Journal of Advances and Scholarly Researches in Allied Education
Vol. 21, Issue No. 3, April-2024, ISSN 2230-7540

https://doi.org/10.29070/n3eggx09

Development and Evaluation of Nutraceutical Tablets from Selected Plant Extracts: A Novel Approach to Functional Medicine

Jayashri Behera[1]*, Dr. Ashish Sarkar[2]

[1] Research Scholar, School of Pharmacy, YBN University, Ranchi, Jharkhand, India

Email: jayashri.b89@gmail.com

[2] Professor, School of Pharmacy, YBN University, Ranchi, Jharkhand, India

Abstract - The potential of a seed powder blend made of Cucumis melo, Punica granatum, and Linum usitatissimum in a 1:1:1 ratio is investigated in this study. In order to guarantee a consistent particle size distribution (10–30 µm), the seeds were ground into a fine powder. The parameters of bulk powder showed strong cohesion and poor flow ability. Photo microscopy, however, showed fibrous, capsular structures that were perfect for formulation. Significant growth of Lactobacillus acidophilus and Bifidobacterium bifidum indicated that the mix had superior prebiotic potential compared to individual seed powders and the conventional chicory powder. It also showed remarkable antioxidant activity. Studies on animals verified the blend's effectiveness and safety. A 40-day feeding trial in Albino Wistar rats demonstrated its haematinic effects, raising haemoglobin levels from 12.1% to 16.5% and red blood cell counts from 7.46 to 9.55 million/mm³. Acute toxicity trials revealed no negative effects. Additionally, the combination decreased VLDL and triglycerides and raised serum protein levels, suggesting possible cardiovascular advantages. Its safety for those with diabetes is supported by the fact that blood glucose levels stayed within the usual range.

The product, which was made as capsules, complied with pharmacopoeial requirements for disintegration time and weight variation. A shelf life of nine to twelve months is suggested by stability studies conducted under accelerated settings. Over the course of three months, the blend's prebiotic activity stayed steady, maintaining its capacity to promote probiotic growth. This nutraceutical formulation is a good option for treating anemia, malnutrition, and metabolic disorders since it provides a promising blend of antioxidant, prebiotic, and health-promoting qualities.

Keywords: Plant extracts; Prebiotic activity; Microbiological quality standards; Bioactive compounds; Nutraceutical tablets

----------X----------

INTRODUCTION

Dr. Stephen L. DeFelice came up with the phrase "nutritraceuticals," which combines the words "pharmaceutical" and "nutrition" to refer to non-toxic food ingredients that have health benefits, such preventing or treating disease (De Felice, 1989). Based on the idea that "food is medicine," as proposed by Hippocrates, this quickly expanding multidisciplinary discipline is based on human nutrition and seeks to improve health by research and creative formulations. All mental and physical functions are fueled by nutrition, with vital nutrients such as fiber, vitamins, minerals, proteins, carbs, and fats playing important roles. Maintaining health requires both macronutrients (proteins, fats, and carbs) and micronutrients (vitamins and minerals). Millions of people worldwide suffer from malnutrition, which the World Health Organization defines as an inadequate, excessive, or unbalanced nutrient consumption.

1.9 billion people are overweight, and 462 million are underweight. Of children under five, 15 million are stunted and 41 million are overweight. Furthermore, iron supplements help more than half of the 528 million women of reproductive age who suffer from anemia. However, compared to inorganic sources of iron, which can result in gastrointestinal distress, plant-based iron is frequently better absorbed (Khera et al., 2017; Singh et al., 2018).

The significance of dietary supplements, prebiotics, probiotics, and plant-based nutrients in supporting health is highlighted by the fact that unhealthy eating patterns and nutritional deficiencies are contributing

factors to diet-related disorders. While nutraceuticals hold promise as possible medications, antioxidants are essential in preventing oxidative damage to biomolecules. To maximize their use, more study is necessary (Reddy et al., 2019; Patel et al., 2020).

There has been a modest but steady decline in the percentage of children suffering from malnutrition in India, in contrast to other nations. However, women, the primary caregivers of children, often suffer from poor health and limited access to clean water and sanitation, contributing to inadequate nutrition. India has the highest incidence of underweight children globally, with rates nearly double those of sub-Saharan Africa, particularly in states such as Maharashtra, Madhya Pradesh, Rajasthan, Orissa, Uttar Pradesh, and Bihar. Melghat in Maharashtra is especially affected by chronic malnutrition. As dietary and health changes impact children's physical development, growth assessment remains the gold standard for determining their nutritional and health status (Khera et al., 2017; Singh et al., 2018). About two billion people worldwide suffer from vitamin and micronutrient deficiencies, with one-third of them living in India, which is home to one-sixth of the world's population. Although trace levels of these micronutrients are necessary for many body functions, new research indicates that shortages in certain of these nutrients may be at an all-time high (Reddy et al., 2019; Patel et al., 2020).

REVIEW OF LITERATURE

The Function of Nutraceuticals in Health

Nutraceuticals are compounds that provide health advantages by preventing and treating a variety of illnesses. Dr. Stephen L. DeFelice, the term's creator, claims that nutraceuticals serve as a functional food to cure medical disorders or preserve health, bridging the gap between pharmaceutical therapy and nutrition (DeFelice, 1994). These chemicals contain bioactive molecules that have therapeutic properties, are generally produced from natural sources, and are non-toxic. The potential of plant-based extracts to enhance public health outcomes is demonstrated by their growing application in the creation of nutraceuticals (Wang et al., 2015).

Nutraceuticals Using Plant Extracts

For generations, people have used plants as a source of therapeutic treatments. Bioactive substances with potential health advantages, such as alkaloids, flavonoids, polyphenols, terpenoids, and essential oils, are found in many plant species. The pharmacological effects seen in the plants are caused by these chemicals (Patel et al., 2018). For example, because of their anti-inflammatory, antioxidant, and immune-modulating qualities, Curcuma longa (turmeric), Withania somnifera (ashwagandha), Ocimum sanctum (holy basil), and Camellia sinensis (green tea) are frequently utilized in the creation of nutraceutical formulations (Pandey et al., 2016).

