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Nutritive Assessment of Composite Flour Biscuit Incorporated with Herbal Plant Powder

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ABSTRACT

A cookie is a baked product, which can be prepared by using wheat flour with non-wheat materials enriched with nutritional value. The study aims to formulate herbal cookies incorporating *Asparagus racemosus* and *Aerva lanata* stem and leaf powder enriched with mungbean (*Vigna radiata*) and wheat flour. Each of the herbal plant powder and flour mixture was formulated at a ratio of 1:1 by drying and grinding into a fine powder. Three different cookie formulas by varying combinations of plant powder and flour mixture were evaluated based on the 5-point hedonic scale on sensory attributes by a 30 member untrained panel. According to the results of sensory evaluation, the sample enriched with 4 % plant powder and 55 % flour mixture was accepted. The phytochemical analysis indicated the presence of secondary metabolites including Alkaloids, Saponins, Flavonoids, Tannins, Phenols, Terpenoids, Glycosides and Steroid in the accepted cookie sample. The cookies were rich in carbohydrate (59.3%), fat (14.3%), protein (13.4%) and the calculated energy value was 419.5 kcal. Peroxide value and acid value of stored cookies were lower than the maximum recommended level. The IC50 value for the methanol extract of the herbal cookies was 16.69±0.88 mg/ml. The bacterial count of cookies was 6.0 ×10^2 CFU/g after 6 weeks of storage and this was well below the safe level of 1×10^4 CFU/g. There was no yeast and mold growth observed during the storage. Overall analysis proved that herbal cookies were acceptable for human consumption after storing it under room temperature within six weeks.

Keywords: Antioxidants, Herbal cookies, Herbal plant powder, Mungbean flour, Sensory evaluation

INTRODUCTION

In the last few years there has been an exponential growth in the field of herbal medicine and functional foods. The population everywhere desires to eat a healthier diet without changing their conventional dietary patterns (Thorat *et al*., 2017). Sri Lankan tradition is steeped with natural medicine that is enjoyed as food in our daily life as sages of ancient times recognized the benefits of valuable herbal plants such as Hathawariya and Polpala. 'Hathawariya' (*Asparagus racemosus*) and 'Polpala' (*Aerva lanata*) are common medicinal plants throughout Sri Lanka (Robert, 1998). Although these herbal plants are common, the plants are not much incorporated into instant foods such as cookies or biscuits apart from herbal biscuits made using few plants such as curry leaves (*Murraya koenigii*). On the other hand herbal porridge is the most common food item in which these plants are applied with a number of nutritive properties (Gunasekera, 2015).

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'Hathawariya' (*Asparagus racemosus*) is a species of asparagus with a long history of use in India and other parts of Asia as folk medicine. Many medicinal qualities of this plant have been associated with phytonutrients present in its roots, and especially one type of phytonutrients called saponins. Saponins in food have repeatedly been shown to have anti-inflammatory and anti-cancer properties, and their intake has also been associated with improved blood pressure, improved blood sugar regulation, and better control of blood fat levels. And also almost all parts of both *Asparagus racemosus* and *Aerva lanata* plants can be consumed as food (Gunasekera, 2015).

A cookie is small sweet, usually containing refined flour, eggs, sugar, and either butter or fat. It may include other ingredients such as raisins, oats, chocolate chips, or nuts. There are minor differences between cookies and biscuits in the percentage of raw materials, methods and the external and internal qualities. The biscuits or cookies are prepared either by machine or manually. Cookies can be classified as ready to eat and convenient foods. They are popular for morning and evening tea-time snacks. Low-cost nutritive cookies made of herbal components plant powder is an alternative option to rejuvenate immunity (Upadhyay *et al*., 2017).

Processed food has a great shelf life and easy to carry in traveling, office and house. Working families have alternate options for readymade food to fulfill their nutritional requirements. Generally, bakery food is high in starch calories. Herbs based cookies have an alternate option to replace conventional cookies. Cookies are a well shelf-stable bakery product. The economic importance of cookies is higher in all areas. Conventional processed foods have been replaced by functional processed food (Upadhyay *et al*., 2017).

