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Formulation Development and Evaluation of Anti-diabetic Nutraceutical Instant Soup Powder Formulation

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Abstract

The research study was conducted to develop anti diabetic nutraceutical instant soup powder formulation with the use of jamun seed powder, fenugreek seed powder, gurmar leaves powder for anti-diabetic activity and garlic powder, curry leaves powder, black pepper powder, and black salt for spices, and mushroom, tomato powder for flavoring, and figer millet flour for thickening of soup the soup powder in different ratios of the ingredients F1, F2, F3 and F4, the prepared formulations were evaluated for sensory evaluation and result reviles F4 found to be best with overall acceptability, hence F4 select ed for further study The results of nutritional analysis indicate that, formulated instant soup powder was enhanced with protein and carbohydrate and low in fat with high energy value. Antioxidant and antimicrobial content of formulated instant soup powder was found to significant, outcome of alpha amylase inhibition assay indicate that formulated instant soup powder retained low glycemic index and can be supplemented to diabetic patient the shelf-life properties were found to be good and it retained flavor and test after 3 months of storage. Hence, this formulated anti diabetic nutraceutical instant soup powder can be easily manufactured at low cost and it can definitely meet out the need of growing functional food market.

Keywords: Diabetes; Nutraceutical; Instant soup powder; Functional food

Introduction

Diabetes

Diabetes is "one of the largest global health issue in the 21st century" the International Diabetes Federation (IDF) In 2017, estimated 424.9 million people 20–79 years globally are suffering from diabetes where 1 in 11 people are affected by the disease with a prevalence rate of 8.8%; 1 in 2 adults is undiagnosed (212 million); and 12% of global health expenditure is spent on diabetes (USD727 billion [1]. It is the second leading

cause of years of life lost to premature death and the fourth leading cause of years lived with disability.

Diabetes mellitus is a metabolic disorder characterised by hyperglycaemia or high blood sugar. The characteristic symptoms of diabetes are polyuria (excessive urine production), polydipsia (thirst and increased fluid intake) and blurred vision; these symptoms may be absent; if the blood sugar is only mildly elevated. The World Health Organisation (WHO) recognises three main forms of diabetes mellitus: Type 1, type 2 and gestational diabetes (occurring during pregnancy), which have similar signs, symptoms and consequences, but different causes and population distributions. Ultimately, all forms are due to the beta cells of the pancreas being unable to produce sufficient insulin to prevent hyperglycaemia [2]. Type 1 is usually due to autoimmune destruction of the pancreatic beta cells, which produce insulin. Type 2 is characterised by tissue wide insulin resistance, but impairment of beta cell function is necessary for its development. Gestational diabetes is similar to Type 2 Diabetes (T2D), in that it involves insulin resistance due to predisposal of pregnancy hormones.

Diabetes is considered in the top five of the most significant diseases in the developed world. Although extensive armamentarium has been developed to combat this ancient disease but now a days, focus has been shifted to identify effective agents that can be used along with developed drug to treat this disease synergistically.

Nutraceutical in treatment of diabetes

Food and drugs from nature are playing a quite significant role in public healthcare system throughout the world [3]. Human inquisitiveness and search for specific constituents of plants, animals, minerals and microbial origin, which are beneficial to our overall health, has coined terms like 'functional food' or 'nutraceuticals'. Dr. Stephen L. Defelice defined nutraceuticals as any substance that may be considered as food or part of food, which in addition to its normal nutritive value; provide health benefits including prevention of disease. The term 'nutraceuticals' came from a combination of 'nutrition' and 'pharmaceuticals' and can be defined as a food or part of a food that provides medical or health benefit including the prevention and treatment of a disease.

Perhaps no other disease is as closely linked to nutrition as diabetes. Although nutrition plays a significant role in its development; it is also one of the most powerful tools in treatment of diabetes. The use of nutritional supplements in the treatment of the diabetes like vitamins, such as vitamin C and B, minerals such as chromium, as well as herbs like *Gymnema sylvestre*, is well documented as safe and effective way to lower blood sugars as well as for prevention of diabetic complications [4]. More importantly, combined of scientifically validated diabetic formula to work synergistically for effective management of diabetes and related complications.

