Formulation and evaluation of polyherbal cookies for immunity booster

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Abstract

Herbal based nutraceuticals have been increasingly popular in recent years among peoples of all ages. Cookies are the easiest way to use at our daily routine work. Based on the review, the phytochemicals such as polyphenols and sterols have reported to possess good immunomodulatory action. On support of thorough literature review and folklore claims, Ashwagandha, Moringa, and Triphala are the good source of macronutrients, micronutrients and nutraceutical components. So, the current study was focused to improve nutraceutical components of herbal source by including traditional herbal ingredients and also to analyze the nutrient composition. Out of the tested formula, the sensory scores of B3 (4.5% polyherbs + 95.5% multi grain flour blends and other ingredients) was found to be good in terms of overall acceptability of the product. Formulated polyherbal cookies were tested for physical evaluation, identification of phytocompounds by preliminary chemical test, sensory evaluation, nutritional analysis, antioxidant activity by TLC bioautography and free radical scavenging activity by 2, 2- diphenyl 1-picryl hydrazyl (DPPH) method. From all observed evaluation parameters, the polyherbal cocoa based cookies was found as a good alternative nutraceutical for immunity booster with enriched nutritional value in any normal individual rather than normal marketed cookies/biscuits.

1. Introduction

Our immune system defends our body against cancer, infections, diseases, and invasive pathogenic microorganisms. The body's system creates an immune response to protect you from foreign substances, cells, and tissues. Macrophages and lymphocytes (B cells, and T cells) are major immune system components (Turvey and Brodie, 2010). The first line of defence against infection is provided by innate immunity. It is a quick reaction that takes minutes but it is not pathogen-specific. It has no memory and does not give the host enduring immunity. It has four fundamental components which are present in every class of plants and animals (Bonilla and Oettgen, 2010). The inflammatory response actively drives immune cells, thereby increasing blood flow to the infection site and marks them for destruction (Murphy *et al.,* 2007). When re-exposure to the same foreign organism induces a memory response with a more rapid immune reaction that eliminates the pathogen and prevents disease is found only in vertebrates (Hofmeyr, 2001). Cell-mediated immune response carried out by B cells and T cells which are activated to fight off the invading pathogen (Puri *et al*., 1994). The sources of immunity are antibodies, mother's milk or synthetic methods antivenom antibodies (Alfons, 2001). Because immune system cells have a variety of receptors on their surfaces, some of which chemically connect to pathogens and others of which bind to other immune system cells or substances to enable the complex immune response

and chemical bonding is required for both detection and elimination (Sheikh, 2008). Immunosuppressants are a class of drugs with a wide range of physical and functional properties that are frequently administered in combination **regimens** to treat various forms autoimmune diseases (Agarwal and Singh, 1999). Till now about 4000 phytochemicals have been identified. When consumed by human, phytochemicals have a wide range of potent biological effects such as antioxidants, anti-inflammatory and immunomodulatory qualities. The therapeutic effects of phytochemicals on treating a range of diseases and enhancing the quality of life have been extensively studied over the past two decades (Thatte and Dahanukar, 1997).

Herbal plants are more effective because they support the body's natural repair processes. Because of the bulk of currently available medications are expensive and pose issues like allergy and drug resistance (Jayakumari *et al*., 2023), there is crucial need for the globe to have increased research on the alleged medicinal plants and its phytochemicals that are economical, effective and safe. The aim of the current study was to formulate polyherbal cookies and to screen its antioxidant potential in order to support the folklore claims. The objective of the present study was to formulate and evaluate polyherbal cookies as per standard protocol. The current study was focused to improve nutrition and nutraceutical components of herbal source by including traditional or unique ingredients, analyzing nutrient composition and determining consumer acceptability of the most popular value-added nutraceutical product. The findings of this study could be a promising platform for herbal based nutraceuticals as immunity enhancer.

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2. Materials and Methods

2.1 Selection of herbs

Triphala powder, Moringa leaf (*Moringa oleifera*), and Ashwagandha root (*Withania somnifera*) were selected based on earlier literature and folklore claims for the development of herbal cookies.

