Analytical Studies

Assay of Nutritive Values

The following parameters were assessed chemically by standard Indian Pharmacopeia's guided method:

1. Ash Value (%).
2. Carbohydrate (%).
3. Protein (%).
4. Fat (%).
5. Fibre (%).
6. Energy (Kcal/100 g).
7. Sodium (mg/100 g).
8. Potassium (mg/100 g).
9. Calcium (mg/100 g).
10. Specific Gravity (Final Product only).
11. HPLC (Final Product only).
12. HPTLC (Final Product only).

Methods

Estimation of Ash. Five gm of each sample was weighed in a silica crucible and heated in muffle furnace for about 5-6 h at 500°C. It was cooled in a desiccator and weighed. It was heated again in the furnace for half an hour, cooled and weighed. This was repeated consequently till the weight became constant.

Estimation of Moisture. Two gm of each sample was taken in a flat-bottom dish and kept overnight in an air oven at 100-110°C and weighed. The loss in weight was regarded as a measure of moisture content.

Estimation of Crude Fat. Two gm moisture free of each sample was extracted with petroleum ether (60-80°C) in a Soxhlet apparatus for about 6-8h. After boiling with petrol, the residual petrol was filtered using Whatman No. 40 filter paper and the filtrate was evaporated in a pre-weighed beaker. Increase in weight of beaker gave crude fat.

Estimation of Crude Fibre. Two gm of moisture and fat-free material of each sample was treated with 200 ml of 1.25% H_2SO_4. After filtration and washing, the residue was treated with 1.25% NaOH. It was the filtered, washed with hot water and then 1%HNO_3 and again with hot water. The washed residue was dried in an oven at 130°C to constant weight and cooled in a dessicator. The residue was scraped into a pre-weighed porcelain crucible, weighed, ashed at 550°C for two hours, cooled in a dessicator and reweighed. Crude fibre content was expressed as percentage loss in weight on ignition.

Estimation of Crude Protein. The crude protein was determined using micro Kjeldahl method. Two gm of each sample compound was decomposed by digestion with concentrated sulphuric acid in the presence of a catalyst, ammonium sulphate is produced. An excess of sodium hydroxide solution was added to the diluted reaction mixture, the liberated ammonia was distilled in steam and absorbed in a measured excess of standard sulphuric acid. Titration of the residual mineral acid with standard

sodium hydroxide gives the equivalent of ammonia obtained from the weight of the sample taken. From this the percentage of nitrogen in the compound can was calculated. On the basis of early determinations, the average nitrogen (N) content of proteins was found to be about 16 percent, which led to use of the calculation N x 6.25 (1/0.16 = 6.25) to convert nitrogen content into protein content.

Estimation of Carbohydrate. Percentage of available carbohydrate was given by:
100 - (ash% + fat% + protein% + crude fibre%).

Estimation of Nutritive Value (Energy). The three components of foods which provide energy are protein, carbohydrate and fat. One gram carbohydrate and protein yield 4 kcal energy whereas one gram fat yield 9 kcal energy. The energy content of each plant samples were determined by multiplying the values obtained for protein, fat and available carbohydrate by 4.00, 9.00 and 4.00 respectively and adding up the values.

Estimation of Minerals. Sample was taken in a silica crucible and heated in a muffle furnace at 400°C till there was no evolution of smoke. The crucible was cooled at room temperature in a desiccator and carbon-free ash was moistened with concentrated sulphuric acid and heated on a heating mantle till fumes of sulphuric acid ceased to evolve. The crucible with sulphated ash was then heated in a muffle furnace at 600°C till the weight of the content was constant (~2-3 h). One gram of sulphated ash obtained above was dissolved in 100 ml of 5% HCl to obtain the solution ready for determination of mineral elements (sodium, potassium and calcium) through atomic absorption spectroscopy (AA 800, Perkin- Elmer Germany).

HPLC Chromatographic Analysis of Phenolic Compounds. HPLC analyses were performed with Dionex Ultimate 3000 liquid chromatograph (Germany) with four solvent delivery system quaternary pump (LPG 3400 SD) including a diode array detector (DAD 3000) with 5 cm flow cell, a manual sample injection valve equipped witha 20 µl loop and Chromeleon 6.8 system manager as data processor. The

separation was achieved by a reversed-phase Acclaim^{TM}120 C_{18} column (5 µm particle size, i.d. 4.6 x 250 mm). The mobile phase contains 1% aqueous acetic acid solution (Solvent A) and acetonitrile (Solvent B), the flow rate was adjusted to 0.7 ml/min, the column was thermostatically controlled at 28°C and the injection volume was kept at 20 µl. A gradient elution was performed by varying the proportion of solvent B to solvent A. The gradient elusion was changed from 10% to 40% B in a linear fashion for duration of 28 min, from 40 to 60% B in 39 min, from 60 to 90 % B in 50 min. The mobile phase composition back to initial condition (solvent B : solvent A : 10 : 90) in 55 min and allowed to run for another 10 min, before the injection of another sample. Total analysis time per sample was 115 min. HPLC Chromatograms were detected using a photo diode array UV detector at 280 nm according to absorption maxima of analysed compounds. Each compound was identified by its retention time and by spiking with standards under the same conditions. The quantification of the sample was done by the measurement of the integrated peak area and the content was calculated using the calibration curve by plotting peak area against concentration of the respective standard sample. The data were reported with convergence limit in six times. According to the USP and ICH guidelines, there are various parameters to validate the reproducibility of the method viz. the effectiveness, the limit of detection (LOD), the limit of quantitation (LOQ), the linearity, the precision and the accuracy.

