and uses in herbal medicine. The aim of the present study was to investigate the nutrients in terms of proximate contents (moisture and ash content and total carbohydrate and protein) and the trace elements (Na, K, Cu, Cr, Zn and Fe) in *G. biloba* leaves collected from different locations in three seasons from Indian Himalayan region (IHR). Statistically significant variations in the proximate contents and the trace elements were estimated with respect to locations and seasons. The proximate contents and the trace elements analyzed from different locations showed that substantial amount of these contents were present with slight variation, in respect to each location. The best results for proximate contents and the trace elements were obtained in autumn season (S3). Interactions between locations x seasons for all the parameters were significant ($p<0.05$) for proximate contents and the trace elements. The predominant geographic indicator, i.e. altitude, showed significant ($p<0.01$) correlation with respect to the moisture content of *G. biloba* leaves.

Keywords: *Ginkgo biloba,* Proximate composition, Trace elements, Medicinal value, Indian Himalayan region (IHR)

Medicinal plants (MPs) play an important role in health care of majority of people all across the globe. India has one of the richest and most diverse cultural traditions associated with the cultivation and use of medicinal plants. As per the traditional medicine program of the World Health Organization (WHO), nearly 80% of the world population, particularly rural communities, rely on the plant based natural products, including antimicrobials (Dubey *et al.*, 2004, Pandey & Agnihotri, 2015). These

natural products are used orally, therefore, knowledge of the proximate composition of these products and raw material used herein play an important role in assessing nutritional implication and health effects (Taiga, 2008). As far as the herbal drug's standardization is concerned, WHO has also emphasized on the need and importance of determining proximate and nutrient analysis and the formulation of natural product have to pass through the standardized process (Rajani & Kanaki, 2008).

Ginkgo biloba L. (common name-maiden hair tree), often referred as the living fossil, is well known for its active ingredients namely terpene trilactone (ginkgolides and bilobalide) and flavonoid glycosides. The leaves and seeds of *Ginkgo* have been in use in Traditional Chinese Medicine (TCM). *Ginkgo* leaves (Figure 1) have unique type of bilobed structure and contain various types of primary and secondary metabolites along with the micro and macronutrients. *Ginkgo* leaf extracts in particular, has received much attention with respect to the treatment of demential disorder, such as concentration difficulties and memory impairment (Alzheimer's disease) and also for antioxidant, antiasthmatic, wound healing and neuroprotective properties (Oken *et al*., 1998; Packer, 1999; Bradly *et al*., 2000). Besides, *Ginkgo* leaf extracts have also been studied for their antimicrobial potential against a range of microorganisms (Sati *et al*., 2012). The use of *Ginkgo* has been growing at a very rapid rate worldwide at 25% per year in the open world commercial market. Presently, there are around 142 *Ginkgo* products in the global market (Singh *et al*., 2008; van Beek & Montoro, 2009). In view of the existence of limited number of established trees of *Ginkgo* in India and its medicinal importance, the species is receiving due attention towards its propagation and conservation (Kumar *et al*., 2009; Pandey *et al*., 2009; Pandey *et al*., 2014; Gopichand & Meena, 2015).

