Development of Poly Herbal Granules and its Anti-Fatigue Efficacy in Swiss Mice

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A B S T R A C T

The current study sought to investigate the anti-fatigue efficacy of poly herbal granule (PHG) formulations of the extracts of Asparagus racemosus, Chlorophytum borivilianum, Tinospora cordifolia, Tribulus terrestris and Withania somnifera enriched with general tonic and health promoting properties based on Aloe vera gel and soyabean whey in Swiss mice. The nutrient compositions of the selected PHG exhibited that the carbohydrates were the major constituents in both AVII and SBWII, followed by protein and total ash. Fat contents were 0.96 and 1.18 g100g-1 in AVII and SBWII, respectively. The energy levels of AVII and SBWII were 378 and 377 kcal100g-1, correspondingly. Higher contents of Ca (70.20 mg100g-1), Mg (85.27mg100g-1) and P (61.55 mg100g-1) were observed in SBWII. The trace elements revealed that, Zn and Fe content were 1.84 and 8.63mg100g-1 in AVII and 1.85 and 7.85mg100g-1 in SBWII, respectively. The retention and stability of Vitamin C, total sugar, reducing sugar and non-reducing sugar for 270 days illustrated the storage life of the product in ambient condition. The body weight of mice, swimming time and corresponding biochemical parameters including haemoglobin, serum protein and blood glucose level were measured by standard method. The oral administration with PHG supplement for 30 days markedly increased the body weight (11.40-11.80%), haemoglobin (11.82-12.46%), serum protein (3.77-6.02%) of the mice. Following forced swimming test (FST), prolonged exhaustive exercise time of 231.02 (AVII), 237.50 (SBWII) minutes were recorded for PHG supplemented groups which were 36.23 (AVII) and 37.97 (SBWII) per cent longer than that of the control group. The minute increase of blood glucose levels (4.51-4.37%) in PHG supplemented Swiss mice after FST as compared to tremendous increase in blood glucose level (40.07%) in the control group illustrated the anti-fatigue activity of PHG.

Keywords
Polyherbal granules, Haemoglobin, Forced swimming tests, Blood glucose.

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Introduction

Fatigue is the consequence of exertion beyond one’s normal ability which is defined as an exercise induced inability to perform the expected or desired work output (Ament and
Physiological, psychological and disease theories are the three major aspects of fatigue (Ament and Verkerke, 2009) which leads to depletion of energy source and accumulation of excess metabolites (Coombes et al., 2002) including reactive free radicals resulting to tissue damage (Nybo, 2003; You and Zhao, 2011). Energetic deficiency both during dynamic and static exercise plays a major role in the aetiology of muscle fatigue. The available therapies or pharmacological drugs for the treatment of fatigue are very limited (Uthayathas et al., 2007), which necessitate potential alternatives from the extracts of traditional herbs and their respective performance of action are worth investigating (Tharakan et al., 2005). Herbs or other botanicals and their extracts or concentrates mentioned in literature as dietary supplements (Sen and Chakraborty, 2016) have the potential to mitigate fatigue, accelerate the elimination of fatigue-related metabolites and improve exercise performance (Tharakan et al., 2005, Lee et al., 2012 and Zink 2016). The herbal plants viz., Aloe vera (Aloe barbadensis Miller), Asparagus racemosus, Chlorophytum borivilianum, Tinospora cordifolia, Tribullus terrestris and Withania somnifera have been found to exhibit therapeutic properties like adaptogenic, immunostimulant, general tonic and health promoting property the present study reports the preparation of poly herbal formulation, its nutritional characteristics and to evaluate in vivo efficacy in alleviating fatigue in Swiss mice.

Materials and Methods

Extraction and preparation of poly herbal mixture

The botanical raw material consisting of five medicinal plants W. somnifera L. Dunal (roots), T. terrestris Linn. (dried spiny fruit), T. cordifolia (T.) Miers (stem), C. borivilianum (tuberos roots) and A. racemosus Wild. (roots), were procured from the authenticated local market of Parbhan, Maharashtra, India. Each raw material was washed separately, dried, powdered (75μm sieve) and was packed in airtight container for further analysis.

The individual plant extracts were taken in equal parts (w/w), mixed together and passed through 75μm sieve to obtain a homogenized poly herbal mixture (PHM). The base material for development of poly herbal supplement was done using A. vera gel and soybean whey.
Preparation of base materials

The leaves were collected, washed and the latex was drained out to obtain the aloe fillet and kept in the freezer (4°C). The freeze-dried aloe fillets were crushed in a grinder and filtered to obtain the A. vera gel extract.

Fresh soybean seeds were cleaned, washed and soaked in water (3:1 ratio) for 6 hours. The soaked beans were dehulled and ground with hot water (1:8 ratio) in a grinder to obtain the milky pulp. The pulp was boiled for 20 minutes and strained through sterilized muslin cloth to extract the soymilk which was curdled by addition of citric acid (0.2%). The whey water was collected by straining the curdled soymilk. The A. vera gel and soyabean bean whey were used for formulation of supplement and its nutritional analysis.