One of the alternatives to overcome these problems is producing an herbal cookie using herbal plants consisting of natural ingredients. Thus, producing that kind of fully nutritional cookies is a good chance for consumers to save time for cooking and money. Also, it will give instant herbal food with high nutritional value, enriched with herbal value and beneficial health effects such as anticancer, decreasing high blood pressure, etc. Same time, it is easy to eat at any time and at any place where most of the children usually like to eat. Therefore, the present study aims to produce herbal cookies incorporating *Asparagus racemosus* and *Aerva lanata* plant powder enriched with mungbean flour, formulated based on the optimal level of selected ingredients for the acceptable level.

MATERIALS AND METHODS

Formulating Plant Powder and Mungbean Flour

Wheat flour, mungbean, margarine, sugar, milk, water, salt, baking powder, vanilla and young leaves and stems of *Aerva lanata* and *Asparagus racemosus* were applied for the sample preparation.

Approximately, equal amounts of dried plant parts were taken and were ground using a laboratory-scale grinder into a fine powder. The plant powder was stored in an airtight bottle in a dry place under room temperature (Ikechukwu *et al*., 2017). Collected 250 g of mungbean were washed, de-stoned, sun-dried and roasted. Then roasted grains were ground using a laboratory-scale grinder to produce flour and the mungbean flour was stored in an airtight bottle under room temperature for preservation.

The Methodology of Producing Herbal Cookies

Each ingredient (wheat flour, mungbean flour, plant powder, margarine, sugar) was measured into a bowl. Approximately equal amounts of mung bean flour and wheat flour were added to the bowl. The ingredients were beaten and mixed by using a food mixer. The mixture was then kneaded until it became dough. The dough was rolled and flattened into a uniform thickness of about 3.5 mm before cutting out to shapes using a hand-cutter. It was baked at 170ºC for 30 minutes in the oven. After baking, the cookies were cooled to room temperature, packed in low-density polyethylene (LDPE) bags and sealed in a plastic transparent container (Thorat *et al*., 2017). Three different treatments (T1, T2 and T3) for sensory evaluation were prepared by varying the quantities of the flour mixture and plant powder mixture (*Aerva lanata* and *Asparagus racemosus*) (Table 1). Results of the sensory evaluation were analyzed using Kruskal-Wallis nonparametric method in MINITAB statistical software version 18.1. As per the results, the best herbal cookie formula was selected for further development of the study.

Determination of Physio- Chemical Characteristics

Phytochemical Screening

The methanol extract of the product was obtained using the method described by Jayashree *et al.* (2013). The presence of saponins, flavonoids, steroids, terpenoids,

Table 1: Formulation of composite ingredients (%) of cookies in three treatments

Ingredients	T1	T2	T3
Flour mixture (Mungbean + Wheat flour)	53.0%	54.0%	55.0 %
Plant powder mixture	6.0%	5.0 %	4.0 %
Sugar	20.0 %	20.0 %	20.0 %
Margarine	20.0 %	20.0 %	20.0 %
Miscellaneous*	1%	1%	1%

**salt, baking powder and vanilla*

glycosides, phenols, tannins and alkaloids was determined using the method described by Oeung *et al*. (2017). The presence of anthraquinones was determined using the method described by Jayashree *et al.* (2013)

pH Value

The pH values of the cookies were determined by suspending 10 g of cookie sample in 100ml of distilled water in 250 ml beaker using digital scale pH meter under room temperature (25 °C). This procedure was triplicated within a week and the average pH value was calculated. After that, the same sample was analyzed for three weeks.

Titratable Acidity and Peroxide Value

Titratable acidity of the product was determined using the method described by Kajala *et al*. (2015). NaOH solution was titrated against the water extract of cookies and the titration was triplicated. The peroxide value of the product was determined using the AOAC. (2006).