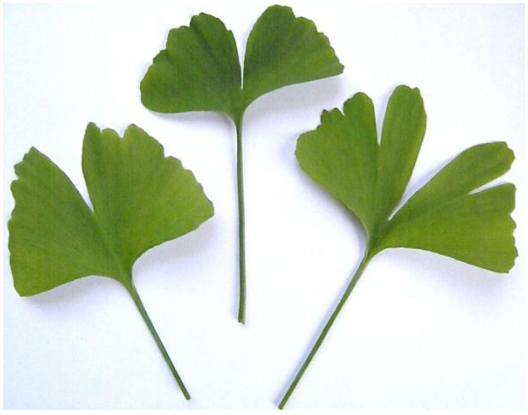

Figure 1. *Ginkgo biloba* leaves

Proximate composition, including moisture, ash and volatile matter contents and carbohydrates, fats and proteins, gives valuable information and help to access the quality of the plant sample. It provides the overall nutritional information of the plant part used in drug preparation or as food. Carbohydrates, proteins and many mineral elements are either initiating the synthesis of important medicinal ingredients or indirectly affecting their synthesis in the plant. While the phytochemicals (total phenolic and flavonoid contents) and antioxidants in leaf extracts of *Ginkgo* with reference to location, seasonal variation and solvent system have been reported in a recent study (Sati *et al*., 2013), the present study provides a picture of quality / nutritional aspects of the plant species from the same locations (GB1 to GB5) in IHR.

The *Ginkgo* leaves were collected from five different locations: GB1 (Kalika, Almora, 1742 amsl), GB2 (Chaubatia, Almora, 2040 amsl), GB3 (Snowview, Nainital, 2260 amsl), GB4 (Highcourt, Nainital, 2046 amsl) and GB5 (Glenthorn, Nainital, 2002 amsl) in Uttarakhand, India (Figure 2). The leaves were collected in three different seasons (spring (S1; April, 2014), rainy (S2; July, 2014) and autumn (S3; October, 2014).

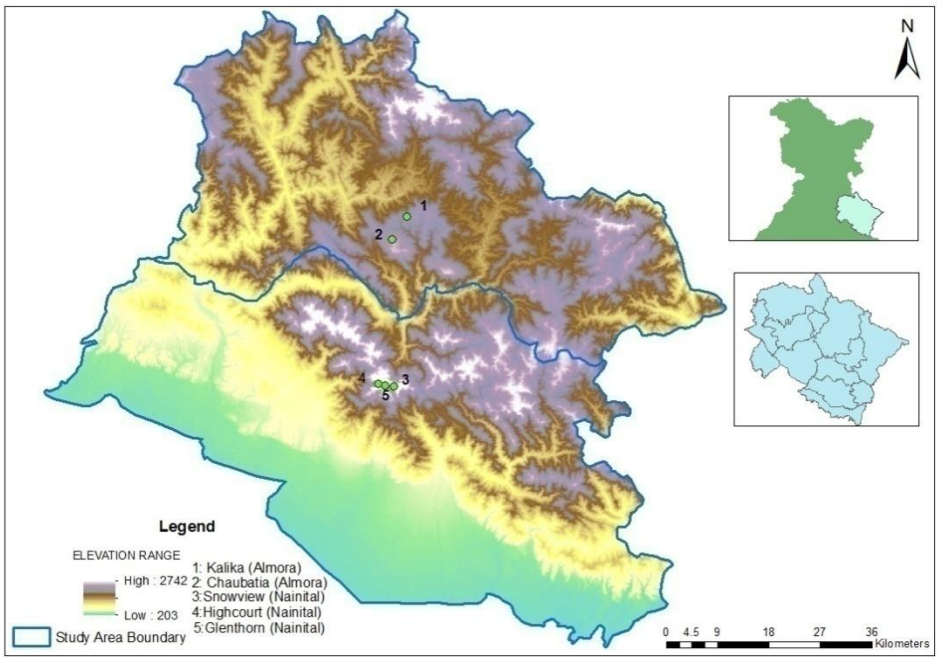

Figure 2. Location Map: *Ginkgo biloba* trees in Uttarakhand

Ginkgo leaves were analyzed for chemical composition (moisture, proteins, carbohydrates and ash) using the AOAC procedures (AOAC 1995). The nutritional analyses (ash, proteins, and total carbohydrates) of all the samples were determined in triplicate. Briefly, the moisture and ash were determined using weight difference method. For estimation of moisture content, leaves were oven dried at 105°C for 24 h. Crude protein was estimated by Lowry's assay (Lowry *et al*., 1951). Total Carbohydrate was determined by Anthrone method (Sadasivam & Manickam, 2005).