Formulation of poly herbal granules

Six different variants with three replications of granules were prepared by dissolving water (20mL), base materials (20mL; A. vera gel/soya whey), citric acid (3.0 %) and sugar at different concentration and heated to obtain a syrup (two thread consistency) to which PHM was added and stirred continuously for 3-5 minutes over low flame. Further, the granular mixture was dried at 45°C for 1 hour and passed through 500 µm sieve to obtain uniform sized granules and stored in air-tight labeled containers. The granules were dissolved in water at 7.1 % for organoleptic evaluation (Table 1).

Organoleptic evaluation

A Five-Point Hedonic Rating Scale (Amerine et al., 1965) was used for rating the attributes, viz., colour, taste, flavour and overall acceptability and the evaluation was carried out by 10 semi trained panelists based on their sensitivity to different tastes.

Chemical properties

The individual plant extracts and the formulated poly herbal granules (PHG) were analyzed in triplicates for moisture, protein, fat, total mineral, iron (Fe), copper (Cu), zinc (Zn), calcium (Ca), magnesium (Mg) and phosphorus (P) content. Moisture, protein and fat were estimated by AOAC (1996) method while the total carbohydrates content was found out by difference method [100 - (proteins + fats + moisture + ash in percentage)] (NIN, 1983) and vitamin C by titration method (AOAC, 1996). Energy value was computed indirectly using energy value for total carbohydrate, protein and fat. The content of Fe, Cu, and Zn were analyzed by atomic absorption spectrophotometer (Perkin R Elner Model 3110). Further, analysis of Ca, Mg and P was carried out by procedure given by Gupta (2000).

Assessment of shelf life of granules

The shelf life of the developed granules on storage was studied at 0, 30, 60, 90,180 and 270 days following organoleptic as well as analytical evaluation. Under analytical evaluation the products were analyzed for total sugar, reducing sugar, non-reducing sugars and vitamin C. The total sugars were determined following the method of Dubois et al., (1956). Reducing sugars and non-reducing sugars were determined by Nelson-Somogyi method (Somogyi 1952), whereas vitamin C was determined by AOAC method (AOAC, 1996).

In vivo evaluation in Swiss mice

Twenty six male Swiss mice weighing 25±5g with specific pathogen-free conditions were purchased from M/S. Shree Farms, Tirpude Bhawan, Z.P. Square, NH-6, Bhandara (M.S.) and maintained in the Department of Veterinary Pharmacology and Toxicology,
College of Veterinary and Animal Sciences, M.A.F.S.U., Parbhani (M.S.). The Swiss mice were given one week to accustomize to the environment and were maintained on *ad libitum* with free access to clean fresh drinking water and housed at 12-h light/dark cycle at room temperature (23 ± 2 °C). Dried clean husk was used as bedding material which was changed on alternate days. The due approval was obtained for the planned protocol from the Institutional Animal Ethical Committee (IAEC) which conformed to the recommended guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animal (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. In the experiment, the animals were drawn at random into three groups as treatment (Group I, Group II and Group III) in which group I consisted of 6 mice and group II and group III consisted with 10 mice each as replication, respectively. The treatment was administered through oral gavage for Group II and Group III with developed PHG *viz*., AVII and SBWII respectively while Group I received distilled water @ 500 mgkg⁻¹ body weight per day in volumes of 0.15 mL per mouse in addition to their normal feeding once a day for 30 consecutive days.

**Assessment of anti-fatigue activity**

The mice were habituated to laboratory housing for 1 week prior to the start of the experiment. The mice were forced to perform swimming exercise in water tank (25cm x 35cm x 30 cm) containing fresh water up to a depth of 25 cm at a temperature of 30⁰C ± 1⁰C. The animal were considered fatigued when they were unable to rise to the surface for 5 seconds (Ikeuchi *et al.*, 2006) and were unable to sustain themselves, so rescued at that stage. Actual time of swimming of individual animals was recorded. The swimming exercise test was carried out following supplementation of mice for a period of 30 days.

**Biochemical analysis**

Blood volumes of 1.0 -2.0mL were drawn from the retro-orbital plexus of eye (inner orbit) of mouse on the 1st and 30th day experimental period and measured for haemoglobin and serum total protein. Blood collected before and after the swimming exercise was analyzed for blood glucose level, collected blood was transferred into vials containing EDTA and analyzed within 24 hours. Haemoglobin was measured as per the method described by Jain (1986). The other biochemical estimations were carried out by using Ambica Diagnostics Reagent Kits on Autoanalyzer Slim (SEAC).

**Statistical analysis**

Statistical analysis was performed with SPSS software (version 16, SPSS Inc., Chicago). Duncan multiple range test were used to test the differences between means (P< 0.05).

