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Camellia sinensis and *Euphorbia prostrata* Nutraceutical Powder Formulation and Evaluation for Osteoarthritis Management

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Abstract

Osteoarthritis is becoming a common disorder among all age groups. Meanwhile bioactive nonfood matrices are realized to display desired therapeutic actions. Nutraceutical blends of *Camellia sinensis* and *Euphorbia prostrata* aqueous extract were prepared and evaluated as fast disintegrating granules. Formula optimization was achieved by a 2^3 Taguchi Orthogonal Arrays on Design Expert© Version 8.0 (freeware). Powder mix was then prepared by mixers then granules' particle size, friability and densities for flowability evaluated. Anti-arthritic activity was tested biochemically by Bovine Serum denaturation and Egg Albumin Denaturation methods. Ethanol Soxhlet extractions yielded 22.8% and 17.8% percentage yields for *C. sinensis* and *E. prostrata*, respectively. The optimized blend ratio was 3:1 for *C. sinensis : E. prostrata* at a concentration of 2.5 $\mu g.mL^{-1}$. This gave protein denaturation of only 6.0% implying a 94% protection compared to the control at 89.95%. An inhibition of 65% was recorded in egg albumin protein method. Angle of repose of 27° implied good flowability. Additionally, bulk and tapped densities were significantly low, indicating suitable powder particle packing. The *C. sinensis* - *E. prostrata* nutraceutical powder stands as a novel herbal-based solid dosage formulation with optimum rheological characteristics for rapid disintegration and osteoarthritis management adequacies.

Keywords: *Nutraceuticals; Osteoarthritis; Powder; Flowability; Biochemical*

Introduction

Osteoarthritis is a progressive degenerative joint disease that has a major impact on joint function and quality of life. Pathological changes like excessive production of pro-inflammatory molecules, such as interleukin 1β (IL-1β) and tumor necrosis factor α (TNFα), usually shift the balance between the synthesis and degradation of matrix components. This has been shown to result in progressive destruction of the joint tissue [1]. This erosion of articular cartilage, inflammation of synovial membrane, and resorption of the underlying subchondral bone is the manifestation of osteoarthritis [2].

Disability due to musculoskeletal disorders is reported to have increased worldwide by 45% between 1990 and 2010, largely due to osteoarthritis which is listed as the fastest growing major health condition and ranked second as cause of disability [3]. Up to 10% of the population 60 years or older worldwide have symptomatic problems attributable to osteoarthritis. Knee, hip, hand and spine are typically the affected joints and are associated with profound clinical and public health burden [4]. In sub-Saharan Africa, particularly in Kenya, studies have shown that osteoarthritis is the most common rheumatic disorder [5].

Pursuit for osteoarthritis cure continues to be intensified but currently there is high reliance on non-pharmacological management. This entails interventions like physical therapy, aerobic exercises, muscle strengthening, weight reduction, walking aids, knee braces,

footwear and insoles, electromagnets, thermal modalities and acupuncture [6]. Meanwhile, the pharmacological management of osteoarthritis targets symptoms of the disease rather than the underlying cause. Analgesics and nonsteroidal anti-inflammatory drugs (NSAID's) are applied as the main treatment regimens. These drugs generally improve function by just decreasing pain and stiffness of the joints [7].

The pre-formulation stage is a proactive phase during the drug to medicine conversion that deals with transformation of new chemical entity into a safe, effective and, most importantly, stable pharmaceutical formulation [6]. It focuses on characterization of the excipients to enable prediction of properties and therapeutic performance. An array of key aspects are investigated such as partition coefficient; dissociation constant; chirality; solubility; stability; crystallinity; polymorphism; deliquescence; particle size; density; flow properties and incompatibilities [8].

Nutraceuticals in form of food or part of the food continue to contribute to therapeutic actions by providing medical and health benefits, including the prevention and treatment of diseases. The bioactive chemical compounds occur in form of a nonfood matrix. Although with no regulatory definition, the US Food and Drug Administration (FDA), considered 'dietary supplements' as a perfect paradigm in pathology control and treatment of osteoarthritis as a chronic disease [2]. Nutraceuticals and dietary supplements based on phytochemicals have long been used in traditional medicine and there is considerable evidence that they may play an important remedy role in inflammation and joint destruction. Nutraceuticals provide safer alternatives as their use is devoid of adverse effects. There is a growing public interest in the nutraceuticals benefits with an array of plants including *Punica granatum, Camellia sinensis, Uncaria tomentosa, Harpagophytum procumbens, Zingiber officinale, Boswellia serrata, Curcuma longa* and *Ananas comosus* reported variously to be efficacious [9-11].

