Evaluation of Nutrient, Mineral Analysis and Quality Characterization of *Gymnema sylvestre* Multi Grain Cookies for Diabetes

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RS and DI conceptualized and discussed the methodology and results in the final manuscript. Gathered the data with regard to this work. Author PSS analysed all the given data gathered. All authors discussed the methodology and results and contributed to the final manuscript. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Diabetes is a group of metabolic disorders with various etiologies that are defined by persistent hyperglycemia and worsening carbohydrate, lipid, and protein digestion due to flaws in insulin discharge and insulin activity. Indians account for one out of every five diabetics of the world. Around 35 million Indians suffer from diabetes, which affects around 150 million individuals worldwide. Because health is a major concern in people's daily lives, foods that are healthy and beneficial to health are becoming increasingly popular. Natural food variations are widely used for the prevention and treatment of a variety of healthy issues. Supplements such as protein, iron, and calcium can be found in plenty of treats. The goal of this study was to develop solid treats using *Gymnema sylvestre* leaf powder, an Ayurvedic component with increased nutraceutical value. Multigrain powder, heating powder, palm sugar, cardamom powder, Margarine, salt, bubbling blend, and *G.sylvestre* leaf powder were among the ingredients used to make the solid snacks. The powder was created by drying the leaves in a plate dryer at 50°C for 2 hours before crushing in a

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home processor. *G.sylvestre* leaf powder was fused at different percentages: 0.25 percent, 0.50 percent, 0.75 percent, and 1.00 percent. Supplement testing, mineral analysis, and tactile testing are not set in stone. Atomic Absorption Spectroscopy was used to determine mineral composition. Over a variety of examples, test 0.50 percent sound snacks were found to be organoleptically superior.

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterised by a lack of the chemical insulin in the blood, resulting in abnormalities in the body's absorption of carbs. In view of the fact that the age of onset isn't the underlying predictor of the type of diabetes, the phrases 'adolescent onset' and 'developmental onset diabetes have been replaced with 'Type I and Type II' [1]. Heredity, incorrect dietary proclivities, lack of movement, the effects of pharmaceuticals and poisons, the impact of chemicals, mental factors, and diabetes as a complication of other illnesses such as pancreatitis and cardiovascular failures are the most generally acknowledged causes of diabetes [2]. Polydipsia, polyuria, and polyphagia are the most common symptoms of diabetes mellitus. Vision blurring, skin irritation, general weakness, and a lack of solidarity are some of the negative effects. Finally, it causes water and electrolyte imbalances, ketoacidosis, and a trance-like state [3]. Retinopathy, nephropathy, neuropathy, and repeating myocardial dead tissue are among the persistent problems, which are associated with an increase in the frequency of congestive cardiovascular breakdown, ulceration, foot infection, and even gangrene [4]. For diabetics, a high-complex-sugar, low-fat diet that includes a variety of soil-based products would be ideal [5].

One of the nibbling things, a treat, contains refined flour, sugar, and spread at first. Because health is such a major concern in our daily lives, nutritious and beneficial things are in high demand. Treats are the most well-known bread shop items due to their excellent nutritional value, ready-to-eat nature, and easy availability in a variety of shapes and sizes at a fair price. Treats are popular because they are high in carbohydrates, lipids, and calories. Currently, the fusion of a few elements in treats has evolved to improve its nutritive and beneficial qualities. Among other things, treats are known to be a good source of energy, protein, iron, calcium, and calories, among other things. Treats are not considered to be staple foods like bread, but they

can be considered fibre transporters because of their longer shelf life, allowing for a wider range of manufacturing and distribution [6]. Margarine is made from vegetable oils, therefore it contains polyunsaturated and monounsaturated fats, as well as unsaturated "excellent" fats. When filling in for submerged fat, these fats aid to lower low-thickness lipoprotein (LDL), or "bad," cholesterol. Cookies' useful features can be enhanced by altering and supplementing them with health-promoting ingredients such as "Gulmar" leaves, mixed sugar (fake + regular) sugars, fat substitutes, and wheat flour [7].