**Results and Discussion**

**Proximate composition and mineral content of the selected plant material**

The proximate composition consisted of moisture, protein, total ash, crude fibre, carbohydrates and energy varied significantly among the selected plant extracts (Table 2). Significantly highest moisture content (98.50g100g⁻¹) was recorded in the *A. vera* gel followed by soybean whey (94.70g100g⁻¹), while highest amount of protein (14.59g100g⁻¹) and total ash (14.33g100g⁻¹) contents were recorded in fruits of *T. terrestris*. Eschun and He (2004) also reported 98.4 per cent water in raw pulp of *A. vera* while discussing the use of *A. vera* in connection with pharmaceutical and cosmetic industries. Although the protein
content of the soybean whey in the present study is low (1.61%), soya whey is considered as a good source of nutrient because of the presence of several essential amino acids which are in balanced amount resulting in high protein efficiency ratio (Kovalenko et al., 2006). The roots of C. borivilianum and the stem of T. cordifolia exhibited better values for protein (8.41 and 12.91, respectively) which are in conformity as reported by Singh et al., (2003). The presence of significantly highest amount of protein (14.59) and ash (14.33) in the fruit extract of T. terrestris in the present study is similar with the findings of Dastagir et al., (2014) who reported 14.20% and 10.90% of protein and ash in the fruit extract, respectively. The mineral content of W. somnifera in the present study was 8.35 (g100g⁻¹) which is fairly nearer to the values given by Jabeen et al., (2010). The roots of C. borivilianum and T. cordifolia contained significantly highest amounts of fat (8.18g100g⁻¹) and crude fibre (18.6 g 100g⁻¹), respectively. In another study, Chauhan et al., (2014) reported 1.03% and 14.83% of crude fat and fibre, respectively in the stem of T. cordifolia. Mahima et al., (2014), however, reported the higher amount of crude fibre (56.42%) in stems of T. cordifolia. Significantly, highest carbohydrate content was recorded in the roots of W. somnifera (63.78 g 100g⁻¹) followed by T. cordifolia (58.42 g 100g⁻¹). Khanna et al., (2006) similarly reported little higher content of carbohydrates (88.7 mg/100g) in the roots of W. somnifera. The energy level in the plants extracts varied between 2.00 and 302.00 (kcal 100g⁻¹) of which the significantly highest was recorded in the root extracts of T. cordifolia (302.00 kcal100g⁻¹). The mineral and trace element composition also varied significantly among the plant extracts (Table 2). Except Cu, the estimated minerals (P, Ca and Mg) and trace elements (Fe and Zn) were significantly highest in the root extracts of T. cordifolia. The P content was 7.84 times higher in the roots of T. cordifolia (643.45mg100g⁻¹) compared to the fruits of T. terrestris (82.12mg 100g⁻¹). Calcium content of T. cordifoliais 48.78 times higher compared to the C. borivilianum in the root extract. Similarly, Mg (34.33 mg 100g⁻¹), Fe (98.82mg100g⁻¹) and Zn (22.13mg 100g⁻¹) contents were also significantly highest in T. cordifolia among all the extracts. Jabeen et al., (2010) in his study on determination of major and trace elements in ten important folk therapeutic plants from Pakistan recorded much higher values for W. somnifera. The variations between his values and the recorded values could be due to different soil conditions in parts of Indian and Pakistan or due to analytical procedures followed. Similar quantities of minerals and trace elements like Ca (102.23ppm), P (24.81ppm), Fe (26.06ppm), Cu (3.73ppm) and Zn (7.34ppm) were also reported by Mahima et al., (2014), in the stem of T. cordifolia. The Cu content was however found significantly highest in the root extract of W. somnifera (1.81 mg 100g⁻¹). Gupta (2013), elucidated the presence of Cu in the stem of W. somnifera to the tune of 0.58ppm.

Organoleptic evaluation

The results of organoleptic evaluation of the six PHG based on A. vera gel and soya bean whey as base materials exhibited significant variation in colour, flavor, taste and overall acceptability (Table 3). In A. vera based PHG the formulation AVII scored highest in colour (4.6), flavor (4.6), taste (4.5) and overall acceptability (4.6) at the end of 270 days. The second formulation based on soybean whey similarly, showed significant variations and the formulation SBWII recorded highest scores in colour (4.5), flavor (4.5), taste (4.5) and overall acceptability (4.5). Sahu et al., (2005) assessed the acceptability of beverages prepared from whey and mango and reported better acceptability up to 60 days. However,
in the present study the PHG based on A. vera gel and soybean whey exhibited better acceptability up to a period of 270 days without significant variations in colour, flavor, taste and overall acceptability. This could be due to the lower moisture content of the dry granules and use of sugar syrup in the formulations. It is convincingly established that higher moisture content in food product plays a vital role in food spoilage (Ananthanarayana and Panikar, 2005). Use of sugar syrup in the present study acts as a preservative by binding moisture and thus positively influenced the acceptability for a longer period (Srilakshmi, 2004). Based on the organoleptic evaluation, two variants viz., AVII and SBWII were finally selected for further analysis.