Camellia sinensis is the tea widely consumed as a beverage throughout the world. It exhibits health-promoting effects that has been attributed to its vast phytochemical profile [12,13]. Reports indicated a content of proteins (15%), amino acids (4%), fiber (26%), other carbohydrates (7%), lipids (7%), pigments (2%), minerals (5%), and phenolic compounds (catechins; 30%) [28]. The catechins found in green tea have been shown to possess anti-inflammatory as well as neuroprotective effects [14]. The catechin (-)epigallocatechin, a flavan-3,3',4',5,5',7-hexol, reportedly lowered the effect of total immunoglobulins (IgG) and type II collagen-specific IgG levels in serum and arthritic joints of green tea-fed mice [15].

Euphorbia prostrata plant belongs to the Euphorbiaceae family and is traditionally used worldwide as medicine for management of diseases like diabetes mellitus, dysentery, asthma and early grades of hemorrhoids [16]. Due to its bioactive compounds, *E. prostrata* has recently been reported to exhibit significant analgesic activity [17]. The Euphorbiaceae family members have been severally reported to exhibit significant anti-inflammatory, analgesic activities [17,18]. In addition to (-)epigallocatechin, *E. prostrata* was found to contain the Hesperetin 7-rutinoside, a flavanone glycoside exhibiting analgesic activity [17]. This makes it a potential therapy for arthritis.

The concept of formulating fast disintegrating solid dosages offers a suitable and practical approach in serving desired objectives of faster dissolution characteristics with potentially increased bioavailability. Different types of mucilage are currently widely used as adjuvant in the manufacturing of different pharmaceutical dosage forms. Mucilages swell in aqueous environments to a greater extend making them suitable for orally administered therapeutics. Several plants have been explored for mucilages including *Trigonella foenum-graceum, Salicornia fruticose, Hibiscus rosa sinensis,* all which have been found to exhibit disintegrant properties [19,20]. *Plantago ovate* mucilage displayed even super-disintegrant performance [21] which makes it an ideal candidate for application in oral solid dosage forms.

To meet the needs of advanced drug delivery systems, this paper presents novel strategies for continuous improved modified excipients application. The work entails *C. sinensis* - *E. prostrata* nutraceutical powder as an innovative herbal-based solid dosage formulation exploiting disintegrant properties of *Plantago ovata* mucilage for the first time. The nutraceutical powder rheological characteristics for rapid disintegration and biochemical evaluations for osteoarthritis management adequacies is also reported.

Materials and Methods

Reagents and instruments

Hexane, dichloromethane, acetone and methanol were all GPR grade and hence distilled before use for extraction. All the other chemicals utilized for biochemical assays were of Analytical grade hence used as obtained without further processing. *Plantago ovata* mucilage powder was obtained from Mehta Pharmaceuticals, Mumbai. The chemicals were all sourced from Sigma Aldrich Company, supplied by Kobian Laboratory supplies, Nairobi Kenya. The instruments used were a Shimadzu UV/VIS Spectrophotometer (Shimadzu, Japan), and Micro-centrifuge (REMI, RM-12C).

Plant collection and identification

Camellia sinensis (green tea) plant samples were collected from the Nandi Hills tea estates (0°3'62" N and 32°15'0" E). *Euphorbia prostrata* samples were got by the road sides at the foot of Mt. Kenya in Murang'a area (34° 7'26"E and 0°57'17"S). Sampling was done during the month of March (short rains season) plucking only the first two leaves from each stem. The plant materials were cleaned with water to remove dirt before carrying in polythene bags to the laboratory. The plant samples were identified, voucher specimen deposited at the United States International University - Africa herbarium and accession numbers allotted.

Sample processing

The fresh cut plant leaves were spread onto cardboards for air drying in the laboratory separately for each plant. The dry plant material was then ground into fine powder using a hammer mill to increase the surface area to volume ratios of the sample and enhance the contact between the solvent and the sample during the extraction process. The obtained plant leaves powder materials was then stored in air tight containers.