Instant soup powder

Nutritional rich convenience foods formulation will have definite uprising and satisfies the market demands. Canned foods, convenience foods, frozen foods, dried foods, preserved foods, etc. comes under instant soup powders are similar kind of instant foods which have extended popularity in the recent years, by way of providing convenience, hygienic, extensible shelf life. A balanced nutrition is obtained by including whole cereals, vegetables and pulses in soup formulations.

Materials and Methods

Selection of raw material

Jamun, gurmar powder, fenugreek seed and other spices and ingridient including finger millet powder, tomato powder, garlic, curry leaves, black paper, black salt etc. was collected from local market, oyster mashroom were collected from local cultivator from Kampthee, Nagpur, and stevia from Bhagyashee lab Nagpur.

Processing for fenugreek seed powder

Fenugreek seed were obtained from the residential area of Nagpur, India the processing of fenugreek seed was carried out, washing and draining of seed were carried out and allow to complete drying under the sunlight in sunny days, after complete drying of seed, the grinding of seed carried out in to fine powder, sieving of powder by sieve no. 44 and powder were subjected for defatting, 100 gm of fenugreek seed powder extracted with sufficient amount of petroleum ether for overnight, after that fenugreek seed powder filter out and draining of powder were carried out and the defatted fenugreek seed powder were obtained.

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Preparation of spice powder

Firstly pealing of garlic were carried out and washing and draining of garlic and curry leaves were takes place, were then cutting of garlic and curry leaves were carried out and allowed for sundrying in very sunny days till complete drying, after complete drying grinding of individually garlic and curry leaves were carried and passing though sieve (sieve no. 44) to obtained fine powder, after that garlic powder, curry leaves powder, black paper powder, black salt and stevia powder were mix completely to obtained spice powder.

Preparation and formulation of antidiabetic nutraceutical instant soup powder formulation

Antidiabetic nutraceutical instant soup powder mixture prepared by mixing of jamun powder, defatted fenugreek powder, gurmar powder as antidiabetic active herbs with other ingredient tomato powder for flavouring the soup mixture, finger millet flour and mashroom powder for thickening the soup and prepared spice powder, the prepared instant soup powder were then sealed in a translucent or coloured polythene bags and used for further analysis like sensory evaluation, proximate analysis metal content and microbial contamination, etc.

Cooking procedure for prepared instant soup powder

Ttake 100 ml of water and add Ten gram of the prepared soup mix powder in it then allow to boil for 3 to 5 min then Pour in to soup bowl, stir it and serve it hot (Figure 1 and Table 1).



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2	Fenugreek	650	550	450	350
3	Gurmar	600	500	400	300
4	Mashroom	1000	1000	1000	1000
5	Finger millet	1500	1500	1500	1500
6	Tomato	3000	3000	3000	3000
7	Garlic	1000	1000	1000	1000
8	Curry leaves	500	500	500	500
9	Black paper	500	500	500	500
10	Black salt	1000	1000	1000	1000
11	Stevia	450	450	450	450

Evaluation of antidiabetic nutraceutical instant soup powder formulation

Physicochemical parameter of formulated instant soup powder: Physicochemical parameter like pH, viscocity, moisture content and ash values were determined according to standard procedure as fallows

Moisture content and ash values for active anti diabetic herb and formulated instant soup powder: Moisture content ash values of the the formulated instant soup powder was determined by standard procedure given by WHO.

pH of formulated instant soup powder: A definite amount of instant soup powder (10 gm) was dissolved in 100 ml water and the pH of formulation was recorded using digital pH Meter.

Viscocity of formulated instant soup powde: The viscosity of the instant soup formulation was measured using Brookfield viscometer the measurement was carried out at $25^{\circ}C \pm 1^{\circ}C$, 0.5 rpm speed using spindle no. 62 in triplicate [5].

Sensory evaluation: In sensory evaluation, each sample was subjected to five point hedonic scale test (5-excellent, 4-very good, 3-good, 2-poor, 1-very poor) and acceptability of sample was judged by 27 untrained members. From the institute the panelists judged the sensory characteristic such as appearance, odor, color, taste, consistency and overall acceptability of the samples.