Estimation of Nutritional Composition

The moisture content of the herbal cookies was determined using the Oven Drying Method described by ISO 712: 2007 (Sri Lanka Standards, 2007). The total ash content of the herbal cookies was determined using the Dry Ash Method described by ISO 1575:2007 (Sri Lanka Standards, 2007). The water-soluble ash content of the herbal cookies was determined using the ISO 1576: 1988 (Sri Lanka Standards, 1988). The crude fat content of the herbal cookies was determined using the solvent extraction method described by Kirk *et al*. (1991). The crude fiber content of the herbal cookies was determined according to AOAC (2006) method. The crude protein content of the herbal cookies was determined according to the Kjeldahl method described by SLS 735: 2012. The carbohydrate content of the herbal cookies was determined according to the Nitrogen free method described by AOAC (2006), i.e. % Carbohydrate (NFE) = 100 - (M + P + F + A + F2), where, M = Moisture, P = Protein, F = Fat, A = Ash, F2 = Crude fibre.

Calorific Value

The caloric value (EV) was calculated using the method described by Grah *et al*. (2014), i.e. % EV = (% Protein × 4) + (% Lipid × 9) + (% Carbohydrate × 4).

Determination of Antioxidant Activity (DPPH Radical Scavenging Assay)

The antioxidant capacity of the cookie extracts was studied through the evaluation of the free radical-scavenging effect on the DPPH radical. The determination was based on the method proposed by Uthumporn *et al*. (2014) with modifications using the following equation, i.e.

$$\text{Inhibition } \% = \frac{(A_0 - A)}{A_0}\ x\ 100\ \%,$$

where 'A_0' is the absorbance of the control (without sample) and 'A' is the absorbance of the sample.

The scavenging ability of the samples was expressed as IC_{50} value which is the effective concentration where 50% of the DPPH radicals were scavenged. The sample concentration providing 50% inhibition (IC50) was calculated from the graph of inhibition percentage against sample concentration.

Evaluation of Microbial Stability of the Cookies

Total Plate Count and Enumeration of Yeast and Mold

The total plate count was determined according to the pour plate method described by ISO 4833: 2013 (Sri Lanka Standards, 2013). The yeast and mold count was determined according to the pour plate method described by ISO 21527-2: 2008 (Sri Lanka Standards, 2008).

RESULTS AND DISCUSSION

Overall Comparison of Sensory Evaluation

For three different herbal cookie formulae, from Kruskal- Wallis test conducted with five-point hedonic scale sensory evaluation, H_{stat} value for smoothness and crunchiness were larger than H_{table} values where significant differences were at $p < 0.05$. There were significant differences among treatments for smoothness and crunchiness while, color, sweetness, mouth-feel and overall likeness were not significantly different among the treatments. However, fondness for crunchiness, mouthfeel and overall likeness were relatively higher in the treatment T3. Therefore, T3 was selected as the most preferable herbal cookie formula out of the three treatments (Figure 1).