G. sylvestre is a prominent Indian medicinal plant that is widely used in the treatment of diabetes mellitus. *G. sylvestre* is a basic therapeutic woody climber that belongs to the Asclepiadaceae ('Milk Weed Family') family. 'Wonder natural product' is one of the unique names for this plant species. The name *'Gymnema sylvestre'* comes from a Latin word that means 'bare' and, which means 'from the woods' [8]. *G.sylvestre* can be found in India, Malaysia, Sri Lanka, Australia, Indonesia, Japan, Vietnam, tropical Africa, and western China in general [9]. *G.sylvestre* is thought to have a potential anti-diabetic effect. When you bite on the *Gymnema* leaves, the lack of capacity tastes good. Because of qualities that lower and balance glucose levels, concentrates of its leaves and roots are used as a typical treatment for diabetes in India and parts of Asia. Antimicrobial, antihyphal, antihyper-cholesterolemic, and hepato-defensive exercises are also performed by the plant. It also acts as a repellent to the caterpillar *prodenia eridania*, prevents dental cavities caused by *Streptococcus mutans*, and is used in cosmetics. *Gymnemic* corrosive is found in *G.sylvestre* leaves, and it is known to suffocate the glucose transporter from the digestive system to the circulatory system. It could potentially restore or regenerate the pancreas' insulin-producing beta cells, as well as reduce cholesterol and glucose absorption from the GI tract. Supplementing with *G.sylvestre* appears to enhance glucose control in type 2 diabetic patients. Reduced postprandial

blood glucose resulted in a decrease in HbA1C, lowering the risk of diabetic complications [10]. This plant's leaves create a zero-calorie, non-nutritive, high-power sugar that can be used as a sucrose alternative. Sorbitol, one of the polyols, is a good sugar substitute that has no effect on the quality of the treat. For diabetics, sorbitol is preferable to sorbitol as a sugar alternative to a variety of foods [7].

2. MATERIALS AND METHODS

2.1 Raw material Preparation

Multigrain and *G. sylvestre* leaves were purchased from the local market and necessary pre -treatments such as washing, drying, grading, sorting, incorporation etc. was carried out. Palm sugar, margarine, baking powder and essence were purchased from local market. Margarine was stored in the refrigerator at -40°C.

2.2 Cookies Preparation and Optimization

Cookies were prepared with some modifications. For the optimization of multigrain powder, a varying proportion of margarine and palm sugar free sweetener by varying proportion of two was carried out (Table 1).

2.3 Proximate Composition

Proximate composition (moisture content, carbohydrate, ash, fat, protein, fibre content and sugar content) was determined by using different methods [11,12,13].

2.4 Mineral Determination

The minerals such as sodium and potassium content of sample were determined by using the Atomic Absorption Spectroscopy as described by [6].

2.5 Determination of Metals

All the atomic measurements are carried out with PerkinElmer model 400/HGA 900/AS 800 coupled with Mercury Hydride System-15 (MHS-15) and Flame Photometer. The Hallow cathode lamps (HCL) for Na and K analyses are used as a light source to provide specific wavelength of the elements to be determined and high purity (99.999 %) Acetylene and Nitrous oxide are used to provide constant thermal energy for atomization process and Argon gas used for carrier gas removal purposes for Graphite furnace.

2.6 Atomic: Absorption Spectrophotometer (AAS)

After calibrating the instrument with prepared working standard, the 10 ml of digested liquid sample is pipette out to a specific container of Mercury Hydride system analyzer followed by adding 1.5% Hcl of 10ml as diluents for each flask and blank, 3 % of $NaBH_4$ solution in 1% of NaOH is run through the reaction flask to quartz cell without heating against the calibration curve obtained from concentration vs. absorbance of the prepared known concentration on the day of the analysis.

