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Nutraceutical Analysis of *Rasnadi Yusha*

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ABSTRACT

Introduction: *Rasnadi Yusha* is described in Ayurveda classical texts as a recipe for dietic intervention in *Swasa* (Bronchial asthma). The ingredients of *Rasnadi Yusha* includes *Mudga* (Seeds of *Vigna radiata*), *Rasna* (Rhizome of *Alpinia calcarata*), *Brihati* (Root of *Solanum indicum*), *Kantakari* (Root of *Solanum xanthocarpum*), *Prishniparni* (Root of *Uraria picta*), *Shalaparni* (Root of *Desmodium gangeticum*), *Gokshura* (Fruit of *Tribulus terrestris*), *Bala* (Root of *Sida cordifolia*) and *Chitraka* (Root of *Plumbago zeylanica*) in various proportion cooked in water.

Aim and Objective: To estimate the nutraceutical potential of *Rasnadi Yusha*.

Materials and Methods: The study was conducted at CSIR-CFTRI, Mysuru. The methodology employed includes testing of macronutrients like carbohydrates, protein, fat and dietary fiber. Among micronutrients, minerals like calcium, magnesium, copper, iron, manganese and zinc were tested by atomic absorption spectroscopic method. Antioxidant activity was tested by DPPH method. Different solvents were used for extraction to estimate total polyphenols, tannins and flavonoids.

Results: The nutritive analysis of *Rasnadi Yusha* showed low energy value (293.64kcal/100g) and fat value (2.93g/100g). It contains high amount of dietary fiber (20.47g/100g), proteins (21.39g/100g) and minerals. It also showed high content of polyphenol (1.94g/100g), flavonoid (644.7 mg/100g) and tannin (511.5mg/100g).

Conclusion: The nutritional profile of *Rasnadi Yusha* revealed a dietary intervention that is low in energy, carbohydrate, fat values and high in dietary fiber, proteins and minerals. The preparation showed high polyphenol, flavonoid and tannin contents.

KEYWORDS

Ayurveda, Bronchial asthma, Rasnadi Yusha, Swasa



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INTRODUCTION

Health of an individual depends upon the food he/she consumes¹. The body is the result of food and so are the diseases. Adaptation of right food and the dietary pattern are among lifestyle modifications to keep one healthy. The phrase "Let food be thy medicine and medicine be thy food," coined by Hippocrates over 2500 years ago is receiving a lot of interest today as food scientists and consumers realize the many health benefits of proper quantity, quality of food and timely food intake. It is widely accepted that the health-promoting properties of foods are not necessarily due to single components, but rather a few or several active ingredients².

Dr Stephen DeFelice coined the term "Nutraceutical" from "Nutrition" and "Pharmaceutical" in 1989. According to DeFelice, nutraceutical can be defined as, "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease". When functional food aids in the prevention and/or treatment of disease(s) and/or disorder(s) other than anaemia, it is called a nutraceutical³.

Nutraceuticals are represented for use as a conventional food or as the sole item of meal or diet³. The health benefits of nutraceuticals are generally focused on

several areas, including prevention and treatment of cardiovascular diseases, various types of cancer, diabetes and inflammations, and enhancement of immune response as well as retardation of aging process and extension of a healthy lifespan⁴. Phytochemicals (bioactive non-nutrient plant compounds), have raised interest in human nutrition because of their potential effects as antioxidants, antiestrogens, anti-inflammatory, immunomodulatory, and anticarcinogenic⁵. However, foods, particularly those of plant origin, contain a wide range of non-nutrient phytochemicals that are elaborated by plants for their own defence and for other biological functions. When man ingests these plant foods to meet his nutritional needs, he also ingests a wide variety of these non-nutrient phytochemicals. These phytochemicals present in commonly consumed plant foods are normally non-toxic and have the potential for preventing chronic diseases. Such an association between consumption of certain foods and low prevalence of non communicable disease was derived initially from epidemiological observations⁶.

Medical Nutrition Therapy (MNT) is a therapeutic approach to treat medical conditions and their associated symptoms via the use of a specifically tailored diet



devised and monitored by a medical doctor, physician, registered dietician, or professional nutritionist⁷. This approach helps to reduce the risk of developing complications in pre-existing conditions. The basic theory behind this approach is that many medical conditions either develop or are made worse by an improper or unhealthy diet.

