

Original article

Effect of drying on phytochemical composition of lemongrass (*Cymbopogon citratus* (DC.) Stapf) powder

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Abstract

In the present investigation, the effect of drying on phytochemical composition of lemongrass powder was evaluated. The lemongrass powder was prepared by traditional (Sun and shade) drying and cabinet drying (45°C). The qualitative determination of phytochemicals such as alkaloid, flavonoid, tannin, saponin, phenol, carbohydrate and glycoside in aqueous, ethanol, acetone and chloroform extract were carried out. It was revealed that, next to ethanolic extract, aqueous extract showed the presence of rich variety of secondary metabolites. The total phenolic content of cold and hot extract was 2.6 ± 0.02 and 3.8 ± 0.02 mg of gallic acid equivalent/g dw basis. The total flavonoid content in cold and hot percolation were found to be 7.6 ± 0.03 and 8.1 ± 0.03 µg/g of quercetin equivalent dw basis. The alkaloids, saponins, steroids and tannin contents were 1.64 ± 0.05 , 1.11 ± 0.05 , 0.45 ± 0.05 and 0.93 ± 0.05 g/100 g. The minimum reduction of total phenol content observed in shade drying treatment in cold (15.38%) and hot (13.15%) aqueous extract, whereas maximum reduction in cold (38.46%) and hot (26.31%) extract by cabinet drying. The minimum reduction in flavonoid contents were found in shade drying in cold (3.94%) and hot (3.70%) aqueous extract, whereas maximum reduction in cold (13.15%) and hot (13.58%) aqueous extracts by cabinet drying. The alkaloids, steroid, saponin and tannin recorded their highest values in cabinet drying, i.e., 2.61, 0.72, 2.21 and 2.03 g/100 g, respectively over sun and shade drying.

Key words: Lemongrass, powder, phytochemical, total phenol, flavonoid

1. Introduction

The plants are variable raw materials as many factors contribute to this variation (Butt *et al.*, 2018). The use of herbal medicine started to decline after the 1960 as large quantities of resources and money were used to promote synthetic medication and their immediate action. Synthetic medications offer fast relief in dose dependent (Zulfa *et al.*, 2018). In view of human health, there is an urgent need to utilize the medicinal plants properly and expeditiously throughout the world. Authentic good quality medicinal plants and plant products including extracts are only scarcely available for the present day world population, particularly for the urban population (Muhammed Majeed, 2017).

Lemongrass (*Cymbopogon citratus* (DC.) Stapf) is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root belongs to family Poaceae. It is locally known by different names such as 'Gawati Chah', 'Nibugrass', 'Puthiganda', *etc.*, in different languages. Lemongrass has numerous health benefiting essential oils, chemicals, minerals and vitamins that are known to have anti-oxidant and disease preventing properties. Some plants having the medicinal value in the form of chemical substances that produce a definite physiological action on the human body are called

phytochemicals. The presence of secondary metabolites in plants is characterized by their ability to provide defences against biotic and abiotic stress (Ballhorn *et al.*, 2009). The phenolic compounds present in *C. citratus* leaves are quercetin, kaempferol and apigenin, catechol, chlorogenic acid, caffeic acid and hydroquinone (Tapia *et al.*, 2007). Recently, the five flavonoids compounds isolated from *C. citratus* are C-glycosylflavonoids (orientin, isoorientin, isoscoparin, swertiajaponin and isoorientin 2"-O-rhamnoside) (Cheel *et al.*, 2005). The *C. citratus* leaves also had two terpenoids (cymbopogonol and cymbopogone) in coating (Avoseh *et al.*, 2015).

The lemongrass has a very wide demand in nutritional, medicinal and flavoring industry. But, it is not stored as fresh for long time at ambient condition because it rotten after long periods (Mukherjee, 2002). Hence, the aim of this study to evaluate the effect of drying methods on phytochemical composition of lemongrass powder.

2. Materials and Methods

The dark green coloured lemongrass var. *Cymbopogon citratus* (DC.) Stapf leaves were collected and authenticated from the Department of Botany, College of Agriculture, Vasant Rao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra.