2.7 Organoleptic Analysis

Sensory evaluation was carried out by a panel of ten semi trained panel members. Hedonic rating test was employed using a 9-point hedonic scale. Sensory parameters such as colour, taste, texture and overall acceptability were evaluated [13]. The following were the numerical scores assigned: 9: Like extremely 8: Like very much 7: Like moderately 6: Like slightly 5: Neither like nor dislike 4: Dislike slightly 3: Dislike moderately2: Dislike very much 1: Dislike extremely.

Table 1. Optimization for control and multigrain cookies

Composition	Control	0.25%	0.50%	0.75%	1%
Multi Grain Powder (Cup)	1	1	1	1	1
Baking Powder (Tablespoon)	1/4	¼	1/4	¼	1/4
Palm Sugar (Cup)	1/4	¼	1/4	¼	1/4
Cardamom Powder (Tablespoon)	1/2	½	1/2	½	1/2
Margarine (Cup)	1/4	¼	1/4	¼	1/4
Salt (Tablespoon)	1/8	1/8	1/8	1/8	1/8
Boiled Mix (Tablespoon)	2	2	2	2	2
G. sylvestre Leaf Powder (%)	-	0.25	0.50	0.75	1

2.8 Isolation of Microbes

The sample is mixed with 1L pure water to make solution sample. Then this solution sample was taken for process. The sterile Petri dish was labeled. Afterwards, 1 ml of the inoculum was evenly spread over the entire surface of the nutrient agar plates until the medium no longer appears moist. The flaming and spreading for each of the remaining plates was repeated. The plates were inverted and incubated at room temperature of at least 48 hours. The plate's colonies were found to be statistically valid [14].

2.9 Biochemical Tests

The biochemical tests were carried out for different methods such as Gram staining [14], Methyl red and Voges- Proskauer test [15], Oxidase test [16], Catalase test [16], Indole test [16], Urease test [16], Nitrate reduction test [16], Glucose fermentation test [16], Motility test [15].

2.10 Statistical Analysis

All experiments were carried out in triplicates. Data obtained were analysed by one-way analysis of variance (ANOVA) and means were compared by Duncan's New Multiple Range test (SPSS 21.0 version). Differences were considered significant at $p<0.05$.

3. RESULTS AND DISCUSSION

In light of the relevant scientific literature, the results collected during this inquiry are addressed under appropriate areas. Raw materials, control cookies, and healthy cookies were analysed at varied concentrations (0.25 percent, 0.50 percent, 0.75 percent, and 1.00 percent). The data collected during the experiments are shown in the tables below, along with a description of the findings. The amount of *G.sylvestre* leaves per kilogramme was determined using an electronic digital balance with a sensitivity of 0.01 gm [17].

3.1 Nutritional Analysis

The nutritional analysis (ash, moisture, fiber, carbohydrates, protein, fat and sugar) were analyzed for control and different concentration (0.25%, 0.50%, 0.75% and 1.00%). The ash value was 1.76%, 1.95%, 2.05%, 1.84% and 1.74 for control, 0.25%, 0.50%, 0.75%, 1.00% cookies samples, respectively. The residue left

after all the moisture has been removed, as well as the organic material (fat, protein, carbs, vitamins, organic acid, and so on) has been burnt at a temperature of around 500 ^{o}C. This, is referred as ash in food. The mineral content of the original food is often measured by ash content [18]. The moisture content was found to be 3.65%, 3.84%, 4.23%, 3.97% and 3.89% for control, 0.25%, 0.50%, 0.75%, 1.00% cookies samples, respectively. Because the fresh plant has a short shelf life and is susceptible to microbial attack, protracted storage would result in deterioration. This encourages users to store their items in dry state. In the processing, preservation, and storage of food, moisture content is one of the most important and often used measurements [18].