Nutraceutical Tablet Formulation

There are several steps involved in making nutraceutical tablets from plant extracts, such as choosing the right plant material, extracting the bioactive ingredients, and formulating the tablets with the right excipients. To separate the active ingredients from plant materials, extraction methods like maceration, Soxhlet extraction, and ultrasonic-assisted extraction are frequently employed (Chaudhary et al., 2019). Following the extraction process, the formulation of tablets is the next stage, which calls for careful consideration of variables like dose, stability, and bioavailability. The effective delivery of bioactive substances to the intended areas in the body is ensured by the use of natural binders and carriers in tablet formulations (Gurav et al., 2020).

Assessment of Nutritional Tablets

Both in vitro and in vivo investigations are used to evaluate the safety, effectiveness, and therapeutic potential of nutraceutical tablets. While in vivo research focuses on how the tablet affects animal models or human clinical trials, in vitro testing evaluate antioxidant activity, antibacterial qualities, and enzyme inhibition (Gupta et al., 2017). One important factor affecting the effectiveness of the nutraceutical tablets is the bioavailability of the active ingredients. According to research, some formulations can increase the bioavailability of plant-based substances, such as those containing nanoparticles or improved absorption strategies (Mohan et al., 2018).

Uses of Nutraceutical Tablets in Therapeutic Settings

Nutraceutical tablets made from plants have demonstrated potential in the treatment and prevention of a variety of illnesses. Nutraceuticals made from ginseng, for instance, have been shown to improve cognitive performance and lessen fatigue (Lee et al., 2019), while extracts from ashwagandha have shown anti-stress and anti-anxiety properties (Choudhary et al., 2017). Additionally, anti-inflammatory nutraceuticals, including those made from green tea and turmeric, are frequently used to treat long-term illnesses like cancer, heart disease, and arthritis (Ghosh et al., 2018).

OBSTACLES AND PROSPECTS

Although there is a lot of promise in the creation of plant-based nutraceutical tablets, there are a number of obstacles to be addressed. These include addressing concerns about the stability and shelf life of the tablets, standardizing plant extracts, and guaranteeing the constant strength and quality of the active substances. Furthermore, the absence of precise standards for the safety and effectiveness of plant-based nutraceuticals makes regulatory approval difficult in many nations (Singh et al., 2020). To determine the therapeutic effects of these products, future research should concentrate on

advancing extraction techniques, increasing bioavailability, and carrying out additional clinical trials.

MATERIALS AND METHODOLOGY

The first step in creating nutraceutical tablets from plant extracts is choosing plants such as Ocimum sanctum (holy basil), Curcuma longa (turmeric), and Withania somnifera (ashwagandha) for their anti-inflammatory and antioxidant qualities (Patel et al., 2018). These plants undergo cleaning, solvent extraction, and powder concentration (Chaudhary et al., 2019). HPLC and GC-MS are used to identify and quantify the bioactive chemicals (Gupta et al., 2017). Extracts and excipients are combined to create tablets, which are then made by wet granulation or direct compression (Gurav et al., 2020).

The physicochemical characteristics of the tablets, including their hardness and rate of dissolution, are assessed (Patel et al., 2020). Their effectiveness is evaluated using in vivo animal experiments and in vitro bioactivity tests (Mohan et al., 2018).

Monitoring the absorption of bioactive compounds allows for the measurement of bioavailability (Mohan et al., 2018). For their nutritional and practical qualities, essential ingredients such as milk powder, cocoa powder, sodium glycolate, and tomato and ginger powders are utilized (Patel et al., 2020; Weier et al., 1982).

GC, HPLC, and drying muffle furnaces are among the apparatus utilized (Gurav et al., 2020). The stability of the tablets is safeguarded by appropriate storage conditions (Chaudhary et al., 2019).

Prebiotic Activity (Direct Inoculation Method)

The direct inoculation method was used to evaluate the prebiotic activity of seed powders derived from Linum usitatissimum, Cucumis melo, and Punica granatum. The medium was solidified using plain agar, and the seed powder samples were inoculated with either Lactobacillus acidophilus or Bifidobacterium bifidum. The apparatus consisted of a standard chicory powder, a positive control (MRS medium for Lactobacillus acidophilus and specialized nutritional media for Bifidobacterium bifidum), and a negative control (plain agar without seed powder). To assess bacterial growth and prebiotic potential, all petri dishes were incubated anaerobically for 48 hours at 37°C. Colony counts were then reported (Patel et al., 2020; Mohan et al., 2018).

Microbiological Load Determination

In accordance with I.P. 2014 recommendations for herbal products, microbiological quality standards were evaluated. The material was diluted in sterile distilled water and incubated on Soybean Casein Agar (TAC) and Sabouraud Dextrose Agar (TFC) at 37°C for 24 hours in order to measure the total aerobic count (TAC) and total fungal count (TFC). When Salmonella, Shigella, and Escherichia coli were examined for their presence in the samples, no growth was found (I.P., 2014). In order to ensure compliance with the permissible limits—TAC < 10^7 CFU/g, TFC < 10^5 CFU/g, and absence of pathogens—the findings were examined by averaging duplicate counts.

RESULT AND DISCUSSION

Reduction in size

The seeds of Cucumis melo, Punica granatum, and Linum usitatissimum were cleaned, dried, and crushed to guarantee uniform weight and particle size. In accordance with the United States Pharmacopoeia National Formulary (2000), which defines powders with this mesh size as fine, the resultant powders were filtered through an 85 mesh screen. For nutraceutical formulations to behave consistently, including uniform dissolving and sedimentation rates, homogeneous particle size distribution is necessary to avoid segregation. To make a homogenous mixture for the investigation, the powders were combined.

Photographic microscopy

Using a Motic optical polarizing microscope at 4X magnification, photomicroscopy photographs of the nutraceutical powder blend (in a 1:1:1) were obtained. The particles had a fibrous, capsular structure and ranged in size from 10 to 30 micrometers.

FEATURES OF BULK POWDER

The individual powders of Cucumis melo, Punica granatum, and Linum usitatissimum, as well as their combination, showed poor flow and compressibility features when compared to bulk powder characteristics. The limited flowability and high cohesiveness were indicated by the angle of repose exceeding 40 degrees and the Carr's index above 25%.