2.1 Preparation of lemongrass (*C. citratus*) powder

2.1.1 Sun drying

The lemongrass leaves were placed in a tray one layer deep on a table. Air was allowed to circulate below as well as above the leaves to speed up drying time. The leaves in the tray on the table were

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placed in direct sun and turned occasionally. These leaves were placed in direct sun for several hours and the weight of the leaves was being measured at intervals of 1 h until the weight became constant.

2.1.2 Shade drying

In shade drying, the lemongrass leaves were spread on filter papers and kept in the room which was well ventilated. Natural current of air was used for shade drying of the lemongrass leaves. The weight of leaves was measured at interval of 1 h until the weight became constant.

2.1.3 Cabinet tray drying

Cabinet tray drying was carried out in the Department of Food Engineering, College of Food Technology, VNMKV, Parbhani. It consists of a 0.8 kW axial flow fan blowing at air velocity of 3.5 m/s over the heating elements into a drying chamber with perforated trays. The dryer casing is lagged with cushion to give it a compact look. The special arrangement was designed for loading and unloading in the dryer. The prepared lemongrass leaves were spread on the tray and placed into the cabinet tray drier at 45°C temperature and the weight was being measured at interval of 30 min until a constant weight was being recorded.

2.2 Qualitative determination of phytochemicals

2.2.1 Preparation of extract from lemongrass leaves powder

The powdered lemongrass leaves samples (50 g/250 ml) were extracted successively with chloroform, ethanol, acetone and water using soxhlet apparatus at 55-85°C for 8-10 h in order to extract the polar and non-polar compounds (Elgorashi and Staden, 2004). The powdered material was air dried and then used for solvent extraction. The solvents of the respective extracts were reduced under room temperature and stored at 4°C for further use.

Chloroform, ethanol, aqueous and acetone extracts were used for preliminary phytochemical analyses (Harbone, 1973; Kokate *et al.*, 1995). The following qualitative tests were used for lemongrass extract.

i. Test for alkaloid

Wagner's test: 10 mg of extract was taken and few drops of Wagner's reagent (Dissolve 2 g of iodine and 6 g of KI in 100 cm³ of water) was added. The indication of reddish brown precipitate showed the presence of alkaloids.

ii. Test for flavonoid

Lead acetate test: 10 mg of extract was taken and few drops of 10% lead acetate solution was added. The presence of flavonoids indicated by formation of yellow colour precipitate.

iii. Test for tannin

Ferric chloride test: To 5 ml of the sample, a few drops of 0.1% ferric chloride were added. The presence of a brownish green or blue black colour indicated that the material possessed tannins.

iv. Test for saponin

Foam test: 0.5 mg of extract was diluted with 20 ml distilled water and shaken well in a graduated cylinder for 15 min. After shaking, the foam formed to the length of 1 cm showed presence of saponins.

v. Test for phenol

Sodium hydroxide test: 5 mg of extract was dissolved in 0.5 ml of 20% sulphuric acid solution. The addition of aqueous sodium hydroxide solution which forms blue colour due to presence of phenols.

vi. Test for carbohydrates

Fehling's test: 5 ml of Fehling's solution was added to 0.5 mg of extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing power.

vii. Test for glycosides

Glycoside test: 0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. The presence of glycosides gives yellow colour.

2.2.2 Quantitative determination of phytochemicals

i. Alkaloid

5 gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 min, the sample was filtered and obtained extract was concentrated with water bath upto ¼ of its original volume. The ammonium hydroxide was added dropwise to extract till the precipitation process will stop. The obtained solution was kept for settle and formed precipitates were collected and washed with dilute ammonium hydroxide and then filtered. The alkaloid was obtained in the form of residue which was dried and weighed (Harbone, 1973).

$$\text{Alkaloid (\%)} = \frac{W_3 - W_2}{W_1} \times 100$$

where,

W_1 = Initial weight of sample,

W_2 = Weight of the extract and

W_3 = Final weight of the residue

ii. Steroid

1 ml of test extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4 N, 2 ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70 ± 2°C for 30 min with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank (Madhu *et al.*, 2016).