**Proximate composition, mineral and trace elements in the selected polyherbal granules**

Carbohydrates were the major constituents in both AVII and SBWII, followed by protein and total ash (Table 4). Fat contents were low, being higher in SBWII (1.18 g 100g⁻¹) and lower in AVII (0.96 g 100g⁻¹). The energy levels of AVII (378 kcal 100g⁻¹) and SBWII (377 kcal 100g⁻¹) were higher and similar, due to their higher contribution of carbohydrates and fats, respectively. Poly herbal formulations like Garlicare tablet, ginger capsule consisting the extracts of Hypericum perforatum, Allium sativum, Zingiber officinalis and Valeriana officinalis has been shown to contain the energy levels of 330.10 kcal 100g⁻¹ and 372.40 kcal 100g⁻¹ respectively, (Hussain et al., 2009). Gupta et al., (2012) similarly exhibited that the rice flakes mix developed with incorporation of polyherbal (Mentha asiatica, Ocimum basilicum, Moringa oligfera, Zingiber officinalis, Allium longicuspis and Nelum bonucifera) extract at 16% yielded the energy level of 330.00 kcal 100g⁻¹. On comparing to the energy level (390 kcal 100g⁻¹) of Boletus edulis Bull., one of the tastiest and most cultivated mushrooms worldwide (Jaworska and Bernas, 2009; Helenoa et al., 2015), the energy levels of PHG in the present study are close to Boletus edulis. It was also observed that the drink prepared by addition of 7.10% of the formulated granules yielded approximately 54 Kcal of energy which is higher than the values reported by other sports drinks that are developed in India (Sahu et al., 2005).

**Table.1 Formulation of poly herbal granules**

| Variants | Base materials | Basic components |
|----------|----------------|------------------|
|          | Aloe vera gel  | Soybean whey     | Poly herbal mixture | Sugar | Citric acid |
|          | mL (%) | mL (%) | mL (%) | mL (%) | mL (%) |
| AVI      | 20 35 | 62 3   | AVII   | 20 37 | 60 3     |
| AVIII    | 20 39 | 58 3   | SBWI   | 20 35 | 62 3     |
| SBWII    | 20 37 | 60 3   | SBWIII | 20 39 | 58 3     |
Table 2: Proximate composition and minerals and trace elements of the selected plant materials for poly herbal formulations

| Plant extracts          | Proximate composition (g/100g) | Mineral and trace elements (mg/100g) |
|-------------------------|--------------------------------|-------------------------------------|
|                         | Moisture | Protein | Total Ash | Fat | Crude fibre | Carbohydrates | Energy | P  | Ca | Mg | Fe | Zn | Cu |
| **Aloe vera** (gel)     | 98.50 ± a | 0.11 ± a | 0.25 ± a | 0.02 ± a | 0.06 ± a | 0.41 ± a | 2.00 ± a | 1.35 ± a | 1.15 ± a | 3.91 ± a | 3.90 ± a | 0.02 ± a | 0.02 ± a | 0.01 ± a | 0.01 ± a |
| **Asparagus racemosus** (roots) | 29.40 ± d | 5.88 ± d | 4.45 ± c | 0.39 ± b | 2.11 ± c | 36.67 ± c | 168.00 c | 0.02 ± a | 11.00 b | 0.21 ± b | 2.32 ± b | 0.20 ± b | 0.02 ± b | 0.05 ± b | 0.01 ± b |
| **Chlorophytum borivilianum** (roots) | 37.02 ± c | 8.41 ± e | 3.02 ± b | 8.18 ± f | 3.49 ± d | 42.18 ± e | 276.00f | 0.02 ± a | 18.00c | 18.89e | 2.21c | 1.98b | 0.02 ± a |
| **Tinospora cordifolia** (root) | 18.12 ± b | 12.91f | 8.76d | 1.85c | 18.6e | 58.42f | 302.00g | 643.45d | 878.00d | 34.33f | 98.82f | 22.13c | 0.02 ± a |
| **Tribulus terrestris** (fruit) | 15.36 ± a | 14.59f | 14.33c | 1.39d | 3.41d | 28.18c | 211.00d | 82.12c | 15.50c | 9.18c | 2.81c | 0.10 ± a | 1.28b |
| **Withania somnifera** (root) | 22.68c | 4.77c | 8.35d | 1.21c | 2.18c | 63.78f | 285.00f | 0.14 ± a | 0.15 ± a | 19.91f | 1.42b | 0.48 ± a | 1.81 ± a |
| **Soybean (Whey)** | 94.70f | 1.61b | 0.19a | 1.12c | 0.55b | 1.69b | 23.00b | 39.42b | 15.60d | 13.62d | 0.02 ± a | 0.27 ± a | 0.02 ± a |