The amount of non-digestible carbohydrate and lignin in a food or plant is measured as crude fibre. The crude fibre obtained for cookies was found to be 8.72%, 10.13%, 10.46%, 10.33% and 10.03% for control, 0.25%, 0.50%, 0.75%, 1.00% cookies samples, respectively. This low amount is thought to be optimal since it improves glucose and fat absorption. Although crude fibre improves digestion, too much of it can produce intestinal discomfort, reduced digestibility, and reduced nutritional utilisation [19]. Crude fibre is primarily made up of cellulose, with a little amount of indigestible lignin [18]. The carbohydrate content of healthy cookies were found to be 62.14%, 63.77%, 63.95%, 63.53% and 63.66% for control, 0.25%, 0.50%, 0.75%, 1.00% cookies samples, respectively. When compared to the Recommended Dietary Allowance (RDA) of 130g, the plant is a moderate source of carbohydrate [20]. The crude protein of healthy cookies were found to be 8.44%, 9.34%, 10.96%, 10.16% and 9.96% for control, 0.25%, 0.50%, 0.75%, 1.00% cookies samples, respectively and represented in Table 2 and Fig. 1. The RDA for protein is 56 grammes for adults weighing 70 kilogrammes and 46 grammes for adults weight 50 kilogrammes; children may ingest 2 kilogrammes per day [21].

The plant has a moderate protein content. Plant-based proteins have a lesser nutritional value, according to [20], but when combined with many other protein sources, such as animal protein, they can provide appropriate nutritious value. Protein is required for a variety of bodily processes, including body development, fluid balance, hormone production, enzyme production, and maintaining a robust immune

system [22]. The fat content of healthy cookies were found to be 21.25%, 21.04%, 20.97%, 19.34 % and 19.23 % for control, 0.25%, 0.50%, 0.75%, 1.00% cookies samples, respectively. The low fat content of 1.83mg/100g is lower than the range (8.3 percent -27.0 percent) observed for various Nigerian green vegetables [23]. Carr's Index and Hausner's ratio for *G. sylvestre* were 20.37 and 1.255, respectively, showing fair compressibility. However, [11,17] obtained similar results for Bulk Density, Tapped Density, Carr's Index, and Hausner's Ratio. The results of proximate composition revealed that multigrain is a good source of carbohydrate, protein, and crude fibre, while *G. sylvestre* powder is a good source of carbohydrate, crude fibre, and crude protein. Moisture content in multigrain and *G. sylvestre* powder was found to be higher while fat content was lower as concentration increased. Multigrain and *G. sylvestre* powder contained higher amount of carbohydrate in 0.50% (63.95 ± 0.04) when compared to other concentrations. Crude fibre, protein and ash content of wheat flour and *G. sylvestre* powder were found to be 10.46, 10.96 and 2.05 per cent respectively.

3.2 Mineral Analysis

Minerals are inorganic elements that serve as structural components and process regulators in the body. The sodium and potassium content of multigrain and *G. sylvestre* powder are shown in Table 3 and Fig. 2. Table 3 shows the mineral contents of the healthy cookie samples. The sodium content in healthy cookies were found to be 53.16 mg, 57.51 mg, 61.25 mg, 59.86 mg and 58.73 mg for control, 0.25%, 0.50%, 0.75%,