iii. Saponin

2 gram of the sample was weighed into a 250 ml beaker and 100 ml of isobutyl alcohol was added. The mixture was shake well with shaker for 5 h to ensure uniform mixing. The mixture was filtered using No 1 Whatman filter paper into 100 ml beaker containing 20 ml of 40% saturated solution of magnesium carbonate. The mixture obtained again was filtered using Whatman filter paper No 1 to obtain a clean colourless solution. 1 ml was added into 50 ml volumetric flask using pipette, 2 ml of 5% iron (iii) chloride (FeCl₃) solution was added and made up to the mark with distilled water. It

was allowed to stand for 30 min to develop the colour. The absorbance was read against the blank at 380 nm (Bruneton, 1999).

$$\text{Saponin} = \left[\frac{\text{Absorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard}} \right]^{-1}$$

iv. Tannin

The sample was weighed (0.2 g) into a 50 ml sample bottle. 10 of 70% aqueous acetone was added and covered. The bottle was put in an ice bath shaker and shaken for 2 h at 30°C. The solution was then centrifuge and the supernatant was collected, 0.2 ml of the solution was pipetted into test tube and added 0.8 ml distilled water. Standard tannin acid solution was prepared from a 0.5 mg/ml of the stock and the solution made up to 1 ml with distilled water, 0.5 ml of Folin-ciocateau reagent was added to the sample and standard followed by 2.5 ml of 20% Na₂CO₃, the solution was then vortexed and allowed to incubate for 40 min at room temperature, its absorbance was read at 725 nm against a reagent blank concentration of the same solution from a standard tannic acid (Markkar and Goodchild, 1996).

2.3 Determination of total phenolic and total flavonoid content

2.3.1 Preparation of cold and hot percolations

Fifty-gram (50 g) of fresh lemongrass in triplicates were put in brown envelopes and dried at a temperature of 65°C to constant weight. Using mortar and pestle, the crispy leaves were homogenized into powder. Using cold (30°C) temperature of percolation, a 0.7 g powder from each sample was added separately to 25 ml of double distilled deionized water and maintained at this temperature for 4 h with continuous shaking. The same procedure was followed for hot (60°C) percolation and extracts were filtered. Another 25 ml of double-distilled deionized water was added to the mark and the extraction process repeated. The filtrates were pooled to provide a total of 50 ml extract for each sample (Godwin *et al.*, 2014).

i. Determination of total phenolic content

Total phenolic contents (TPC) from the extracts were quantified using Folin-Ciocalteu's method (Mohan *et al.*, 2017). First, 5 ml

Folin-Ciocalteu reagent was added to 1 ml sample in tube. Then, 4 ml of 7.5% (w/v) sodium carbonate was added to mixture. After 60 min of incubation at room temperature (32 ± 1°C), the absorbance was read at 765 nm against blank. The results were expressed as mg gallic acid equivalent per gram dry weight basis of fresh sample (mg GAE/g dw basis). The total phenolic content was calculated by using the following formula;

$$C = c V/m$$

where, C = total phenolic content mg GAE/g dry extract

C = concentration of gallic acid obtained from calibration curve in mg/ml

V = volume of extract in ml

m = mass of extract in gram.

ii. Determination of total flavonoid content

The total flavonoid content of cold and hot extracts were determined using a slightly modified method reported by Mohan *et al.* (2017). A 0.5 ml of appropriately diluted sample was mixed with 0.5 ml methanol, 50 µl of 10% AlCl₃, 50 µl of 1 mol l⁻¹ potassium acetate and 1.4 ml water and allowed to incubate for 30 min at room temperature. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm. The total flavonoid was calculated using quercetin as standard using formula,

$$\text{TFC} = \frac{A \times \text{DF}}{A^{1\%}_{1\text{cm}} \times (w - \text{ld})}$$

where, A = Absorbance

DF = Dilution Factor

A^{1%}_{1cm} = Specific absorption by AlCl₃

w = Mass of plant material

ld = Loss on drying

3. Results

3.1 Phytochemical screening of lemongrass (*C. citratus*) leaves

In the present investigation, secondary metabolites were qualitatively analysed using lemongrass leaves. The results are presented in Table 1.