Table 1 : Properties of *Punica granatu, Cucumis melo, Linum usitatissimum*, and a combination of the three as a powder

Seed powder	Mesh size	% Compressibility	Angle of repose (')	Flowability
Linum usitatissimum	85	25.55	44.15	Poor
Punica granatum	85	22.3	45.00	Poor
Cucumis melo	85	28.58	40.95	Poor
1:1:1 mixture	85	23	43.36	Poor

Proximate analysis

Table 2: Estimating the distance between two points in the seed mixture

Parameter	Units	Result	Test method
Proteins	g/100g	35.5	AOAC 920.152
Crude fibre	g/100g	17.75	IS 2234.2011
Fat	g/100g	22.44	IS 12711.2010
Carbohydrates	g/100g	33.42	IS 1656.2012
Calcium	mg/100g	1620	944.02,320109 and 999.10
Iron	mg/100g	22.67	AOAC
Soluble dietary fibre	g/100g	4.1	IS 11062.2010
Fatty acid profile Saturated fat	g/100g	1.93	AOAC 996.01
Trans fat	g/100g	Not detected	
Polysaturated fat	g/100g	14.82	
Monosaturated fat	g/100g	5.69	
Omega 3 fatty acids	g/100g	34.13	

Antioxidant Activity Determination

The information is displayed as percentage inhibition, IC50, and quercetin equivalent. All three of the active nutraceutical ingredients demonstrated extremely high antioxidant potential, and the synthesized nutraceutical product exhibited remarkable antioxidant activity. The outcomes of the three seed powder combinations are shown in the following table.

Table 3 : The DPPH assay of nutritional powders as a percentage of inhibition

Name	% inhibition					Equation (Squared co relation coefficient) R^2	IC50 (µg/ml)
	Concentration in µg/ml						
	10	20	30	40	50		
Quercetin (standard)	59.02	76.23	81.4	87.01	90.23	Y=53.666x+2.9925 R^2=0.9925	7.51
Cucumis melo	70.54 0.83	80.74 0.94	85.02 0.95	91.20 0.95	94.74 0.95	y = 57.438x +3.696 R^2 = 0.9749	6.3322
Punica granatum	51.78 1.13	65.30 1.16	78.75 1.03	88.95 0.95	93.82 0.96	y = 61.584x - 11.562 R^2 = 0.9847	4.2087
Linum usitatissimum	61.49 0.95	68.36 1.11	75.76 1.07	84.41 1.03	91.52 0.98	y = 42.198x + 16.564 R^2 = 0.9351	6.1995
Seed Powder mixture Quercetin equivalent	45.35 1.08	62.21 1.22	74.35 1.09	86.25 1.00	92.65 0.97	Y=68.436x -24.729 R^2 = 0.9902	2.3402

It is more effective than utilizing either seed powder alone since the mixture of the seeds has a higher antioxidant capacity than the seeds alone. When evaluated at dosages ranging from 10 to 50 ppm, all of the seed powders demonstrated high antioxidant activity.

Prebiotic likelihood

For additional research, we chose seeds of Linum usitatissimum, Punica granatum, and Cucumis melo. A considerable colony growth was seen in the 1:1:1 seed powder mixture of these seeds when tested with Lactobacillus acidophilus ATCC 4356 (Sample). The positive control, on the other hand, showed growth, whilst the negative control showed none. The seed powder mixture's prebiotic potential is confirmed by the profusion of Lactobacillus acidophilus growth on it.

Table 4: Determination of prebiotic potential for a single seed powder sample using Lactobacillus Acidophilus ATCC 4356 on MRS as a positive control and plain agar as a negative control in a controlled environment.

Sample	Type	No of colonies
combined with normal agar, 0.2 g of *Cucumis melo* seed powder	Test	67
combined with normal agar, 0.2 g of *Punica granatum* seed powder	Test	41
0.2 g of chicory powder	Positive control	59
0.2 g of a seed powder combination 1:1:1. mixed with regular agar,	Test	94
0.2 grammes of *Linum usitatissimum* seed powder mixed with ordinary agar	Test	57
The MRS agar (De Man, Rogosa, and Sharpe)	Positive control	47
Simple agar	Negative control	0

Table 5: Analysis of the prebiotic potential of a single seed powder sample by means of the *Bifidobacterium bifidum* ATTC 29521on As a positive control, Bifidobacterium agar, and as a negative control, plain agar

Sample	Type	No of colonies
combined with normal agar, 0.2 g of *Cucumis melo* seed powder	Test	57
0.2 grammes of *Linum usitatissimum* seed powder mixed with ordinary agar	Test	62
combined with normal agar, 0.2 g of *Punica granatum* seed powder	Test	42
0.2 g of a seed powder combination 1:1:1. mixed with regular agar,	Test	77
Simple agar	Negative control	0
Food for Bifidobacterium.	Positive control	47
0.2 g of chicory powder	Positive control	35

The two probiotic bacteria, Lactobacillus acidophilus ATCC 4356 and Bifidobacterium bifidum ATCC 29521, showed greater colony counts than the positive control when 0.2 grams of seed powder were added to each petri dish. Any development that was seen can be ascribed to the prebiotic activity of the seeds because the petri dishes only contained the seed combination on a solid agar base. The seed powder blend outperformed chicory powder in terms of colony-forming units (CFUs) of Bifidobacterium bifidum and Lactobacillus acidophilus, demonstrating higher prebiotic potential. In contrast, the well-known prebiotic chicory powder was less successful.

Nutraceutical powder combination microbiological contamination limit test

Table 6: Maximum allowable microbial contamination in a nutraceutical powder blend1:1 ratio

Microorganism	Nutraceutical Powder Mixture 1:1 (CFU)
Escherichia coli	2×10^2 per g
Shigella	Absent in 10g
Total Fungal Count (TFC)	4×10^4
Salmonella	Absent in 10g
Total Aerobic Count (TAC)	5×10^5

The microbiological load for the nutraceutical powder mixture1:1;1 was determined to be under the limit and safe for human consumption according to the IP guidelines. I.P. 2014 states

TAC: Acceptance criterion: 10^7 CFU per g

TFC: Acceptance criterion: 10^5 CFU per g

***Escherichia coli:* Acceptance criterion: 10^3 CFU per g**

***Salmonella:* Absent in 10g**

Shigella: Absent in 10g

ASSESSING BIOLOGICAL FACTORS

Animal studies were performed to evaluate the potential biological effects of a nutraceutical mix of *Cucumis melo*, *Punica granatum*, and *Linum usitatissimum* seeds at a ratio of 1:1:1.