One g of the dried leaf powder was used for making ash for further analysis. Ash of dried leaf powder was produced through muffle furnace (MAC, MSW 251) at 550 ºC temperature for 4 h. The ash was dissolved in 5 ml of 20% HNO_3 and was analyzed for metal ions (Sodium (Na) and Potassium (K) using flame photometer (Systronics Mediflame 127) and Copper (Cu), Manganese (Mn), Zinc (Zn), Iron (Fe) and Chromium (Cr)) using Electrothermal- Atomic absorbance spectrometer (Varian, AA2802). The percentage of different elements in these samples was determined by the corresponding standard calibration curve.

All the experiments were carried out in triplicate. Means and standard error for each

sample was calculated through two way ANOVA and significance level was determined at $p<0.05$ and significant difference was separated using Duncan's multiple Range Test (DMRT) using SPSS (version 16).

The proximate compositions (moisture content, protein and total carbohydrate) of the samples significantly varied ($P<0.05$; Table 1) with respect to three seasons (S1= spring, S2= rainy and S3= autumn) in all the five locations. The moisture content of leaves varied from 56.19 to 78.18%, with the highest values in rainy season followed by spring and autumn and the highest moisture content was recorded in GB3 (69.17%). The total ash content was recorded from 6.43- 13.87 % in three seasons, however, the variations were not statistically significant and the total ash content was observed highest in GB4 (14.44%). These results are in line with the earlier reports (Pharmacopeia Slovaca 1997a,b). Ash is the inorganic residue, remaining after water and organic matter have been removed by heating, which provides a measure of total amount of minerals (Zhou *et al*., 2013). Minerals are not destroyed by heating and they have a low volatility as compared to other components (Sunggyum, 2005).

Protein and carbohydrate concentration ranged between 217-487 mg/g and 0.610-2.164 mg/g, respectively. The high value of protein was observed in GB1 (413 mg/g), while the lowest concentration was found in the GB2 (261mg/g). Proteins are the primary metabolites and their quality and quantity in the seeds and leaves are considered for the selection of plants as medicine (Hussain *et al*., 2009). Zhou *et al*. (2013) has reported antioxidant properties of proteins present in *G. biloba*, which may serve as a good source of desirable antioxidants for nutraceutical and pharmaceutical ingredients. Total carbohydrate content was observed maximum in GB2 (1.70 mg/g) followed by GB5 (1.34 mg/g). Carbohydrates, especially oligosaccharides, polysaccharides, and glycoconjugates make relevant part of the bioactive components of the natural products exploited in therapeutics, diagnostics, food additives, and biomaterials (Thakur *et al*., 2012; Li *et al*., 2013).

Carbohydrate analysis is, therefore, very important for quality control (QC) of herbal medicine. The *Ginkgo* samples were found to be a good source of protein and to some extent of carbohydrate as well. Medicinal plants are mostly rich in various trace elements like zinc, iron, copper, chromium, manganese etc (Karak & Bhagat, 2010) that play important role in metabolic processes of human being (Iyengar 1989). Most of these elements work as co-factor activators in metal-ligand enzyme complexes (Valkovic, 1975) and pair up with vitamins in the metabolism of carbohydrate, fat and protein.

The trace elements showed a great level of variation ($p<0.05$) with respect to harvesting seasons of *Ginkgo* leaves in all the five locations (Table 2). The concentration of sodium and potassium varied from 112.83-281.33 mg/l and 1111.70-4533.70 mg/l, respectively. In case of location specific variations the highest Na concentration was observed in GB4 (227.17 mg/l), while the lowest concentration was observed in GB5 (139.78 mg/l). Similarly, the highest concentration of K (4095.40 mg/l) was found to be in GB3 with the lowest values in GB2 (1348.30 mg/l). The highest content of Na and K were found in autumn (S3) followed by spring (S1) and rainy (S2) seasons. The potassium content in *Ginkgo* leaves, in the present study, is on the lower side as compared to the earlier report from Bratislava, Slovakia, from different vegetation period (Czigle *et al*.,

2013). In the cited study, the K content has been reported higher in early May (22508.05 mg/l).