Nutrient analysis of formulated instant soup powder: Moisture content, ash values and nutrient analysis such carbohydrate, protein, fat, and energy value was evaluated for formulated instant soup AOAC method (2000).

Heavy metal determination of formulated instant soup powder: Lead, arsenic, cadmium and mercury of formulated instant soup power were determined by flame atomic absorption spectrometric method.

Antimicrobial, antioxidant and antidiabetic activity of formulated instant soup power: Antimicrobial activities of

formulated instant soup power were determined by agar well diffusion method [6]. Antioxidant activity of formulated instant soup power was analyzed by DPPH assay. Antidiabetic activity of formulated instant soup power and marketed anti diabetic nutraceutical formulation was assessed through α -amylase inhibition assay Methanolic extract of sample was dissolved in DMSO in order to obtain concentrations of 10, 20, 40, 60, 80, and 100 µg/mL. Then, 0.2 mL of sample of particular concentration was added to the tube containing the substrate solution [7]. In addition, 0.1 mL of porcine pancreatic amylase in Tris–HCl buffer (2 units/mL) was added to the tube containing the extract and substrate solution. The reaction was carried out at 37°C for 10 min. The reaction was stopped by adding 0.5 mL of 50% acetic acid in each tube. The reaction mixture was centrifuged at 3000 rpm for 5 min at 4°C. The absorbance of resulting supernatant was measured at 595 nm using spectrophotometer Acarbose, a known α -amylase inhibitor was used as a standard drug. The experiments were repeated thrice. The α-amylase inhibitory activity was calculated by using following formula.

%Inhibition=Control-Sample/Control)*100

Results and Discussion

Physicochemical parameter

Moisture content and ash values: The physicochemical quality standardization like moisture content and ash values of herbal crude drug and formulation batches (pre and post stability) were determined, Moisture (loss on drying) is one of the major factors responsible for the deterioration of the drugs and formulations [8]. Low moisture content is always desirable for higher stability of herbal drugs (Table 2). Moisture contents of the individual Herbal drug were found in the range 1.39%-2.52%, w/w and different Formulation batches F1, F2, F3,

F4 pre and post stability (Table 3) in the range of 1.44-2.48% w/w, all the values were less that 5%, were formulation F1, F2, F3 shows change in moisture content and F4 shows 1.44%-1.57% w/w moisture shows better stability, and high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the drug or drug combinations for marketing [9]. All the individual drugs and different Formulation batches F1, F2, F3, F4 pre and post stability were determined. Ash values of the individual Herbal drug found to be total ash value in the range 4.53%-10.82% w/w, water-soluble ash in range of 2.43%-3.24% w/w and acid-insoluble ash in the range of 1.32%-2.46% w/w and ash values of different formulation batches F1, F2, F3, F4 pre and post stability were found to be, total ash value in the range of 9.32%-8.25% w/w, water soluble ash in the range of 2.98%-4.45% w/w, acid insoluble ash in the range of 0.9%-2.1% w/w, These values were found to be reasonably low indicating low contamination (Table 4). Moisture and ash values of the formulations matches with the average total ash values of the individual drugs [10]. Were formulation F1, F2, F3 shows change in values and F4 shows total ash 9.1-9.3,

water soluble ash 4.43-4.45 and acid insoluble 1.9-2.0 having better stability.

pH and viscocity: pH and viscocity of the formulated instant soup powder was determined and found to be respectively.

Sensory analysis: Sensory evaluation is an essential concept in food product development as it reduces the risk of product failure and links the consumer perception about quality of food. Even though formulated food products are nutritious, without taste and odour the product cannot reach market in successfully. The results of sensory evaluation different formulation batches F1, F2, F3, F4 are illustrated in the Table 6. The data reveals that formulation F4 was found to be highly acceptability with significant difference when compared to other formulation F4 having better taste, odour, consistency and colour of soup [11-15]. From this study we finalize the F4 instant soup powder formulation as optimized formulation.

 Table 2: % LOD, total ash value water soluble ash acid insoluble ash for antidiabetic.

