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Research Article

PREPARATION AND EVALUATION OF NUTRACEUTICAL FORMULATIONS OF LEAVES OF *TRIGONELLA FOENUM GRAECUM, CORIANDRUM SATIVUM, RAPHANUS RAPHANISTRUM* AND *ANETHUM GRAVEOLENS*

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ABSTRACT

Introduction - In recent years there is a growing market for nutraceuticals which are proven to have health benefits and preventive in certain diseases. Nutraceuticals are developed and are being developed to target specific disease. Scope-The nutraceutical market escalated and has reached to its maturity in USA, Europe and in Japan. In India it's in infancy, though the scope is huge, especially owing to the vast variety of vegetables, fruits and grains which are grown in India Objective-This research has made an attempt to evaluate four rich green leafy vegetables *namely Trigonella foenum graecum, Coriandrum sativum*, *Raphanus raphanistrum* and *Anethum graveolens* for nutraceutical, prebiotic and antioxidant potential individually and in combination. The results were encouraging which prompted preparation of two formulations of the four leafy vegetable mixture. Methodology- The whole powder mixture was analysed for nutrient content, prebiotic and antioxidant potential. The mixture was designed into dry powder health drink formula to be mixed with warm water. The ethanol extract of four leaves mixture was obtained to get more concentrated nutrients and was developed into capsule dosage form. Both formulations complied with the required in-vitro pharmaceutical parameters of evaluation. Conclusion-Nutraceuticals developed under this research are rich in antioxidants, essential nutrients, proteins, soluble fibres and thus can have applications in treatment and prevention of many illnesses that can be helped with prebiotic and antioxidant content.

Keywords: Nutraceutical, *Trigonella foenum graecum, Coriandrum sativum*, *Raphanus raphanistrum , Anethum graveolens,* prebiotic, antioxidant.

INTRODUCTION

Today foods are not intended to only satisfy hunger and to provide necessary nutrients for humans, but also to prevent nutrition-related diseases and improve physical and mental well-being of the consumers. In this regard, nutraceuticals play an outstanding role and the market for them is increasing.[1, 2]

Nutraceuticals are a broad umbrella term used to describe any product derived from food sources that provides extra health benefits in addition to the basic nutritional value found in foods. The term Nutraceutical is a hybrid of nutrition and pharmaceutical technology, coined in 1989 by Stephen Defelice.[3]

Nutraceutical-rich vegetables and fruits are an important component of a healthy diet. There has been an explosion of consumer interest in the health enhancing role of physiologically-active specific nutraceuticals.[4]

Individually *Trigonella foenum graecum* (Fenugreek leaves) *Coriandrum sativum* (coriander leaves) *Raphanus raphanistrum (* Radish leaves*)* and *Anethum graveolens* (Dill leaves) have been reported to contain rich macro and micro nutrients. Under this research these four leaves in dried powder form are used in equal proportion as the basic nutraceutical mixture. The combination increases nutraceutical contents, prebiotic potential and antioxidant properties which are marker qualities of any nutraceutical. Two teaspoonful of dry powder of leafy vegetable is actually equal to one vegetable serving. When cooked all vegetable loose some nutrition. In the nutraceutical formulations the chances of nutrition preservation are more, which serves the larger purpose of health improvement and prevention and cure of diseases.[5]

Trigonella foenum graecum -Fenugreek leaves reported to contain - 4.4% protein, 0.9% fat, 1.5% minerals, 1.1% fiber, and 6% carbohydrates. The minerals and vitamins include calcium, zinc, iron, phosphorous, riboflavin, carotene, thiamine, niacin, vitamin C and β-carotene about 19 mg/100 g. The reported medical benefits are that it improves heart function as prevents atherosclerosis, lowers cholesterol level, helpful in anaemia as rich in iron along with prebiotic potential [6,7,8,9]

Coriandrum sativum,-Coriander leaves reported to contain - Crude protein 10.61 %, Crude fiber 5.97 %, Minerals- Calcium 2.11%, Potassium 4.29% Iron 0.091% The reported medical benefits are that it lowers cholesterol, stimulates the insulin secretion, antiseptic, anti-inflammatory, along with prebiotic potential [10,11,12]