2.2 Procurement of raw material

Triphala powder, Ashwagandha root powder, Moringa leaf powder and finger millet were purchased from the authenticated herbal shop and other ingredients like wheat, butter, country sugar, baking soda, milk, cocoa powder, almonds, and walnuts were purchased from the local market in Chennai.

2.3 Formulation

Ragi was made into sprouted naturally and dried ground into a powder. The ragi flour was dry roasted until to get good aroma. Triphala powder, Moringa leaf powder (*Moringa oleifera*), Ashwagantha root powder (*Withania somnifera*), roasted millet, wheat flour and baking powder were measured and taken in the glass bowl. Cardamom was weighed and ground into a coarse powder and added to the all the dry ingredients. All were sieved to remove any larger debris. The cold butter was chopped into small cubes. The chopped cold butter was added to the dry ingredients rubbed using the finger. Country sugar was added in required quantity and milk was added and kneaded to get dough. The dough was refrigerated for 10 to 15 min. Meanwhile the oven or cooker is preheated for 10 min. The dough was made into small balls and flattened. Almonds and walnuts were sprinkled at the top. The butter paper was placed in the baking tray and greased with butter. The flattened dough was placed in the tray. The baking tray was placed in the oven or pressure cooker and heated at 180°C for 15-20 min. After baking, cookies were cooled to room temperature and packed in an air tight container.

2.4 Physical evaluation

A vernier calliper scale is used to measure the dimensions of prepared cookies and the spread ratio was calculated. The spread ratio of herbal cookies was determined using a formula:

Spread ratio = diameter (mm) / thickness (mm)

2.5 Phytochemical evaluation

The prepared plant extracts were subjected to preliminary phytochemical screening for its phytoconstituents such as saponins, glycosides, phytosterols, tannins, flavonoids, carbohydrates, triterpenoids and alkaloids as per standard procedure (Vijayalakshmi *et al.*, 2023). Phytochemicals such as phenols, flavonoids, glycosides, alkaloids and carbohydrates were found to present. Whereas saponins, steroids and tannins were absent.

2.6 Sensory evaluation

Sensory evaluation of the cookies for their acceptability was done by a panel of 10 on the basis of evaluation of attributes like colour and appearance, body and texture, taste and flavour and overall acceptability on a Hedonic scale based on 9 points. The average sensory ratings for the various parameters in the treated and control cookies clearly demonstrate that B3 received the highest rating and that the cookies showed improved in all metrics. So, it was chosen for final formulation (Podder *et al.,* 2015).

2.7 Analysis of nutritional value

The proximate analysis like fat, carbohydrate, protein, ash, fibre was carried out following AOAC (1996, 2000) procedures. Protein content was determined by Kjeldahl method, fat by Soxhlet method, ash content was determined by Muffle furnace method, fibre content by enzymatic-gravimetric method and carbohydrate by Molisch's test. All tests were analysed and presented in the Table 5.

2.8 Evaluation of antioxidant activity

2.8.1 TLC bioautography method

The extracts were run on TLC silica plate (Merck, F245) using toluene: ethyl acetate: formic acid (4:5:1) as mobile phase. Plates were air dried and then sprayed with 0.2% DPPH in methanol. The plate is examined in daylight after 30 min.

2.8.2 DPPH method

Antioxidant study was performed for the free radical scavenging property by DPPH antioxidant assay. The free radicals scavenging potential of the immune booster formulation was evaluated using DPPH assay's radical scavenging. The effect of given samples on DPPH radical was estimated according to the procedure (Rasool and Varalakshmi, 2006). The decrease of absorbance at 515 nm was continuously recorded in a spectrophotometer for 16 min at room temperature. Methanolic solutions of pure compound (quercetin) were tested at 1 mg/ml concentration. The scavenging effect (decrease of absorbance at 515 nm) was plotted against the time and the percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16 min in duration (Bouic, 2002).

3. Results

Based on the thorough literature review, folklore claims and day-to-day usage the commonly used herbs. Triphala powder, Moringa leaf powder, Ashwagandha root powder were selected. All were collected, authenticated and processed as per standard methods. The millet used as a binding agent as well as base. From the 13 trials carried out, 3 trials were optimized. Three batches were formulated. The formulation ratio of ingredients was shown in Table 1.