Table 1: Phytochemical screening of lemongrass (*C. citratus*) leaves

Sr. No.	Phytochemical constituents	Extracts				Name of the test
		Chloroform	Ethanol	Acetone	Aqueous	
1	Alkaloid	+	+	+	+	Wagner's test
2	Flavonoid	+	+	+	+	Lead acetate test
3	Tannin	+	+	+	+	Ferric chloride test
4	Saponin	-	-	+	+	Foam test
5	Phenol	-	+	-	+	Sodium hydroxide test
6	Carbohydrate	-	+	-	-	Fehling's test
7	Glycoside	-	+	-	-	Glycoside test

Table 1 revealed that chloroform, ethanolic, acetonetic and aqueous extract of leaves contained alkaloid, flavonoid, tannin. However, carbohydrate and glycosides were detected in ethanolic extract of lemongrass leaves. Aqueous extract also showed the presence of saponin and phenol. Whereas, saponin is visible in acetone extract and phenol in ethanolic extract. Next to ethanolic extract, aqueous extract showed the presence of rich variety of secondary metabolites. Chloroform and acetone extract showed the less variety of these secondary metabolites. The similar observations were also detected by Umar *et al.* (2016) who reported that, phytochemicals such as flavonoids, carbohydrates, tannins, alkaloids, steroids and phytosteroids were detected except glycosides and phenol that were absent in the acetone and chloroform leaf extracts.

3.2 Phytochemical composition of lemongrass (*C. citratus*) leaves

Phytochemical analysis is of paramount importance in identifying new source of therapeutically and industrially valuable compounds from medicinal plants have been chemically investigated. In the present study, phytonutrients are quantitatively analysed and results are presented in Table 2.

Table 2: Phytochemical constituents of lemongrass (*C. citratus*) leaves

Sr. No.	Phytonutrients	Values (g/100 g)
1	Alkaloid	1.64 ± 0.05
2	Steroid	0.45 ± 0.05
3	Saponin	1.11 ± 0.05
4	Tannin	0.93 ± 0.05

Each value is average of three determinations.

The alkaloids and saponins contents were 1.64 ± 0.05 and 1.11 ± 0.05 g/100 g, respectively. Alkaloids are formed as metabolic by-product and have been reported to be responsible for the antibacterial activity (Okwu, 2004). Saponins are glycosides which has hypocholesterolemic effects and, thus may aid in lessening the metabolic burden on liver (Olivebever, 1986). The data showed that, lemongrass leaves contain lower amount of steroids (0.45 ± 0.05 g/100 g) as compared to other phytonutrients. Steroids play critical roles in a number of disorders, including malignancies like prostate cancer, where steroid production inside and outside the tumour promotes cancer cell aggressiveness (Lubik *et al.*, 2016). Tannins are plant polyphenols which have medicinal properties such as antimicrobial, anti-inflammatory and astringent activity (Chung *et al.*, 1998). The tannin content in lemongrass leaves was 0.93 ± 0.05 g/100 g. This supports the use of lemon juice in herbal medicines for the treatment of varicose ulcer, haemorrhoids, frostbite and burns by the native (Okwu, 2004). The results obtained for phytoconstituents in lemongrass leaves are in agreement with the report of Uraku *et al.* (2015).

3.3 Total phenolic and total flavonoid content of lemongrass leaves

The total phenolic and flavonoid content were determined by using cold and hot aqueous extract of lemongrass and results are presented in Table 3.

Table 3: Total phenolic and total flavonoid content of lemongrass leaves

Sr. No.	Sample	Parameter	
		Total phenol ^a	Total flavonoid ^b
1	Cold	2.6 ± 0.02	7.6 ± 0.03
2	Hot	3.8 ± 0.02	8.1 ± 0.03

Each value is average of three determinations.

^a mg of gallic acid equivalent/g dw basis

^b µg/g of quercetin equivalent dw basis

The results indicated that the total phenolic content of cold and hot extract was 2.6 ± 0.02 and 3.8 ± 0.02 mg of gallic acid equivalent/g dw basis respectively. Similar results were also reported by Godwin *et al.* (2014) and found phenolic content in cold and hot percolations ranged from 1.3 to 4.7 mg of gallic acid equivalent/g dw and 2.6 to 7.3 mg of gallic acid equivalent/g dw. The lemongrass containing phenolic compounds may have high antioxidant activity, which means that they could have positive effects on the preservation of foods quality and human health (Becker *et al.*, 2004).