1.00% cookies samples, respectively. Sodium is a vital mineral that aids in the transmission of nerve impulses as well as the preservation of cell osmotic balance. The Recommended Daily Allowance for sodium for humans is 1100-3300 mg/100g, according to the National Research Council [24]. Dehydration or muscle cramps can result from a sodium deficiency [25]. The potassium was the most abundant element in the healthy cookie samples. The highest potassium content (257.95 mg) was recorded in 0.50% healthy cookie sample. The potassium content in healthy cookies was found to be 173.44 mg, 238.15 mg, 257.95 mg, 241.24 mg and 231.65 mg for control, 0.25%, 0.50%, 0.75%, 1.00% cookies samples, respectively. According to the National Research Council [24], the Recommended Dietary Allowance for potassium for adults is 1875-5625 mg/kg. Potassium is essential for the management of water and electrolyte balance and acid-base balance in the body, as well as nerve action and muscular function. Potassium deficiency causes muscle paralysis [26]. The sensory analysis was found to be good in every of the examined parameters. A panel of ten semi-trained judges evaluated the control and healthy cookies for sensory qualities using a 9 point Hedonic scale method. Several parameters such physical appearance, texture/mouth feel, taste, colour, and overall acceptability were tested. The quality was assessed using the mean values of ten semi-trained assessors. Sensory evaluation revealed that the control sample cookies 0.50 percent scored well on all metrics when compared to the others. As a result, sample 0.50 percent was chosen for the creation of nutritious cookies.

Table 2. Nutrient analysis for cookies

Nutrients Parameters	Control	0.25%	0.50%	0.75%	1.00%
Ash (%)	1.76 ± 0.03	1.95 ± 0.03	2.05 ± 0.02	1.84 ± 0.04	1.74 ± 0.02
Moisture (%)	3.65 ± 0.03	3.84 ± 0.03	4.23 ± 0.02	3.97 ± 0.02	3.89 ± 0.02
Fiber (%)	8.72 ± 0.01	10.13 ± 0.02	10.46 ± 0.02	10.33 ± 0.02	10.03 ± 0.02
Carbohydrates (%)	62.14 ± 0.04	63.77 ± 0.02	63.95 ± 0.04	63.53 ± 0.02	63.66 ± 0.03
Protein (%)	8.44 ± 0.01	9.34 ± 0.03	10.96 ± 0.02	10.16 ± 0.02	9.96 ± 0.03
Fat (%)	21.25 ± 0.03	21.04 ± 0.03	20.97 ± 0.03	19.34 ± 0.03	19.23 ± 0.02
Sugar (g/ml)	18.44 ± 0.03	11.25 ± 0.03	11.36 ± 0.02	11.06 ± 0.03	10.96 ± 0.02