Solvent extraction

The soxhlet method of extraction was carried out serially using hexane, acetone, dichloromethane and methanol solvents with filtering and re-soaking the plant residue and changing the solvent in an increasing polarity sequence. Some 50 grams of the sample powder was extracted with 250 ml of each of the solvents for 12 hours till the thimble cleared. After filtration, the excess solvent was removed through rota evaporation under vacuum. The crude extracts were then left in an open container to completely eliminate the solvents. Percentage yields were then determined after 48 hours by the formula (i) below:

$$\text{Percentage yield} = \frac{\text{Extract weight}}{\text{Plant powder}} \text{X } 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(i)}$$

Taguchi orthogonal array (TOA)

An L8 (2^3) TOA was applied in the current study to establish the optimal ratios for blending *C. sinensis* with *E. prostrate.* The concentration of the blend in the Nutraceutical formulae was also optimized as a factor to produce a formulation that efficiently inhibited protein denaturation. In this regard therefore the response factor for the TOA was set as the percentage protein denaturation. In the design, factors were identified as amount of *C. sinensis,* amount of *E. prostrata* and concentration of blend being evaluated at two levels. The array yielded 8 experiments, each being performed in triplicate, corresponding to a total of 24 tests. The mean value for percent protein denaturation was recorded as the response of the respective experiment.

Preparation and characterization of the nutraceutical blends

The Nutraceutical was formulated as rapid disintegrating granules. This was achieved by the incorporation of super disintegrant *Plantago ovata* mucilage powder as an ingredient. The ingredients were weighed as per formula and building up the amount of material in a laboratory scale tumbling mixer sequentially. After the full duration of mixing, the powder perceived a random mix was evaluated in terms of the following powder properties.

Granules particle size

The granules particle size distribution was evaluated by sieve analysis using a Zeus circular sieve shaker (Filtra-Vibracion, Barcelona Spain) set at amplitude 7 for a period of 10 minutes. Blend powder was subjected to vibration over an array of sieves with mesh aperture of 1000 µm to 600 µm to 300 µm in a series. On completion, powder at each sieve was weighed and percentage of the initial total weight calculated.

Angle of repose

Newman's funnel method was applied in the determination of the angle of repose. Accurately weighed 10g of the powder was poured through a glass funnel from a height of 6 centimeter onto a level bench top. The angle that the side of the conical heap made with the horizontal plane was then recorded as the angle of repose.

Angle of repose was then calculated by applying the relationship in equation (ii):

$$Tan\ \theta = \frac{h}{r} \quad \text{..... (ii)}$$

Where θ^0 = Angle of repose while h and r are the height and radius of the powder cone, respectively.

The obtained value for θ^0 was then evaluated in comparison with previous reports as:

Angle of repose (θ^0)	Type of flow
< 25	Excellent
25 - 30	Good
30 - 40	Fair/passable
> 40	Very poor

Table A: *Adopted from Sravani and Sailaja (2016) [22].*

Bulk density/apparent bulk density (pb)

The random mix blend powder was placed into a graduated cylinder then the volume (Vb) and weight (M) determined and used in the equation (iii) for bulk density calculation:

$$Apparent\ bulk\ density = \frac{M}{Vb} \quad \text{..... (iii)}$$

Tapped density

A measuring cylinder containing a known mass of blend was tapped for 15 minutes. The minimum volume (Vt) occupied in the cylinder and the weight (M) of the blend was then applied in establishing the tapped density (pt) using following formula in equation (iv):

$$Tapped\ density = \frac{M}{Vt} \quad \text{..... (iv)}$$

Powder flowability

Inter-particulate interactions during flow was evaluated by investigating the propensity of nutraceuticals blend powder to be compressed. This was established by determination of the Carr Compressibility Index and Hausner Ratio as two measures for predicting flowability. The two indices are calculated similar to Elmarzugi and group (2013) [23] as indicated in equation (v) and (vi) respectively:

$$Carr'sIndex = \frac{(Tapped\ density - Bulk\ density)}{Tapped\ density} X\ 100 \text{ ... (v)}$$

$$Hausner's\ Index = \frac{Tapped\ density}{Bulk\ density} \text{ ... ... (vi)}$$