The total flavonoid content of lemongrass expressed in µg/g of quercetin equivalent in dw basis. The total flavonoid content in cold and hot percolation were found to be 7.6 ± 0.03 and 8.1 ± 0.03 µg/g of quercetin equivalent dw basis, respectively. According to Godwin *et al.* (2014), the flavonoid content in cold and hot percolations were in the range from 6.9 to 11.3 and 6.9 to 12.9 µg/g of quercetin equivalent dw basis.

3.4 Effect of drying on phytochemical content of lemongrass (*C. citratus*) powder

The drying effects on phytochemical content of powder which was determined and tabulated in Table 4.

Table 4: Effect of drying on phytochemical content of lemongrass (*C. citratus*) powder

Sr. No.	Drying method	Phytochemicals (g/100 g)			
		Alkaloid	Steroid	Saponin	Tannin
1	Sun	1.88	0.57	1.38	1.14
2	Shade	2.16	0.64	1.82	1.76
3	Cabinet	2.61	0.72	2.21	2.03
	SE ±	0.1774	0.0274	0.038	0.0627
	CD at 5%	0.5675	0.0877	0.1216	0.2006

The alkaloids, steroid, saponin and tannin recorded their highest values when samples were cabinet dried *i.e.* 2.61, 0.72, 2.21 and 2.03 g/100 g, respectively. The alkaloid, steroid, saponin and tannin content after sun drying were 1.88, 0.57, 1.38 and 1.14 g/100 g which was less as compared to shade drying as, 2.16, 0.64, 1.82 and 1.76 g/100 g, respectively. In all drying processes, the intercellular spaces of tissues collapse, liberating more bioactive secondary metabolites such as steroidal alkaloids and polyphenols (DiCesare *et al.*, 2003). More intact cell wall structures and presence of degradation enzymes could be the reason for the lowest steroidal alkaloid content in the fresh samples (Yousif *et al.*, 1999).

This may be attributed to the absence of sunlight and low oxygen in the cabinet which may have favoured the formation and development

of saponins, tannins, steroid and alkaloids. The thermal processing can affect the phytochemicals by thermal breakdown that affect the integrity of the cell structure which then results in the migration of components, leading to losses by leakage or breakdown by various chemical reactions involving enzymes, light and oxygen (Schieber *et al.*, 2001).

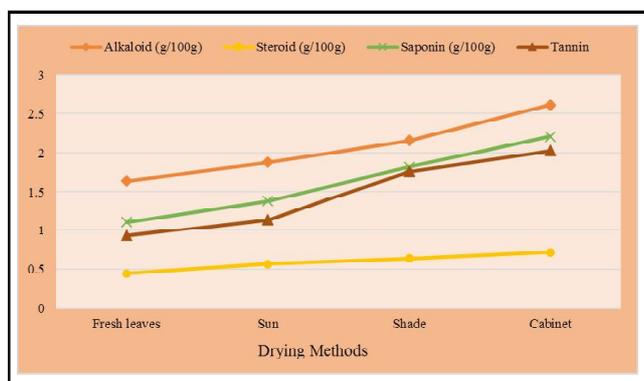


Figure 1: Effect of drying on phytochemical content of lemongrass powder.

These changes in phytochemical constituents during different drying techniques were found to be similar with the findings of Mbah *et al.* (2012), who found that the antinutrient tannin contents were increased by drying treatments. According to Rwubatse *et al.* (2014), the cabinet dried orange peel flour had higher values for alkaloid, saponin and tannin over sun and solar drying.

3.5 Effect of drying on total phenol and total flavonoid content of lemongrass (*C. citratus*) powder

The total phenol and total flavonoid were affected by drying methods and results obtained were tabulated in Table 5.