Raphanus raphanistrum -Radish leaves. The green parts of a radish contain more nutrients than the entire radish itself and are eaten as vegetable in India. It contains minerals like iron, calcium, folic acid, vitamin C and phosphorous. It's proximate analysis – shows to contain Crude Fat 4.03 %, Protein 3-3 % , Crude fibre 13.5%, Ash 20.63%, Carbohydrate 12.16%, and antioxidants 326.80± mg/g. Medical benefits include –It treats constipation, increases immunity and reduces fatigue, It is diuretic and anti-scorbutic, it treats piles, helps to prevent diabetes:, and it is detoxifying agent: [13,14]

Anethum graveolens - Leaves of Dill, is a green, leafy vegetable, rich in minerals like calcium, magnesium, iron. As per its proximate analysis it is reported to contain carbohydrates 7%, dietary fiber 2.1 %, fat 1.1 %, protein 3.5%. It is rich in vitamin A, Thiamine B1), Riboflavin B2), Pantothenic acid (B5), Vitamin B6, Folate (B9), and vitamin C. The reported medical uses are- It protects from infections due to its anti-bacterial and anti-inflammatory activity. Helps in digestion lowers blood sugar levels, induces sleep, maintains hormonal balance, it is heart protective agent- due to its ability to lower blood cholesterol levels in the body, it is one of the best plant sources of calcium[15,16,17].

RATIONALE - The mixture of above leafy vegetables could be a rich mixture and can be developed into prebiotic and antioxidant rich nutraceutical. So, under this research work was done to study the nutraceutical, prebiotic and antioxidant potential of the selected leafy vegetables and their mixture. The leaf powders were individually and as a mixture were studied for proximate analysis, prebiotic potential, DPPH assay for antioxidant potential. The promising results lead to development of powder formulation to be used as soup and further lead to more potent extract-based product.

MATERIALS AND METHODS

Preformulation Study

Procurement of leafy vegetables needed for the research, drying and size reduction-
Trigonella foenum graecum, Coriandrum sativum, *Raphanus raphanistrum* and *Anethum graveolens* are four common green leafy vegetables abundantly available in India. Fresh vegetables were purchased; leaves were separated, washed and dried to constant weight in shade.[18] The dried vegetables were ground in a mixer grinder and passed through 85 number sieve. The leaf powders were mixed in equal proportion as the basic raw material for the nutraceutical product.

Determination Of Moisture Content Of Powders And Bulk Powder Characteristics[19]

Method used was gravimetric. The nutraceutical powder mixture mentioned above was dried in an oven at 60°C for 3 hours. The sample was dried to constant weight and the petriplates were cooled and put in a desiccator before weighing. Moisture content was then calculated using the following equations.

Moisture weight = Initial weight (before drying) – Final weight (after drying)
Moisture content = (Moisture weight / Initial weight) 100%

Bulk Powder characteristics

Following bulk powder characteristics were determined.
Bulk density, Tapped density, % compressibility and flowability- (using Carr's index method), [20]
A pre-weighed, pre-sieved quantity of leaf powder mixture was poured into a graduated cylinder, and the volume was recorded (V_0). The cylinder was secured in its holder and was tapped till the final volume remained constant. The bulk and tapped densities were then calculated using the following equations.

Bulk density (poured density) = m / V_0, in g per cm^3.
Tapped density = m / V_t g per cm^3.
Where m = weight of the powder and V_0 = bulk volume
Carr's compressibility index was calculated using formula -
Carr's index (%) = Tapped density – Bulk density / Tapped density x 100
The Carr's index is related to flowability of the powder.

Proximate Analysis Of Nutraceutical Powder Mixture [21,22]

Nutraceutical powder mixture was analyzed for moisture, crude protein, fats, crude fibres, carbohydrates, calcium and iron. The recommended methods of the Association of Official Analytical chemists (AOAC, 1999) were used for the determination of crude proteins, carbohydrates, fats, fibres, calcium, iron, moisture. This study was conducted at National Agriculture and Food Analysis and Research Institute (NAFARI) NABL, Pune, India an accredited laboratory as per ISO/IEC 17025:2005.

Determination Of DPPH Radical Scavenging Properties [23-27]

All the Nutraceutical herbal powders were evaluated for antioxidant activity by DPPH assay. DPPH- 1, 1-diphenyl-2-picryl hydrazyl, is a stable free radical and contains a delocalized spare electron. The delocalisation also gives rise to the deep violet colour, characterised by an absorption band in methanol solution at about 520 nm. When a solution of DPPH is mixed with a solution of substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour. Thus DPPH represents the free radicals produced in a system and antioxidant suppresses their formation.

