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In vitro digestive activity and stability study of *Rhus chinensis* Mill. fruit and its value-added products

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Rhus chinensis Mill. belonging to the family Anacardiaceae is a highly acidic fruit-bearing tree abundantly grown in the North-Eastern part of India. Due to its high medicinal values, the local medical practitioners of this region have widely used the fruit in various treatments like indigestion, dysentery, and gastrointestinal disturbances since ancient times. However, because of its 'underutilized status', there is an acute shortage of scientific literature thereby undermining its high potential use in developing many commercial functional foods or food ingredients. Therefore, this study was undertaken to address the same by formulating natural digestive tablets, *R. chinensis* tablets and *R. chinensis* candy from the fruit with minimal expenses. *R. chinensis* fruit and its products' efficacy for the digestive property was determined *in vitro* by amylolytic, proteolytic and lipolytic activity and compared other commercial samples of the herbal digestive, Gasex and allopathic digestive, Unienzyme. The formulation was evaluated for a period of zero to 90 days. Physical parameters *viz*. extractive values, total titratable acidity, total soluble solids, pH, and reducing sugar were determined. The fruit and its products showed significant amylolytic, proteolytic, and lipolytic activities in comparison with other commercial samples. Interestingly in all the samples, no significant changes in digestive activities were observed during the storage period. The formulations were found to possess the property of breaking down starch, protein, lipid into simpler units hence, substantiating the traditional practice of digestive activity. This is the first scientific record in terms of *in vitro* digestive property of *R. chinensis* fruit.

Keywords: Digestive activity, Functional food, *Rhus chinensis* Mill., Traditional medicine.

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Introduction

North-Eastern India falls within the world's famous biodiversity hotspot 'Indo-Burma'. So, the region is inundated with rich medicinal plants and trees, but most of them remain unexplored or under-explored. *Rhus chinensis* Mill. (syn. *Rhus semialata Murray*) belonging to the family Anacardiaceae, common name Nutgall tree or Chinese sumac is one such deciduous tree abundantly found in this region. It is a fruit-bearing tree and the fruit is extensively and effectively used in traditional medicine by local medicinal practitioners in curing a variety of ailments like diarrhoea, dysentery, gastrointestinal problems, urinary complaints, stomach ulcer and many other health problems[1-4]. In addition, the ripe fruit is used traditionally in various culinary purposes for its effectiveness in indigestion. The ethnic people have enormous indigenous knowledge to prevent and combat various diseases. The inhabitants mainly depend on plant resources for food, medicine, fuel, fibre and other purposes[5-8]. Since the digestive system is the core of energy, susceptibility, and well being of human health, its maintenance is of prime importance. However, due to the fast-changing lifestyle, the general population is confronted with many health issues. The direct or indirect effect on the digestive system due to these changes led to manifold increase in acute digestive health issues like constipation and distension. Therefore, to mitigate these health issues, we generally spend a lot on pharmaceutical products like antacids, laxatives, and enzymes. But these pharmaceutical products have cascading effects on our health in the long run, hence replacement with natural products is the most feasible and beneficial way.

To address some of the issues, the study has been conducted holistically to develop value-added digestive products with minimal expenses. The present study examined the favourability of *R. chinensis* fruit as a digestive product with or

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without the addition of other spices which is said to have digestive properties. Since the fruit has high acidic content, to improve its palatability and enhancing the digestive activity, other natural ingredients like garlic (*Allium sativum*), pepper (*Piper nigrum*) and jaggery were added in the formulation. Garlic has been described as a gastric stimulant. It aids in digestion and relieving flatulence[9]. Dietary addition of garlic is also reported to increase and enhance the activity of the digestive enzyme and improve meal digestion[10,11]. The bioactive component, melanoidin present in black garlic decreased significantly when treated with hydrochloric acid or α-amylase. It was found to be stable and retained over 60% after adding pancreatin and pepsin. Thus, black garlic can be used in the production of food additives and functional foods[12]. Black pepper is not just utilized commonly as a household spice but also has immense therapeutic potentials. Pharmacologically, pepper has been investigated for antimicrobial, antioxidant, digestive, neuroprotective, antidiarrheal, analgesic, anticancer, hypoglycaemic, anti-inflammatory, hypolipidemic, and antihypertensive activities[13,14]. It also helps in promoting weight loss and improving digestion as well. Jaggery is used as an Indian traditional sweetener and is reported to have various health benefits. It has a potential antioxidant activity, supplements the requirement of calcium and iron in children and women, prevents anaemia and boosts the vitality in men, and also helps in the digestion process[15,16]. Jaggery also activates the digestive enzymes in our body, thus helping in the proper digestion of food and thereby preventing constipation.