HPTLC Chromatographic analysis

Densitometric HPTLC fingerprint of Body Revival was carried out. Body Revival was diluted into 1 mg/ml in methanol and spotted (10 µl) in the form of bands with on a pre-coated silica gel plates (Merck, 60 F 254, 20x20 cm) by automated Camag Linomat 5. The plates were developed in a mobile solvent system (toluene : ethyl acetate : formic acid = 4.5:3:0.2) for 30 min and scanned at 254 nm suing Camag TLC Scanner3.

Results

Nutritive Values

Product No.	001
Sample Name	*Aegle mermelos*
Form	Dry Extract
Study	April-May, 2016

	Mean ± SD	Range
Ash (%)	7.10±0.01	7-7.3
Carbohydrate (%)	74.66±0.21	72.14-76.25
Protein (%)	2.40±0.15	2.1-2.8
Fat (%)	0.49±0.01	0.45-0.52
Fibre (%)	15.25±0.04	15-16.1
Energy (Kcal/100 g)	314.30±1.01	310-318.5
Sodium (mg/100 g)	3.64±0.01	3.51-3.83
Potassium (mg/100 g)	24.96±0.05	24-25.52
Calcium (mg/100 g)	43.54±0.48	42.1-44.5

N=6 in each test

Results

Nutritive Values

Product No.	002
Sample Name	*Acorus calamus*
Form	Dry Extract
Study	April-May, 2016

	Mean ± SD	Range
Ash (%)	12.43±0.20	12.1-12.84
Carbohydrate (%)	73.71±0.06	72.6-75.62
Protein (%)	4.21±0.04	4-4.54
Fat (%)	4.07±0.05	3.8-4.28
Fibre (%)	5.61±0.02	5.4-5.78
Energy (Kcal/100 g)	348.03±0.27	344.6-350.9
Sodium (mg/100 g)	6.17±0.04	6-6.38
Potassium (mg/100 g)	31.56±0.30	31.1-32.6
Calcium (mg/100 g)	35.34±0.14	35-36.8

N=6 in each test

Note: As per Sponsor's information, Acorus calmus extract has been prepared according to Ayurvedic sodhona (detoxification) process. Investigator has tried to estimate B-asarone form the extract and final formulation (Body Revival) according to HPLC method, but it could not detected in the supplying sample. (Ref. Pharm Methods, 2015; 6: 94-99)

Results

Nutritive Values

Product No.	003
Sample Name	*Rumex vesicarius*
Form	Dry Powder
Study	April-May, 2016

	Mean ± SD	Range
Ash (%)	11.80±1.01	10.9-12.2
Carbohydrate (%)	76.64±0.13	75.3-77.6
Protein (%)	4.55±0.03	4.4-4.6
Fat (%)	4.32±0.04	4.2-4.4
Fibre (%)	2.91±0.02	2.8-3.1
Energy (Kcal/100 g)	363.01±0.45	360.4-36.7
Sodium (mg/100 g)	5.01±0.07	4.9-5.2
Potassium (mg/100 g)	30.52±0.08	29.6-31.2
Calcium (mg/100 g)	33.74±0.06	32.8-34.5

N=6 in each test

Results

Nutritive Values

Product No.	004
Sample Name	*Blumea lacera*
Form	Dry Powder
Study	April-May, 2016

	Mean ± SD	Range
Ash (%)	25.66±0.25	25.1-26.4
Carbohydrate (%)	60.21±0.40	59.3-61.8
Protein (%)	7.06±0.05	6.9-7.2
Fat (%)	0.74±0.03	0.7-0.78
Fibre (%)	7.20±0.26	7-7.5
Energy (Kcal/100 g)	274.45±0.43	270-280
Sodium (mg/100 g)	3.45±0.02	3.3-3.5
Potassium (mg/100 g)	6.16±0.04	6-6.2
Calcium (mg/100 g)	41.67±0.18	40-42

N=6 in each test

Results

Nutritive Values

Product No.	005
Sample Name	*Cucumis melo*
Form	Dry Powder
Study	April-May, 2016

	Mean ± SD	Range
Ash (%)	14.50±0.21	14-15
Carbohydrate (%)	8.40±0.02	8.2-8.5
Protein (%)	23.86±0.10	23-24
Fat (%)	47.51±0.12	47-48
Fibre (%)	6.14±0.03	6-6.4
Energy (Kcal/100 g)	555.40±1.26	550-560
Sodium (mg/100 g)	2.93±0.04	2.9-2.98
Potassium (mg/100 g)	11.56±0.05	11-11.8
Calcium (mg/100 g)	14.37±0.06	14-14.6