The highest concentration of all the macronutrients was estimated in the autumn season (Cu (18.88-88.17 mg/l), Mn (12.60-17.84 mg/l), Zn (12.77-18.95 mg/l), Fe (193-529 mg/l) and Cr (7.70-22.07 mg/l)) followed by spring (S1) and rainy (S2) seasons. In case of locations the Cu concentration was extremely high in GB4 (59.86 mg/l) as compared to GB1 (13.17 mg/l). For Mn, it was the highest in GB5 (15.34 mg/l) followed by GB1 and GB3 (14.92 and 14.12 mg/l). Zn contents were the highest in GB2 (16.86 mg/l), followed by GB4 (13.76 mg/l), GB1 (11.55 mg/l) and GB5 (10.70 mg/l). Fe content was highest in GB2 (445 mg/l) and lowest contents were found in GB3 (171 mg/l). Values for Cr contents of GB1 (10.90 mg/l) was slightly higher in comparison to other locations. In the present study, the Cu and Cr concentration were higher in comparison to the values reported by Yu *et al*. (1992) where the concentration of trace elements ranged from 1.1-6.6 mg/l for Cr, 15-73 mg/l for Mn, 74-399 mg/l for Fe, 2.8-6.9 mg/l for Cu, 6.1-17.1 mg/l for Zn in *Ginkgo* leaves collected from the Chinese province of Zheijang. However, the values for the rest of the elements (Mn, Fe and Zn) were as in case of this cited study (Yu *et al*., 1992). In general, it may be mentioned that interrelationship of several elements in medicinal plants suggest synergistic or antagonistic effects, thus providing various elements to the body in bio-available form in a balanced manner with almost no harmful effects (Paranjpe, 2001), except some environmental contaminants that can be avoided by collecting herbs grown in a clean and well controlled environment.

For studying the effect of location and seasons on the ash content, moisture content, carbohydrate, proteins and minerals of the *Ginkgo* plant, leaves were analyzed through factorial analysis, and bi-variable Pearson's correlation co-efficient were performed (Table 3&4). Factorial analysis revealed a significant interaction ($P< 0.05$, $P< 0.01$) of proximate and elemental analysis results among different locations and seasons. Altitude was positively correlated with moisture content ($p<0.01$) as compared to trace elements. Whereas, moisture content and different trace elements did not reveal any significant relationship with altitude. The variation in the trace elemental concentration in different environment can be attributed to several factors like absorption ability of the plants for respective minerals of the soils and climatic conditions (Hamurcu *et al*., 2010; Subramanian *et al*., 2012).

Conclusions

The growth season as well as the location significantly influenced the proximate content and trace elements of *Ginkgo.* These differences can be accredited to the seasonal changes in terms of temperature and humidity, also to different stage of plant metabolism. Autumn (S3) was the best season for the proximate contents and the trace elements of *Ginkgo.* The plants, which have been used for herbal drug preparation or as phytomedicine, should contain right amount of minerals and other important components as even in trace quantities these may control, trigger, or stop the biochemical reactions in the living organisms and modulate pharmacological activity of medicinal plants. This study can contribute to the future investigations on the interactions between macro and micro-minerals in this plant and the rest of its biologically active components.