Table 5: Effect of drying on total phenol and total flavonoid content of lemongrass powder

Sr. No.	Drying method	Total phenol ^a		Total flavonoid ^b	
		Cold	Hot	Cold	Hot
1	Sun	2.0	3.1	6.8	7.4
2	Shade	2.2	3.3	7.3	7.8
3	Cabinet	1.6	2.8	6.4	7.0
	SE ±	0.113	0.0289	0.0419	0.1787
	CD at 5%	0.3615	0.0924	0.134	0.5717

^a – mg of gallic acid equivalent/g dw basis

^b – µg/g of quercetin equivalent dw basis

The minimum reduction of total phenol content after shade drying treatment was observed in cold (15.38%) and hot (13.15%) aqueous extract, whereas it showed the maximum phenolic content reduction in cold (38.46%) and hot (26.31%) extract by cabinet drying. The total phenol contents after drying were reduced from 2.6 to 1.6 mg of gallic acid equivalent/g in cold extract of lemongrass powder. In case of hot extract, total phenol content was reduced from 3.8 to 2.8 mg of gallic acid equivalent/g. During drying process, drying time was extended at lower temperatures, such that the samples had a longer duration of oxygen exposure resulting in increased redox activity and degradation of phenolic compounds (Hung and Duy, 2012).

Total phenol content (TPC) loss after air drying may be caused by enzymatic processes. Air drying did not immediately deactivate degraded enzymes such as polyphenol oxidases; therefore, they are able to degrade phenolic compounds before the plant materials are completely dry. Cabinet drying at 60°C temperature was shown to rapidly inactivate polyphenol oxidases present in herb materials; however, some of their initial activities may have occurred earlier and degraded some polyphenols (Lim and Murtijaya, 2007).

The minimum reduction in flavonoid contents were found after shade drying in cold (3.94%) and hot (3.70%) aqueous extracts, whereas, it showed the maximum reduction in flavonoid content in cold (13.15%) and hot (13.58%) aqueous extracts by cabinet drying (Table 5). The total flavonoid content of lemongrass powder from cold extract was reduced from fresh 7.6 to cabinet drying 7.6 µg/g of quercetin equivalent. In case of hot extract, total flavonoid content found in the range 7.0 to 8.1 µg/g of quercetin equivalent. In case of total phenol and flavonoid, not much statistical variations were observed.

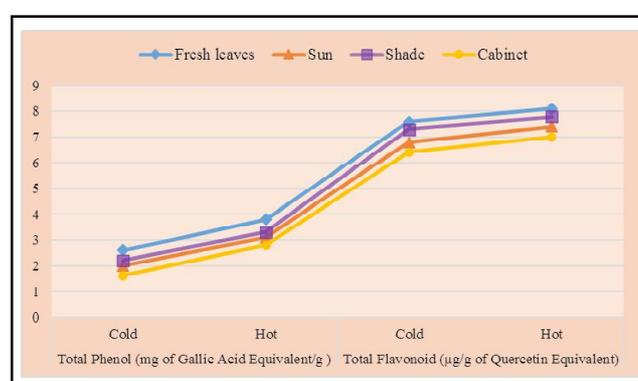


Figure 2: Effect of drying on total phenol and flavonoid content of lemongrass powder.

The losses in flavonoid content may due to drying time and temperature. Heating may breakdown some phytochemicals which affect cell wall integrity and cause a migration of some flavonoids component. In addition, the loss in flavonoids may due to breakdown or leakage by chemical reactions includes oxygen, enzymes and light (Davey *et al.*, 2000).

The reduction in total phenol and total flavonoid contents were also found by Mansour (2016), who found that drying process significantly decreased the phenolic and flavonoid contents of *Thymus vulgaris* extract. Fresh thyme has the highest contents of total phenolic (291.23, 322.12 mg GAE/100 g) and flavonoids (210.11, 296.22 mg QE/ 100 g), whereas the lowest levels were found in oven dried (200.10, 231.30 mg GAE/100 g, 100.11, 111.42 mg QE/100 g). Dry processing significantly decreased the phytochemical contents of thyme. The air shade drying contained more total phenolic, and flavonoids than air sun drying of thyme.

4. Conclusion

The current research has been carried out to analyse the phytochemical constituents by both qualitatively and quantitatively methods. The aqueous extract of lemongrass was showed the presence of alkaloid, flavonoid, tannin, saponin and phenols. The cabinet drying was found to be best method of drying for retention of phytochemicals. In case of cabinet drying, the total phenol, total

flavonoid, alkaloid, steroid, saponin and tannin content were found to be increased without affecting on the overall acceptability. Due to the presence of these phytochemicals, the lemongrass is known as herb which provides medicinal benefits. This study identified the ecotypes with high secondary metabolite which can help herbal drug manufacturer industries.

Conflict of interest

We declare that we have no conflict of interest.

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