Rasnadi yusha is prescribed under the treatment protocol for asthma in Ayurveda classical texts^{8,9}. Bronchial asthma is a major public health problem worldwide and the morbidity of asthma has increased in last few decades¹⁰. Bronchial asthma is the most common chronic respiratory disease, with a case burden of approximately 358.2 million in 2015. In 2015, about 0.40 million people died from asthma, a decrease of 26.7% from 1990, and the age-standardized death rate decreased by 58.8%. The prevalence of asthma increased by 12.6%, whereas the age-standardized prevalence decreased by 17.7%¹¹.

Even though, *Rasnadi Yusha* has been mentioned in *Ayurveda* classical texts for the treatment of asthma patients, its therapeutic potential is still not studied. Thus, it is desirable to study the information on macronutrient and micronutrient contents of this particular *Yusha* including its therapeutic components such as polyphenols, flavonoids, and tannins.

MATERIALS

Ingredients of *Rasnadi Yusha*:

The materials used for preparation of *Rasnadi Yusha* were *Mudga* (Figure 1. *Vigna radiata*; Seed), *Rasna* (Figure 2. *Alpinia calcarata*; Rhizome), *Brihati* (Figure 3. *Solanum indicum*; Root), *Kantakari* (Figure 4. *Solanum xanthocarpum*; Root), *Prishniparni* (Figure 5. *Uraria picta*; Root), *Shalaparni* (Figure 6. *Desmodium gangeticum*; Root), *Gokshura* (Figure 7. *Tribulus terrestris*; Fruit), *Bala* (Figure 8. *Sida cordifolia*; Root) and *Chitraka* (Figure 9. *Plumbago zeylanica*; Root)^{8,9}. Green gram was procured from local market and other herbal ingredients were procured from A.V.Traders, Indian Drug Merchants, Calicut, Kerala.



