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Effect of lemongrass powder on proximate and phytochemical content of herbal cookies

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Abstract
Lemon grass (*Cymbopogon citratus*) is a perennial grass belonging to family *Graminaceae* and grouped under genus *Cymbopogon*. In this study, the herbal cookies were prepared by incorporating lemongrass powder at 1, 3 and 5% level. The proximate and phytochemical analysis of fresh lemongrass leaves were analyzed. The effect of drying on proximate and phytochemical composition were also determined. The moisture, fat and fiber content were found decreased while protein, carbohydrate and ash content were increased. The phytochemical constituents get reduced when subjected to cabinet drying. The herbal cookies were found to increase in proximate composition with increase in level of lemongrass powder. The herbal cookies contain alkaloid (0.27%), saponin (0.20%), tannin (0.16%), steroids (0.10%), phenols (0.08%) and flavonoids (0.28%) in sensorially selected sample. The sample with 3% lemongrass powder was found to be highly acceptable over other sample. The required daily dose of was about 0.7 mg/kg of bodyweight/day.

Keywords: lemongrass, drying, phytochemicals, proximate composition, herbal cookies

Introduction
Lemon grass (*Cymbopogon citratus*) is a C4 tropical and sub-tropical grass. The lemongrass (*Cymbopogon flexuosus* (Steud) Wats) is a perennial grass belonging to family *Graminaceae* and grouped under genus *Cymbopogon*. It is of indigenous origin and is a medicinal and aromatic plant. It is locally known by different names such as 'Gawati Chah', 'Nibugrass', 'Puthiganda' etc. in different languages. The three species of lemongrass are found in India. *Cymbopogon flexuosus* is grown in East Indian States which is famous for its oil and has a good market. *Cymbopogon citrates* found in the West Indian States contain less citral. *Cymbopogon pendulus* in Jammu region contain higher high citral but its cultivation is limited (Tovar *et al*., 2010) [24].
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. Hence, lemongrass powder is preferred and it has huge demand in the world market. Traditionally, lemongrass powder is prepared by grinding the dried leaves.
In India, bakery products especially cookies are gaining wide popularity as processed foods in rural as well as in urban areas among people of all age groups. Some of reasons for such wide popularity of cookies varied best and acceptable returns profiles to suit easy market. Cookies are palatable and provide an excellent source of fat, carbohydrates, proteins, minerals and some vitamins. Cookies could conveniently supplement protein in diet of children who live in poverty areas. They could be prepared at central location and distributed to school children.
Cookie is chemically leavened product, also known as biscuit. Generally, the term biscuit is used in the European countries and cookies in the USA. Biscuits and biscuit like products have been made and eaten by man for centuries. Cookies are ideal for nutrient availability, palatability, compactness and convenience. They differ from other baked products like bread and cakes because of having low moisture content, ensure comparatively free from microbial spoilage and confer a long shelf life of the product (Wade, 1988) [25].
The aim of the present research was to prepare the herbal cookies with incorporation of lemongrass powder and investigate the effect of its addition on proximate composition and phytochemical content of cookies. Also to optimize the level of incorporation of lemongrass powder in preparation of cookies.

Materials and Methods
The Lemongrass (*Cymbopogon citratus*) was collected from the Department of Botany, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. The research was carried out in College of Food Technoogy, VNMKV, Parbhani.

Preparation of lemongrass powder

Evenly matured, disease free and sound leaves of lemongrass have to select. The leaves are washed with clean water and subjected to the treatments like cutting, blanching and drying (Lonkar *et al*., 2013) [12]. The lemongrass leaves will dry using cabinet drying at 45^{0}C for 7 h (Hanna *et al*., 2012) [8]. The dried leaves were grinded, sieved (80 mesh), packaged and stored.

Preparation of herbal cookies

Firstly, oven was preheated at 170°C. The butter was beaten in a food mixer and sugar was added. Then, sifted flour sample was added in the mixture. The mixture was then mixed and kneaded until it became dough. The dough was moulded into balls, placed on the greased tray and finally, baked in the oven for 15 minutes until a golden brown colour was obtained. They were allowed to cool before being packed in polyethylene bag and stored in cold and dry place until further analysis being done (Norhidayah *et al*., 2014) [18].