Determination of total phenolic content

Methanol extracts of *C. sinensis,* and *E. prostrata* were each separately evaluated and then nutriceutical blend powder evaluated for total phenolic content utilizing the Folin-Ciocalteu method [24]. A working solution of Folin-Ciocalteu reagent was first prepared by diluting it with distilled water (1:10) Accurate volume of 0.5 mL of was mixed with a 0.1 mL of each methanol extract and the nutriceuticals blend solution. After 5 minutes to each of the solutions, 1.7 mL of 20% aqueous sodium carbonate solution was added and vortexed. To each of the obtained mixture, 10 mL of distilled water were added then incubated for 20 min of with agitation at room temperature before determination of absorbance at λ = 735 nm. Results were expressed in mg of gallic acid equivalents (GAE) per 100g of fresh sample.

Biochemical evaluations

The biochemical evaluations involved assaying for *in vitro* anti-arthritic activity by Bovine Serum denaturation method and confirmation using Egg Albumin Denaturation method.

Bovine Serum Denaturation assays were performed similar to Rahman and group (2012) [24] with little modifications. Some 500 mg of Bovine Serum Albumin (BSA) was dissolved in 100 mL distilled water to prepare a 0.5% solution. A 6.5 pH Phosphate Buffer Saline (PBS) was then prepared by dissolving 8g of sodium chloride, 0.2g of potassium chloride, 1.4g of dihydrogen phosphate, and 0.2g potassium dihydrogen phosphate in 800 mL distilled water. This was made to 1000mL mark before adjusting the pH to 6.5 by drops of 1N hydrochloric acid.

A 0.5 mL test solution was then prepared to comprise of 0.45 mL BSA spiked with 0.05 mL of the nutraceutical blends at concentrations of 50 µg mL^{-1}, 100 µg mL^{-1} and 200 µg mL^{-1}. The positive control comprised of 0.45 mL BSA with 0.05 mL Diclofenac sodium similarly at concentrations of 50 µg mL^{-1}, 100 µg mL^{-1} and 200 µg mL^{-1}. While the negative control was 0.45 mL BSA with 0.05 mL distilled water.

The samples of the test solution, positive and negative controls were incubated at 37°C for 15 minutes before rapidly increasing the temperature to 55°C and holding for 3 minutes. They were then cooled and 2.5 mL of PBS added to each sample before reading the absorbance at 255 nm on a Shimadzu UV/Vis spectrophotometer.

The negative control was regarded as 100% protein denaturation from which the percentage protein denaturation was calculated using equation (v):

$$\%\ inhibition = 100 - \left[\frac{(optical\ density\ of\ test\ sample - optical\ density\ of\ control)}{optical\ density\ of\ test\ sample}\ X\ 100\right] \text{..... (v)}$$

The Egg Albumin Denaturation method was performed as a confirmatory test. In the setup, a 5 mL reaction mixture consisted 0.2 mL egg albumin, 2.8 mL PBS and 2 mL of test samples at concentration of 50 µg mL^{-1}, 100 µg mL^{-1} and 200 µg mL^{-1}. Similarly distilled water was utilized as the control while the Diclofenac sodium at the respective concentrations was used as the positive control.

The mixtures were incubated at 37°C for 15 minutes then heated at 70°C for 5 minutes. These were then cooled and absorbance determined as before but now at 660 nm. The equation (vi) was then used to determine the percentage inhibitions:

$$\%\ inhibition = \left[\frac{absorbance\ of\ test\ sample}{(absorbance\ of\ control - 1)}\right]\ X\ 100 \text{ ..... (vi)}$$

The experiments were all conducted in triplicates, the mean values determined and tabulated accordingly.