Sr. no.	Name of drug powder and formulation	% LOD Mean (n=3) ± SD (% w/w)	Total ash Mean (n=3) ± SD (% w/w)	Water soluble ash Mean (n=3) ± SD (%w/w)	Acid insoluble ash Mean (n=3) ± SD (%w/w)
1	Jamun	1.50 ± 0.17	4.53 ± 0.12	2.76 ± 0.35	1.72 ± 0.02
2	Fenugreek	2.52 ± 0.04	9.68 ± 0.24	2.43 ± 0.03	1.86 ± 0.02
3	Gurmar	1.39 ± 0.24	10.82 ± 0.14	3.24 ± 0.06	2.46 ± 0.02

Table 3: % LOD and total ash value for instant soup powder formulations.

Formulations	Pre stability		Post stability		
	% LOD Mean ± SD (% w/w)	Total ash value Mean ± SD (% w/w)	% LOD Mean ± SD (% w/w)	Total ash value Mean ± SD (% w/w)	
F1	2.48 ± 0.015	8.25 ± 0.065	3.13 ± 0.081	8.12 ± 0.095	
F2	2.38 ± 0.055	9.35 ± 0.045	3.19 ± 0.034	9.11 ± 0.078	
F3	1.78 ± 0.035	8.59 ± 0.11	2.27 ± 0.045	8.43 ± 0.065	
F4	1.44 ± 0.040	9.32 ± 0.075	1.57 ± 0.057	9.11 ± 0.076	

Table 4: water soluble and acid insoluble ash values for instant soup powder formulations.

Formulations	Pre stability	Post stability (After 1 month)		
	WatersolubleashAcidinsolubleashMean ± SD (% w/w)Mean ± SD (% w/w)	WatersolubleashAcidinsolubleashMean ± SD (% w/w)Mean ± SD (% w/w)		

F1	4.10 ± 0.11	1.4 ± 0.043	3.87 ± 0.43	0.98 ± 0.32
F2	3.65 ± 0.17	1.97 ± 0.43	3.28 ± 0.12	1.45 ± 0.11
F3	3.15 ± 0.11	1.81 ± 0.21	2.98 ± 0.54	1.45 ± 0.098
F4	4.45 ± 0.34	2.0 ± 0.32	4.43 ± 0.01	1.99 ± 0.087

Table 5: pH and viscosity of instant soup powder formulation.

Formulations	Pre stability		Post stability (After 1 month)		
	Viscosity (cPs) Mean ± SD	pH Mean ± SD	Viscosity (cPs) Mean ± SD	pH Mean ± SD	
F1	711 ± 3.51	4.53 ± 0.010	789 ± 4.5	4.19 ± 0.01	
F2	792 ± 4.58	4.43 ± 1.025	822 ± 2.08	5.72 ± 0.01	
F3	738 ± 5.10	4.16 ± 0.20	710 ± 3.512	4.97 ± 0.02	
F4	607 ± 4.93	4.13 ± 0.020	615 ± 2.00	4.26 ± 0.02	

Table 6: Sensory analysis.

	Parameter	Very poor	Poor	Good	Very good	Excellence
F1	Odour	10	16	-	-	-
	Taste	12	7	7	-	-
	Consistancy	10	8	7	2	-
	Color	15	6	6	-	-
F2	Odour	9	11	7	-	-
	Taste	10	15	2	-	-
	Consistancy	8	7	7	5	-
	Color	15	6	3	3	-
F3	Odour	3	13	8	3	-
	Taste	2	9	10	6	-
	Consistancy	-	5	14	8	2
	Color	-	7	13	7	-
F4	Odour	-	2	8	15	2
	Taste	-	1	10	13	3
	Consistancy	-	2	9	10	6
	Color	-	3	7	13	4

Estimation of nutritional values

Formulated instant soup powder was prepared using antidiabetic herb's, spices, flavor and finger millet flour for thickening of soup and optimized formulation subjected for nutritional analysis and it was found to highly acceptable. The nutrient analysis of the formulation is represented in the Table 8 [16]. Protein content was found to be 10.55 gm/100 gm shows high in protein which required for diabetic patient, carbohydrates was found to 64.97 gm/100 gm which was in the safety range required for diabetic patient, total fat 1.06 gm/100 gm shows low total fat essential for diabetic patient, total fat 1.06 gm/100 gm shows low total fat essential for diabetic patient, energy value (calories) was found to 311.62 Kcal/100 gm shows acceptable calorie value [17]. The above study shows prepared formulation is in superior and safe nutritional content (Table 7).