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Table 1. Proximate composition of *Ginkgo biloba* leaves

Plant locations	Seasons	Moisture (%)	Ash (%)	Protein (mg/g)	Carbohydrate (mg/g)
GB1	S1	58.41±0.03b	10.00±0.62a	331±0.40c	0.686±0.007c
	S2	58.86±0.01a	10.03±1.51a	422±0.29b	0.932±0.010b
	S3	56.19±0.00c	13.87±1.23a	487±0.24a	1.263±0.008a
	Mean	**57.82±0.41**	**11.41±0.85**	**413±22.65**	**0.96±0.008**
GB2	S1	57.97±0.03a	11.00±1.93a	221±0.13c	1.048±0.012c
	S2	58.29±0.00a	9.00±1.72a	261±0.29b	1.873±0.003b
	S3	56.91±0.17b	10.73±2.40a	300±0.37a	2.164±0.166a
	Mean	**57.72±0.21**	**10.24±1.07**	**261±11.39**	**1.70±0.035**
GB3	S1	71.44±0.00b	6.43±1.60a	200±0.18c	0.779±0.014c
	S2	78.18±0.16a	10.60±2.55a	218±0.47b	0.986±0.004b
	S3	57.90±0.00c	10.17±1.44a	410±0.31a	1.147±0.009a
	Mean	**69.17±2.98**	**9.07±1.17**	**276±33.54**	**0.97±0.005**
GB4	S1	58.95±0.05b	12.90±0.10b	217±0.29c	0.610±0.007c
	S2	60.96±0.06a	9.53±2.23a	292±0.18b	1.090±0.004b
	S3	58.45±0.00c	14.23±0.90a	473±0.31a	1.174±0.009a
	Mean	**59.45±0.39**	**14.44±1.89**	**327±37.99**	**0.96±0.004**
GB5	S1	57.40±0.00b	8.17±1.16a	204±0.29c	1.073±0.007c
	S2	58.15±0.00a	11.13±2.42a	278±0.24b	1.336±0.009b
	S3	56.55±0.00c	11.83±1.63a	319±0.31a	1.610±0.007a
	Mean	**57.36±0.23**	**10.38±1.07**	**267±16.75**	**1.34±0.004**

GB1 Kalika; GB2 Chaubatia; GB3 Snow-view; GB3 Highcourt; GB5 Gleinthorn; S1 Spring; S2 Rainy; S3 Autumn; Values are mean± standard error, Mean values followed by same letter (s) in a column are not significantly different ($p<0.05$) based on DMRT