Different lower case letters within each column indicate significant differences between plant extracts at 5% level of significance as par DMRT
Table 3: Organoleptic scores of poly herbal granules with Aloe vera gel and Soyabean whey as base material

| Organoleptic traits | Day of evaluation | Poly herbal granules with Aloe vera gel | Poly herbal granules with Soyabean whey |
|---------------------|-------------------|----------------------------------------|----------------------------------------|
|                     |                   | AVI         | AVII        | AVIII        | SBWI     | SBWII        | SBWIII       |
| Colour              | 0                 | 4.3<sup>a</sup> | 4.7<sup>a</sup> | 4.4<sup>a</sup> | 3.8<sup>a</sup> | 4.7<sup>b</sup> | 3.8<sup>a</sup> |
|                     | 30                | 4.0<sup>a</sup> | 4.6<sup>a</sup> | 4.3<sup>a</sup> | 3.8<sup>a</sup> | 4.6<sup>b</sup> | 3.9<sup>a</sup> |
|                     | 60                | 4.2<sup>a</sup> | 4.6<sup>b</sup> | 3.8<sup>a</sup> | 3.7<sup>a</sup> | 4.6<sup>b</sup> | 3.6<sup>a</sup> |
|                     | 90                | 4.3<sup>a</sup> | 4.6<sup>b</sup> | 3.8<sup>a</sup> | 3.9<sup>a</sup> | 4.6<sup>b</sup> | 3.8<sup>a</sup> |
|                     | 180               | 4.1<sup>a</sup> | 4.6<sup>a</sup> | 4.1<sup>a</sup> | 3.7<sup>a</sup> | 4.6<sup>b</sup> | 3.8<sup>a</sup> |
|                     | 270               | 3.7<sup>a</sup> | 4.6<sup>b</sup> | 3.9<sup>a</sup> | 3.6<sup>a</sup> | 4.5<sup>b</sup> | 3.9<sup>a</sup> |
| Flavor              | 0                 | 4.3<sup>a</sup> | 4.7<sup>a</sup> | 4.2<sup>a</sup> | 4.3<sup>a</sup> | 4.6<sup>a</sup> | 4.2<sup>a</sup> |
|                     | 30                | 4.2<sup>a</sup> | 4.6<sup>a</sup> | 4.0<sup>a</sup> | 4.2<sup>a</sup> | 4.6<sup>a</sup> | 4.0<sup>a</sup> |
|                     | 60                | 4.3<sup>a</sup> | 4.6<sup>a</sup> | 4.2<sup>a</sup> | 4.3<sup>a</sup> | 4.6<sup>a</sup> | 4.1<sup>a</sup> |
|                     | 90                | 4.3<sup>a</sup> | 4.6<sup>a</sup> | 4.3<sup>a</sup> | 4.3<sup>a</sup> | 4.5<sup>a</sup> | 4.3<sup>a</sup> |
|                     | 180               | 4.0<sup>a</sup> | 4.6<sup>a</sup> | 4.1<sup>a</sup> | 4.1<sup>a</sup> | 4.6<sup>a</sup> | 4.1<sup>a</sup> |
|                     | 270               | 4.0<sup>a</sup> | 4.6<sup>b</sup> | 3.9<sup>a</sup> | 4.1<sup>a</sup> | 4.5<sup>b</sup> | 3.7<sup>a</sup> |
| Taste               | 0                 | 4.4<sup>a</sup> | 4.8<sup>a</sup> | 4.3<sup>a</sup> | 4.4<sup>a</sup> | 4.8<sup>a</sup> | 4.3<sup>a</sup> |
|                     | 30                | 4.3<sup>a</sup> | 4.7<sup>a</sup> | 4.2<sup>a</sup> | 4.3<sup>a</sup> | 4.7<sup>a</sup> | 4.2<sup>a</sup> |
|                     | 60                | 4.3<sup>a</sup> | 4.6<sup>a</sup> | 4.2<sup>a</sup> | 4.3<sup>a</sup> | 4.7<sup>a</sup> | 4.3<sup>a</sup> |
|                     | 90                | 4.1<sup>a</sup> | 4.6<sup>b</sup> | 3.9<sup>a</sup> | 4.3<sup>a</sup> | 4.6<sup>a</sup> | 4.3<sup>a</sup> |
|                     | 180               | 4.1<sup>a</sup> | 4.6<sup>b</sup> | 3.9<sup>a</sup> | 4.2<sup>a</sup> | 4.6<sup>a</sup> | 3.9<sup>a</sup> |
|                     | 270               | 3.9<sup>a</sup> | 4.5<sup>b</sup> | 4.0<sup>a</sup> | 3.8<sup>a</sup> | 4.5<sup>b</sup> | 3.9<sup>a</sup> |
| Overall Acceptability | 0               | 4.3<sup>a</sup> | 4.8<sup>a</sup> | 4.4<sup>a</sup> | 4.3<sup>a</sup> | 4.7<sup>a</sup> | 4.4<sup>a</sup> |
|                     | 30                | 4.3<sup>a</sup> | 4.7<sup>b</sup> | 4.1<sup>a</sup> | 4.3<sup>a</sup> | 4.7<sup>a</sup> | 4.3<sup>a</sup> |
|                     | 60                | 4.0<sup>a</sup> | 4.6<sup>b</sup> | 4.0<sup>a</sup> | 4.0<sup>a</sup> | 4.6<sup>b</sup> | 4.0<sup>a</sup> |
|                     | 90                | 4.0<sup>a</sup> | 4.6<sup>a</sup> | 4.1<sup>a</sup> | 4.0<sup>a</sup> | 4.6<sup>a</sup> | 4.1<sup>a</sup> |
|                     | 180               | 3.9<sup>a</sup> | 4.6<sup>a</sup> | 4.0<sup>a</sup> | 4.1<sup>a</sup> | 4.5<sup>a</sup> | 4.0<sup>a</sup> |
|                     | 270               | 4.0<sup>a</sup> | 4.6<sup>b</sup> | 3.9<sup>a</sup> | 4.0<sup>a</sup> | 4.5<sup>b</sup> | 3.8<sup>a</sup> |