Based on the report of digestive property for the above-mentioned ingredients, digestive candies and tablets from *R. chinensis* fruit were formulated and evaluated for the digestive enzyme activities namely amylolytic, lipolytic, and proteolytic in comparison with commercial samples namely, herbal digestive, Gasex and allopathic digestive, Unienzyme. Gasex is a herbal formulation used for control and relief from dyspepsia/indigestion, flatulence, abdominal distension, and belching. Unienzyme is used for digestion, intestinal gas, flatulence, stomach gas, and other conditions.

Materials and Methods

Chemicals

Glucose, tyrosine, dinitrosalicylic acid, sodium potassium tartrate, casein, folin-ciocalteu reagent were procured from Sigma Aldrich, Bengaluru, India. Gasex and Unienzyme were purchased from the local market, manufactured by The Himalaya Drug Company and Unichem Labs, respectively.

Collection and authentication of plant sample

The fully ripe *R. chinensis* fruit was procured from the local market of Bishnupur, Bishnupur District, Manipur. The plant species was identified and authenticated by Dr. A. A. Mao, Scientist-F, Head of Department and Dr. Chaya Deori, Scientist-D, Herbarium Incharge, Botanical Survey of India, Eastern Region Centre, Shillong, India. A voucher specimen was submitted bearing accession number BSI/ERC/Tech/2017-18/699.

Preparation of value-added products

The whole fruits were cleaned and dried at 55±5 °C for 4 hours in a hot air oven. The dried fruits were deseeded in a plate mill and passed through an aspirator for separation of seeds from the fruit. The samples were stored in an airtight container at 4 °C until they were required for formulation and analysis.

After subsequent trial experiments, value-added products namely, *R. chinensis* candy (RCC) and *R. chinensis* tablet (RCT) were formulated in fixed ratios to formulate the commercially palatable products. The candy and tablet were packed in an airtight glass container and stored at room temperature (29±2 °C) for various physicochemical changes during zero to 90 days of storage. Microbial evaluation for total viable counts, coliform, yeast and mold were determined[17]. Sensory analysis of the value-added products was carried out by Ranking test (Quantitative differences) using 5-Point Hedonic Scale to determine the consumer preferences and acceptability of the products[18,19].

Preliminary phytochemicals screening

The sample extracts were assessed for the existence of the phytochemical analysis *viz*. tannins, saponins, flavonoids, polyphenols, glycosides, terpenoids and steroids by using the following standard methods[20-22].

Physico-chemical analysis (fruit, value-added products and commercial samples)

Water-soluble extractive value

The extractive value was determined according to the method by Chamundeeswari[23]. Two g of each sample were accurately weighed and macerated with 100 mL of chloroform water. It was then filtered and about 25 mL of the filtrate was transferred into a crucible and allowed to evaporate to dryness. It was then dried at 105 °C, cooled and weighed.

Alcohol-soluble extractive values

Ethanol was used as a solvent in place of chloroform water. The remaining procedures were the same as in the water-soluble extractive method.

Determination of TSS, pH, acidity, and reducing sugar

Decoctions of the samples with water (2 g/10 mL) were used for the evaluation of physicochemical parameters. Total soluble solids TSS (Brix) was determined by using a digital refractometer (RX-5000 ATAGO). The pH of the samples was measured using pH testr30 (Eutech Instruments, OAKTON). Total tritatable acidity TTA% of the samples was determined by titration method[24], expressed as anhydrous citric acid equivalent (% w/w). Reducing sugar was estimated by the Dinitrosalicylic acid method and absorbance was read at 510 nm with glucose as a standard for the calibration curve[25].

Colour measurement

Colour measurement of the samples was done in Minolta CM 5 (Hunter) in three sets.

Determination of *in-vitro* digestive property

Dialysis of R. chinensis and value-added products

The samples were dialysed using a Dialysis Tubing membrane (10,000 MWCO) in phosphate buffer (pH 7.5) of 20 mM overnight to remove the sugar content from the samples[26].