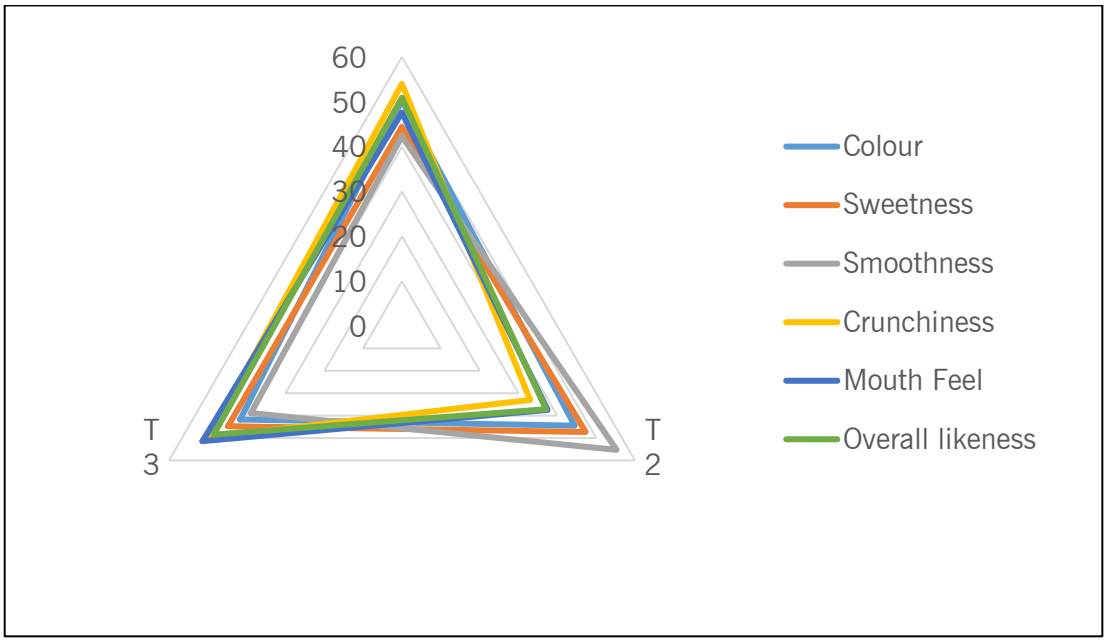

Figure 1: Sensory profile developed by panellists' score for the attributes of each treatment

Phytochemical Screening of Herbal Cookie Sample

Qualitative phytochemical screening of herbal cookies indicated the presence of alkaloids, saponins, flavonoids, tannins, phenols, terpenoids, glycosides, and steroid while only the negative response implied the absence of anthraquinones (Table 2). The methanol extract was used as the control.

Table 2: Phytochemical constituents of the powdered herbal cookies

Phytochemical	Results
Alkaloids	+
Saponins	+
Flavonoids	+
Tannins	+
Phenols	+
Terpenoids	+
Glycosides	+
Anthraquinones	-
Steroids	+

Presence of the compound (+), absence of the compound (-)

Determination of Physicochemical Characteristics

pH and Titratable Acidity

The pH is an important parameter in the formulation of biscuits. The result of the pH and TTA of herbal powder incorporated wheat flour are presented in Table 3. The pH and TTA values were ranged from 6.0±0.05 to 6.1±0.05 and 0.347% to 0.383% respectively during the storage period of cookies. The pH of the most bakery products was ranged from 3.5 to 8.0. And also, the pH of the most biscuits was ranged from 7.0 to 7.2 (BAKERpedia, 2019). The low TTA and pH in the cookies may be due to the departure of volatile acidity and organic compounds during baking (Akoja and Coker, 2018). Even though the acidity was higher than the reported biscuits, acidic products were more shelf-stable than non-acidic counterparts and an acidic pH was associated with the development of a pleasant taste (Ikechukwu *et al*., 2017). Therefore, the selected cookie formula was more shelf-stable.

Peroxide Value

It can be seen that the peroxide values of the cookie sample tend to increase gradually as the number of storage days increased from day 1 to day 14 (Table 4). As shown by Showkat *et al.* (2018), in our study also, the application of mungbean flour along with wheat flour has slightly reduced the rancidity level (peroxide value) between the day 10 to day 14 (Showkat *et al.*, 2018). Oxidation processes are of particular significance in cookies as they contain a high amount of fat (Rinku *et al*., 2017). Overall, the peroxide value was found to be increased from 7.164±0.25 meqO_2/kg to 7.919±0.15 meqO_2/kg. This increase might be due to the oxidation of unsaturated fatty acids in the oil. This was in complement to the fact that the higher substitution of mungbean flour which will practically reduce the peroxide value due to the improvement of stability toward oxidation

Table 3: pH, titratable acidity, moisture content and microbial analysis of herbal cookies during storage

Storage Time (Week)	Average pH (25°C)	Titratable acidity % (TTA %) (meq/100g)	Total plate count (CFU/g) × 10^2	Yeast and mold count (CFU/g)	Moisture %
0	-	-	2.6±0.08	Not Detected	5.9
1	6.0±0.05	0.383±0.002	-	-	-
2	6.1±0.05	0.365±0.005	-	-	-
3	6.0±0.05	0.347±0.001	3.6±0.05	Not Detected	5.0
6	-	-	6.0±0.05	Not Detected	4.2

Each value is expressed as mean ± standard deviation (n =3)

(Uthumporn *et al*., 2014). Therefore, the peroxide value of herbal cookies was within the range throughout 15 days of storage and it was within the standard of 10 meqO_2/kg reported for herbal biscuit (Srivastava and Raman, 2017).