1. Acute toxicity study.
2. Gain in weight.
3. When analysing blood samples.

a) Complete blood count (WBC, RBC, Hb).

b) Concentration of all serum proteins.

c) Anaerobic glycogen.

An animal study was carried out to ascertain the impact of a combination of nutraceutical powders on weight gain or loss and hemogram-based health benefits. The experimental diet was given to the experimental animal group, and the amount of feed they consumed each day was calculated." The results were compared to those of the control group. We compared the readings after zero and forty days for the control group, and after forty days for the test groups, with the control group's readings. (So they can evaluate the hemogram and other aspects.)

For Acute Toxicity Study

For the first half hour following dosing and then at regular intervals for the first twenty-four hours, each animal is closely observed separately; no deaths occurred during the first four or twenty-four hours.

Then, each day for the following fourteen days. After 14 days of observation, the meal showed no negative effects on the animals. This toxicological study's findings showed no signs of acute toxicity.

Data from a 40-day animal research comparing the effects of nutraceuticals on weight and blood parameters

Table 7: Control group dietary habits, hydration levels, and weight increase

Sr. No	Date	Food intake (g)				Water intake (mL)				Body Weight (g)			
		H	B	T	HB	H	B	T	HB	H	B	T	HB
1	27/02/2013	43	40	41	48	28	28	25	21	139	157	165	145
2	28/02/2013	41	40	43	46	27	21	23	23				
3	29/02/2013	38	49	40	45	22	29	24	26				
4	01/03/2013	42	46	43	44	24	25	26	21				
5	02/03/2013	48	44	41	49	25	20	21	23				
6	03/03/2013	41	40	49	42	23	24	25	28				
7	04/03/2013	43	42	46	41	24	25	24	21	140	161	170	150
8	05/03/2013	40	40	40	48	28	23	28	29				
9	06/03/2013	43	50	38	45	28	24	27	25				
10	07/03/2013	41	41	40	45	21	23	29	23				
11	08/03/2013	42	48	43	40	29	24	25	28				
12	09/03/2013	47	40	41	41	25	25	28	20				
13	10/03/2013	45	41	49	49	20	26	21	24				
14	11/03/2013	40	42	49	43	20	20	29	28	155	170	176	164
15	12/03/2013	49	46	46	40	27	24	25	27				
16	13/03/2013	42	41	41	48	28	28	21	24				
17	14/03/2013	48	49	49	43	21	27	23	20				
18	15/03/2013	49	43	42	41	29	22	28	25				
19	16/03/2013	40	41	49	44	25	27	24	25				
20	17/03/2013	50	49	41	43	29	24	28	23				
21	18/03/2013	44	49	48	45	28	28	27	24	162	182	182	173
22	19/03/2013	46	50	43	48	27	21	28	26				
23	20/03/2013	43	44	49	43	24	23	29	21				
24	21/03/2013	40	46	46	41	24	26	25	28				
25	22/03/2013	43	44	43	42	25	21	23	24				
26	23/03/2013	41	40	40	47	21	23	24	25				
27	24/03/2013	46	42	43	41	28	24	26	23				
28	25/03/2013	45	41	48	48	27	26	21	24	173	191	193	180
29	26/03/2013	43	42	43	45	20	22	28	28				
30	27/03/2013	44	47	41	40	27	22	21	20				
31	28/03/2013	45	40	49	42	26	25	26	22				
	29/03/2013	45	43	49	49	25	21	23	24				
33	30/03/2013	42	43	40	43	22	24	24	25				
34	31/03/2013	48	46	46	45	24	25	26	25				
35	01/04/2013	43	42	40	41	20	28	21	23	185	198	201	197
36	02/04/2013	41	43	42	43	25	24	28	24				
37	03/04/2013	44	43	47	42	26	28	23	21				
38	04/04/2013	44	42	41	40	20	18	24	29				
39	05/04/2013	46	41	40	45	26	25	23	27				
40	06/04/2013	44	41	45	40	25	29	21	26	198	205	208	210

Table 8: Diet, hydration, and growth in mass for experimental subjects

Sr. No	Date	Food intake (g)				Water intake (mL)				Body Weight (g)			
		H	B	T	HB	H	B	T	HB	H	B	T	HB
1	27/02/2013	47	44	49	35	20	23	27	21	143	163	173	145
2	28/02/2013	41	40	43	46	25	28	22	23				
3	29/02/2013	40	41	40	45	28	20	24	26				
4	01/03/2013	38	48	50	40	21	24	25	21				
5	02/03/2013	48	43	44	49	29	28	23	23				
6	03/03/2013	43	41	46	42	25	27	24	28				
7	04/03/2013	41	46	44	41	20	24	26	21	150	168	176	152
8	05/03/2013	49	45	40	48	24	25	21	29				
9	06/03/2013	46	43	42	45	28	23	23	25				
10	07/03/2013	43	40	41	48	25	24	29	28				
11	08/03/2013	40	38	49	43	23	28	25	27				
12	09/03/2013	41	40	42	41	24	26	28	22				
13	10/03/2013	40	41	47	49	26	23	21	24				
14	11/03/2013	38	49	40	45	21	23	29	25	158	175	182	160
15	12/03/2013	48	46	44	40	25	28	25	23				
16	13/03/2013	43	41	43	48	24	25	21	24				
17	14/03/2013	48	49	43	43	28	23	23	28				
18	15/03/2013	43	42	41	41	27	24	28	21				
19	16/03/2013	41	47	49	49	20	21	24	25				
20	17/03/2013	49	45	41	40	24	29	28	23				
21	18/03/2013	49	40	48	50	28	25	27	24	170	189	195	175
22	19/03/2013	50	49	43	44	27	20	24	26				
23	20/03/2013	44	42	49	46	24	20	25	21				
24	21/03/2013	46	41	46	43	28	27	26	28				
25	22/03/2013	44	41	43	40	21	28	20	24				
26	23/03/2013	40	48	40	38	23	21	24	25				
27	24/03/2013	42	45	43	41	26	29	28	23				
28	25/03/2013	41	48	48	48	21	25	27	24	183	193	203	188
29	26/03/2013	45	42	43	45	23	29	22	28				
30	27/03/2013	40	47	41	48	28	24	30	20				
31	28/03/2013	48	40	49	43	29	26	23	25				
32	29/03/2013	43	44	49	41	25	21	24	26				
33	30/03/2013	41	43	40	44	23	28	25	20				
34	31/03/2013	49	45	43	44	24	23	21	25				
35	01/04/2013	49	48	41	46	26	28	28	23	195	202	211	197
36	02/04/2013	46	43	46	44	21	24	27	24				
37	03/04/2013	41	41	45	40	28	28	20	21				
38	04/04/2013	49	42	43	42	27	18	24	29				
39	05/04/2013	42	47	44	49	24	25	23	27				
40	06/04/2013	47	41	40	43	25	29	21	26	210	218	220	210