Figure 1. *Mudga*- *Vigna radiata*



Figure 2 *Rasna*-*Alpinia calcarata*



Figure 3 *Brhati-Solanum indicum*

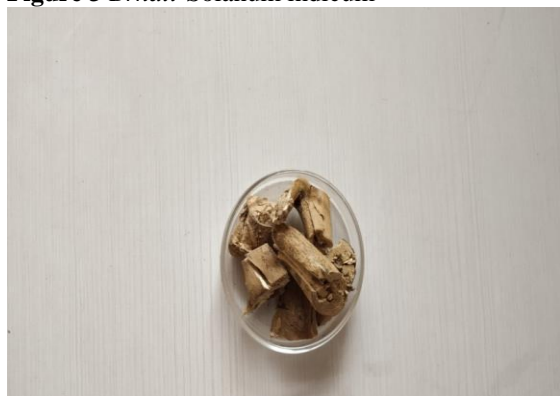


Figure 4 *Kantakari-Solanum xanthocarpum*

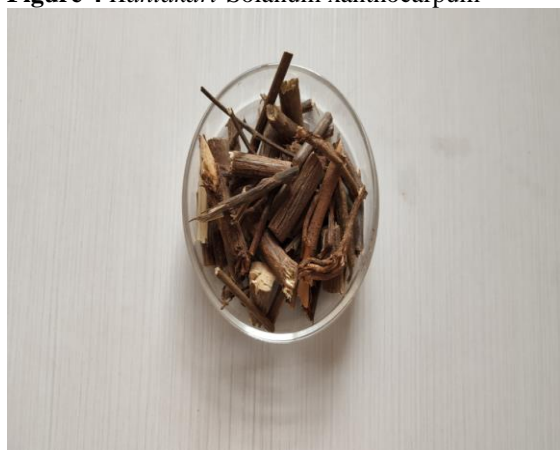


Figure 5 *Prshniparni-Uraria picta*

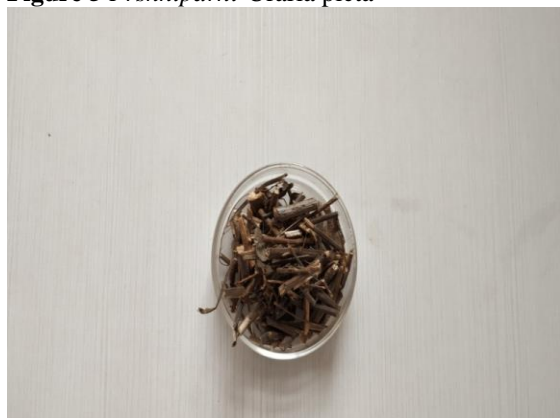


Figure 6 *Shalaparni-Desmodium gangeticum*

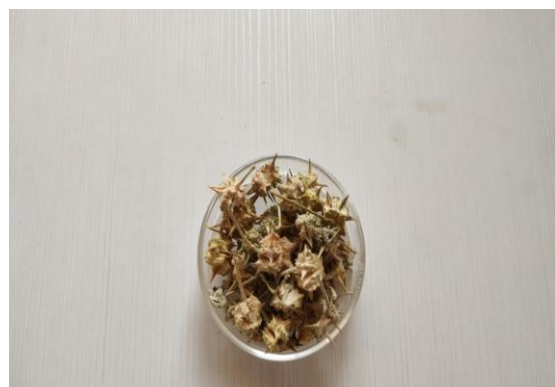


Figure 7 *Gokshura-Tribulus terrestris*



Figure 8 *Bala-Sida cordifolia*

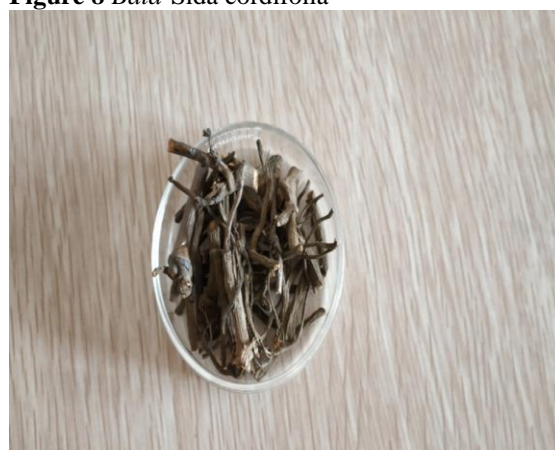


Figure 9 *Chitraka-Plumbago zeylanica*

METHOD OF PREPARATION

Green gram was soaked overnight. Each of the herbal coarse powder was taken in equal amounts (30g) and 16 parts of water (3840 ml) was added to it. The contents were boiled in a low flame till the volume reduces to half (1920 ml)¹². To the filtered



herbal kashaya (Figure 10), soaked green gram (106 g) was added in a ratio of 18:1 and cooked till the green gram was completely cooked and a semisolid consistency was formed (Figure 11)¹³. The soup thus prepared was freeze dried (Figure 12) to remove the liquid and the dry powder was stored in refrigerator for further analysis. The yield of the freeze dried powder was 89.7 mg.



Figure 10 Freshly prepared *Rasnadi kashaya*

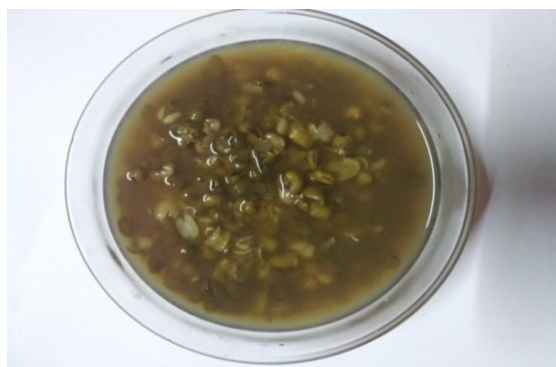


Figure 11 Freshly prepared *Rasnadi Yusha*

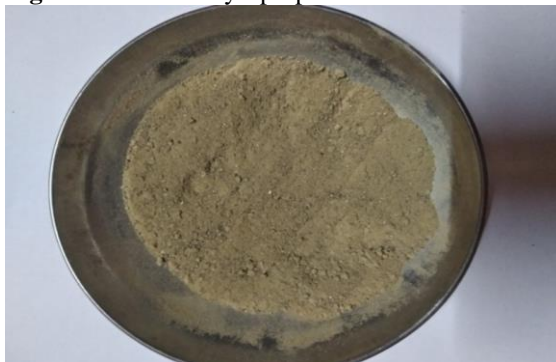


Figure 12 Freeze dried sample of *Rasnadi Yusha*

PROXIMATE ANALYSIS

pH

The pH of the product was measured using a freshly prepared liquid sample using a pH meter.