Table 4: Effect of storage of herbal cookies on peroxide value

Storage period (Days)	Peroxide Value (meq O_2/kg)
Fresh	7.164±0.25
07	7.907±0.26
14	7.919±0.15

Each value is expressed as mean ±standard deviation (n =3)

Proximate Composition of the Cookies

The level of moisture, ash, total fat, fiber, carbohydrate and protein content of the developed herbal cookies are in the range of USDA National Nutrient Database for Standard Reference (USDA Food Composition Databases, 2019). Akoja and Coker (2018) reported that moisture content was an indicator of shelf stability, thus an increase in moisture content can enhance microbial growth which leads to deterioration in foods. The moisture content (6.0%) of the product was lower than the range recommended by USDA National Nutrient Database for Standard Reference which is 11.5%. The low moisture content of the herbal cookies reduced the possibility of microbial attack. High moisture content is not desirable in a product such as biscuits, because it has an inverse relationship with the texture of the product, which is an important attribute with consumer preference (Akoja and Coker, 2018). Taste and flavor of cookies are directly associated with the amount and quality of fat (Upadhyay *et al*., 2017). The total fat content of the cookies (14.3%) was within the range of standard value reported by USDA which is 24.4% (Table 5). According to Banusha and Vasantharuba. (2014), the protein content of cookies is only 11.5% including 100% wheat flour compared to the protein content in herbal

cookies (Table 5). The increase in protein content might be due to the appreciable amount of protein present in mungbean powder (Showkat *et al*., 2018). Because, mungbean powder contains the highest percent of protein according to the literature (Swaminathan *et al*., 2012). Therefore, herbal cookies enriched with the composite mungbean flour can serve as a cheap source of protein to the consumers (Akoja and Coker, 2018). The crude fiber content (3.3%) was also slightly higher than the recommended value of 2.1% by USDA (Table 5). This may be due to greater fiber present in herbal plant powder and mungbean powder. The increased fiber content aids good bowel movement and it is necessary for foods generally as it reduces food retention time in the digestive tract (Akoja and Coker, 2018). A slight increase in ash content (3.7%) of the product compared to the standard value of 3.3% shows that herbal plant powder increases the ash content in the cookies. This also might be due to the mineral content present in mungbean powder (Showkat *et al*., 2018). Ash content of a food material is a non-organic compound containing mineral content of the food. Nutritionally, it aids in the metabolism of other organic compounds such as fat and carbohydrates (Akoja and Coker, 2018). This might be due to the mineral content present in mungbean powder (Showkat *et al*., 2018). The carbohydrate content (59.3%) of the herbal cookies is slightly higher than the standard value of USDA which is 58.15% (Table 5). Herbal cookies consisted of carbohydrates that may contributed from wheat flour and mungbean flour. The increased value is not as high as 67.67% of carbohydrate reported by Banusha and Vasantharuba (2014) who have used 100% wheat flour.

Calorific Value

The reported calorific value for 100% wheat flour cookies is 402.9 kcal (Omran *et al*., 2016). The calculated calorific value for the

Table 5: Proximate composition of herbal cookies produced from wheat flour and enriched with mung bean flour

Parameter	**Herbal cookies (%)**	**USDA standard for cookies (%) (USDA Food Composition Databases, 2019)**	**Wheat flour biscuit (%) (Banusha and Vasantharuba, 2014)**
Crude Protein (%)	13.4±0.18	8.92	11.5±0.5
Ash (%)	3.7±0.06	3.3	1.10±0.12
Moisture (%)	6.0±0.06	11.5	5.85±0.25
Crude Fiber (%)	3.3±0.28	2.1	0.15±0.04
Total Fat (%)	14.3±0.12	24.4	19.49±0.38
Carbohydrate (%)	59.3±0.33	58.15	67.67±0.38

Each value is expressed as mean ±standard deviation (n =3)

herbal cookies was 419.5 kcal. The energy values which is the total available energy in any food samples are ranged from 455.15 to 419.06 kcal (Akoja and Coker, 2018). According to USDA standard, commercially prepared cookie enriched with butter includes 467 kcal (USDA Food Composition Databases, 2019). Therefore, the developed herbal cookie enriched with mungbean flour secure a considerable amount of carbohydrate, protein and fat compared to the cookies consisted of 100% wheat flour.