The Albino Wistar rats were given a nutritional supplement that was composed of 33.42% carbohydrates, 22.44% fat, and 35.5% protein. The rats' body weight varied somewhat after 40 days of receiving the nutraceutical powder mixture of seeds in a 1:1:1 ratio as the main product mix (Group I). The study discovered that despite being fed the nutraceutical mix, the test rats' weight increase was lower than that of the control group. This might be explained by the seed combination's moderate fat levels and comparatively high protein content when compared to regular rat chow.

In order to analyse blood samples

Table 9: Blood cell count, haemoglobin, triglyceride, and very low density lipoprotein (VLDL) analysis during a 40-day animal trial

Parameters	Zero day's reading		Forty day's reading	
	Control group	Test group	Control group	Test group
RBC count (million/mm3)	6.38	7.46	4.82	9.55
Haemoglobin	11.5	12.1	14.5	16.8
Serum Protein(g%)	6.1±0.1750	6.325±0.025	6.3±0.2000	6.5±0.1750
VLDL (%mg)	19.2	21.6	23	15.4
Serum triglycerides (%mg)	96`	108	115	77
Blood sugar (mg%)	79.33±0.1707	82.66±3.500	92±3.928*	107±5.626***

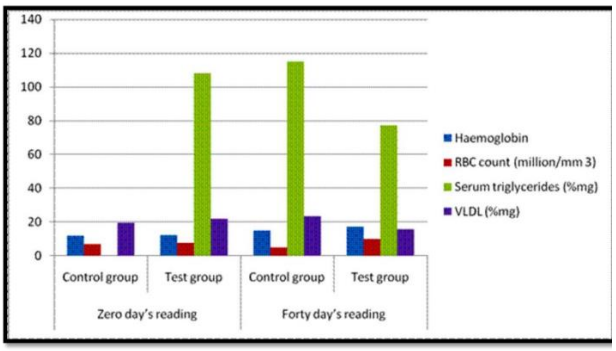


Figure 1: Effect on Red Blood Cell, Haemoglobin, Triglyceride, and VLDL Measuring in a 40-Day Animal Trial

Significant results were obtained from laboratory analyses of hemoglobin, triglycerides, red blood cells, and very low-density lipoprotein during the 40-day animal study. The test group's hemoglobin level rose from 12.1% to 16.5% by the end of the trial, whereas the control group's climbed from 11.5% to 14.5%. This improvement suggests a haematinic impact, which is frequently linked to anemia treatment. The test group's red blood cell (RBC) counts increased from 7.46 million/mm³ to 9.55 million/mm³ due to the nutraceutical blend, while the control group's RBC counts decreased to 4.82 million/mm³. This nutraceutical combination may be used as a supplement because the iron, proteins, and vitamins included in the three seed components are thought to promote the synthesis of red blood cells and raise hemoglobin levels.

Serum Protein (mg)

An increase in the test group's plasma total protein concentration (6.45 mg/dl) was associated with a noticeable improvement in health. Since proteins are essential for many body functions, serum protein levels are a significant predictor of overall nutritional status. Serum protein concentration is frequently measured using a total protein test; low levels might be caused by dilution, increased loss, decreased production, or starvation. Mildly low protein levels might not show any signs, but extremely low levels might cause fluid to leak from the circulation into tissues, resulting in limb weakness, exhaustion, and swelling. By raising serum proteins in vivo, this nutraceutical combination may aid in the treatment of malnutrition.

Triglycerides and VLDL

Serum triglyceride levels dropped to 77% in the experimental group and rose to 115% in the control group from 96%. The test group's very low-density lipoprotein (VLDL) levels decreased from 21.6 mg to 15.4 mg, whereas the control group's LDL levels rose from 19.2 mg to 23.2 mg. Elevated LDL and VLDL levels are associated with plaque accumulation in arteries, which exacerbates heart disease. Given that it can lower triglyceride and VLDL levels, this nutraceutical may help patients with diabetes and heart disease by lessening the negative effects of these lipoproteins.

Blood Glucose Levels

The test group's blood sugar level climbed to 107±5.626 mg/dl, which was statistically significant ($p<0.05$), while the control group's blood sugar level increased from 79.33±0.1707 mg/dl to 82.66±3.500 mg/dl. The test group's glucose levels stayed within the usual range in spite of this increase, indicating that the nutraceutical formulation did not result in a blood sugar surge. These findings suggest that diabetics can safely use this nutraceutical. The moisture content

Weight before drying minus weight after drying equals moisture weight.

0.82 g is 10 g minus 9.18 g.

Moisture content is equal to 100% (moisture weight divided by initial weight).

8.2% is 0.82 / 10 x 100.

It was discovered that the seed combination had an 8.2% moisture content.