Procedure-Jasco 630 UV-Spectrophotometer was used for the assay. Fresh DPPH stock solution (0.004%) was prepared and stored in dark place until used. As a positive control, quercetin (50 mg/50 mL) was prepared in distilled water and serial dilutions 10, 20, 30, 40, 50 µg/mL were made. Test solutions of leafy powder was made in DMSO and allowed to react with DPPH solution at room temperature in dark place and filtered after 30 minutes .The absorbance values were measured at 517 nm against blank. The radical scavenging activity (% inhibition) was expressed as % of DPPH radical elimination and was calculated according to the equation,

% Inhibition = [(A0-A1)/A0] × 100
Where A0 is the absorbance of the control; A1 is the absorbance of test samples.

The reaction kinetics can get affected by time, solvent used, so as reported by Brand-Williams W *et al.* The concentrations were transformed to log concentrations and were plotted against % inhibition for five different concentrations.

Determination Of Prebiotic Value Of Nutraceutical Powders

Preparation of nutrient medium for prebiotic evaluation [28,29]
Modern day research in the area of nutraceuticals also includes the concept of prebiotics. These are foods for probiotic bacteria which grow in intestine and improve gastrointestinal health. Prebiotic potential of *Trigonella foenum graecum, Coriandrum sativum*, *Raphanus raphanistrum* and *Anethum graveolens*, their powder mixture and extract were determined by microbial method using *Lactobacillus acidophilus* ATCC 4356 and *Bifidobacterium bifidum* ATTC 29521. In the method for *lactobacilli*- De Man, Rogosa and Sharpe (MRS) nutrient media was used as positive control, whereas plain agar medium was used as negative control and for *Bifidobacterium bifidum.* The following media were prepared for the prebiotic evaluation using lactobacilli-
1) De Man, Rogosa and Sharpe (MRS) nutrient media 25mL
2) Plain agar medium (25mL)
3) Plain agar (25mL) + powder *Trigonella foenum graecum,* (0.1g.)
4) Plain agar (25mL) + *Coriandrum sativum* powder (0.1g.)
5) Plain agar (25mL) + *Raphanus raphanistrum* powder (0.1g.)
6) Plain agar (25mL) + *Anethum graveolens* powder (0.1g.)
7) Plain agar (25mL) + 1:1:1 leaf powder mixture (0.1g)
8) Plain agar (25mL) + 1:1:1 leaf powder extract. (0.1g)

For *Bifidobacterium bifidum* above protocol remained the same except in place of MRS, *Bifibacterium* agar was used.
All nutrient media were sterilized in an autoclave for 15 min at 121^0 C. The suspension of lactobacillus acidophilus was prepared. Aseptically, one mL bacterial suspension was added in all prepared nutrient media in Petri plate. All Petri plates were transferred in the BOD incubator for 48 hrs & temperature of 37^0 C was maintained with anaerobic conditions. The growth of *lactobacillus* bacteria was observed after 48 hours.

Microbial Load Determination [30]

1g nutraceutical powder mixture was mixed and stirred in 100 mL sterile distilled water. One mL top clear liquid from this was pipette out and diluted to 10 mL with sterile distilled water. This procedure was repeated to get a dilution of 10^{-5}.. One mL of this final dilution was poured on to sterile petri plate containing soybean casein agar medium. Aseptic conditions were maintained during this operation. The plates were incubated at 37°C for 24h. and the numbers of colony forming units were counted. The microbial content was taken as the mean of duplicate determinations.

Extraction

All four nutraceutical ingredients as equal proportion mix were extracted using 70% ethanol as solvent in Soxhlet extractor. The extract was dried in rota-evaporator and further dried in desiccator. The resultant extract was a slightly sticky mass which was mixed with lactose to get a dry powder. 15 g of dry powder mixture gave 136 mg of dry extract.

Formulation Development

Two Formulations as health soup drink (using whole leaf powder) and capsule (using extract mentioned above) were developed.
Nutraceutical powder mixture as a health drink was prepared by mixing *Trigonella foenum graecum, Coriandrum sativum, Raphanus raphanistrum and Anethum graveolens* using following formula. As seen in Table 1.