N=6 in each test

Results

Nutritive Values

Product No.	006
Sample Name	*Symplocos racemosa*
Form	Dry Extract
Study	April-May, 2016

	Mean ± SD	Range
Ash (%)	16.56±0.20	16.4-16.6
Carbohydrate (%)	73.16±0.09	72-73.5
Protein (%)	1.88±0.03	1.8-1.9
Fat (%)	0.40±0.10	0.38-0.42
Fibre (%)	8.35±0.05	8-8.8
Energy (Kcal/100 g)	302.85±0.32	300-318.3
Sodium (mg/100 g)	3.37±0.03	3.31-3.48
Potassium (mg/100 g)	13.91±0.04	13.6-14.1
Calcium (mg/100 g)	17.76±0.15	17.3-18.4

N=6 in each test

Results

Nutritive Values

Product No.	007
Sample Name	*Withania somnifera*
Form	Dry Powder
Study	April-May, 2016

	Mean ± SD	Range
Ash (%)	10.46±0.25	10.2-10.8
Carbohydrate (%)	80.93±0.04	80.4-81.2
Protein (%)	1.90±0.10	1.8-1.9
Fat (%)	0.86±0.02	0.83-0.9
Fibre (%)	6.15±0.03	6-6.4
Energy (Kcal/100 g)	338.84±0.65	335.4-342.1
Sodium (mg/100 g)	4.72±0.06	4.68-4.75
Potassium (mg/100 g)	27.58±0.03	27.3-28.2
Calcium (mg/100 g)	43.73±0.11	43.4-44.2

N=6 in each test

Results

Nutritive Values

Product No.	008
Sample Name	*Rubia cordifolia*
Form	Dry Extract
Study	April-May, 2016

	Mean ± SD	Range
Ash (%)	13.63±0.15	13.2-13.8
Carbohydrate (%)	81.43±0.06	80.8-81.9
Protein (%)	1.10±0.01	1-1.3
Fat (%)	0.44±0.02	0.4-0.48
Fibre (%)	3.66±0.03	3.2-3.8
Energy (Kcal/100 g)	335.21±0.70	331-329
Sodium (mg/100 g)	3.34±0.04	3.3-3.5
Potassium (mg/100 g)	36.88±0.03	36.6-36.9
Calcium (mg/100 g)	36.43±0.35	36.1-36.9

N=6 in each test

Results

Nutritive Values

Product No.	009
Sample Name	Honey
Form	Semi Liquid
Study	April-May, 2016

	Mean ± SD	Range
Ash (%)	0.32±0.01	0.3-0.35
Carbohydrate (%)	88.08±0.71	87.6-89.1
Protein (%)	0.13±0.01	0.1-0.15
Fat (%)	0.0006±0.00001	0.0005-0.0007
Fibre (%)	10.66±0.08	10.2-10.9
Energy (Kcal/100 g)	356.61±0.54	353.8-359.6
Sodium (mg/100 g)	2.96±0.02	2.9-2.99
Potassium (mg/100 g)	0.95±0.03	0.92-0.96
Calcium (mg/100 g)	3.54±0.02	3.5-3.7

N=6 in each test

Results

Nutritive Values

Product No.	010
Sample Name	Body Revival
Form	Liquid / Suspension
Study	April-May, 2016

	Mean ± SD	Range
Ash (%)	8.35±0.02	8.2-8.5
Carbohydrate (%)	82.26±0.08	81.6-82.8
Protein (%)	0.18±0.01	0.16-0.19
Fat (%)	0.0004±0.00006	0.0003-0.0005
Fibre (%)	9.16±0.01	8.8-9.6
Energy (Kcal/100 g)	330.79±0.65	328-333
Sodium (mg/100 g)	4.18±0.005	4-4.3
Potassium (mg/100 g)	1.15±0.01	1-1.18
Calcium (mg/100 g)	3.76±0.03	3.7-3.8

N=6 in each test

Note: As per Sponser's information, Acorus calmus extract has been prepared according to Ayurvedic sodhona (detoxification) process. Investigator has tried to estimate B-asarone form the extract and final formulation (Body Revival) according to HPLC method, but it could not detected in the supplying sample. (Ref. Pharm Methods, 2015; 6: 94-99)

Results

Special Tests

Product No.	010
Sample Name	Body Revival
Form	Liquid / Suspension
Study	April-May, 2016
Specific Gravity	1.31±0.002

	Mean ± SD	Range
HPLC Analysis		
Gallic acid (µg/g)	18.40±0.015	18.3-18.4
p-Coumaric acid (µg/g)	2.41±0.02	2.4-2.44
Apigenin (µg/g)	0.29±0.0004	0.28-0.30

N=6 in each test

	Peaks (Rf)	Area %
HPTLC Analysis	Mean ± SD	Mean ± SD
Peak 1	0.06±0.001	1.18±0.002
Peak 2	0.11±0.004	5.72±0.006
Peak 3	0.2±0.003	1.04±0.001
Peak 4	0.26±0.006	2.59±0.003
Peak 5	0.54±0.012	84.19±0.015
Peak 6	0.71±0.002	5.27±0.004

N=6 in each test