The selected all the three batches were subjected to physical evaluation and the results showed that batch 3 have very good results as compared to batchs 1 and 2. The average thickness was 728 mm and diameter is 7.16 cm and spread ratio was found to be 9.83. The results were exhibited in Table 2.

The phytochemical test was carried for prepared cookies to identify the presence of active compounds. Phytochemical test revealed the presence of alkaloids, glycosides, flavonoids, phenol and carbohydrates which are mainly responsible for immunity enhancement. The results of qualitative phytochemical analysis of all tested batches were shown in Table 3.

Based on data and average sensory score of different parameters of both blank and prepared product, the batch 3 (B3) was found to possess highest score shown in Table 4 and Figure 1. So, batch 3 was selected for the final formulation of the product.

Table 1: Composition of the polyherbal cookies

S. No.	Ingredients	Batch 1 (for 20 cookies)	Batch 2 (for 20 cookies)	Batch 3 (for 20 cookies)
1.	Triphala powder	5 mg	8 mg	15 mg
2.	Moringa leaf powder	5 mg	8 mg	15 mg
3.	Ashwagandha root powder	5mg	8 mg	15 mg
4.	Ragi or finger millet	20 mg	30 mg	60 mg
5.	Wheat flour	40 mg	75 mg	125 mg
6.	Cocoa powder	5 mg	8 mg	10 mg
7.	Cardamom	5 mg	7 mg	10 mg
8.	Jaggery powder	50 mg	70 mg	125 mg
9.	Baking powder	0.5 mg	1mg	1 mg
10.	Cold butter	40 mg	60 mg	100 mg
11.	Almond	5 mg	5 mg	5 mg
12.	Walnuts	5 mg	5 mg	5 mg
13.	Milk	0.5 ml	1 ml	1 ml

Table 2: Physical parameters of polyherbal cookies

Sample	Thickness (mm)	Diameter (mm)	Spread ratio
Batch 1	7.23	71.6	9.90
Batch 2	7.34	71.1	9.68
Batch 3	7.28	72.2	9.91

Table 3: Qualitative phytochemical analysis of polyherbal cookies

Phytoconstituents	Presence/Absence
Alkaloids	Presence
Glycosides	Presence
Flavonoids	Presence
Phenols	Presence
Saponins	Absence
Steroids	Absence
Tannins	Presence
Carbohydrate	Presence

Table 4: Results of sensory evaluation of three batches of polyherbal cookies

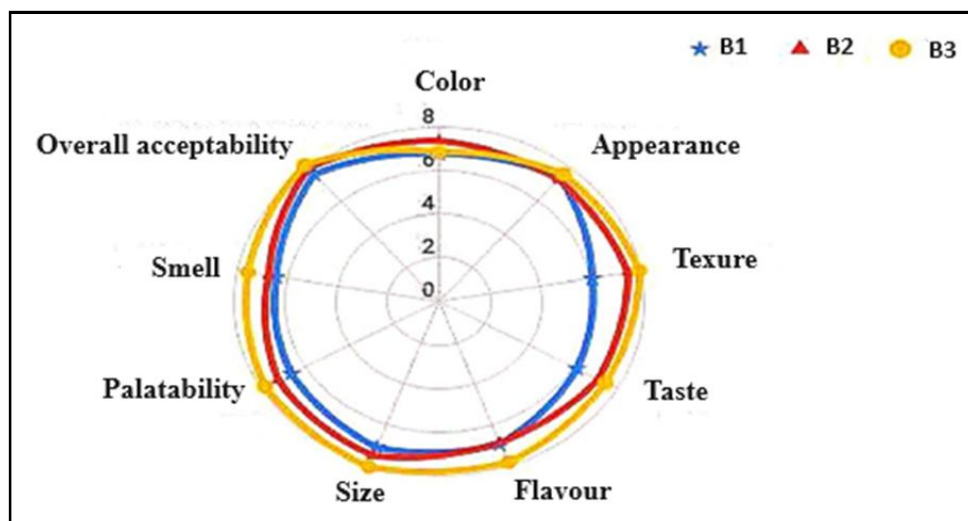| Properties	Batch 1	Batch 2	Batch 3
Colour	6.8	7.4	6.9
Appearance	7.4	7.3	7.6
Texture	6.1	7.5	8
Taste	6.2	7.2	7.5
Flavour	7	6.9	7.9
Size	7.2	2.6	8
Palatability	6.7	7.3	7.9
Odour	6.5	6.8	7.6
Overall acceptability	7.6	8	8.2

Figure 1: Radar chart representing sensory analysis score.