Table 1: Formula I (for powder to be mixed with warm water)

Ingredients	Use	Qty. in g
Trigonella foenum graecum	Active nutraceutical ingredient	18
Coriandrum sativum	Active nutraceutical ingredient	18
Raphanus raphanistrum	Active nutraceutical ingredient	18
Anethum graveolens	Active nutraceutical ingredient	18
Zingiber officinale (ginger powder)	For flavor and taste	9
Solanum Lycopersicum(tomato powder)	For taste	9
Sodium starch glycolate	Dispersing agent	2
Sucrose	Sweetener	8
Total		100

Table 2: Formulation of Nutraceutical Composition (for capsules)

Ingredient in extract form	Qty in mg
Trigonella foenum graecum	34
Coriandrum sativum	34
Raphanus raphanistrum	34
Anethum graveolens	34
Lactose	164
Total	300 mg

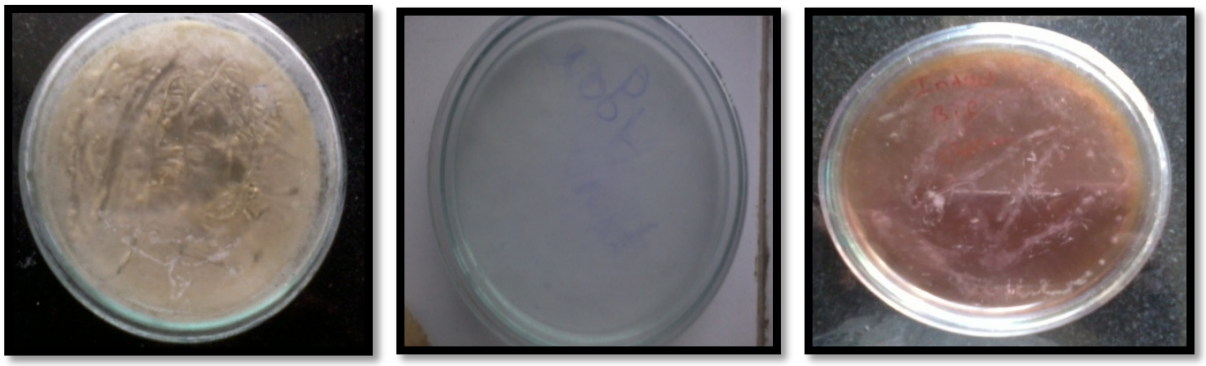
Table 3: Proximate analysis of leaf mixture sample

Sr No	Parameter	Result	Units	Test method
1	Proteins	18.95	g/100g	AOAC 920.152
2	Carbohydrates	58.96	g/100g	IS 1656.2012
3	Fat	3.15	g/100g	IS 12711.2010
4	Crude fibre	11.23	g/100g	IS 2234.2011
5	Soluble dietary fibre	2.6	g/100g	IS 11062.2010
6	Iron	23.27	mg/100g	AOAC 944.02,320109
7	Calcium	440	mg/100g	AOAC 984.27 and 999.10

Table 4: Determination of Antioxidant activity of individual leaf powder, mixture and extract

log c	% inhibition	log c	% inhibition	log c	% inhibition
1	54.7	1	45.03	1	51.36
1.301	64.92	1.301	79.72	1.301	63.05
1.4771	86.89	1.4771	83.64	1.4771	85.47
1.602	91.89	1.602	90.09	1.602	90.25
1.6989	96.89	1.6989	93.25	1.6989	95.55
leaf powder mixture		***Coriandrum sativum* leaf powder**		***Trigonella foenum graecum***	
log c	% inhibition	log c	% inhibition	log c	% inhibition
1	38	1	42	0.1	51.80
1.301	56.48	1.301	56.89	0.1301	65.30
1.4771	68.21	1.4771	69.52	0.14771	78.79
1.602	81.31	1.602	88.8	0.1602	89.05
1.6989	90.01	1.6989	92.75	1.6989	93.2
Anethum graveolens		*Raphanus raphanistrum*		**Extract of four vegetable powder mixture**	

1) Sample- mixture -Growth 2) Negative control – No growth 3) Positive control- MRS Growth
Figure 1: Prebiotics potential determination using Probiotic - *lactobacilli.*



1)Sample- mixture -Growth 2) Negative control – No growth 3) Positive control- *Bifibacterium* agar. Growth
Figure 2: Prebiotics potential determination using Probiotic - *Bifidobacterium bifidum*

Manufacture of Capsules [31]

15 g of dry powder mixture gave 136 mg of dry extract. Two capsules containing 272 mg extract therefore can be equivalent to 30 g of dry powder mixture. Because as per literature one serving of vegetables is equal to 30 g of dry powder of vegetables, two capsules containing 272 mg extract can be logical dose.