Statistical analysis of contribution of factors in the experimental design in percentage

All the factors subjected to optimization using Taguchi orthogonal design were statistically evaluated for percent contribution to nutraceutical blend powder formulae. Statistical analysis of data was performed by Analysis of Variance (ANOVA) on Statistical Package for Social Sciences (SPSS). The various factors contribution was also calculated by the formulae in equation (vii):

$$Percentage\ Contribution\ (PC) = \frac{Sum\ of\ Square\ (SS_F)}{Sum\ of\ Squares\ Total\ (SS_{Total})}\ \text{X}\ 100 \text{..... (vii)}$$

Results and Discussion

Extraction yields

The percentage yields in the extraction processes were calculated and tabulated in table 1. Highest extraction percentage yields were realized with the methanol solvents for both plants. These were 22.8% and 17.8% for *C. sinensis* and *E. prostrata,* respectively. The bulkiness of leafy part for the *C. sinensis* justifies this higher percentage as compared to *E. prostrata* whose morphology is characteristically small and not bulky. Comparison of the solvents gave a uniform trend with hexane yielding higher amounts of the extract than dichloromethane for both plants.

Plant	Extraction solvent	Extract weight (g)	Percentage yield
C. sinensis	Hexane	5.2 ± 1.6	10.4%
	Dichloromethane	4.1 ± 1.2	8.2%
	Acetone	6.3 ± 2.3	12.6%
	Methanol	11.4 ± 2.2	22.8%
E. prostrata	Hexane	3.6 ± 3.1	7.2%
	Dichloromethane	2.8 ± 2.1	5.6%
	Acetone	4.5 ± 3.2	9.0%
	Methanol	8.9 ± 2.6	17.8%

Table 1: *Percentage yield of plant extracts.*

These mainly being non-polar components that easily dissolved in hexane as the least polar solvent used. Dichloromethane gave minimal extract yields due to intermediate polarity with the percentage yields rising as the extraction solvent polarity increased to acetone and ultimately to methanol, the most polar solvent. The plant materials can therefore be regarded to be endowed with highly polar constituents, comparable with previous findings [25]. Consecutive experiments were therefore performed utilizing the methanol extracts for both *C. sinensis* and *E. prostrata.*

Optimization of blend by a 2^3 Taguchi orthogonal array

The Experimental Design Expert© array applied for optimizing the ratio of blend constituents and its concentration in the formulae gave the following 8 runs with respective responses as shown in table 2.

Blend	Run	Factor 1: *C. sinensis*	Factor 2: *E. prostrata*	Factor 3: Concentration ($\mu g.mL^{-1}$)	Response 1: % denaturation
1	1	7.5	2.5	2.5	6.0
2	2	2.5	7.5	5.0	13.6
6	3	7.5	2.5	5.0	6.9
4	4	7.5	7.5	2.5	21.6
5	5	2.5	7.5	2.5	42.3
3	6	2.5	2.5	5.0	32.5
8	7	2.5	2.5	2.5	28.6
7	8	7.5	7.5	5.0	7.0

Table 2: *Experimental design Expert© array.*

The *C. sinensis : E. prostrate* (3:1) blend illustrated significant activity in terms of inhibiting protein denaturation as seen from the low percentages in denaturation of protein. The response in the orthogonal array indicated that doubling the concentration of blend in the formulae from 2.5 $\mu g.mL^{-1}$ to 5.0 $\mu g.mL^{-1}$ decreases the denaturation inhibition capability.

The optimum blend was thus 3:1 *C. sinensis : E. prostrate* respectively at a concentration of 2.5 $\mu g.mL^{-1}$. This gave a percent protein denaturation of as low as 6.0% implying a protection to the level of 94%. Interchange of the ratios in the blend at the same concentration was noted to result in severe protein denaturation.

Nutraceutical blend powder characterization

Particle size distribution

Particle size analysis revealed that the granules fall in the fraction between 1000 µm and 600 µm. Weighed amounts indicated only 12% of the granules were in the fraction of 600 µm to 300 µm (Table 3).

Sieve mesh size	Percent of particles	Cumulative percentage of particles
1000 µm	7%	7%
600 µm	81%	88%
300 µm	12%	100%

Table 3: *Percentages of powder particles collected at sieve mesh sizes.*

The data in table 3 indicate that the highest percent realized was 81% sieve level collection. This revealed that the average particle size of the nutraceuticals blend powder was approximately 600 µm. This is a sufficiently large surface area desirable for rapid dissolution.

Powder bed properties

The perceived random mix of the blend powder was characterized in terms of angle of repose, bulk density and tapped density to assure rapid disintegration. The values for these were recorded in table 4 as follows.

