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FORMULATION AND EVALUATION OF NUTRACEUTICAL DRY POWDER SUSPENSION USING *MORINGA OLEIFERA* LEAVES AND *CELASTRUS PANICULATUS* SEEDS EXTRACTS

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ABSTRACT: Herbs, which have been used to cure numerous ailments since ancient times, play an important role in building the foundation of modern medications. The present study aimed to investigate the antioxidant potential of nutraceutical dry powder suspension formulated with *Moringa oleifera* leaves and *Celastrus paniculatus* seeds extract against DPPH (2,2-diphenyl-1-picryl-hydrazine-hydrate). Plant extracts are prepared using ethanol as a solvent in the Soxhlet apparatus and extracts are evaluated for antioxidant activity using ascorbic acid as standard and best % inhibition of free radicals the dose is selected for formulation. Further evaluation studies are carried out for all the batches to finalize the optimized batch showing good flow properties and drug release profile. Both plant extracts showed good % Radical scavenging activity (%RSA) compared to ascorbic acid's scavenging activity. The optimized formulation showed acceptable evaluation results, including pH, angle of repose, carr's index, hausner ratio, sedimentation volume, redispersibility, viscosity and drug release. The formulated dry powder suspension formulation showed good antioxidant activity. Thus, the present investigation revealed that the developed nutraceutical antioxidant formulation is formulated and all evaluation criteria are within the limit.

INTRODUCTION: The term nutraceuticals comprise nutritionally and medicinally enhanced foods with health benefits. From the consumer's point of view functional foods and nutraceuticals offer some brilliant advantages such as increasing the health value of the routine diet; compared to standard medicine, it might be perceived as being more natural and less likely to have adverse side effects.

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Some benefits of nutraceuticals

- Helps to stay away from a particular medical condition
- Easily available and economically affordable
- No side effects
- Helps in increasing longevity [1].

Plant Profile:

Moringa oleifera: *Moringa oleifera* belongs to the Moringaceae family. This plant is a fast-growing, deciduous tree that can reach a height of 10–12 meters the tree has fragile branches, and the leaves are bi- or tripinnately compound [2]. It contains chemical constituents including Alkaloids, Flavonoids, terpenoids, tannins, saponins and

anthraquinones [3]. The plant shows various pharmacological activities such as Neuroprotective, Anti-asthmatic, anti-diabetic, hepatoprotective, anti-inflammatory, anti-cancer, anti-oxidant, cardiovascular and anti-ulcer [4].

FIG. 1: *MORINGA OLEIFERA* LEAVES POWDER

Celastrus paniculatus: The deciduous vine *C. paniculatus* has stems that can grow up to 10 cm (3.9 in) in diameter and 6 m (20 ft) in length. Its rough, pale brown exfoliating bark is covered heavily in small, elongated lenticles and its seeds are completely encased in red arillus, which are ovoid in shape and brown in colour. It contains chemical constituents, including Alkaloids, sterols, triterpenoids, fatty acids, tannins, saponins and phenolic compounds [5]. It possesses various pharmacological activities such as Anti-Alzheimer, nootropic, anti-arithmetic, wound healing, antioxidant, antibacterial and antiviral [6].

FIG. 2: *CELASTRUS PANICULATUS* SEEDS

MATERIALS AND METHODS:

Plant Material: The *Moringa oleifera* leaves powdered form was purchased from 'Yucca Enterprises' Wadala East, Mumbai 400037. *Celastrus paniculatus* seed was purchased from 'Natucare Herb essence Private Limited' Mulund East, Mumbai 400081. Alarsin House authenticated all drugs. A/32, Street No. 3 M.I.D.C. Andheri (East). Mumbai, India.

Herbal powders were stored in an airtight container. All chemicals and reagents used were of LR grade.

Preparation of Extracts: The individual drug powder weighing 100 g each of *Moringa oleifera* leaves and C*elastrus paniculatus* seeds were extracted with ethanol by hot continuous percolation method in the Soxhlet apparatus. The extracts were filtered, evaporated and dried under reduced pressure with a rotary evaporator to get it in dry powdered form. The dried extract weighing 17.56 g of *Moringa oleifera* and 24.38 g of *Celastrus paniculatus* was used further for the formulation of dry powder suspension.

Evaluation of Antioxidant Activity of Plant Extracts Using DPPH [7]:

The DPPH (2, 2 – di (4 – tert - octylphenyl) – 1 - picrylhydrazyl) Test: Using the DPPH assay, the radical scavenging capacity of various extracts was assessed. At 517 nm, the reduction in DPPH solution absorption following the addition of an antioxidant was observed. The reference used was ascorbic acid (10 mg/ml DMSO).

Principle: 1, 1 Diphenyl 2- Picryl Hydrazyl (DPPH) is a free radical, stable (in powder form), and has a red colour that changes to yellow when scavenged. This characteristic is used in the DPPH assay to demonstrate free radical scavenging activity. The scavenging reaction between an antioxidant (HA) and (DPPH) can be expressed as follows:

(DPPH) + (H-A) DPPH-H + (A)

Antioxidants convert DPPH to DPPH-H by a reaction, which lowers absorbance. The degree of discolouration reveals the antioxidant compounds' or extracts' scavenging capacity in terms of hydrogen-donating capacity.