RESEARCH ON CELL LINES FOR ANTI-CANCER AND ANTI-EXPOSURE AGENTS

Preparation for formation.- Dispersible powder formulation

Table 10: Model 3^2 iterative Cinnamon, *Punica granatum*, and lutein are the main ingredients.1:1 ratio

Dispersible powder formula (*Punica granatum*,*Cucumic melo*, ,*Linum uisitatissimum*1:1:1)NI with Excipients EI			
NI-X1	Y2 %Comressibility	EI-X2	Y1 Dispersion time in seconds
50	25	30	40
60	26	30	35
50	28	50	50
50	23	40	35
60	27	40	40
70	31	40	40
70	30	30	35
60	28	50	50
70	33	50	55

Table 11: Summary of Responses for Year 1 (see tables for details below)

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Cubic	0.7559	0.8049	-3.4451	Aliased
Quadratic	0.1023	0.8862	0.5956	
2FI	0.2890	0.6878	-0.0164	
Linear	0.0156	0.6667	0.3575	Suggested

The dependent variable, dispersion time in seconds, and the interactions between the independent variables, N-X1 and E-X2, are described using a linear model.

The physical blend of nutraceutical seed powder with excipients (milk powder and cocoa powder) does not include any chemical contact, therefore the relationship between the two is linear. If the additional terms are truly important, we select a linear polynomial. Predicted R2 and Adjusted R2 are the best choices in this case.

Table 12: 1-D.T.(Sec) Analysis of variance table for Response Surface Linear Model [Partial sum of squares - Type III

Source	Sum of Squares	Mean Square	df	F-value	p-value	
Model	341.67	170.83	2	9.00	0.0156	significant
A-A	4.17	4.17	1	0.2195	0.6559	
B-B	337.50	337.50	1	17.78	0.0056	
Residual	113.89	18.98	6			
Cor Total	455.56		8			

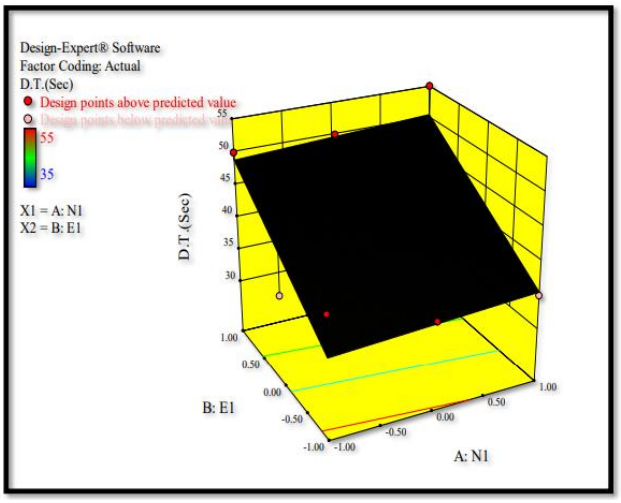
The model is statistically significant with an F-value of 9.00. With a probability of only 1.56%, a "Model F-Value" of this size is extremely unlikely to be the product of chance.The linear model excels in many ways. When "Prob > F" is less than 0.0500, model terms are deemed significant. In this case, B is a key model term. The terms in the model are considered non-significant when the values exceed 0.1000.

The Last Equation Relating to Coded Factors

D.T.(Sec) = +42.22 + 0.83 * A + 7.50* B Final Equation in Terms of Actual Factors:D.T.(Sec)

= + 42.22222 + 0.83333 * N1 + 7.50000 * E1

The dispersion time coefficient is positive for both the fraction of nutraceutical seed combination N1 and the proportion of excipient mixture E1. The coefficient for the effect of excipient percentage on dispersion time is 7.5," which is more indicative than the coefficient for the seed powder proportion, which is 0.8333.



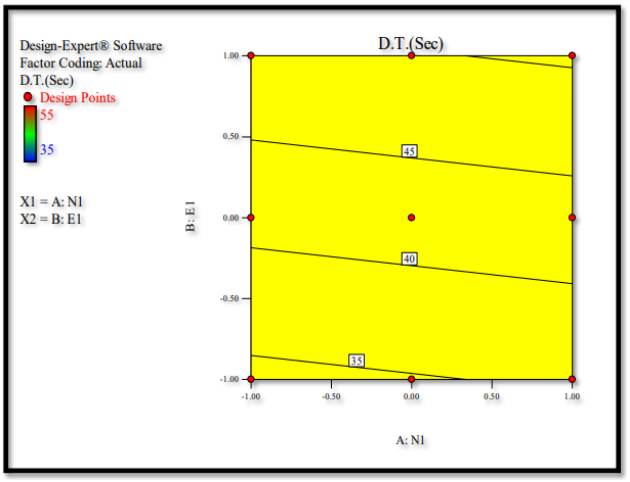


Figure 2: If the dispersion time is 55 seconds at its highest and 35 seconds at its lowest, as shown in of the response surface graph for D.T. Product 1, then the projected value is 45 seconds, and the linear link between N1 and E1 for dispersion time is also evident.

Table 13: Model 3^2 iterative For Y2, cucumber, *Punica granatum*, and Linum uisitatissimum in a ratio of 1:1:1.

Dispersible powder formula (*Cucumic melo,Punica granatum,Linum uisitatissimum*)			
N-X1	Y1 Dispersion time in seconds	E-X2	Y2 %Comressibility
50	40	30	25
50	35	40	23
50	50	50	28
60	35	30	26
60	40	40	27
60	50	50	28
70	40	40	31
70	35	30	30
70	55	50	33

Table 14: Below are the comprehensive tables displaying the response summary for Y2.

R2-Response 2 Y2- C (%)

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	**0.0040**	**0.7881**	**0.5979**	**Suggested**
Quadratic	0.2704	0.8227	0.2995	
2FI	1.0000			
Cubic	0.5898	0.8150	-3.2139	Aliased

According to the model, N-X1 and E-X2 are the independent variables, while the percentage of compressibility is the dependent variable.

The physical blend of nutraceutical seed powder with excipients (milk powder and cocoa powder) does not include any chemical contact, therefore the relationship between the two is linear. If the additional terms are truly important, we select a linear polynomial. Predicted R2 and Adjusted R2 are the best choices in this case.

Table 15: Linear model ANOVA for Response 2 C (%): R2

(Third Type Partial Sum of Squares) Analysis of Variance Table

Source	Sum of Squares	Mean Square	df	p-value	F-value	
Model	64.67	32.33	2	0.0040	15.87	significant
A-A	54.00	54.00	1	0.0021	26.51	
B-B	10.67	10.67	1	0.0621	5.24	
Residual	12.22	2.04	6			
Cor Total	76.89		8			

An F-value of 15.87 indicates a significant model. This kind of "Model F-Value" could only occur by chance (with a little 0.40% chance).When "Prob > F" is less than 0.0500, model terms are deemed significant. In this case, the model terms A are crucial. The terms in the model are considered non-significant when the values exceed 0.1000.