Characteristic	Mean value
Angle of repose	$27^{o} \pm 2$
Bulk density	0.58 ± 0.4
Tapped density	0.65 ± 0.9
Hausner ratio	1.12 ± 0.1
Carr's Index %	10.7%

Table 4: *Nutraceutical powder properties.*

Powder flowability

The Hausner Ratio for guaifenesin was found to be 1.12 ± 0.1, which indicated that the nutraceuticals powder exhibits a good flow. This was further confirmed from the compressibility as depicted from the calculated Carr's Index for the nutraceutical blend powder found to be approximately 10.7%. Therefore, the powder would be described as exhibiting suitable flowability.

The mean values indicated powder of desirable properties. The angle of repose of 27^{o} was noted to fall within a range found to exhibit very good flowability [22]. In addition, the powder density values for both the bulk and tapped were found to be as low as 0.58 and 0.65 respectively implying desirable powder particles packing. The Nutraceutical blend powder could be regarded to have optimum rheological characteristics required to potentiate rapid disintegration. These findings are reasonable and agreeable with the established course particle size distribution of the nutraceutical blend, since course powders generally exhibit good degrees of flowability [29].

Total phenolic content

The total phenol content of the extracts and nutriceuticals blend were found to be as expressed in table 5.

Sample/extract	Total phenolic content as *GAE (mg per 100g)
C. sinensis	80 ± 4.3
E. prostrata	51 ± 2.9
Nutraceutical blend powder	99 ± 58

Table 5: **GAE = Gallic Acid Equivalents.*

The total polyphenolic content realized varied considerable with *C. sinensis* being higher than *E. prostrata*. The registered amounts were 80 mg per 100g of GAE and 51 mg/100g of GAE for *C. sinensis* and *E. prostrata* respectively. The results is consistent with finding of previous studies that reports polyphenols being a major constituent of phytochemicals in *C. sinensis* alongside caffeine [26]. Blending yielded a nutraceutical blend with phenolic content incremental to values of up to 99 mg/100g GAE. The high phenolic content thus presents the great potency of the blend for desired activity as it has been reported that polyphenols are secondary metabolites of plants play important roles in human health particularly for diseases related to oxidative stress and free radical-induced damage of tissues like osteoarthritis [27].

Biochemical evaluations

The biochemical assays revealed the anti-arthritic potentials of the nutraceutical blend powder from the inhibition of denaturation of protein. Bovine serum protein denaturation was inhibited by 64.15%, 73.91% and 82.64% at 50, 100 and 200 µg.mL^{-1} respectively of the nutraceutical blend powder (Table 6). This compares well to the positive control at equal concentrations illustrating 89.95%, 93.50% and 95.72% respectively.

Concentration (µg/mL)	Absorbance			Percent denaturation			Percent Inhibition		
	Negative control	Positive control	Nutra-blend	Negative control	Positive control	Nutra-blend	Negative control	Positive control	Nutra-blend
50	0.019	0.189	0.053	100.00	10.05	35.85	0.00	89.95	64.15
100	0.018	0.277	0.069	100.00	6.50	26.09	0.00	93.50	73.91
200	0.021	0.491	0.121	100.00	4.28	17.36	0.00	95.72	82.64

Table 6: *Bovine serum protein denaturation inhibition. Negative control = distilled water; Positive control = Diclofenac sodium; Nutra-blend = Nutraceutical blend powder.*

The mechanism of protein denaturation has been reported to involve alteration in electrostatic, hydrogen, hydrophobic and disulfide bonds [28]. This contributes to various electronic transitions involving π-π bonding and anti-bonding electrons. In this study, it was adequately demonstrated from the extent of ultraviolet spectrum interaction as seen from the absorbance. Absorbance of the samples were noted to be directly proportional to the concentration of test solutions at 50, 100, and 200 μg.mL^{-1} eliciting absorbance of 0.053, 0.069 and 0.121, respectively, for the nutraceutical blend. This absorbance indicates sustained electronic transition across energy levels indicating stability to denaturation even with thermal shock. This therefore could be regarded a confirmation of the stabilization of bovine serum protein secondary structure by the nutraceutical powder.