Measurement of the Antioxidant Activity: The antioxidant activity of the ethanolic extracts was determined based on their scavenging activity of the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and is usually utilized for detecting the radical scavenging activity in chemical analysis. 1 ml of each solution of different concentrations (20, 40, 60, 80 & 100

µg/ml) of the extracts was added to 2 ml of 0.5mM ethanolic DPPH free radical solution. The reaction mixture was incubated in dark condition at room temperature for 30 min.

After 30 minutes the absorbance of the preparations was read at 517 nm by UV spectrophotometer which was compared with the corresponding absorbance of standard ascorbic acid concentrations (20, 40, 60, 80 & 100 µg/ml) and 3ml of DPPH was taken as control(blank).

The method described was used to measure the absorbance with some modifications. Then the % inhibition was calculated by the following equation:

% Radical scavenging activity = (Absorbance of blank-absorbance of sample) / (Absorbance of blank) × 100

Formulation Development:

Dose Selection: The dose of *Moringa oleifera* leaves and *Celastrus paniculatus* seeds extracts for the formulation was selected based on the antioxidant activity of individual extracts and the activity in combinations with varying extract ratios both extracts. Individually *Moringa oleifera* leaves extract shows antioxidant activity more than *Celastrus paniculatus* seeds extract. Using a selected extract dose, various batches of dry suspension were prepared and evaluated. A dry suspension was prepared using the dried ethanol extracts of both herbs.

Optimization of Batches: Ingredients used for the formulation of dry powder suspension are given in **Table 1.**

TABLE 1: PRELIMINARY FORMULATION DEVELOPMENT

Ingredients	Batches (in grams)									
	S1	S2	S3	T4	T5	T6	P7	P8	P9	P10
M. oleifera extract	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
C. paniculatus extract	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Crospovidone	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
CMC sodium	0.008	0.012	0.016	0.02	0.024	-	-	-	-	-
Sod. Starch glycolate	-	-	-	-	-	0.02	0.04	0.16	0.08	0.12
Polysorbate 80	0.08	0.16	0.24	0.32	0.4	0.48	0.56	0.64	0.72	0.8
Sucrose	1.2	1.2	1.2	1.4	1.4	1.4	1.6	1.6	1.6	1.6
Potassium citrate	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Sorbic acid	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
Aerosil	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032
Ascorbic acid	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
Lactose	0.856	0.856	0.856	0.856	0.856	0.856	0.856	0.856	0.856	0.856

Manufacturing Procedure [8, 9, 10]:

1. All the ingredients were sifted through sieve #60.
2. Sucrose was milled and sifted through #80.
3. Dried extract of drugs equivalent to 400mg and 100mg was triturated with sucrose using a mortar and pestle.
4. Above blend was geometrically mixed with all ingredients in Step 1.
5. Geometrically mixed polysorbate 80 & sodium lauryl sulfate to the powder blend.
6. The powder was blended thoroughly.
7. In the Final step, the powder blend was sifted and passed through sieve #100.
8. Weighed and dispensed in amber-colored bottles.

Evaluation of Batches [9, 11]:

Organoleptic Evaluation: Visual and olfactory tests were performed on the formulation.

Flow Property: The flow property of powder determines its flow from the hopper while manufacturing. This is measured regarding angle of repose, bulk density, tapped density, compressibility index, hausner ratio.

Rheological Behaviour: The rheological characteristics of the reconstituted suspension are ascertained using the Brookfield viscometer.

Deposit Behaviour:

Redispersibiltiy: Within a week of seven days of storage, the Redispersibility of preparation is determined by measuring the number of strokes necessary to redisperse the created sediment. (NMT 100 strokes = Redispersibility).

Sedimentation Volume Ratio (SVR): The sedimentation volume of suspension is simply the ratio of the balance capacity of the sediment, Vu, to the overall volume, Vo, of the suspension, i.e., F= Vu/Vo. For any pharmacological solution, F is usually between 0 and 1. The F value represents qualitative data on the physical stability of a suspension.

pH Measurement: Using a pH meter, the pH of the suspension was determined.

***In-vitro* Drug Release:** The *in-vitro* dissolution studies of dry powder suspension is performed using USP type II dissolution apparatus (paddle type). The dissolution medium consisted of 900ml of 0.07M phosphate buffer (pH=7.0) maintained at 37 ± 0.2°C. The paddle was rotated at a rate of 50 rpm. Aliquot of samples (5ml) were withdrawn at specific time intervals of 5 min.

Drug Content: The drug content of dry powder suspension formulation was determined by taking 1 gm of dry powder in 100 ml volumetric flask. Quantity sufficient (for extraction of drug from formulation) of methanol was added in volumetric flask and the flask was kept in a warm water bath for extraction of drug with intermittent shaking. After cooling to room temperature, volume was made up to 100 ml with methanol.