The Last Equation Relating to Coded Factors

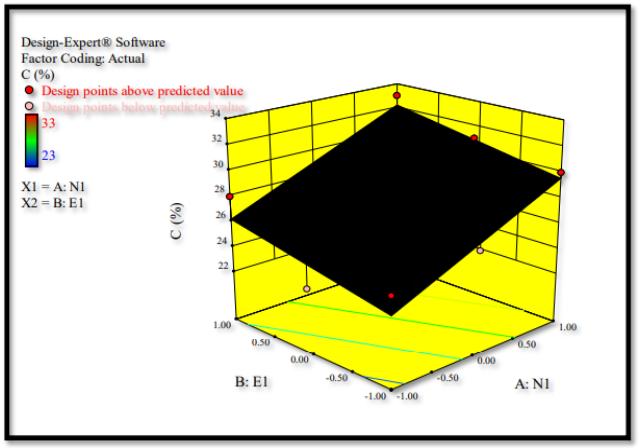
C (%) = +27.89 + 3.00* A + 1.33 * B Final Equation in Terms of Actual Factors: C (%)

= +27.88889 + 3.00000 *N1 + 1.33333* E1

The percentage of compressibility is positively correlated with the percentage of excipient mixture E1 and the fraction of nutraceutical seed combination N1. The effect of the percentage of the seed powder mixture on the percent compressibility is more strongly reflected by the coefficient of 3.00 than by the coefficient of 1.3333 for the excipient proportion.

Power transformations display the box-cox plot

The response surface graph reveals a linear relationship between N1 and E1 for % compressibility, and if 33% is the maximum and 23% the lowest, then the projected value is 28%.



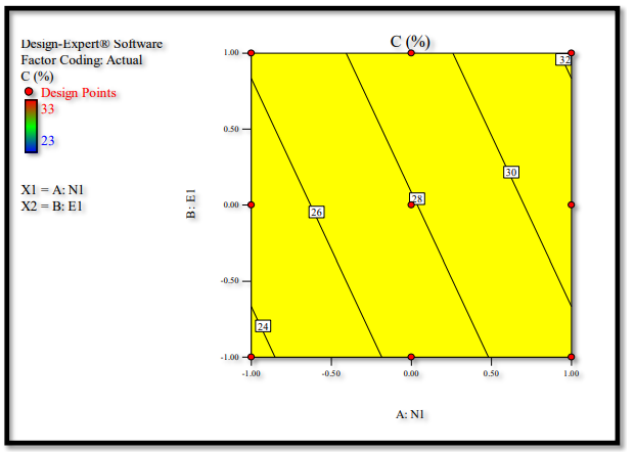


Figure 3: The graph showing the reaction surface for $C Product 1

Table 16: Batch product optimisation phase I

N-X1	E-X2	Y2 (%C)	Acceptability For (%C)	Y1 Dispersion time (D.T.) in seconds	Acceptability For (D.T.)	Organoleptic preference
50	50	28	Poor	50	Fair	+++
50	40	23	Passable	35	Very good	++
50	30	25	Poor	40	Good	+
60	50	28	Poor	50	Fair	+++
60	40	27	Poor	40	Good	++
60	30	26	Poor	35	Very good	+
70	30	30	Poor	35	Very good	+
70	50	33	Very Poor	55	Poor	++
70	40	31	Poor	40	Good	++

Batch 5, with slightly greater D.T. and % C, is regarded optimal as it contains 50% of the nutraceutical combination, however the programme suggests batch 2 as the best since it has the least D.T. and % C.

Batch number five is the best option.

This dispersible powder mixture is expected to taste like a hot health drink with a pleasant, pleasant flavor. The powder is not meant to flow freely; it will be combined with either milk or hot water. The flavor score is correlated with the dispersion time. Different concentrations of milk and cocoa powder as excipients in the finished composition are indicated by scores of +++/++/+. Batch 5 is the best since it has five times as many nutraceutical components as the legal minimum. However, because of its best result of % C and lowest dispersion time, batch no. 2 is considered ideal by the algorithm. However, this specific batch, B. No. 2, has a low flavor score.

Development of Formulation. - Capsule (for those with diabetes)

a) **Capsules**

Measurements were made of the capsules' weight change and disintegration time. Weight variationEach of the twenty full capsules had 1,127 milligrammes of gelatin, while each empty capsule contained 127 milligrammes.

Because the pills' percentage of weight fluctuation fell within the parameters defined by the Pharmacopoeia, they were considered to have passed the weight variation test. The hard gelatine capsules' disintegration time fell within the IP limit, and all of the capsules' weights were standardized with low standard deviations. The results are shown in the following table.

Table 17: Assessment of pills

Parameters	Result
Degradation duration	4 mins 47secs.
Variation in mass	within limits

b) **Prompted Stability Investigations**

For three months, the Accelerated Stability Studies were carried out in a harsh storage environment with 40°C and 75% relative humidity. If the product is kept in a dry, cold place as recommended, it will stay stable for a long period. The majority of nutraceutical products on the market have a shelf life of nine to twelve months. According to preliminary stability tests, this product might fit the requirements. The prebiotic potential, antioxidant activity, and stability testing method of a nutraceutical powder blend were evaluated.

Table 18: Nutraceutical product combination stability research I: DPPH test, fresh seed mixture, and three months later

Name	% inhibition					Equation (Squared co relation coefficient) R^2	IC50 (µg/ml)
	Concentration in µg/ml						
	10	20	30	40	50		
Fresh powder mixture	45.35	62.21	74.35	86.25	92.65	y = 68.436x - 24.729 R^2 = 0.9902	2.3402
Blend (after three months of rapid stabilisation)	42.56	59.36	73.5	84.56	88.35	y = 68.118x - 26.775 R^2 = 0.9909	2.19

Using *Lactobacillus Acidophilus* ATCC 4356 on De Man, Rogosa and Sharpe (MRS) as a positive control and plain agar as a negative control (after 3 months), the prebiotic potential of various seed powder samples was determined.