Colour Estimation

The colour was measured using a colour measuring equipment Spectrophotometer CM- 5 against a standard white. The L, a, b and ΔE values for colour was measured. The L value indicated the lightness of the sample. Positive 'a' value indicated the redness and negative values greenness while, the positive 'b' values indicated yellowness and negative b values blueness. The total deviation from the standard, ΔE , indicated the quantum of darkness of the sample.

Moisture

A known amount (5 g) of the freeze dried powder was weighed into pre-weighed aluminium cups and dried for 16 hours at 106 ° C. The cups were cooled in a desiccator and weighed. The moisture content was computed with the following formula and expressed as the percentage per 100 g.

$$\text{Moisture} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

NUTRITIVE ANALYSIS

Fat Estimation

The estimation of fat was carried out using a Soxhlet apparatus. The sample (10 g) was



weighed accurately into a thimble and plugged with cotton. The thimble was then dropped into the fat extraction tube of the Soxhlet apparatus. The bottom of the extraction tube was attached to the Soxhlet round bottom flask, and top to a condenser. The extraction was carried out with about 150ml of petroleum ether for about 16-20 hours. The water bath should be regulated so that petroleum ether which volatilizes, condenses and drops continuously on the sample without any appreciable loss. The soluble material is then evaporated and weighed. Total Fat is calculated by the formula.

$$\% \text{ Fat Content} = \frac{W_2 - W_1}{\text{Sample Weight (g)}} \times 100$$

W_1 -Weight of round bottom flask before fat extraction

W_2 - Weight of round bottom flask after fat extraction

Protein Estimation

The protein content was estimated using a protein analyser (Gerhardt Turbotherm, Denmark). To each of the digestion tubes, 0.5 g of sample was weighed along with 0.5 g of CuSO_4 and 0.5 g of K_2SO_4 followed by the addition of 10 ml of Conc. H_2SO_4 . The contents were digested in a digestion unit, cooled and 50 ml of water was added. The tubes were distilled with 40% NaOH and the ammonia released was collected in a flask containing 2% freshly prepared boric acid with 2-3 drops of mixed (bromocresol green and methyl red) indicator. The distillate was titrated against 0.1N HCl. The experiment was repeated with a blank. The protein content was determined using the formula.

$$\text{Protein \%} = \frac{(\text{Sample titre value} - \text{Blank titre value}) \times \text{Normality of HCl} \times 1.4007}{\text{sample weight}}$$

The value obtained was multiplied with correction factor. i.e. 6.25

Ash Value

Accurately weighed 5g of the sample was weighed in to a pre-weighed silica crucible and ignited in a heating coil till the sample

turns black. The crucible was placed in a muffle furnace maintained at 460°C and allowed to ash. The crucible was weighed to calculate the ash content using the formula;

$$\text{Total Ash in \%} = \frac{\text{Wt of the Crucible+ Ash} - \text{Wt of the empty crucible} \times 100}{\text{Sample weight}}$$

Total Dietary Fiber

To 1 (one) g of defatted sample was taken in a conical flask, 25 ml of 0.1M phosphate



buffer (pH- 6) and 0.1ml alpha-amylase was added followed by boiling in a water bath for 15 min. To the contents, 20 ml of distilled water was added and the pH was adjusted to 1.5 with 4M HCl, followed by the addition of 100mg pepsin. The contents were incubated at 40° C in shaking water bath for 60 min. To this 20 ml of distilled water was added and pH was adjusted to 6.8 with 4 M NaOH. Then 100 mg pancreatin was added followed by incubation at 40° C for 60 min in a shaking water bath. The pH was adjusted to 4.5 with 4 M HCl and volume was made up to 100 ml in a volumetric flask.

The sample was filtered using a filtration unit with crucibles containing a celite bed. After filtration, sample was washed with 20 ml distilled water and 40 ml of 95 % ethanol. Crucible was dried in hot air oven for 4-5 hours and weighed followed by incineration in a muffle furnace for 4-5 hours and again weighed. The insoluble dietary fiber was calculated with the formula=

$$\frac{(W_2 - W_1) - (W_3 - W_1) \times 100}{\text{Sample weight}}$$

Sample weight

W₁- Empty weight of the crucible

W₂- Weight after drying in hot air oven

W₃- Weight after incineration in muffle furnace

The filtrate was collected and precipitated for the soluble dietary fiber contents by

adding 4 times the volume of warm 95% ethanol and keeping overnight. The contents were filtered using pre weighed crucible and washed with 20 ml of 75% ethanol and 40 ml of 95% ethanol. Crucible was dried in hot air oven and weighed followed by incineration in a muffle furnace for 4-5 hours and weighed again.

