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Analysis of Antioxidant and Prebiotic Properties of selected seed extracts for Potential Nutraceutical Applications

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Abstract - This research evaluates the antioxidant and prebiotic potentials of the seed extracts obtained from Cucumis melo (musk melon), Punica granatum (pomegranate) and Linum usitatissimum (flax seeds) with a view of establishing nutraceutical functionality. Some of the merit making activities included preparation and characterization of the seed powders, physical characteristics, proximate composition and determination of antioxidant activity using DPPH assay. Furthermore, the ability of seed extracts to support the growth of beneficial bacterial strain was checked using Lactobacillus Acidophilus ATCC 4356 and Bifidobacterium bifidum ATTC 29521. The outcomes suggested that the seed powders' combination held higher antioxidant activity than the seed powders alone and had substantial prebiotic functionality even superior to that of chicory powder well-known as a prebiotic. Based on the findings of this study, the seed extracts have immense potential to be used in the formulation of functional food-utritional supplements with antioxidant and prebiotic characteristics.

Keywords: Prebiotic, Antioxidant, Bifidobacterium, Lactobacillus Acidophilus, Linum Usitatissimum.

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INTRODUCTION

The combination of "nutrition" and "pharmaceutical" forms the "nutraceutical" term.A nutrient is defined as a feed component that helps support the life of either a human or an animal, while a nutraceutical is defined as any non-toxic food component that has health benefits, such as the prevention or treatment of diseases, according to the Association of American Feed Control Officials (AAFCO), 1996. Hippocrates rightly stressed the need of letting food be medicine and medicine be food some two millennia ago. The importance of "nutraceuticals" in improving health has just come to light, and as a result, there is a surge of interest throughout the world. 1. The study of nutraceuticals and related formulations is a relatively young and rapidly developing multidisciplinary discipline. Human nutrition provides a foundation for understanding the field's history and the research required to advance it. [1]

Symptoms of sickness brought on by lack of food Eating the wrong foods, The consequences of unhealthy eating habits, include diet-related and lifestyle-related illnesses, Food regimens and nutritional gaps The importance of dietary supplements,"The Function of Plant-Based Nutrients, The function of antioxidants in the diet is avoid oxidative damage to biomolecules, The health benefits of prebiotics and probiotics, Uses for nutritional supplements,Varieties of dietary supplements, The present state of nutraceuticals pros and cons, The promising future of nutritional supplements, Research is necessary And nutritional supplements as medicines .[2]

All of the mental and physical processes that make up a human existence get their energy from the food we eat, which is a process known as nutrition. Proteins, carbs, lipids, vitamins, and minerals make up the essential nutrients." These staples, in addition to fibers, are essential for the food we consume. [3] It is also crucial to know how much of these to consume. Nutrients are categorized as either macro or micro nutrients according to this theory. Some examples of macronutrients include carbohydrates, proteins, and lipids; micronutrients include vitamins, minerals, and trace elements. The food we consume should include fiber in addition to macro and micro nutrients. [4]

Inadequate, excessive, or imbalanced consumption of calories and nutrients is what the World Health Organization calls malnutrition. Worldwide, undernourishment causes: Every nation has its fair share of malnutrition cases. Approximately 462 million individuals are underweight, while 1.9 billion are overweight. Nearly forty-one million children under the age of five are overweight or obese, and another fifteen million suffer from stunting. [5] An further 528 million women, or 29% of all reproductive-age women, suffer from anaemia; of these, over 50% would be receptive to iron supplements.3 As is the case with many other vital micronutrients, inorganic iron supplements are supposedly poorly absorbed and induce gastrointestinal distress (gastric discomfort, constipation, or both) as compared to iron found in plants.[6]