Table 19: Evaluation of the seed mixture's prebiotic potential during a three-month stability testing with Lactobacillus Acidophilus

Sample	Type	No of colonies after stability	No of colonies fresh sample
0.2 g of *Cucumis melo* seed powder mixed with ordinary agar	Test	52	67
combined with normal agar, 0.2 g of *Punicagranatum* seed powder	Test	32	41
combined with normal agar, 0.2 g of *Linumusitatissimum* seed powder	Test	46	57
0.2 g of a seed powder combination 1:1:1.	Test	63	94
mixed with regular agar, 0.2 g of chicory powder	Positive control	32	59
Mr. Rogosa, Sharpe, and De Man (MRS)agar	Positive control	45	47
Simple agar	Negative control	0	0

Analysis of the prebiotic potential of a single seed powder sample by means of the *Bifidobacterium bifidum* ATTC 29521on As a positive control, *Bifidobacterium* agar, and as a negative control, plain agar

Table 20: Research on the seed mixture's stability and its prebiotic properties after three months of testing with *Bifidobacterium bifidum*

Sample	Type	No of colonies after stability	No of colonies fresh sample
0.2 g of *Cucumismelo* seed powder mixed with normal agar	Test	37	57
combined with normal agar, 0.2 g of *Punicagranatum* seed powder combined with normal agar,	Test	32	42
0.2 g of *Linumusitatissimum* seed powder	Test	49	62
0.2 g of a seed powder combination 1:1:1.	Test	52	77
2 milligrammes of chicory powder mixed with agar	Positive control	27	35
Food for Bifidobacterium.	Positive control	49	47
Simple agar	Negativecontrol	0	0

The seed powder blend maintains the majority of its prebiotic qualities as compared to ordinary chicory powder. If the product is stored in a cold atmosphere, its prebiotic action might last for a very long time. The prebiotic potential is determined by the stability of the soluble fibers and oligosaccharides that make up the prebiotic diet. The number of colonies remained consistent since the *Bifidobacterium* agar and positive control agar were both fresh.

CONCLUSION

With its rich macro and micronutrient profile, potent antioxidants, and prebiotic potential, the nutraceutical blend of *Cucumis melo*, *Punica granatum*, and *Linum usitatissimum* seeds shows promise in reducing oxidative stress, a major contributor to diabetes, heart disease, and cancer. Additionally, it exhibits promise in treating anemia and malnutrition.

In a similar vein, the combination of powdered leaves from *Trigonella foenum-graecum*, *Coriandrum sativum*, *Raphanus raphanistrum*, and *Anethum graveolens* has remarkable prebiotic and antioxidant properties. Both blends' promise for efficient and reasonably priced disease prevention and health promotion is demonstrated by their development into stable powder and capsule forms. Additional investigation may open up other uses for them as native, affordable nutraceuticals.

REFERENCES

1. Abdul MIM, Siddique S, Rahman SAU, Lateef D, Dan S, Mandal P, Bose A (2018) A critical insight of modern herbal drugs therapy under the purview of toxicity and authenticity. Biomed Res 29(16):3255–3260
2. De B, Singla KR, Bhandari K, Katakam P, Adiki KS, Mitra A (2017) Study the enzyme inhibitory potentialities of a phytocomposite for Type 2 diabetes by in silico GRIP docking. IJOPILS 5(3):34–57
3. Djordjevic SM. From medicinal plants raw material to herbal remedies. Intech Open Science 2017;269–88
4. Ekor M. The growing use of herbal medicines: Issue relating to adverse

reactions and challenges in monitoring safety. Front Pharmacol 2014;4:177
5. Kambizi LGBM, Goosen BM, Taylor MB, Afolayan AJ (2007) Anti-viral effects of aqueous extracts of Aloe ferox and Withania somnifera on herpes simplex virus type 1 in cell culture: research in action. S Afr J Sci 103(9):359–360
6. Katiyar CK, Duggal RK and Rao BVJ, Dabur Research Foundation (2002) Herbal composition and method of manufacturing such composition for the management of gynecological disorders. U.S. Patent 6,455,077
7. Kayani S, Ahmad M, Zafar M, Sultana S, Khan MPZ, Ashraf MA, Hussain J, Yaseen G (2014) Ethnobotanical uses of medicinal plants for respiratory disorders among the inhabitants of Gallies–Abbottabad, Northern Pakistan. J Ethnopharmacol 156:47–60
8. Khanuja SPS, Chaturvedi P, Singh AK, Shasany AK, Agarwal VK, Gupta VK, Gupta SC, Tripathy AK, Pal A, Saikia D and Darokar MP (2007) Anti-dermatophytic preparation and use thereof. U.S. Patent 7,291,349
9. Khanuja SPS, Srivastava S, Shasney A K, Darokar MP, Kumar TRS, Agarwal KK, Ahmed A, Patra NK, Sinha P, Dhawan S, Saikia D and Kumar S (2003) US Patent No 6514541
10. Mahajon B, Chincholikar M, Narayanan R, Venigalla S, Sharma BS, Ahmad A. Basis for the use of substitutes for medicinal flora and harmonization of rational use: A critical appraisal based on “Kitab al-Abdal”: A classical compendium of unani medicine. J Drug Res Ayurvedic Sci 2020;5:121–31
11. Mallare JT, Karabell AH, Mieyer PV, Stender SRS, Christensen ML (2005) Current and future treatment of metabolic syndrome and type 2 diabetes in children and adolescents. Diabetes Spectr 18(4):220–228
12. Parasuraman S, Thing GS, Dhanaraj SA (2014) Polyherbal formulation: concept of ayurveda. Phcog Rev 8(16):73–80
13. Saklayen MG (2018) The Global epidemic of the metabolic syndrome. Curr Hypertens Rep 20(12):1–8
14. Sharma AK, Kumar R, Mishra A, Gupta R. Problems associated with clinical trials of Ayurvedic medicines. Rev Bras Farmacogn 2010;20:276–81
15. Van Zwieten PA, Visser FC (2006) Metabolic syndrome: pharmacological treatment. Heart Metab 30:15–20

Corresponding Author

Jayashri Behera*

Research Scholar, School of Pharmacy, YBN University, Ranchi, Jharkhand, India

Email: jayashri.b89@gmail.com