Different lower case letters within each row separately for Aloe vera gel and Soyabean whey based formulations indicate significant differences between PHF at 5% level of significance as per DMRT.
### Table 4 Proximate composition and mineral and trace elements of the selected poly herbal granules

| Proximate composition          | Formulation AVII | Formulation SBWII |
|-------------------------------|------------------|-------------------|
| Moisture (g/100g)             | 2.37 ± 0.13      | 2.80 ± 0.08       |
| Protein (g/100g)              | 4.39 ± 0.02      | 4.69 ± 0.19       |
| Fat (g/100g)                  | 0.96 ± 0.01      | 1.18 ± 0.18       |
| Total ash (g/100g)            | 3.65 ± 0.02      | 3.65 ± 0.06       |
| Crude fibre (g/100g)          | 0.76 ± 0.01      | 0.76 ± 0.07       |
| Carbohydrate (g/100g)         | 87.87 ± 0.04     | 86.92 ± 0.16      |
| Energy (Kcal/100g)            | 378 ± 0.03       | 377 ± 0.18        |

**Mineral and trace elements (mg/100 g)**

|                          | Formulation AVII | Formulation SBWII |
|--------------------------|------------------|-------------------|
| Calcium                  | 68.13 ± 0.01     | 70.20 ± 0.02      |
| Phosphorus               | 53.67 ± 0.02     | 61.55 ± 0.02      |
| Iron                     | 8.63 ± 0.03      | 7.85 ± 0.02       |
| Magnesium                | 83.33 ± 0.02     | 85.27 ± 0.06      |
| Copper                   | 0.23 ± 0.01      | 0.19 ± 0.00       |
| Zinc                     | 1.84 ± 0.01      | 1.85 ± 0.02       |

### Table 5 Storage study of the developed polyherbal granules with Aloe vera gel and Soyabean whey as base material

| Day of storage | Vitamin C (mg/100g) | Total sugar (gm/100g) | Non-reducing sugar (gm/100g) | Reducing sugar (gm/100g) | Vitamin C (mg/100g) | Total sugar (gm/100g) | Non-reducing sugar (gm/100g) | Reducing sugar (gm/100g) |
|----------------|---------------------|-----------------------|-------------------------------|--------------------------|---------------------|-----------------------|-------------------------------|--------------------------|
| 0              | 6.65^a              | 27.57^a               | 20.90^a                       | 6.62^a                   | 4.44^a              | 24.83^a               | 18.47^a                       | 6.29^a                   |
| 30             | 6.66^a              | 27.52^a               | 20.90^a                       | 6.62^a                   | 4.41^a              | 24.83^a               | 18.47^a                       | 6.29^a                   |
| 60             | 6.62^a              | 27.57^a               | 20.90^a                       | 6.62^a                   | 4.39^a              | 24.85^a               | 18.45^a                       | 6.31^a                   |
| 90             | 6.60^a              | 27.58^a               | 20.89^a                       | 6.65^a                   | 4.38^a              | 24.86^a               | 18.44^a                       | 6.32^a                   |
| 180            | 6.59^a              | 27.60^a               | 20.88^a                       | 6.65^a                   | 4.37^a              | 24.88^a               | 18.42^a                       | 6.34^a                   |
| 270            | 6.57^a              | 27.60^a               | 20.84^a                       | 6.68^a                   | 4.35^a              | 24.88^a               | 18.37^a                       | 6.35^a                   |