Particularly affecting the world's most defenseless people, malnutrition is a major public health crisis. A 2013 assessment by the United Nations Food and Agriculture Organization (FAO) estimated that 842 million people, or 12% of the world's population, are undernourished. Additionally, almost 3.1 million children less than five years old die each year as a result of starvation. [7] According to UNICEF, 8,000 children under the age of five die every day from hunger, and 300 million children go to bed hungry every night. A shortage of essential nutrients also contributes significantly to this health catastrophe, despite the common belief that malnutrition is only related to caloric consumption. An increased mortality risk has been associated with nutritional deficiencies, including those in zinc, iron, and vitamin A. [8]

RESEARCH METHODOLOGY

- **Initiation Research**

Seed sourcing and preparation

Common fruits that are readily accessible in India include Musk melon (*Cucumis melo*) and Pomogranate (*Punica granatum*). [9] We sourced our fresh fruits from the fruit market in Ranchi, India. After removing the seeds from the fruit pulp, washing, and drying them in the shade to a consistent weight, the process was complete. Nutroactive, a food provider based in Mumbai, India, supplied the original *Linum usitatissimum*, often known as flax seeds. [10]

Prescription drug account

Cucumis melo, *Punica granatum*, and *Linum usitatissimum* seeds were analysed from a pharmacognostic perspective. (In accordance with the Pharmacognostic Report on Plant

Reducing size

Shape, surface area, and porosity are some of the associated attributes that may be changed by adjusting the particle size. Pharmaceutical items' bioavailability and shelf stability might be impacted by this. The shape, surface properties, and distribution of particle sizes all have an impact on how a formulation works and how well it suits a given application. [11] Surface area and other surface features may affect hygroscopicity, compaction, flowability, and other attributes. These characteristics may have a major impact on the production methods used for process plant materials, making them crucial. “Almost all size-reduction techniques involve creating new surface area, which requires adding energy proportional to the bonds holding the plant fibrous particles together. [12] For materials like seeds and leaves, this cutting action is achieved on a small scale using a domestic mixer grinder followed by sieving to get a particular size of plant material.” Hardness is the most important characteristic affecting size reduction, but toughness and elasticity of the materials are also important. [13]

Separately, intact seeds of Musk melon (*Cucumis melo*), Pomogranate (*Punica granatum*), and Linseed (*Linum usitatissimum*) were placed in a home mixer grinder. The grinder was then run for ten minutes, with the capacity of the mixer being filled three quarters of the way up, halting occasionally. [14] With the aid of a spatula, the whole pile of seeds was combined, and the grinding process continued for another 10 minutes, pausing every minute. After passing the powder through an 85# sieve, the excess material was crushed again until the whole bulk went through. The remaining two seeds were similarly ground down to size. [15] To prevent temperature-induced deterioration, all powder components were sealed in containers and stored in the refrigerator. [16] Based on the USP24/NF19 Pharmacopoeial standards, it is anticipated that this technique will provide a powder combination that may be described as fine powder.

Powder fineness is defined using descriptive terminology in the USP 24/NF19. You can see the categorization and connection in the table below.

Table 1: Powder categorization according to U.S.P.

Description Term	Mesh Size Number	Mesh Opening Size (microns)
Very Coarse	2 - 10	> 1000
Moderately Coarse	40 - 80	180 - 355
Coarse	20 - 40	355 -1000
Fine	80 - 120	125 - 180

Characteristics of Bulk Powder

Particle density, which is influenced by solid density, particle internal porosity, and the spatial arrangement of the particles in the container, as well as other factors, determines the bulk density of powders. Compressibility is another important property of powders. [17] There are two types of density for powders: "loose bulk density," which is the density when the powder is poured freely into a container, and "compact density," which is the density after the powder is compressed by echanical pressure, vibration, or impact. [18]

density.

A graduated cylinder was used to record the volume (V0) after weighing and sieving a certain amount of seed powder. In order to maintain a consistent ultimate volume, the cylinder was tapped in a bulk density device. The following formulae were used to compute the bulk and tapped densities. [19]

Bulk density (poured density): m/V_0, in g per cm^3.