The soluble dietary fiber was calculated with the formula =

$$\frac{(W_2 - W_1) - (W_3 - W_1) \times 100}{\text{Sample weight}}$$

Sample weight

W₁- Empty weight of the crucible

W₂- Weight after drying in hot air oven

W₃- Weight after incineration in muffle furnace

Total dietary fiber/ 100 g = Insoluble dietary fiber + Soluble dietary fiber

Starch Estimation

To 100 mg of sample taken in a conical flask, 15ml of distilled water and 0.1ml of thermostable alpha-amylase was added and boiled in a water bath for 15 minutes. The contents were cooled and 15ml of 0.05M acetate buffer of pH 4.8 was added followed by the addition of 10 mg glucoamylase. The contents were incubated for 16 hours at 60° C in a shaking water bath and the volume was made up to 100 ml. An aliquot of the sample was filtered and to 2ml of the filtrate taken in a test tube, 2ml of DNS reagent was added and the contents were boiled exactly for 5 minutes. The test



tube was cooled and 16 ml of distilled water was added and the OD was measured at 540 nm against a glucose standard. A sample blank and a reagent blank were prepared in a similar manner. The absorbance was recorded in a spectrophotometer and compared with those of known standard glucose concentrations ($R^2 = 0.999$). The value obtained was multiplied with correction factor. i.e. 0.9.

Available Carbohydrates

The available carbohydrate content of the sample was calculated by difference method.

Available Carbohydrates % = 100- [Moisture + Fat + Protein + Ash + Total dietary Fiber]

Total Energy

Total energy was calculated by using the formula

Total energy (kcal/100 g) = [4 X Protein %] + [9 X Lipid %] + [4 X Carbohydrates %]

Estimation of Minerals

Ash sample(5g) was kept in water bath and 5ml conc. HCl was added to it twice till HCl was completely absorbed. Then 4ml of HCl and 1 ml of distilled water were added and removed from water bath when it was heated. Later it was filtered using Whatmann paper 40 and volume was made upto 100ml, it was analyzed through Atomic Absorption Spectrophotometer

(Atomic Absorption Flame Emission Spectrophotometer AA 670IF, Shimadzu) .

PHYTOCHEMICAL ANALYSIS

Extraction of nutraceuticals

The nutraceutical components such as polyphenols, tannins etc. were extracted using different solvent systems;

- 100 % Methanol
- 80 % Methanol+ 20% Distilled water
- 50 % Methanol + 50 % Ethanol
- 70 % Acetone + 2% Glacial acetic acid +28 % Distilled water

To 5g of the sample, 50 ml of solvent was added and refluxed for 3 hours. The contents were centrifuged and the supernatant was collected. The residue was again refluxed with the solvent and procedure was repeated for 4 times. The extracts were pooled; the volume was noted down and stored in brown bottles till further analysis.

Total Polyphenols Estimation

Total polyphenol content was estimated by Folin-Ciocalteu method (Singleton et al.,1999). To 1 ml of the extract 5 ml of Folin-Ciocalteu reagent(1:1 dilution) was added and neutralized with 10ml of saturated sodium carbonate solution followed by incubation for 30 min at room temperature. The absorbance was recorded in a spectrophotometer at 760nm and against standard gallic acid ($R^2 = 0.999$).



The total polyphenol content was computed and expressed as mg gallic acid equivalents (GAE) per 100g of sample.

Total Flavonoids Estimation

Total flavonoid content was determined according to the method of Zhishen *et al.* (1999). The extract (0.1 ml) was diluted with 4.9 ml of distilled water and mixed with 0.3 ml of (5% w/v) NaNO₂. After 5 min, 0.3 ml of (10% w/v) AlCl₃ and at 6 min, 2 ml of 1 M NaOH were added and immediately the volume was made up to 10ml with distilled water. The mixture was shaken vigorously and the absorbance was read at 510 nm. Standard catechin was used to prepare a calibration curve ($R^2 = 0.99$). The flavonoid concentration was expressed as mg catechin equivalents (CEQ) per 100 g of sample.