It shows that the moisture content of B1 is 4.04%, B2 is 2.36 and B3 is 1.4%. The present study shows that the ash content in B1 is 1.4%, B2 is 1.2% and B3 is 0.8%. The present study showed that the crude fibre content in B1 is 2.5%, B2 is 2%, and B3 is 5%. The increase in crude fibre level is associated with the presence of grains.

The pH of B1 is 7.3, B2 is 7.2, and B3 is 6.9. The decrease in pH level is due to lactic acid production and sucrose hydrolysis. The nutritional values such as fat, carbohydrate, protein, sugar and fiber content were determined for prepared cookies and it was compared with market sample. The result was shown in Table 5.

Table 5: Nutritional comparison of market sample and formulated polyherbal cookies

Parameter	Marketed sample in G/100 gm	Formulated herbal cookies in G/100 gm
Carbohydrate	54.61	61.64
Total fat	24.6	25.14
Total protein	4.8	5.73
Total sugar	23.26	8.94
Crude fibre	2.50	4.59
Energy	499.04	495.74

Physical analysis such as moisture content and ash value were determined for three batches, from the observation values the batch 3 (B3) was found to show the significant values. The crude fiber content was found maximum in B3, due to more amount of fiber. The pH was found to be near neutral in all the tested three trials. All were represented in Figure 2.
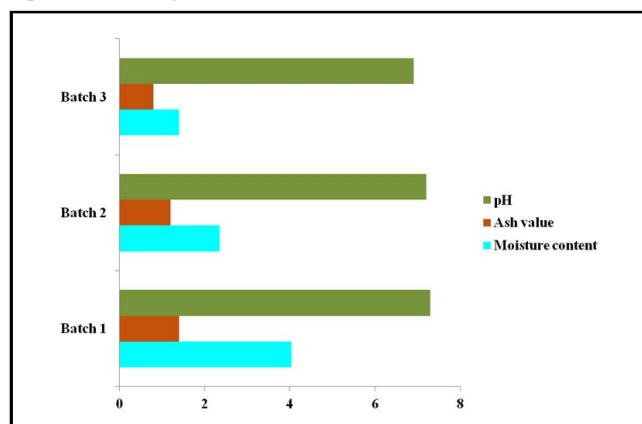

Figure 2: Physical analysis of three batches of polyherbal cookies.
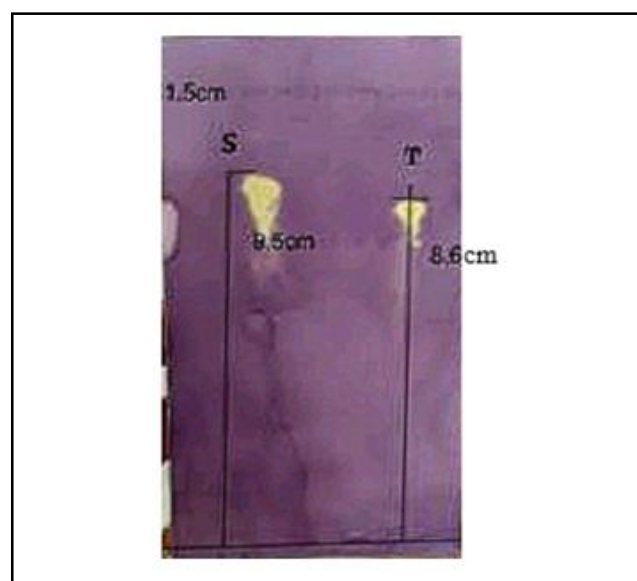

Figure 3: TLC bioautography assay with DPPH reagent. S: Standard quercetin, T: Test sample.