Tapped density: m/V_t g per cm^3.

m= the powder's weight

V0= aggregate size

Carr's index (%) = (Tapped density – Bulk density) / Tapped density x 100

Powder flowability is connected to Carr's index.

Preliminary Examination of a Nutraceutical Powder Blend

One way to find out what foods are good for you is to use the proximate analysis. Around 1850, they were created in Germany. Their techniques rely on the chemical and physical characteristics of a certain class of nutrients. This collection of analyses has the benefit of being inexpensive and straightforward to do. [20]

Analysis of moisture, crude protein, lipids, crude fibres, carbs, calcium, and iron were performed on the whole nutraceutical powder blend. Dry weight, crude protein, carbs, lipids, fibres, calcium, iron, and the Association of Official Analytical Chemists' (AOAC, 1999) suggested procedures were used for these analyses. The research was carried out in an approved laboratory at (ICAR) in Ranchi, India.

- **The DPPH Radical Scanning Capabilities Were Determined**

By using the DPPH test, we were able to determine the antioxidant activity of each of the nutraceutical herbal powders. One stable free radical that has a delocalized spare electron is 1,1-diphenyl-2-picryl hydrazyl, or DPPH. An absorption band in methanol solution at around 520 nm characterises the deep violet hue, which is also caused by delocalization. The violet light-sensitive reduced form of diphenylphthalate (DPPH) is produced when DPPH solutions are combined with substances that may donate hydrogen atoms. [21]

The test was conducted using a Jasco 630 UV-Spectrophotometer. The DPPH stock solution was made and kept in a dark area until it was needed. The concentration was 0.004%. To ensure accuracy, quercetin was diluted in distilled water to a concentration of 50 mg/50 ml and then made into serial dilutions of 10, 20, 30, 40, and 50 µg/ml. "The seeds were dissolved in dimethyl sulfoxide (DMSO) and left to react with DPPH solution for 30 minutes in a dark, room-temperature environment. After filtering, the absorbance values were measured at 517 nm relative to a blank. The equation was used to compute the radical scavenging activity, which was represented as a percentage of DPPH radical elimination.

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} X\ 100$$

For each of the five concentrations, the concentrations were converted to log concentrations and then plotted against the percentage of inhibition. "The concentration needed to produce a 50% reduction of DPPH activity (IC50 in µg/ml) was determined by solving the equation for 50% inhibition and then taking the antilog.

- **Determining The Nutritional Powders' Prebiotic Value**

The strategy called for screening a large number of plant seed materials for prebiotic potential before settling on three seeds *Cucumis melo L*., *Punica granatum*, and *Linum usitatissimum* specifically.No prebiotic potential was found in the other seeds examined, which included Tamarindus indica, Magnifera indica, Ziziphus moritiana (indian ber), and Limonia acidissima (wood apple).

1) *Cucumis Melo* Seeds

Description: Seeds of the *Cucumis melo* plant. The cucumber family includes the *Cucumis melo* plant.

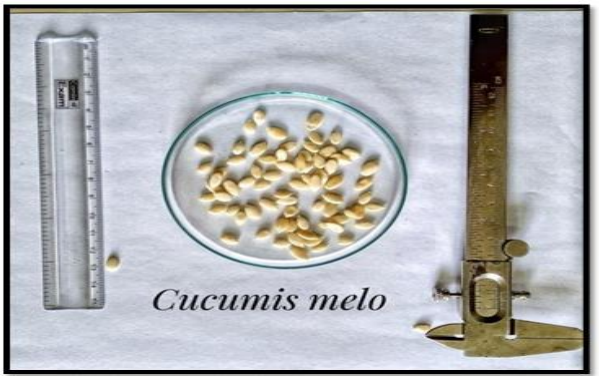

Figure 1: Experimental anatomy Seeds of *Cucumis*

Morpholog: Experimental Seeds The flattened, elliptical seeds have a thickness ranging from 0.25 to 0.3 cm and a length ranging from 0.9 to 1.2 cm.