In the confirmation of inhibition potentials of the nutraceutical by the egg albumin protein method, the curve for the test sample was seen to intersect with that of the control at a concentration of 80 µg. mL^{-1}, a protein denaturation inhibition of 65% (Figure 1). This was regarded as comparative performance by the nutraceutical blend to alleviate arthritic trauma as depicted by the potency to inhibit denaturation of egg albumin protein.

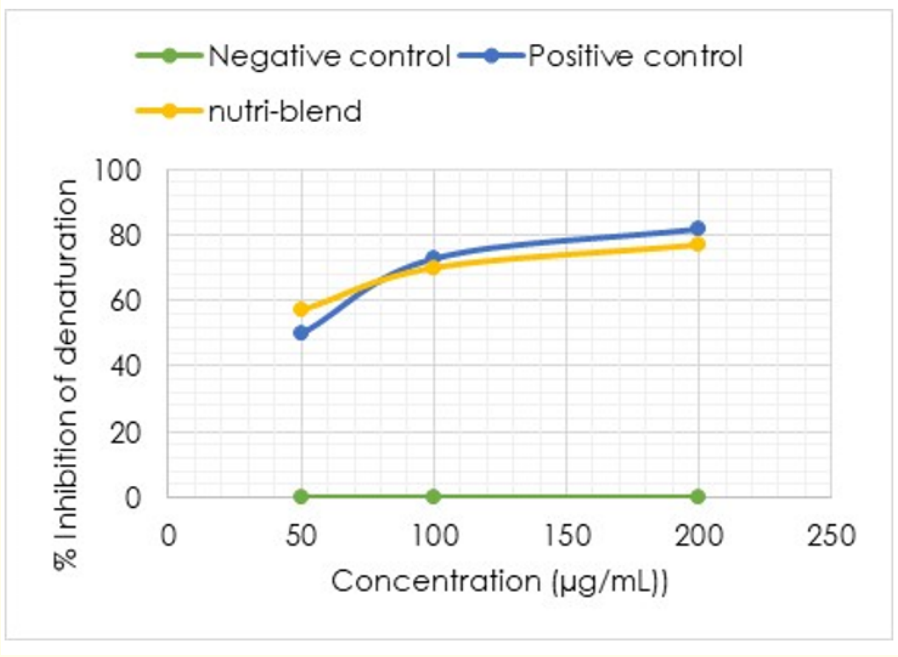

Figure 1: *Egg albumin protein denaturation inhibition (Negative control = distilled water; Positive control= Diclofenac sodium;Nutra-blend = Nutraceutical blend powder).*

The *C. sinensis* and *E. prostrata* have previously been reported as being endowed with a range of phenolic phytochemicals including flavonoids and terpenoids. These groups of compounds are well known for their ability to interfere with the release of phospholipases that trigger the formation of inflammatory mediators through involvement in peroxidase generating reactions *in-vivo*. Particularly the polyphenolics confirmed to be present in nutriceuticals blend powder is considerable evidence of (−)-epigallocatechin-3-gallate (EGCG), the predominant green tea polyphenol activity. This as inhibition of enzyme activities and signal transduction pathways that play important roles in inflammation and joint destruction in arthritis.

ANOVA analysis and factors percent contribution in the experimental design

Analysis was performed and results tabulated in table 7 accordingly for sum of squares, degree of freedom, mean squares and the F-value.

Factor	SS	DF	MS	F	PC (%)
A - *C. sinensis*	9.84	1	9.84	3.34	42.2
B - *E. prostrata*	6.23	1	6.23	7.98	26.7
C - Concentration	2.30	1	2.30	0.19	9.9
Residual (error)	4.93	4	1.23	1.86	21.2
Total	23.30	7			

Table 7: *Factors percent contribution in the experimental design.*

SS - Sum of Squares; DF - Degree of freedom; MS - Mean Square; F - F value; PC - Percent Contribution.

The ANOVA reveals that *C. sinensis* and *E. prostrata* exhibited key therapeutic roles, this is witnessed from their percent contribution determined that were much more than the contribution of the concentration in the blend formulation. This hence confirms their potency as active pharmaceutical agents as their function is not based on quantity but rather is qualitative.

Conclusion

Since production of auto antigen in arthritic disease is attributed to the denaturation of protein and membrane lysis, the obtained results illustrates potency of the nutraceutical Green tea - *E. prostrata* blends to control production of auto antigen as management of the rheumatic disease.

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