Plates were air dried and then sprayed with 0.2% DPPH in methanol. The plate is examined in daylight after 30 min. Bright yellow or cream colour was used for the confirmation of antioxidant property. The Rf value of the sample and standard were recorded. Antioxidants bands were observed in the TLC plate exposed to DPPH. The Rf value of standard (quercetin) was found to be 0.82 and the Rf value of test (cookie sample) was found to be 0.81. The TLC plate after spaying with DPPH has shown in the Figure 3.

On supporting of this, the cookies also evaluated for *in vitro* antioxidant assay by DPPH method, taking minimum concentration of 0.5 mg to maximum of 2.5 mg by calorimetry method 51.05% of inhibition was observed at the concentration of 1.5 mg. So, the results of DPPH activities of the test sample exhibited significant proton donating power and as good free radical scavenger activity as shown in Table 6 and Figure 4.

Table 6: Antioxidant activity of the test sample by DPPH method

S. No.	Sample concentration (in mg)	Optical density (OD)	% Inhibition
1	Blank	0.617	0.00
2	0.5	0.473	23.34
3	1	0.342	44.57
4	1.5	0.302	51.05
5	2	0.214	65.32
6	2.5	0.165	73.26
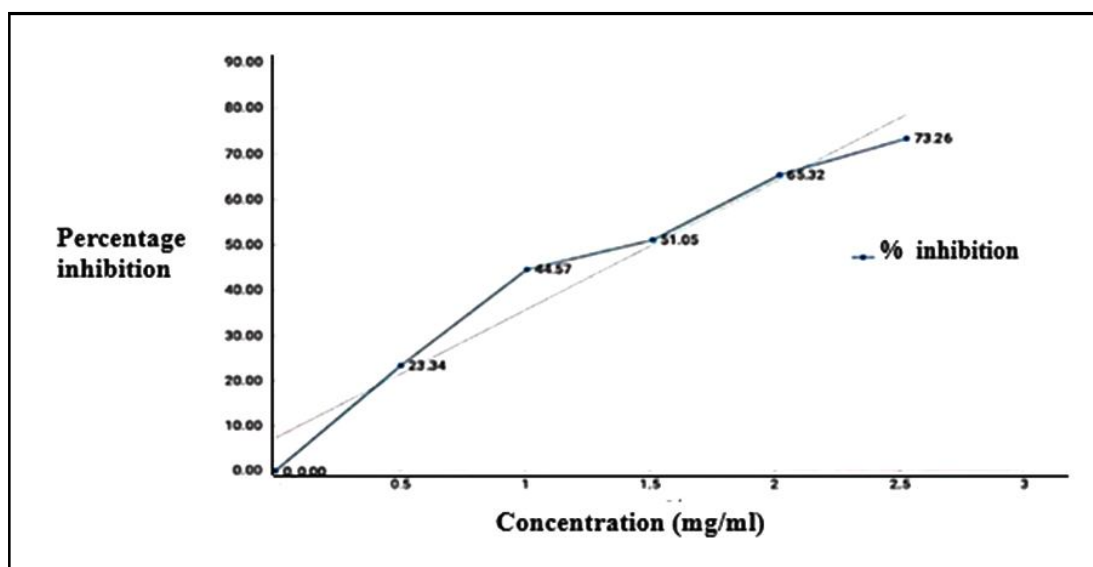
Figure 4: Antioxidant activity by DPPH method.

Figure 5: Formulated polyherbal cookies.

From all observed evaluation parameters, the polyherbal cocoa based cookies was found as a good alternative nutraceutical for nutritional value and as immunity booster in any normal individual rather than normal marketed cookies/biscuits. The formulated cookies were shown in Figure 5.