Table 3. Mineral analysis for control cookies

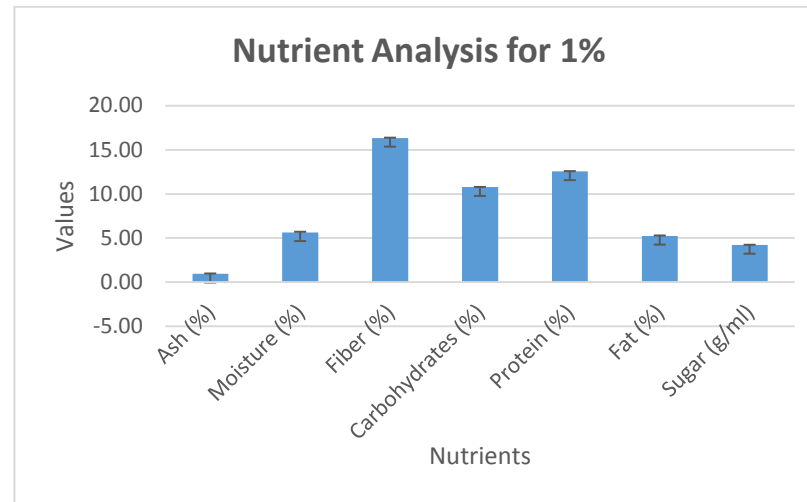| Minerals	Control	0.25%	0.50%	0.75%	1.00%
Sodium (mg)	53.16 ± 0.02	57.51 ± 0.04	61.25 ± 0.02	59.86 ± 0.02	58.73 ± 0.03
Potassium (mg)	173.44 ± 0.04	238.15 ± 0.02	257.95 ± 0.02	241.24 ± 0.03	231.65 ± 0.02