Analysis of Antioxidant Activity (DPPH Radical Scavenging Assay)

DPPH is a highly stable free radical with purple color. After reacting with an antioxidant it turned to a stable yellow color compound (diphenyl-picrythydrazine). Reduction in the color was measured by spectrophotometer as reported by Kumar *et al*. (2013). The IC50 value for the methanol extract of the herbal cookie was found to be 16.69 mg/ml (Table 6). Compared to the IC50 value of ascorbic acid, the IC50 value of the product is higher which means the herbal cookies have lower scavenging activity than the ascorbic acid. The IC50 value of the aqueous extract of *Aerva lanata* is 5.25 mg/ml which means the herbal cookies have lower scavenging activity than the aqueous extract of *Aerva lanata* and similar results are reported by Jayathilake *et al*. (2016). It seems that the scavenging activity has been decreased when the plant is incorporated into the cookies. On the other hand, the plant extract was found nontoxic towards human erythrocytes in the hemolytic assay where the IC50 value of the plant extract was 24.89 mg/mL (Kumar *et al*., 2013). These results concluded that the *A. lanata* stem incorporated into herbal cookies possesses an acceptable and considerable amount of antioxidant activity which can aid in the treatment of degenerative diseases such as cancer and diabetes (Jayathilake *et al*., 2016).

Table 6: IC50 values of different samples in DPPH scavenging assay

Type of samples	IC50 value
Herbal cookie sample	16.69±0.88 mg/ml
Ascorbic acid (Reference)	21.23±0.62 µg/ml
Aqueous extract of *Aerva lanata*	5.25±0.91 mg/ml

Microbial Analysis

Total Plate Count and Mold Count

The total plate count of herbal cookies was found to be increased with the storage period of the cookie sample. The highest TPC was observed at the end of the 6th week as 6.0×10^2 CFU/g (Table 4). The safe level of bacterial count for cookies should be below 1×10^4 CFU/g (Banusha and Vasantharuba, 2014). Therefore, it seems that the product could be acceptable for future consumption.

Yeast and molds were not detected in the product throughout the storage of 6 weeks at one week interval. It might be due to the thermal processing, low water

activity and hygienic practices followed during processing as reported by Agrawal *et al*. (2017).

Shelf Life Determination

It shows that the moisture content of herbal cookies has decreased over 6 weeks of storage (Table 4). It may be due to the decrease in fat content or sugar content with the storage period (Upadhyay *et al*., 2017). However, moisture content was within the standard range specified by USDA which was 4-5% (USDA Food Composition Databases, 2019). Therefore, the microbial activity has been minimized since the moisture is essential for the growth of microorganisms. According to microbial analysis and the moisture content, it indicated that the microbial counts were within the accepted range and cookies made in this study were safe to consume after storing it for 6 weeks.

CONCLUSION

The results of this study revealed that the incorporation of young stems, leaves and flowers of *Aerva lanata* and *Asparagus racemosus* fine plant powder together with mungbean (*Vigna radiata*) flour significantly improved the nutritional, sensorial quality, microbial stability, herbal value and antioxidant activity of cookies compared to the traditional wheat flour biscuit. Qualitative phytochemical screening for the acceptable cookie treatment showed that the product contained phytochemicals such as Alkaloids, Saponins, Flavonoids, Tannins, Phenols, Terpenoids, Glycosides, and steroid which may play roles in anti-cancer, anti-diabetic and anti-inflammatory in the human body.

The pH and TTA values during the storage period of cookies indicated to be more shelf-stable. Peroxide value significantly increased with the increase in storage period, though still, it was lower than the maximum recommended level and thus safe to consume. The level of moisture, ash, total fat, fiber, carbohydrate and protein content of the developed herbal cookies were in the acceptable range and a higher amount of carbohydrate, protein and fat is caused for the richness in energy value of cookies. The *A. lanata* stem incorporated into herbal cookie possesses acceptable and considerable amount of antioxidant activity which can aid in the treatment of many chronic diseases. Microbial study showed that the microbial counts were within the accepted range and cookies made were safe to consume. Hence, it was concluded that cookies supplemented with herbal plant powder enriched with mungbean flour can be stored safely in low-density zip lock polythene under room temperature for 6 weeks without any adverse changes in the organoleptic traits.

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