Table 1: Standard Recipe for Cookies

Ingredients	Amount (g)
Maida	100
Sugar	60
Fat	45
Baking Powder	1.5
Ammonium Bicarbonates	1.5
Milk	As Required

Table 2: Formulation of Composite flour (%) for Cookies

Sample	Maida (%)	Lemongrass Powder (%)
Control	100	0
A	99	1
B	97	3
C	95	5

Proximate Analysis

The proximate composition like protein, carbohydrate, fat, crude fibre and ash were measured as per standard method given in AOAC, 1990.

Phytochemical Analysis

Alkaloid Determination

Five 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, this was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was alkaloid which was dried and weighed (Harbone, 1973).

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

Where, W_1 =initial weight of sample,

W_2 =weight of the extract and

W_3 = final weight of the residue

Total flavonoid determination

Ten gram of the sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered using What man filter paper No. 42 (125 mm). The filtrate was transferred into crucible and evaporated into dryness over water bath and weighed to a constant weight (Bohm, 1994) [4].

Tannin Determination

Finely grounded sample was weighed (0.2g) into a 50 ml sample bottle. Ten of 70% aqueous acetone was added and properly covered. The bottle was put in an ice bath shaker and shaken for 2 hrs at 300ºC. The solution was then centrifuge and the supernatant stored in ice, 0.2 ml of the solution was pipette into the test tube and 0.8 ml of distilled water was added. Standard tannin acid solution was prepared from a 0.5 mg/ml of the stock and the solution made up to 1ml with distilled water, 0.5 ml of Folinciocateau reagent was added to the sample and standard followed by 2.5 ml of 20% Na_2CO_3 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 curve prepared (Markkar, 1996) [14].

Saponin Determination

Two gram of the finely grinded sample was weighed into a 250 ml beaker and 100 ml of Isobutyl alcohol was added. Shaker was used to shake the mixture for 5h 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. One (1 ml) was added into 50 ml volumetric flask using pipette, 2 ml of 5% iron (iii) chloride ($FeCl_3$) solution was added and made up to the mark with distilled water. It was allowed to stand for 30 min for the colour to develop. The absorbance was read against the blank at 380 nm (Bruneton, 1999) [5].

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

Determination of total phenol content

Preparation of acetone extract

The sample was homogenized with chilled phosphate buffer (0.05M, p^H 7.5) using a homogenizer. The homogenate was made up to 80% acetone with respect to acetone by adding chilled acetone and mixed thoroughly and filtered using cheesecloth. The residue was washed with 80% chilled acetone, filtered and air dried (acetone powder). The filtrates (80% acetone extract) were combined and kept in 4^{0}C for further studies. The extractability of 80% ethyl alcohol forth extraction of bioactive compounds from orange and pomace peel also carried out in the same way using ethyl alcohol instead of acetone.

The 80% acetone extract used for the estimation of total phenolic compounds, anthocyanin and carotenoid contents and evaluated for antioxidant activity. The dried powder obtained after filtering was used for the estimation of dietary fibre and enzymes

Determination of total phenolic content

The sample extracts with 80% acetone, 80% ethanol and 0.05M sodium phosphate buffer (pH 7.5) were centrifuged for 15 min at 10,000 X g. The clear supernatants obtained were subjected to total polyphenol estimation using the method of Swain and Hills (1959) [23]. The 0.5 ml of extract, 4.5 ml of

ethanol was added and to this 0.5 ml phenol reagent (Folin-Ciocalteu reagent, diluted 1:2 with water) was added and the contents were incubated at room temperature for 3 min to this 1 ml of saturated Na_2Co_3 was added and the reaction mixture was incubated at room temperature for 60 min. The absorbance was recorded at 675 nm; Gallic acid was used as a standard. The total polyphenol content in the extract was expressed as gallic acid equivalents (GAE).

Sensory Analysis

The prepared herbal cookies were organoleptically evaluated on the basis of 9-point hedonic scale. The level of addition of lemongrass powder in cookies were optimized on the basis of sensory score.