4. Discussion

The whole world facing COVID-19 pandemic, so there has been a lot of interest in ways to boosting our immune system, and thus build a first line of defence against the deadly virus. Since ancient times, medicinal plants, herbs and spices were well known for their medicinal properties. Therefore, the medicinal plants and herbs playing a critical role to boosting our immunity during the COVID-19 pandemic. It is also very important to consume supplements in the form of immune nutrients such as vitamin A, C, E, D, B-complex, Zinc and copper that will support your body to fight against the pathogens. This study presents an analysis of popular immune-boosting medicinal

plants and herbs. Based on the thorough literature review, folklore claims and day-to-day usage, the commonly used herbs, Triphala powder, Moringa leaf powder, Ashwagandha root powder were selected. All were collected, authenticated and processed as per standard methods. The millet used as a binding agent as well as base. Three batches were prepared. All the three batches were formulated by natural traditional method. The main herbal ingredient are: Triphala, Ashwagandha and Moringa for developing immunity. The finger millet (ragi) and wheat flour were used dual purpose as binder as well as source for vitamins and minerals. Jaggery as sweetening agent, cardamom as flavor, cocoa powder as colorant and taste. Almond and walnut were used for elegant purpose as well as nutrition purpose, since it contains rich amount of calcium. Milk and butter added only in negligible quantity as for formulation purpose. The formulated cookies were evaluated for physical, phytochemical and antioxidant study. Phytochemical test revealed the presence of alkaloids, glycosides, flavonoids, phenol and carbohydrates which are many to responded for immunity enhancement. The sensory evaluation was applied to all the three batches. Based on data and average sensory score of different parameters of both control and prepared cookies, the batch 3 (B3) was found to be the highest scores which has all accepted parameters. So, it was chosen for the final formulation of the product. The nutritional values such as fat, carbohydrate, protein, sugar and fibre content were determined for prepared cookies and it was compared with market samples. Moisture content, ash content were determined for three trials, from the observation values, the batch 3 (B3) was found to show the best values. The crude fibre content was found maximum in B3, due to more amount of fibrous herbal ingredients. The pH was found to be near neutral in all three trials. In comparison to the multigrain cookie product currently on the market, the overall carbohydrate and protein values are higher in the formulated polyherbal cookies. Due to its high nutritional content, it helps to achieve its intended goal of increasing overall energy and reducing stress. In the present study, three formulations of sample were estimated for moisture, ash, protein, fat, protein, carbohydrate and energy. Phenols, a group of organic compounds, are recognized for their antioxidative potential. They play a pivotal role in neutralizing free radicals and reducing oxidative stress in the body. Flavonoids another class of bioactive compounds, possess strong antioxidant effects (Falak Bamne *et al*., 2023). In order to determine and evaluate its immunity booster in support of traditional claims, the study was carried out for DPPH antioxidant assay by TLC bioautography method and DPPH *in vitro* antioxidant assay. The bright yellow colour was observed in day highest after 30 min. It was compared with standard, natural antioxidant (quercetin). The Rf value matches with standard value. It indicates that our prepared cookies have significant amount of antioxidant compounds, which may be responsible for immunity enhancement. On supporting of this, the cookies also evaluated for *in vitro* antioxidant assay by DPPH method by taking minimum concentration of 0.5 mg to maximum of 2.5 mg by calorimetry method. 51.05% of inhibition was observed at the concentration of 1.5 mg. So, the observed antioxidant activities of the tested samples possess strong proton donating power and as good free radical scavenger activity. From all observed evaluation parameters, polyherbal cocoa based cookies was found as a good alternative nutraceutical and food supplement for nutritional value and as immunity booster in any normal individual rather than normal marketed cookies/biscuits.

5. Conclusion

Herbal based nutraceuticals have been increasingly popular in recent years among peoples of all ages. Cookies are the easiest way to use at our daily routine work. Based on the thorough literature review and earlier claims, Ashwagandha, Moringa, and Triphala are a good source of macronutrients, micronutrients, and nutraceutical components. Out of the tested formula, B3 (4.5% polyherbs + 95.5 % multi grain flour blends and other ingredients) was found to good in terms of overall acceptability. The polyherbal cocoa based cookies was found as a good alternative nutraceutical with significant nutritional value and may use as immunity booster in any normal individual rather than normal marketed cookies/biscuits. The current study was focused to improve nutrition and nutraceutical components of herbal source by including traditional or unique ingredients, analyzing nutrient composition, and determining consumer acceptability of the most popular value-added nutraceutical product.

Acknowledgments

Conflict of interest

The authors declare no conflicts of interest relevant to this article

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