Tannin Estimation

Tannin content was determined by the modified vanillin-HCl method (Price, Van Scoyoc & Butter, 1978). The extract (0.1ml) was made up to 1 mL with distilled water and 5ml of Vanillin-HCl reagent was added immediately. The samples were allowed to stand at room temperature for 20 min and the colour developed was recorded at 500 nm. A calibration curve was prepared using a standard solution of catechin ($R^2=0.994$). The result was expressed as mg catechin equivalents (CEQ) per 100g of sample.

Free radical scavenging activity

The free radical activity of the extract was determined using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method (Brand-Williams *et al.*, 1995). The extract (0.1ml) was reacted with 3.9ml of 6×10^{-5} mol/L of DPPH solution. The Absorbance was recorded at 0 and 30 min at 515nm. The ability to scavenge DPPH radical was measured by the discoloration of the solution.

$$\text{The DPPH \%} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}} \times 100$$

RESULTS AND DISCUSSION

pH Analysis of *Rasnadi Yusha* : 6.56

The pH is towards neutral, that shows *Rasnadi Yusha* is neither acidic nor alkaline. Studies have shown that acidity potentiates bronchoconstriction¹⁴. So pH is favorable for a patient of Bronchial asthma.

Colour Analysis

Table 1. Colour analysis of freeze dried *Rasnadi Yusha*

Parameters	Value
L* = Brightness	56.72
a* = Red to Green	5.11
b* = Yellow to Blue	16.12
ΔE = Deviation from the Standard	44.09

The L, a, b values in Table 1 for colour analysis indicated that the freeze dried powder is not very dark ($\Delta E = 44.09$). However, the product was slightly yellow in colour as indicated by the b values.



The Macronutrient Analysis of freeze dried *Rasnadi Yusha*

Table 2 The macronutrient analysis of freeze dried *Rasnadi Yusha* (mean \pm SD g /per 100 grams of sample) :

Moisture	3.76 \pm 0.01
Fat	2.93 \pm 0.08
Protein	21.39 \pm 0.12
Ash	6.019 \pm 0.01
Insoluble dietary fiber	17.58 \pm 0.11
Soluble dietary fiber	2.88 \pm 0.03
Total dietary fiber	20.47
Available carbohydrate	45.42

Starch Estimation:(mean \pm SD g/100 g of sample): 53.22 \pm 10.33

Total energy (Kcal / 100 g sample) :293.64

The macronutrient composition in Table 2 of the sample showed that, it is a low fat and high protein product with a good amount of dietary fiber (20.47 g/100g). The moisture content of the sample was very low (3.76 g) which was usual for any freeze dried product. Even though the available carbohydrates were only 45.42 g, the total starch contents appeared to be slightly high (53.22g), which may be due to the high fiber content that slows down carbohydrate digestion.

The Mineral contents of *Rasnadi Yusha*

Table 3 Mineral contents of freeze dried *Rasnadi Yusha*: (mean \pm SD mg/100 g of sample)

Mineral	Estimated Value
Calcium	179.42 \pm 134.32
Magnesium	146.62 \pm 87.52
Iron	24.35 \pm 0.80
Zinc	2.76 \pm 0.04
Copper	1.36 \pm 0.22
Manganese	3.78 \pm 0.04

The Table 3 indicates the mineral contents of the freeze dried product. The product

forms a very good source of minerals with 179 mg of calcium and 24 mg of iron. It is also a good source of magnesium (146 mg). This showed that the herbs used in the formulation contained good amount of minerals.

Bio active compounds

The term "bioactive compound" is not attributed to the nutrients contained in food or, more broadly, to the nutrients that are essential for a living organism, such as primary metabolites. Therefore, a bioactive compound is, too simply, a compound which has the capability to interact with one or more component(s) of the living tissue. Bioactive compounds in the plants can be defined, then, as secondary plant metabolites eliciting pharmacological effects in humans and animals¹⁵.