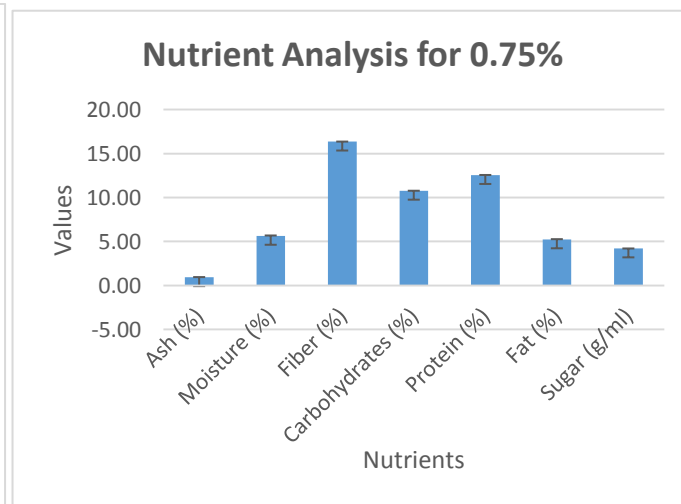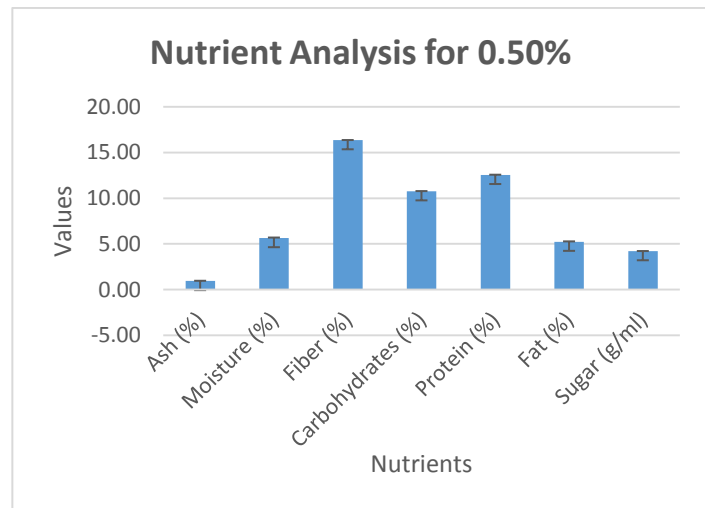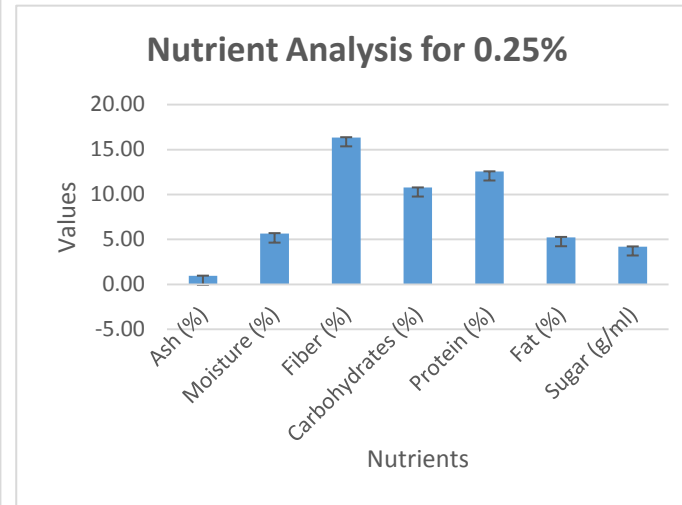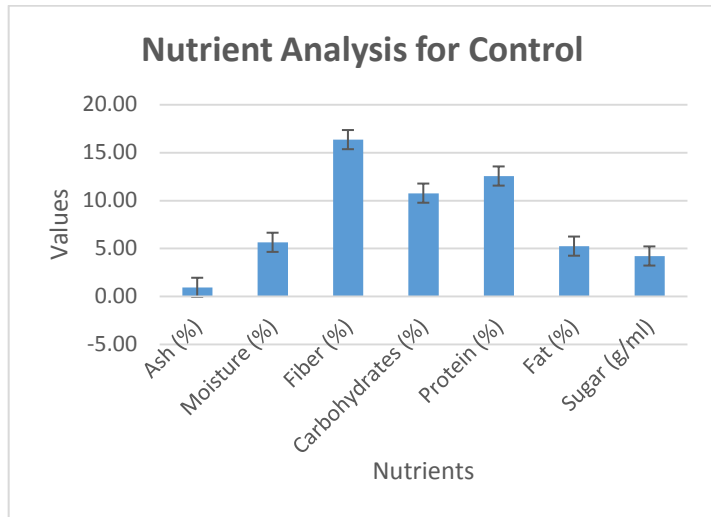
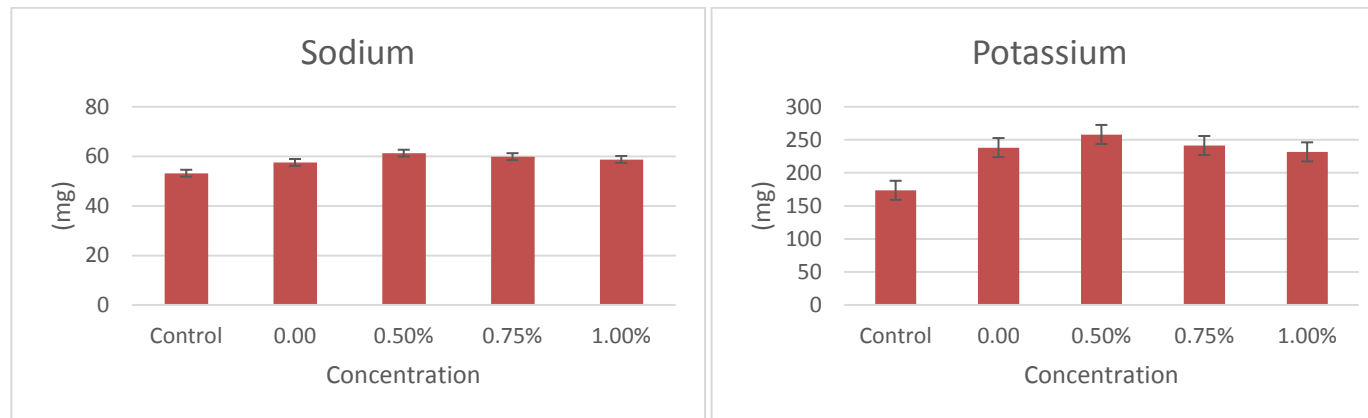**Fig. 1. Nutrient analysis for cookies**