Different lower case letters within each column indicate significant differences between plant extracts at 5% level of significance as per DMRT

### Table 6 Effect of poly herbal granules on the body weight of Swiss mice

| Treatment | Body weight (g) | Per cent increase |
|-----------|-----------------|-------------------|
|           | Initial         | Final             | Weight gain       |                   |
| Control   | 28.40^a±2.05    | 30.20^a±2.03      | 1.27^a±0.59       | 4.23              |
| AVII      | 29.80^a±2.51    | 33.60^b±2.13      | 3.80^b±0.77       | 11.40             |
| SWBII     | 29.20^a±1.92    | 33.10^b±1.78      | 3.90^b±0.30       | 11.80             |

Different lower case letters within each column indicate significant differences between treatments at 5% level of significance as per DMRT (control, n=6; AVII and SWBII, n=10)
**Table 7** Effect of poly herbal granules on haemoglobin and serum protein level of Swiss mice

| Treatment | Haemoglobin (gm/dl) | Per cent increases in hemoglobin | Serum protein (gm/dl) | Per cent increases in serum protein |
|-----------|---------------------|---------------------------------|-----------------------|-----------------------------------|
|           | Before              | After                           | Before                | After                             |
| Control   | 11.84±0.29          | 11.78±0.18                      | -0.50<sup>a</sup>     | 6.09±0.27                        | 0.45<sup>a</sup>                  |
| AVII      | 11.56±0.66          | 13.11±0.71                      | 11.82<sup>b</sup>     | 5.83±0.33                        | 3.77<sup>b</sup>                 |
| SWBII     | 11.39±0.70          | 13.02±0.83                      | 12.46<sup>b</sup>     | 5.64±0.29                        | 6.02<sup>c</sup>                 |

Different lower case letters within each column indicate significant differences between treatments at 5% level of significance as per DMRT (control, n=6; AVII and SWBII, n=10).

**Table 8** Effect of poly herbal granules on swimming performance and blood glucose level of Swiss mice

| Treatment | Swimming time (minutes) | Average Per cent increase in swimming time | Blood glucose (mg/dl) | Percent Increase of blood glucose |
|-----------|-------------------------|------------------------------------------|-----------------------|----------------------------------|
|           |                         |                                          | Before swimming       | After swimming                   |
| Control   | 147.32±1.48            | -                                       | 82.74±1.92            | 138.26±0.04                      | 40.07                           |
| AVII      | 231.01±2.33            | 36.23                                    | 83.18±1.58            | 87.11±1.73                       | 4.51                            |
| SWBII     | 237.50±2.14            | 37.97                                    | 82.98±2.02            | 86.78±2.14                       | 4.37                            |

Different lower case letters within each column indicate significant differences between treatments at 5% level of significance as per DMRT (control, n=6; AVII and SWBII, n=10).

The results pertaining to the minerals of the PHG illustrated that comparatively higher contents of Ca (70.20 mg 100g<sup>-1</sup>), Mg (85.27 mg 100g<sup>-1</sup>) and P (61.55 mg 100g<sup>-1</sup>) were observed in SBWII than the AVII. In AVII the Ca, Mg and P contents were 68.13, 83.33 and 53.67 mg 100g<sup>-1</sup>, respectively (Table 4). The level of Calaid down by WHO is 450-1200 mg per day, which is in agreement to the one found in the present PHG. The analysis of trace elements reveals that, Zn and Fe content were 1.84 and 8.63 mg 100g<sup>-1</sup> in AVII and 1.85 and 7.85 mg 100g<sup>-1</sup> in SBWII, respectively. Fe is an element essential for healthy immune system and energy production (Ullah et al., 2012), while Zn containing metalloenzymes participates in the metabolism, growth and repair of the tissue and cell membrane stabilization and improves the immune response, especially T-cell mediated response (Bhowmik et al., 2010).

The retention of appreciable amount of Zn and Fe in both the PHG viz., AVII and SBWII have the potential in complementing the widespread deficiency of Zn and Fe in humans (Bailey et al., 2015).
Shelf life of the selected poly-herbal granules

Stability of the primary phytoconstituents of PHG has recently been recognized as essential for quality control to support their shelf life. The most important aspect in the evaluation of the stability study of a product is its storage condition. In the present study four parameters viz., vitamin C, total sugar, non-reducing sugar and reducing sugar of the PHF (AVII and SBWII) were determined during the storage which resulted in no significant losses of the phytoconstituents (Table 5). It has been well documented that ascorbic acid is an unstable compound and on long storage it causes degradation (Ancuceanu et al., 2015). However, no significant degradation of vitamin C has been observed for 270 days of storage in the study. Though non-significant, the reducing sugar slightly increased in AVII with progress of storage period, which might be attributed to the hydrolysis of non-reducing sugar. The retention of the determined phytoconstituents in the present study for longer periods (270 days) might be due to the antimicrobial and antioxidative activity of A. racemosus (Alok et al., 2013) and W. somnifera (Chatterjee et al., 2010). Moisture levels in a food material greatly affect its physical, chemical and microbial stability and have a critical effect on product’s shelf-life (Gulati et al., 2015). The extension of shelf life of the formulated PHG might be due to the reduced moisture to the tune of 2.37 and 2.80 %, respectively in AVII and SBWII, coupled with antimicrobial and antioxidant activity of the extracts. Sahu et al., (2005) however reported that due to high moisture content the storage life of whey based mango-herbal (lemongrass) beverage was comparatively low.