Table 7: Heavy metal.

Sr. no.	Heavy metal	Result (mg/kg)
1	Lead (Pb)	Absent
2	Arsenic (As)	Absent
3	Cadmium (Cd)	Absent
4	Mercury (Hg)	Absent

Heavy metal content

Heavy metals are harmful and become toxic for health if they are taken above the limit of daily allowance recommended. In

this study, heavy metals were not found in this antidiabetic nutraceutical formulation which shows formulation is safe (Table 8).

Table 8: Nutritional values.

Sr. no.	Nutritional value (per 100 gm)		
1	Energy value (Calories)	311.62 Kcal/100 gm	
2	Carbohydrates	64.97 gm/100 gm	
3	Total fat	1.06 gm/100 gm	
4	Protein	10.55 gm/100 gm	

Antimicrobial assesment

The standard drug ofloxacin and extract of Instant soup powder sample subjected for antimicrobial assessment for checking susaptability against *S. bacillus*. Were different concentration of sample extract shows zone of inhibition as follows: 50 mg/ml shows 10.50 mm, 100 mg/ml shows 12.50 mm, 150 mg/ml shows 13.50 mm, 200 mg/ml shows 16.00 mm against standard ofloxacin (Figures 2 and 3) were not superiar than std. but good susaptability shown by sample againt *S. bacillus* (Table 9).

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(Ofloxacin).

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Figure 3: Zone of inhibition shown by instant soup powder sample.

Tabel 9: Zone of inhibition shown by standard and sample.

Strain used: <i>S. bacillus</i> zone of inhibition in mm					Standard drug (Ofloxacin) 10 mcg/ml
Concentration	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	
Sample	10.50 ± 0.577	12.50 ± 0.577	13.50 ± 0.577	16.00 ± 0.816	31.25 ± 0.957

Antioxidant activity by DPPH assay

Antioxident activity of standard ascorbic acid and test sampleantioxident study of antidiabetic nutraceutical Instant soup powder the sample shows % inhibition for distinct concentration

in range of 48.798 to 66.996% which was within range (Table 10 and Figure 4, Table 11 and Figure 5).

Table 10: % Inhibition of standard ascorbic acid in DPPH assay.

Concentration in mg/ml	% Inhibition
20	52.741
40	56.36
60	61.513
80	68.969
100	71.711

IC₅₀=11.54



Tabel 11: % Inhibition of sample in HPPH assay.

Concentration in mg/ml	% Inhibition	
20	48.794	
40	53.838	
60	57.346	
80	62.061	
100	66.996	
IC ₅₀ =25.06		



Alpha amylase inhibition assay

Alpha amylase is an enzyme linked to diabetes is in agreement with earlier reports that showed that plant phytochemicals are mild inhibitors of alpha-amylase. A property that confers advantage over synthetic drugs such as acarbose; use by diabetics in the management of postprandial blood glucose, which strongly inhibit alpha-amylase stronger. Were standrd acarbose, sample 1 (Antidiabetic nutraceutical instant soup powder formulation), sample 2 (marketed) subjected to check alpha-amylase inhibition activity were standard shows % inhibition for 10 to 50 (μ g/ml) in range of 47.66%-69.93%, sample 1 antidiabetic nutraceutical formulation (instant soup powder) shows % inhibition for 10 to 50 (µg/ml) in range of 45.68 to 56.58% and sample 2 marketed nutraceutical formulation shows 30.85% to 53% inhibition [18,19]. Were the above study shows formulated sample 1 antidiabetic nutraceutical formulation (instant soup powder) shows greater % inhibition than sample 2 marketed antidiabetic nutraceutical formulation and conform that the formulated antidiabetic nutraceutical formulation having antidiabetic activity (Tables 12-17) [20].