Preparation of extracts

The extracts of *R. chinensis* fruit and its products (candy and tablet) were prepared according to the procedure of Chamundeeswari[23], with slight modifications. About 2 g of dialysed sample was extracted in 25 mL phosphate buffer (pH 7.5) overnight with intermittent shaking and filtered. The filtrate was used as an enzyme source. The commercial samples were prepared similarly to the test sample.

Amylolytic activity

The amylolytic activity was determined according to the procedure of Sadasivam and Manickam[25]. An aliquot of enzyme extract (1 mL) and substrate (1 mL soluble starch 1% in phosphate buffer) was incubated for 15 minutes at 27 °C. The enzyme reaction was interrupted by the addition of a 2 mL DNS reagent and heated in a boiling water bath for 5 minutes. One mL of potassium sodium tartrate solution was added in the warm tubes, cooled to room temperature and volume make-up was done. The absorbance was read at 540 nm with maltose as standard for the calibration curve.

Proteolytic activity

The protease activity was determined using modifications according to the Universal protease activity assay: Casein as a substrate of Sigma-Aldrich[27]. Casein solution (0.65%) was equilibrated in the water bath at 37 °C for about 5 minutes. Then, 0.5 mL of enzyme solution was added and incubated at 37 °C for exactly 10 minutes. After this, 5 mL of the TCA reagent was added to each tube to stop the reaction. About 0.5 mL of enzyme solution was added to each tube, even the blank, then incubated at 37 °C for 30 minutes and filtered. After 30 minutes of incubation, the test solutions were filtered including the blank using a 0.45 µm polyethersulfone syringe filter. About 2 mL of the filtrate was taken, to which 5 mL of sodium carbonate was added. For best results, 1 mL of Folin's reagent was added immediately afterwards, mixed by swirling, and then incubated at 37 °C for 30 minutes. The absorbance was read at 660 nm using tyrosine as a standard for the calibration curve.

Lipolytic activity

Preparation of substrate solution: Two mL of clear vegetable oil was neutralized to pH 7.0 and stirred well with 25 mL of water in the presence of bile salts (sodium taurocholate) till an emulsion was formed. About 2 g gum arabic was added to hasten the emulsification process.

Enzyme source: A known quantity of sample was taken and homogenized with twice the volume of ice-cold acetone. The homogenized powder was filtered and washed with acetone, acetone: ether (1:1), and ether. It was air-dried and stored in the refrigerator. About 1 g of the sample was extracted in 20 mL phosphate buffer (pH 7.0), centrifuged at 3,000 X g for 10 minutes and the supernatant was used as enzyme source.

Experimental procedure: The lipolytic activity was determined according to the assay by Sadasivam and Manickam[25]. Twenty mL of the substrate was taken, to which 5 mL phosphate buffer (pH 7.0) was added. The contents were stirred slowly with a magnetic stirrer maintaining the temperature at 35 °C. The electrodes of the pH meter were dipped in the reaction mixture, and the pH was adjusted to 7.0. The enzyme extract (0.5 mL) was immediately added and a drop of approximately 0.2 units of pH was recorded. The timer was set such that at zero time, the pH was observed as 7.0. As the pH drops by 0.2 units (i.e. pH 6.8), it was brought to the initial pH value (7.0) with

the addition of N/10 NaOH. This titration continued for a duration of 30 to 60 minutes to examine the lipolytic activity of the sample. The total volume of alkali consumed in the entire process was noted.

$$\text{Lipolytic activity (meq/min/g sample)} = \frac{\text{Volume of alkali X Strength of alkali}}{\text{Weight of sample X Time in minutes}}$$

Data analysis

The data were subjected to single ANOVA and the multiple comparison test was done by Tukey's test using GraphPad Prism 7.

Results and Discussion

Preparation of value-added products

R. chinensis fruit is highly acidic in nature and hence, necessary care was taken in the preparation of value-added products to select the right type of ingredients in a proper ratio to ensure that final products were palatable and retained the desired sensorial properties. RCC and RCT were subjected to microbiological analysis for yeast, mold, and pathogenic microorganism (*E. coli*). No microbial contamination was observed in both the products in zero and 90 days of study.