Results and Discussion

Proximate Composition of Lemongrass (*Cymbopogon citratus*) leaves

The proximate composition of lemongrass leaves was carried out and results obtained were tabulated in Table 3.

Table 3: Proximate composition of lemongrass leaves

Content	Value
Moisture (%)	71.03
Protein (%)	3.83
Carbohydrate (%)	20.73
Fat (%)	4.76
Fibre (%)	9.30
Ash (%)	2.94

The fresh green leaves of lemongrass plant were collected and proximate analysis was determined. The moisture content (71.03%) of lemongrass is desirable as it will prevent microbial attack and allow for high storage capacity due to its antioxidant activity. The protein and carbohydrate content of leaves were found to be 3.83 and 20.73%. This shows that *cymbopogon citratus* is a good source of energy. The fat, fibre and ash content of lemongrass leaves were 4.76, 9.30 and 2.94% respectively. These findings are in correlation with the results obtained by Assous *et al*., 2013 [3].

Phytochemical constituents of lemongrass (*Cymbopogon citratus*) leaves

The values of phytochemicals were presented in Table 4.

Table 4: Phytochemical constituents of lemongrass leaves

Constituents	Values
Alkaloid (g/100g)	1.27
Saponin (g/100g)	1.06
Tannin (g/100g)	0.87
Phenols (g/100g)	0.27
Flavonoids (g/100g)	0.84

The data in Table 4, represent that the alkaloid and saponin content in lemongrass leaves were 1.27 and 1.06%. The tannin content (0.87%) which attributed to astringency in prepared product. The antioxidant activity of leaves was dependent on phenol and flavonoid content which was found to be 0,27 and 0.84% in *cymbopogon citratus* leaves. The similar results were also obtained by Ndukwe *et al*. (2013).

Proximate composition of Lemongrass (*Cymbopogon citratus*) Powder

The lemongrass leaves were dried in cabinet dryer and analysed for its proximate composition. The results were presented in Table 5.

Table 5: Proximate composition of Lemongrass Powder

Content	Value
Moisture (%)	7.01
Protein (%)	11.15
Carbohydrate (%)	65.78
Fat (%)	1.45
Ash (%)	11.28

The moisture content of the dried samples was not significantly different and were drastically reduced from 71.03% in the fresh samples to between 7.01%. The protein, carbohydrate and ash content were found in increasing trends in relation with fresh leaf sample. The values for protein, carbohydrate and ash were 11.15, 65.78 and 11.28% respectively. The fat content was reduced from 4.76% to 1.45% in dried powder. These findings are in relation with the findings of Satwase *et al*. (2013) [20] which showed the increasing trends of nutrient in case of drumstick after drying. Abioye *et al*. (2014) [2] reported that the protein, carbohydrate and ash content were increased after drying of baobab leaves.

Phytochemical constituents of lemongrass powder

The effect of cabinet drying on phytochemical constituents were analysed and data were presented in Table 6.

Table 6: Phytochemical constituents of Lemongrass Powder

Constituents	Values
Alkaloids (%)	0.68
Saponins (g/100g)	0.62
Tannin (g/100g)	0.36
Steroids (g/100g)	0.24
Phenol (g/100g)	0.15
Flavonoids (g/100g)	0.56

The phytochemical which were affected by drying conditions. The alkaloids, saponin and steroid were found to be 0.68, 0.62 and 0.24% in lemongrass powder. The astringency in powder is due to the presence of tannin (0.36%). The phenol and flavonoid content were slightly reduced due to drying i.e. 0.15 and 0.56%. As solubility of polyphenols and flavonoids in oil is low, the reduction in TPC and TFC in the inside part of the falafel ball during drying might be due to degradation (Makris and Rossiter, 2001) [13]. These findings are in consistent with the Xu *et al*., (2007) [26]; Price and Rhodes, (1997) [19].

Proximate composition of herbal cookies

The proximate composition of herbal cookies was analysed at various level of lemongrass powder (1, 3 and 5%). The values were tabulated in Table 7.