Total Polyphenol Content:

Table 4 The polyphenol content of freeze dried *Rasnadi Yusha*(mean \pm SD mg/100g)

Extract	Total polyphenol
100 % Methanol	1,596.17 \pm 241.97
80 % Methanol: 20% Distilled Water	1,941.48 \pm 308.46
50% Methanol : 50% Ethanol	1,324.53 \pm 510.002
70% Acetone:2% Glacial acetic acid:28% Distilled water	875.85 \pm 6.99

Total Polyphenol Content analysis

The total polyphenol present in the different extracts are presented in Table 4. The table indicated that, the solvent combination of 80% methanol with 20% distilled water extracted highest polyphenols (1.941 g)



followed by 100% methanol (1.596 g). The solvent system containing acetone, glacial acetic acid and water was a poor extractor for polyphenols. Here, the methanolic solvent system proved to be the suitable one.

Total Flavonoids:

Table 5 The flavonoid content of freeze dried *Rasnadi Yusha* (mean \pm SD mg/100g)

Extract	Total Flavonoid
100 % Methanol	606.24 \pm 25.83
80 % Methanol: 20% Distilled water	644.75 \pm 47.46
50% Methanol : 50% Ethanol	550.21 \pm 6.32
70% Acetone:2% Glacial acetic acid:28% Distilled water	405.80 \pm 48.15

Total Flavonoids analysis

Similar to polyphenols, the total flavonoids showed in Table 5, were also maximum for 80% methanol with 20% distilled water extract, followed by 100% methanol. The least value was shown by acetone, glacial acetic acid and water solvent system .

The Tannin Content

Table 6 The Tannin content of freeze dried *Rasnadi Yusha* (mean \pm SD mg/100g)

Extract	Total Tannins
100 % Methanol	651.61 \pm 16.26
80 % Methanol: 20% Distilled water	511.57 \pm 14.86
50% Methanol : 50% Ethanol	522.38 \pm 5.24
70% Acetone:2% Glacial acetic acid:28% Distilled water	481.07 \pm 27.67

Tannin content analysis

Unlike polyphenols and flavonoids, the total tannins showed in Table 6, were high for the extract with 100% methanol, followed by methanol and ethanol

combination solvent system. However, the lowest values were shown by acetone, acetic acid and water solvent indicating that, this solvent system is not suitable for extraction of nutraceutical components from *Rasnadi Yusha*.

Free Radical Scavenging Activity:

Table 7 The antioxidant activity of freeze dried *Rasnadi Yusha* (mean \pm SD %)

Extract	DPPH %
100 % Methanol	67.85 \pm 1.18
80 % Methanol:20% Distilled water	70.16 \pm 2.26
50% Methanol : 50% Ethanol	61.09 \pm 2.26
70% Acetone:2% Glacial acetic acid:28% Distilled water	57.52 \pm 1.02

The antioxidant activity presented in Table 7, is also highest for 80% methanol with 20% distilled water and 100% methanol solvent system. Thus, 80% methanol with 20% distilled water forms a potential solvent system for the extraction of nutraceuticals from *Rasnadi Yusha*.

CONCLUSION

Asthma is defined as a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. Asthma was regarded primarily as a problem of bronchospasm and measures to prevent or reverse bronchospasm comprised the mainstay of therapy. However, during early 1980s when asthma emerged as an inflammatory rather than primarily a bronchospastic disorder, the basic approach switched from control of



symptoms to control of underlying airway inflammation¹⁶. While analysing the nutrient and bioactive components, it was estimated that *Rasnadi Yusha* has an anti-inflammatory potential with the help of information provided by the Dietary Inflammatory Index. The oxidative stress plays an important role in aggravation of bronchial asthma. So the high content of polyphenols, flavonoids and tannins help in providing antioxidant activity that can reduce the symptoms and stop the progress of the disease. The immunity is compromised as the disease progress. So immuno-modulation can also help in the treatment of bronchial asthma. High content of dietary fiber, tannins and proteins help in that context.

Thus *Rasnadi Yusha* can help in reducing the symptoms of bronchial asthma or prevent the progress and onset of bronchial asthma through its high content of protein, dietary fiber, polyphenols, flavonoids, tannins and high anti-oxidant activity. The low content of carbohydrates and fat also favours the condition. Low energy level and being a non refined food intervention *Rasnadi Yusha* can be an ideal food intervention in asthma.

Further clinical study in human subjects is essential to confirm the mode of action of *Rasnadi Yusha*.

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