Kingdom:	Plantae
***Clade*:**	Angiosperms
***Clade*:**	Eudicots
***Clade*:**	Rosids

Order: Cucurbitales

Family: Cucurbitaceae

Genus: *Cucumis*

Species: ***C. melo***

Table 2: composition of 100 g Cucumis melo seed powder

Component	g /100 g
Proteins	27.41
Carbohydrates	29.96
Ash	4.83
Fibres	25.32
Fats	30.65
Moisture	7.16

Chemical constituents: linoleic acid and oleic acid. flavonoids, β and γ-tocopherol , phytosterols

Uses: The antihyperlipidemic impact and the amount of monounsaturated fatty acids contribute to the improvement of heart health. In addition to lowering the incidence of type 2 diabetes and promoting bone density, it acts as an antioxidant, anti-atherosclerotic, probiotic, and colon cleanser.

2) Punica Granatum Seeds

Description: One member of the Lythraceae family is the deciduous shrub or small tree known as the pomegranate (*Punica granatum*).

Experimental Morphology of Seeds: Measurements range from 0.35-0.39 cm in thickness and -0.6 to 0.8 cm in length.

Figure 2: Experimental shape *Punica granatum Seeds*

Kingdom: Plantae

***Clade*:** Angiosperms

***Clade*:** Eudicots

***Clade*:** Rosids

Order: Myrtales

Family: Lythraceae

Genus: Punica

Species: ***P. granatum***

Proximate analysis of powder of seeds of *Punica granatum*

Table 3: Component of Punica granatum /100 g

Component	g /100 g
Fats	26.03
Proteins	14.06
Carbohydrates	23.96
Moisture	6.84
Ash	1.55
Fibres	27.59

Molecular building blocks

The chemical make-up of pomegranate seeds has been the subject of research. Total fats, protein, crude fibres, and other nutrients abound in the seeds." "The oleic and linoleic acids, as well as the iron, copper, sodium, magnesium, and zinc.

Among its many potential applications are the following: anti-inflammatory, anti-cancer, antimicrobial, memory-enhancing, and lowering blood pressure and LDL oxidation.

***Linum Usitatissimum* Seeds**

Description: Linseed, or *Linum usitatissimum*, belongs to the Linaceae family and is a member of the *Linum genus*.

Experimental Morphology: The dimensions range from a thickness of 0.33 to 0.36 cm and a length of -0.4 to 0.7 cm.

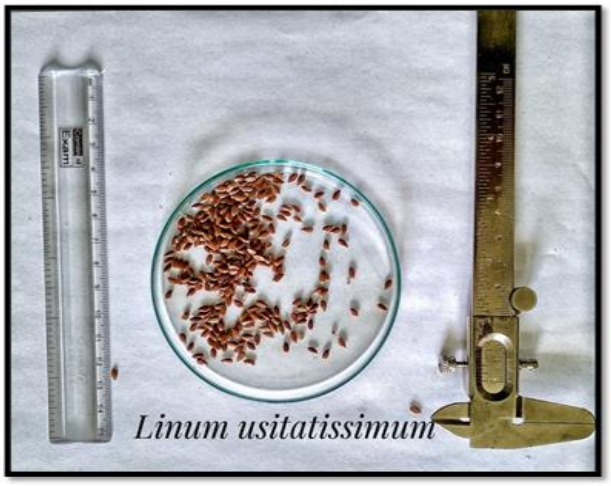

Figure 3: *Linum usitatissimum seeds* morphology

Kingdom: Plantae

***Clade*:** Angiosperms

***Clade*:** Eudicots

***Clade*:** Rosids

Order: Malpighiales

Family: Linaceae

Genus: Linum

Species: *L. usitatissimum*

Table 4: Component of Linum usitatissimum - per 100 g

Component	g /100 g
Proteins	18.3
Carbohydrates	28.9
Fats	42.2
Moisture	7
Ash	4
Fibres	27.3

Chemical constituents: B-group vitamins, minerals, tocopherols", dietary fibres, phytoestrogen lignans, and polyunsaturated fatty acids (PUFAs)

Functions: hypolipidemic and antiatherogenic functions

RESULTS

The three powders, both alone and together, exhibited low flow and compressibility properties, with an angle of repose more than 40 degrees and a Carr's index greater than 25%.