Table 7: Proximate composition of Herbal Cookies

Treatment	Moisture (%)	Ash (%)	Crude fat (%)	Protein (%)	Crude fiber (%)	Carbohydrate (%)
T_0	4.43	2.54	24.01	6.68	0.22	62.12
T_1	3.20	2.37	20.64	8.87	2.62	62.30
T_2	2.84	3.86	16.82	9.96	3.10	63.42
T_3	2.42	5.58	13.72	10.53	3.34	64.41

T_0 – Cookies without lemongrass powder
T_1 – Cookies with 1% lemongrass powder
T_2 – Cookies with 3% lemongrass powder
T_3 – Cookies with 5% lemongrass powder

From the above Table, it could be concluded that there was increase in proteins, ash, crude fibre and carbohydrate whereas moisture and crude fat content were reduced (Table 7). The moisture content was reduced from 4.43% to 2.42 due to low water absorption capacity of lemongrass powder as compared to raw cookies. The protein, crude fibre, ash and carbohydrate were increased while crude fat was reduced due to the content of lemongrass powder. These results were found to be similar with Dhillon *et al*., 2013 [6] who found that the fibre content were increased with addition of oregano herb.

Phytochemical constituents of herbal cookies

The lemongrass powder was added at 1, 3 and 5% and analysed for its phytochemical content. The obtained results were presented in Table 8.

Table 8: Phytochemical constituents of Herbal Cookies

Constituents	Content		
	1%	3%	5%
Alkaloids (g/100g)	0.09	0.27	0.45
Saponin (g/100g)	0.06	0.20	0.34
Tannin (g/100g)	0.05	0.16	0.28
Steroid (g/100g)	0.03	0.10	0.17
Phenol (g/100g)	0.02	0.08	0.14
Flavonoid (g/100g)	0.09	0.28	0.48

The phytochemical content was increased as increase in the level of lemongrass powder. The all are found in the range from 0.02 to 0.09 g/100g with 1% powder. The lemongrass powder at 5% level which increased the phytochemical content to range 0.14 to 0.48 g/100g. Similar observations have been made when baking rhubarb, whereby both TPC and FRAP AA were higher during the first 20 min and then decreased to low levels (McDougall *et al*., 2010) [15], and when baking chocolate cookies and chocolate cakes made with baking powder rather than baking soda (Miller *et al*., 2006; Stahl *et al*., 2009) [16, 22].

The baking did not affect the levels of TPC or FRAP AA relative to the flour and dough, but slightly decreased the levels of TFC. This might be due to omission of a pre-soaking stage before baking, and thus no discarding of the baking water. These results strengthen our previous observation that poly- phenols and flavonoids most likely leak into the sur-rounding water during the soaking and cooking processes (Segev *et al*., 2011) [21]. In cv. Zehavit, however, a slight but significant in- crease in TPC and FRAP AA was observed in the bread crust. This might be due to the production of Maillard reaction products in the crust during thermal processing (Lindenmeier *et al*., 2004) [11].

Sensory analysis of herbal cookies

The herbal cookies with incorporation of lemongrass powder were analysed for sensory parameters such as colour, flavour, texture, taste and overall acceptability on the basis of 9-point hedonic scale. It helps in deciding the level of incorporation of lemongrass powder as per sensory acceptance by consumer.

Table 9: Sensory analysis of Herbal Cookies

Samples	Colour	Flavour	Texture	Taste	Overall Acceptability
T_0	8.5	8.0	8.2	7.8	8.4
T_1	8.3	7.8	8.0	7.5	7.9
T_2	8.5	8.0	8.3	8.0	8.3
T_3	8.2	7.5	8.0	7.2	7.8

The Table 9 showed that the level of lemongrass powder effects on sensory parameters. The overall acceptability, texture and taste values are comparable with the control sample. The increased level of lemongrass powder than 3%, found that it adversely effects on sensory quality of prepared cookies.

Conclusion

The addition of lemongrass powder in cookies is better way to providing the nutritional benefits to consumer. The prepared cookies were also found to contain phytochemical which plays the role such as anti-cancer, anti-diabetes, anti-inflammation agent in human body. As suggested safe limit for human (Based on experiments in rats) is 0.7/mg/kg of body weight/day (Fandohan *et al*., 2008) [7].

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