Table 2. Trace elements concentration in *Ginkgo biloba* leaves

Plant locations	Seasons	Concentrations of trace elements (ppm)						
		Macronutrients		Micronutrients				
		Na	K	Cu	Mn	Zn	Fe	Cr
GB1	S1	163.50±0.29b	3384.50±0.58b	10.60±0.66ab	13.74±0.55b	10.19±0.15b	253±0.00b	10.29±0.46a
	S2	142.00±1.00c	3193.30±0.44c	10.03±3.76b	14.24±0.99b	6.70±0.10c	203±14.53c	10.35±1.22a
	S3	273.67±0.17a	3473.30±0.60a	18.87±1.71a	16.78±0.26a	17.75±0.14a	303±0.000a	12.07±0.86a
	Mean	**193.06±20.39**	**3350.40±41.31**	**13.17±2.04**	**14.92±0.60**	**11.55±1.63**	**325±14.9**	**10.90±0.85**
GB2	S1	177.67±0.44b	1456.70±0.83b	23.50±0.90a	8.01±0.42b	17.53±0.10b	426±2.00b	7.20±0.46ab
	S2	137.33±1.20c	936.67±0.44c	12.63±1.14b	8.49±0.48b	14.10±0.10c	376±3.21c	5.87±0.28b
	S3	182.17±0.60a	1651.50±8.87a	22.27±1.16a	12.60±1.80a	18.95±0.21a	529±1.76a	10.29±1.48a
	Mean	**165.72±7.14**	**1348.30±106.68**	**19.47±1.07**	**9.70±0.90**	**16.86±0.70**	**445±22.6**	**7.79±0.74**
GB3	S1	171.17±0.73b	4442±0.1.04b	17.17±4.47b	15.14±2.36a	8.62±0.04b	163±6.67b	11.23±1.28a
	S2	126.50±0.87c	3310.70±0.44c	13.90±0.62b	11.61±0.77a	4.26±0.19c	156±6.67b	3.64±0.07b
	S3	181.83±0.33a	4533.70±0.73a	29.57±1.34a	15.61±0.95a	12.77±0.11a	193±6.67a	11.71±0.74a
	Mean	**159.83±8.48**	**4095.40±193.64**	**20.21±0.14**	**14.12±1.36**	**8.53±1.23**	**171±6.5**	**8.86±0.70**
GB4	S1	228.83±0.44b	1111.70±0.44b	22.73±1.64b	9.99±1.25b	13.54±0.16b	426±1.85b	8.75±0.75a
	S2	171.33±0.73c	1012.20±017c	18.67±1.43c	7.88±0.63b	8.80±0.15c	272±2.08c	4.07±0.43b
	S3	281.33±0.17a	1991.70±1.01a	35.17±1.75a	14.66±1.18a	18.08±2.23a	506±1.00a	9.01±0.47a
	Mean	**227.17±15.88**	**1371.80±155.62**	**25.52±1.61**	**10.84±1.02**	**13.47±1.49**	**401±34.4**	**7.28±0.55**
GB5	S1	138.33±0.60b	3312±0.29ab	20.67±0.74b	16.53±0.48a	9.32±0.10b	357±2.96b	4.14±0.68b
	S2	112.83±0.60c	3255.80±2.20c	20.87±1.76b	11.66±0.08b	9.41±0.21b	293±0.67c	3.94±0.12b
	S3	168.17±0.33a	3486.30±0.73a	25.13±3.07a	17.84±0.71a	13.36±0.07a	395±3.00a	7.70±0.28a
	Mean	**139.78±8.00**	**3351.40±34.70**	**22.22±1.85**	**15.34±0.42**	**10.70±0.67**	**348±14.9**	**5.26±0.36**

GB1 Kalika; GB2 Chaubatia; GB3 Snowview; GB3 Highcourt; GB5 Glenthorn; S1 Spring; S2 Rainy; S3 Autumn; Values are mean± standard error, Mean values followed by same letter (s) in a column are not significantly different ($p<0.05$) based on DMRT

Table 3. Analysis of variance for determining effect of location, seasons and their interaction on nutrients of *Ginkgo biloba* (values of mean squares is given)

Source of variation	DF	Moisture content	Ash content	Carbohydrate	Protein	Na	K	Cu	Mn	Zn	Fe	Cr
Location	4	18220***	3.39**	510.14***	509.55***	8473***	6498000***	242.74***	25.112***	197.32***	1097***	197.56***
Seasons	2	9076***	1.505	187.48***	187.41***	19460***	804400***	77.12***	0.781	8.22**	383.91***	8.26**
Location*Seasons	8	4457***	2.33**	196.35***	196.15***	1456***	113000***	79.80***	12.56***	15.13***	34.74***	15.14***

df = degree of freedom; ***P<0.05; ** P < 0.01

Table 4. Correlation among altitude, moisture content, and trace elements in *Ginkgo biloba*

	Altitude	Moisture content	Na	K	Cu	Mn	Zn	Fe	Cr
Altitude	1								
Moisture content	0.572**	1							
Na	-0.173	-0.259	1						
K	0.083	0.261	-0.157	1					
Cu	0.153	-0.005	0.056	-0.481**	1				
Mn	-0.193	-0.043	-0.137	0.581**	-0.113	1			
Zn	-0.658**	0.065	0.148	0.217	-0.338*	0.274	1		
Fe	-0.183	-0.589**	0.356*	-0.670**	0.19	-0.394**	-0.398**	1	
Cr	-0.658**	0.065	0.148	0.217	-0.338*	0.274	1.000**	-0.398**	1

Level of significance: *p<0.05; **p<0.01