Reading 1		Reading 2		Reading 3	
Conc. (µg/ml)	% Inhibition	Conc. (µg/ml)	% Inhibition	Conc. (µg/ml)	% Inhibition
10	46.99	10	47.44	10	47.66
20	52	20	52.56	20	52.78
30	59.13	30	60.02	30	59.69
40	63.25	40	64.37	40	62.14
50	69.71	50	70.04	50	69.93

Table 12: % Inhibition of alpha amylase for standard acarbose.

Table 13: IC₅₀ for standard acarbose.

No.	IC ₅₀ (μg/ml)
Reading 1	15.5

Reading 2	14.4
Reading 3	14.3
Mean ± SD	14.73 ± 0.665

 Table 14: % Inhibition of alpha amylase for standard acarbose for test sample 1 (Antidiabetic nutraceutical instant soup powder).

Reading 1		Reading 2		Reading 3	
Conc. (µg/ml)	% Inhibition	Conc. (µg/ml)	% Inhibition	Conc. (µg/ml)	% Inhibition
10	45.25	10	44.74	10	45.68
20	47.35	20	48.32	20	47.64
30	51.24	30	52.31	30	51.36
40	54.23	40	54.98	40	54.28
50	56.25	50	56.41	50	56.89

Table 15: IC_{50} for test sample 1 (Antidiabetic nutraceutical instant soup powder).

S. no.	IC ₅₀ (μg/ml)
Reading 1	27.08
Reading 2	25.5
Reading 3	26.03
Mean ± SD	26.21 ± 0.805

 Table 16: % Inhibition of alpha amylase for test sample 2 (marketed).

Reading 1		Reading 2		Reading 3	
Conc. (µg/ml)	% Inhibition	Conc. (µg/ml)	% Inhibition	Conc. (µg/ml)	% Inhibition
10	31.07	10	32.85	10	30.85
20	35.3	20	35.63	20	36.75
30	43.1	30	43.43	30	43.76
40	48	40	48.55	40	48.78
50	55.12	50	56.57	50	53.23

Table 17: IC₅₀ for test sample 2 (marketed).

S. No.	IC ₅₀ (μg/ml)
Reading 1	22.56
Reading 2	21.72
Reading 3	20.4
Mean ± SD	21.56 ± 1.084

Conclusion

Safe and effective antidiabetic nutraceutical formulation of combined antidiabetic herb's and spices, flavor and thickners in the form of instant soup powder was successfully developed, the present investigation revealed that on the basic of sensory evaluation and intial accelerated stability study F4 formulation found to be superior than rest with least moisture content, and taken for further analysis and it shows absence of trace metals like arsenic, cadmium, lead and mercury, it is important to note that this soup is high in protein ash carbohydrate and low fat, high energy value of the soup powder which make it appropriate choice for fulfillment of nutritional demand. Further study for antioxident and antimicrobial. Shows the product is high in antioxident and antimicrobial. These studies for alpha-amylase inhibition assay for in vitro antidiabetic activity carried out and it was found that the formulated antidiabetic nutraceutical formulation (instant soup powder) is superior than the marketed nutraceutical formulation.

Throughout the experimental work experiments carried out under standard and safety measures. The use of conventional treatment of diabetes like insulin and drug like medformin etc. may have side effect in long run use. Now a day nutraceutical formulations is in trend to control various diseases. Nutraceuticals play important role in controlling diabetes.

Nutraceutical herb's like jamun seed, fenugreek seed, gurmar leaves play vital role in controlling diabetes by make use of this antidiabetic herb's antidiabetic nutraceutical formulation were formulated in the form of Instant soup powder formulation by making use of spices like garlic, black paper, curry leaves salt, tevia for sweetening, tomato for flavor, fingermillet for thickening and oyster mashrrom for thickening and flavor all the ingridients are collected from local market having good quality.

The formulated antidiabetic nutraceutical formulation were evaluated for sensory evaluation intitial accelerated stability study, pH, viscocity, moisture, ash values, micromeritics properties, nutritional values, heavy metal content, antioxident and antimicrobial properties and *in vitro* alpha amylase inhibition assay. Were formulation showed satisfactory results. The stability studies were carried on the basis of ICH guideline for 90 days.

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