The methanolic solution of drug was filtered through 0.45 µ syringe-driven filter and 1 ml of filtrate was diluted to 10 ml with methanol and then subjected to HPTLC analysis.

The drug content was calculated from the linear regression equation obtained from the calibration curve.

RESULTS AND DISCUSSIONS:

Antioxidant Activity: The antioxidant activity results of *M. oleifera* and *C. paniculatus* extracts and antioxidant activity in the combination of both extracts are mentioned in **Tables 2** and **3.**

TABLE 2: DPPH SCAVENGING ACTIVITY OF PLANT EXTRACTS AND ASCORBIC ACID

Concentrations (µg/ml)	% Radical scavenging activity (%RSA)		
	Moringa oleifera	***Celastrus paniculatus***	**Ascorbic acid**
20	37.75	29.59	39.79
40	47.95	44.89	43.87
50	57.94	46.43	53.06
60	58.16	47.95	52.04
80	63.26	56.12	57.14
100	66.60	60.20	63.26
150	77.55	65.30*	74.48
200	92.34*	65.26	87.75
250	92.19	65.27	91.83

TABLE 3: DPPH SCAVENGING ACTIVITY OF COMBINATION OF *MORINGA OLEIFERA* LEAVES EXTRACT AND *CELASTRUS PANICULATUS* SEEDS EXTRACT

Concentrations MO: CP 200:150(µg/ml)	%RSA
9:1	87.75
8:2**	91.43
7:3	89.79
6:4	73.46
5:5	60.20
4:1	53.06
3:7	41.83
2:8	61.22
1:9	30.61

According to the antioxidant study, *M. oleifera* extract has shown best radical scavenging activity at a concentration 200 (µg/ml) and *C. paniculatus* extracthas shown best radical scavenging activity at a concentration 50 (µg/ml) **Table 2**. By testing the radical scavenging activity of combination of both the extracts in varying ratios. It has been found that the ratio 8:2 have shown best radical scavenging activity **Table 3**.

Hence, the dose for the formulation was been selected accordingly.

Evaluation of Batches: All 10 batches are evaluated for many parameters among them, an optimized batch P8 is obtained based on evaluation results.

TABLE 4: EVALUATION RESULTS OF BATCHES

Parameters	S1	S2	S3	T4	T5	T6	P7	P8	P9	P10
Bulk density	0.521	0.528	0.520	0.544	0.551	0.575	0.361	0.277	0.422	0.433
Tapped density	0.625	0.786	0.742	0.664	0.681	0.692	0.431	0.317	0.495	0.534
Angle of repose	34º30'	36º18'	40º2'	41º18'	27º6'	31º6'	27º61'	23º29'	28º3'	28º18'
Compressibility index	16.6	32.8	29.9	18.07	19.08	16.9	16.24	9.75	14.7	18.9
Hausner ratio	1.19	1.48	1.42	1.22	1.23	1.20	1.19	1.10	1.17	1.23
pH	6.54	6.58	6.61	6.67	6.70	6.56	6.66	6.76	6.76	6.73
Viscosity (cps)	248	322	378	298	351	342	367	356	361	365
Redispersibility	12	8	9	13	14	12	7	9	11	11
Sedimentation	0.83	0.67	0.69	0.82	0.80	0.82	0.83	0.85	0.87	0.82
Drug content	89.76	92.25	90.31	89.47	91.84	94.78	93.69	95.34	89.42	94.21

Batch P8 has shown good flow properties, and redispersibility among all other remaining 9 batches and all other parameters are within acceptable limits.

***In-vitro* Drug Release Study:** The drug release shown that more than 80% of drug was released after 40 min **Fig. 3.**

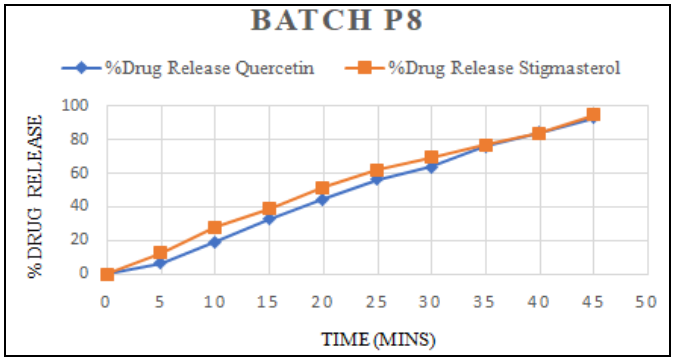

FIG. 3: THE OPTIMIZED BATCH P8 DISSOLUTION PROFILE CHART (% DRUG RELEASE *VS* TIME)

CONCLUSION: In the present work, an attempt has been made to formulate and evaluate the combination of herbal drug extracts and incorporation as a nutraceutical dry powder suspension formulation. Dry powder containing crude extracts of *Moringa oleifera* leaves and *Celastrus paniculatus* seeds extracts with different concentrations was prepared with having good antioxidant activity against DPPH (2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl).It can be summarized that the prepared formulations showed satisfactory rheological characteristics, release behaviour, appearance, pH, and antioxidant activity.

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