Fig. 2. Mineral analysis for cookies

3.3 Microbial Analysis

The isolation of microbes was carried out in 0.50% cookies. In each and every test which was performed the results were good in 0.50% cookies. The healthy cookies of 0.50% concentration were tested in different storages that is AF (Aluminium Foil), LDPE (Low density polyethylene) and HDPE (High density polyethylene). The CFU count was 0.12×10^2, 0.48×10^2 and 0.72×10^2 in day 1, day 15 and day 30 AF samples. However, a higher CFU count was obtained in HDFE samples (0.36×10^2, 1.98×10^2 and 2.52×10^2 in day 1, day 15

and day 30 AF samples, respectively) and it is represented in Table 4 and Figure 3, 4 and 5. The dilution was 10^{-6}. The decrease observed in bacterial load after 48 h liquid fermentation may be as a result of some bioactive substances which might have produced an inhibitory effect on other organisms involved in the fermentation. This is in line with the report of Ouoba and Kalui [27,28,29]. Low microbial growth observed after 48 hrs of solid fermentation may also be as a result of reduction in the moisture content, which is known to be the most important factor in microbial growth [30,31].

Table 4. Counts for colony forming unit in 0.50% cookies

Samples	Day 1		Day 15		Day 30		Dilution
	No. of Colonies	CFU (No. of Cells/ml)	No. of Colonies	CFU (No. of Cells/ml)	No. of Colonies	CFU (No. of Cells/ml)	
AF	2	0.12×10^2	8	0.48×10^2	12	0.72×10^2	10^{-6}
LDPE	7	0.42×10^2	36	2.16×10^2	46	2.76×10^2	10^{-6}
HDPE	6	0.36×10^2	33	1.98×10^2	42	2.52×10^2	10^{-6}

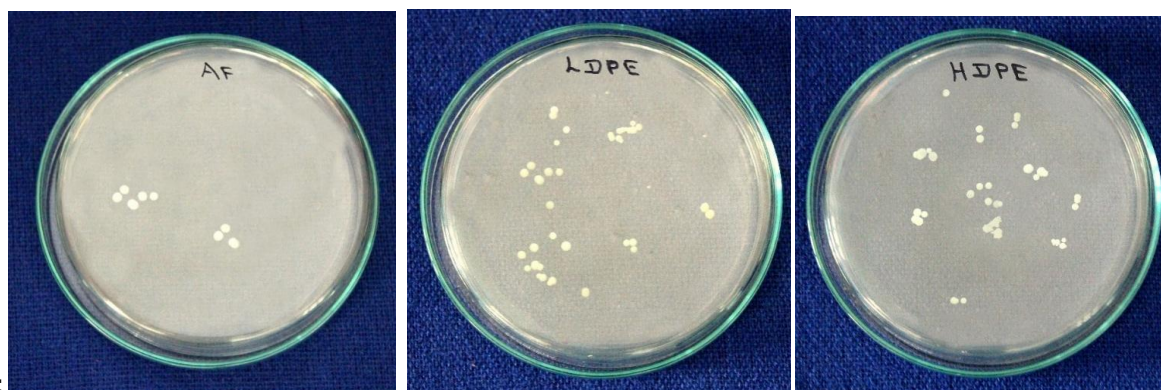**Fig. 3. Microbial plate view day 1**

Fig. 4. Microbial plate view day 15

Fig. 5. Microbial plate view day 30

Table 5. Organisms identified for cookies

Day	Samples	Organisms Identified
Day-1st	AF	*Lactobacillus bulgaricus*
	LDPE	*Lactobacillus lactis*
	HDPE	*Streptococcus thermophillus*
Day-15th	AF	*Lactobacillus casei*
	LDPE	*Pseudomonas mephitica*
	HDPE	*Pseudomonas nigrificans*
Day-30th	AF	*Lactobacillus acidophilus*
	LDPE	*Bifidobacterium longum*
	HDPE	*Bacillus polymyxa*

The presence of these organisms during the fermentation periods confirms that they grow in close association with the food substrate and produce extracellular enzymes [32].

4. CONCLUSION

In recent years, medicinal plant conservation and use have received a lot of attention. It has been utilised by indigenous and marginal people around the world to treat a variety of ailments from time immemorial. The goal of this study was to assess the nutritional value, mineral content, and microbial activity of healthy cookies. When compared to other concentrations and controls, the 0.50 percent concentration of *G.sylvestre* demonstrated superior activity. *G.sylvestre* was a traditional medicinal plant whose leaves had remarkable therapeutic characteristics and were highly efficient for diabetic therapy. As a result, *Gymnema* leaves can be utilised in the preparation of hypoglycemic mix-in foods. According to the findings of the study, these meal preparations may aid in instilling the habit of taking herbal mixtures, which are curative and therapeutic for diabetes patients. As a result, this study showed that we must raise knowledge about the usage of locally available but underutilised medicinal herbs.

NOTE

The study highlights the efficacy of "Ayurvedic component" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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