The formula for capsule as seen in the Table 2.
Above formula was the raw material for capsules. The powder was filled in size 1 capsule. The average weight of capsule was 300 mg. The extract based capsule was more potent (proven by antioxidant DPPH assay) but since yield of extraction process was low the cost of product is high. Capsules were manually filled and were evaluated for weight variation and disintegration test.

RESULTS AND DISCUSSION

Preformulation-Washing ,Drying and Size Reduction
The *Trigonella foenum graecum, Coriandrum sativum*, *Raphanus raphanistrum and Anethum graveolens* were washed and dried till the constant weight was obtained. Powders were passed through 85# sieve

Moisture Content
Moisture content of leafy mixture was found to be- 7.4%

Bulk Powder Characteristics
All the three powders individually and as a mixture showed angle of repose more than 40^{o}, Carr's index more than 25% , Hausner ratio was more than 1.2 ,indicating poor flow and compressibility characteristics.

Proximate Analysis Of The Leaf Mixture Sample – Sample reference number14-15/C**13**325 NAFARI - National Agriculture and Food Analysis and Research Institute (NAFARI) NABL Accredited Laboratory as per ISO/IEC: 17025:2005. Proximate analysis of leaf mixture sample, as seen in Table 3.

Determination of Antioxidant Activity
DPPH free radical scavenging assay gave an idea about antioxidant potential of the four leafy vegetable powders individually and for their powder mixture. The results were obtained on comparison with a quercetin as a standard. The result is expressed as % inhibition. All the four active nutraceutical ingredients showed very high antioxidant potential (from 10-50 ppm concentration range) and the nutraceutical powder mixture shows excellent antioxidant potential. The extract gave excellent result from 1 to 5 ppm range, which indicates that it is more potent than vegetable powders. The result of the four leafy powders, their combination mixture and extract is shown in Table 4.

Prebiotic Potential Determination Of Mixture Of Whole Powders Of Four Leafy Vegetables Under Study
Prebiotics potential determination using Probiotic - *lactobacilli.* As seen in the Figure 1
Prebiotics potential determination using Probiotic - *Bifidobacterium bifidum* As seen in the Figure **2**

Microbial Contamination Limit Test For Nutraceutical Powder Mixture

Total Aerobic count (TAC) = 2 x 10 ^{5} CFU per gram

Extract of Nutraceutical Mixture - The resultant extract was a slightly sticky mass. The extract was more potent antioxidant. (proven by antioxidant DPPH assay)
Since the extract was slightly sticky, was mixed with lactose to get a dry powder.136 mg extract and 164 mg lactose was mixed to get dry powder.

Formulation Development

Powder Soup Drink Formula- Formula was developed for 30g quantity to be mixed with 100 mL hot/ warm water as 30 g of dry powder approximately is equal to one fresh vegetable serving. The angle of repose, Carr's index of this powder formula suggested that it was poor flowing powder with poor compressibility index. It does not hamper the health drink formula as two table spoon quantities can be scooped out easily which can be mixed with warm water. For such powders flowability is not a quality parameter needed. 30 g easily dispersed in warm water with gentle stirring for 30 sec.

Capsule formula -136 mg extract and 164 mg lactose was mixed to get dry powder. The powder was filled in size 1 capsule. The average weight of capsule was 300 mg. The capsules complied with weight variation and disintegration time limit as per I.P .Both results were average of duplicate readings. The extract based capsule was more potent, but since yield of the extraction process was low, the cost of such product will be high.

CONCLUSION

Individually and in combination powders of leaves of *Trigonella foenum graecum, Coriandrum sativum, Raphanus raphanistrum* and *Anethum graveolens* proved to be a potential nutraceutical mixture with rich macro and micro nutrients. The proximate analysis of powder mixture of these four leafy vegetable mixture supports it. Individually all the ingredients showed very good antioxidant potential. The extract of the mixture showed excellent antioxidant potential almost at one tenth concentration compared to whole powders of the individual ingredients. Except *Anethum graveolens* all leafy ingredients showed very good and the mixture showed excellent prebiotic potential with two probiotics *lactobacilli* and *bifidobacterium bifidum* bacteria. The mixture of whole powders was designed into dry powder health drink formula to be mixed with warm water. The extract of four leaf mixture was obtained to get more concentrated nutrients and was developed into capsule dosage form. With more extension to this study, both dosage forms can have commercial applications. Nutraceuticals like these which are rich in nutrients, antioxidants and have prebiotic content, can have applications in treatment and prevention of many illnesses that can be helped with prebiotic and antioxidant content. Being indigenous nutraceuticals these can be economical too.

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