Properties of *Cucumis melo, Punica granatum, Linum usitatissimum*, and a combination of the three as a powder

Table 5: 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

1. Proximate analysis

Table 6: 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	

2. Antioxidant Activity Determination

The data is presented as quercetin equivalent, IC50, and percentage inhibition. The manufactured nutraceutical product had exceptional antioxidant activity, and all three active nutraceutical components showed very high antioxidant potential. Presented in the following table are the results of the three seed powder combination.

Table 7: 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

Because the combination of the seeds has a greater antioxidant capacity than the seeds alone, it is more effective than using either seed powder alone. All of the seed powders exhibited strong antioxidant activity when tested at doses between 10 and 50 ppm.

Probability of prebiotics

We chose *Cucumis melo, Punica granatum, and Linum usitatissimum* seeds for future investigation based on the findings of our prebiotic potential studies, which are shown in the table.

Table 8: Initial evaluation of putative prebiotics in a variety of seed sources

Seed source	Pramotion of growth of *Bifidobacterium bifidum* ATTC 29521on	Pramotion of growth of *Lactobacillus Acidophilus* ATCC 4356
Magnifera indica,	No	No
Limonia acidissima (wood apple)	No	No
Cucumis melo,	Yes	Yes
Tamarindu indica,	No	No
Linum usitatissimum	Yes	Yes
Ziziphus moritiana (Indian ber)	No	No
Punica granatum	Yes	Yes

Tests with Lactobacillus Acidophilus ATCC 4356 on a 1:1:1 seed powder combination of *Punica granatum, Cucumis melo, , and Linum usitatissimum* Sample- Growth B) Negative control – No growth C) Positive control- Growth

Nutraceutical powder combination microbiological contamination limit test

Table 9: 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

Table 10: 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 11: 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

With 0.2 grammes of seed powder per petri dish, the two probiotic bacteria *Bifidobacterium bifidum* ATTC

29521 and *Lactobacillus Acidophilus* ATCC 4356 produce more colonies than the positive control. Since the petri dishes only contained the seed combination on top of a solid agar base, any observed growth must have resulted from the seeds' role as prebiotics.

The seed powder mixture outperforms chicory powder in terms of CFUs of *Lactobacillus Acidophilus* ATCC 4356 and *Bifidobacterium bifidum* ATTC 29521, demonstrating the superior prebiotic potential of the seed mixture." Chicory powder is a well-known natural plant material with good prebiotic activity.

CONCLUSION

The findings of the current study reveal that seed extracts of *Cucumis melo, Punica granatum, and Linum usitatissimum* have antioxidant and potential prebiotic effects, and can be utilised in nutraceutical products. The result of the antioxidant activity measurement in this study also showed that the seed powder mixture possessed higher antioxidant activity than the individual seed powders with IC50 lower than quercetin standard. On the aspect of prebiotic effectiveness the seed powder mixture developed increased more the population of the *Lactobacillus Acidophilus* ATCC 4356 and *Bifidobacterium bifidum* ATTC 29521 than that of chicory powder, a known prebiotic substance. Thus, it points out that the seed mixture can be used as a functional ingredient in prebiotic food products.

The proximate analysis provided the nutritional composition of the seed mixture with fairly good quality proteins, carbohydrate and beneficial fats. In addition to this, the microbiological test as carried out revealed that the seed powder mixture is safe for human consumption. It is proposed that the melting of *Cucumis melo, Punica granatum, and Linum usitatissimum* seed extracts must go ahead because they have prebiotic and antioxidant values that can fit into nutraceutical value addition. Additional studies should be conducted to discover the best combinations and to examine these seed extracts' effectiveness in animal tests as well as clinical trials.

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