In vivo evaluation in Swiss mice

The anti-fatigue activity of the PHG, viz., AVII and SBWII was evaluated by FST in Swiss mice. The FST model in mice was a reliable measure of anti-fatigue treatment as established in both laboratory animals and humans (Jung et al., 2007; Jia and Wu, 2008). Changes in the body weight of mice, swimming time and corresponding biochemical parameters including haemoglobin, serum protein and blood glucose level were observed.

The body weights of mice increased during the experimental period which showed significantly higher body weights and weight gain in both the PHG supplemented mice compared to the control at the final stage (Table 6). Compared to the control, AVII and SWBII might have resulted in the increase in body weight of mice by 11.40 and 11.80 percent, respectively. However, no significant differences were observed between AVII and SWBII with respect to body weight and weight gain of mice. Similar result of increased body weight in mice was also observed due to feeding of water-soluble polysaccharides from Morinda officinalis (Zhang et al., 2009).

Oral administration of PHG in mice for 30 days caused a significant increase in haemoglobin levels compared to control, which resulted in 11.82 and 12.46 percent increase in haemoglobin due to AVII and SWBII, respectively (Table 7). Enhanced haemoglobin and haematological profile in animals has been reported on consumption of root extracts from A. racemosus (Rakhate et al., 2010) and C. borivillianum (Kenjale et al., 2007). The increased haemoglobin levels in mice might be due to the presence of A. racemosus and C. borivillianum extracts in both the PHG (AVII and SWBII). However, no significant difference of serum protein level was observed among the treatments in Swiss mice. In comparison to control (0.45 %), the serum protein levels increased by 3.77 and 6.02 percent during the test period due to the oral administration of AVII and SWBII, respectively (Table 7).

Anti-fatigue activity

The anti-fatigue activity of the Swiss mice carried out by inducing FST exhibited significant variations among the treatments (Table 8). The mean swimming time to exhaustion in the supplemented group and the
control were 231.02 (AVII), 237.50 (SBWII) and 147.32 minutes, respectively. The results also revealed that the swimming times to exhaustion of the supplemented groups were 36.23 (AVII) and 37.97 (SBWII) per cent longer than that of the control group. Reduction in the physiological fatigue and increase in the physical performance due to supplementation of Curcumin for enhanced muscular glycogen content on Swiss mice was also reported (Huang et al., 2015). In the present study the increase in swimming time with concomitant reduction in fatigue might be due to the presence of health promoting bio constituents present in PHG containing various herbs (Jung et al., 2004).

With reference to the blood glucose there was slight increase in the PHG supplemented (AVII and SBWII) groups after swimming. However, the increase in control group was tremendous (40.07%) compared to AVII (4.51%) and SBWII (4.37%), respectively. It was estimated that higher blood glucose level of the control group was due to shorter swimming time than that of the PHG supplemented group. This extreme variation in blood glucose between control and supplemented groups indicated positive and beneficial effects of developed supplement in reducing the fatigue. The blood glucose levels increased during stress (Dominiczak, 1999) is an indication of adaptogenic activity (Sen et al., 1992) due to stress induced hyperglycemia and release of cortisol (Sadock and Sadock, 2003). Increased plasma cortisol influences the mobilization of stored fat and carbohydrate reserves (Tache and Salye, 1976) that influences the increase in blood glucose level which is reversed by anti-stress agents (Sen et al., 1992). The present experiment exhibited the anti-fatigue and adaptogenic activity of PHG which might have induced minute increase of blood glucose levels as compared to tremendous increase in blood glucose level in the control group. Several other scientists also reported similar findings while working on different herbs (Dhuley, 2000 and Kenjale et al., 2007).

In conclusion the PHG (AVII and SBWII) developed from extracts of Asparagus racemosus, Chlorophytm borivilianum, Tinospora cordifolia, Tribulus terrestris and Withania somnifera using Aloe vera gel and soyabean whey as basic components exhibited adequate amount of carbohydrates, proteins, fats, crude fibre, total ash, minerals and trace elements. The oral administration of PHG displayed increase in body weight, haemoglobin and serum protein level in mice. The prolonged exhaustive exercise time and minute increase in blood glucose level following FST in Swiss mice for PHG supplemented groups compared to the control illustrated the anti-fatigue activity of the developed PHG.

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