RCC and RCT were subjected to sensory evaluation using the 5-Point Hedonic Scale for the sensory attributes like appearance, aroma, taste, mouthfeel and overall acceptability. It was observed that both the products were found to have overall acceptability by the panellists.

Phytochemical screening of fruit and value-added products

Preliminary phytochemical screening was done for RCF, RCC, and RCT in different extracts, *viz*. aqueous, acetone, ethanol, and methanol. The present study revealed that different extracts contained tannins, flavonoids, polyphenols, glycosides, and terpenoids. Saponins and steroids were found to be absent. The presence of phytochemicals is considered as active medicinal chemical constituents[28]. Terpenoids are reported to have anti-inflammatory, anti-viral, antimalarial, inhibition of cholesterol synthesis, and anti-bacterial activities[16,17]. Polyphenols and flavonoids possess anti-cancerous, anti-inflammatory, significant antioxidant capacities responsible for adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides which are associated with a lower occurrence and lower mortality rates of several human diseases[29,30]. Plants containing phytochemicals are also known to possess antioxidant, antimicrobial, curative properties, anthelmintic, skin problems, cardiotonic aliments, and chronic bronchitis[31-34].

Physico-chemical parameters of fruit, value-added products and commercial samples

It was found that RCF was highly acidic (19.94±0.47% TTA) and hence formulation was needed to make it consumable. Therefore, value addition was done to retain its maximum nutritional quality and at the same time meet consumers' preferential taste. It can also be used as an acidulant in the preparation of many foods. Considering its high benefits, value-added products namely candy and tablet were developed. They were evaluated for their nutritional characteristics for commercial usage and production. The physicochemical parameters such as extractive values, colour parameters, TSS, TTA, and reducing sugar were also evaluated.

Extractive values give an idea about the nature of chemical constituents present in the food. It is the measure of a certain constituent or group of related constituents contained in the drug often indicating the index of its purity. It plays an important role in the evaluation of different formulations and crude drugs. Less extractive values indicate adulteration, an addition of exhaustive materials or incorrect processing during drying[35]. The water and alcohol extractive value of RCF was found to be higher among the samples. The water extractive value of RCC was found to be high as compared to its alcohol extractive value. However, the water and alcohol extractive values of RCT and commercial samples (HDG and ADU) showed no significant difference. It was also observed that the water-soluble extractive values of all the samples were higher compared to alcohol extractive values (Table 1).

The colour of a product plays an important role in the psychological perception of the food and

Table 1 — Extractive values of *R. chinensis* fruit (RCF), value-added products (RCC and RCT) and commercial samples (HDG and ADU)

Sample name	Alcohol extractives (%)	Water extractives (%)
RCF	21.42±0.02^{a}	50.30±0.06^{a}
RCC	16.88±0.57^{b}	41.67±1.94^{a}
RCT	9.02±0.19^{b}	29.43±0.92^{b}
HDG	11.84±0.03^{b}	27.22±0.1^{b}
ADU	14.36±0.1^{b}	26.70±0.1^{b}

*Values are given in mean±standard deviation of three independent determinations. Values in the same row followed by the same letter are not significantly different at $P \leq 0.05$ as measured by Tukey's test.

acceptance by the consumers. In the colour parameter, L* value indicates black and white (L= 0, black to L=100, white), a* and b* values indicate redness and yellowness. It was observed that L* value of RCF and RCC was inclined towards black colour showing a lower intensity range (20-28). Whereas, RCT was neither black nor white, showing middle-intensity range (44-51). Furthermore, RCC was slightly reddish yellow with a* and b* values ranging from 3 to 4 and 4.1 to 4.9 respectively. The a* value of RCF and RCT was slightly reddish with a range of (6.35-11.05) whereas, the b* value for both the samples was observed to be high ranging from 14 to 20, indicating more yellowness.

It was observed that the values of TSS (°Brix), reducing sugar (%) and TTA (%) were found to be significantly high in RCF, RCC, and RCT as compared to the commercial samples, whereas the pH value was found to be high in the commercial samples. However, no significant changes were observed in the biochemical parameters throughout the storage period. The values are summarized in Table 2.

Determination of *in vitro* digestive properties of the fruit, value-added products, and commercial samples

The *in vitro* digestive activities of RCF, RCC, and RCT were evaluated by determining amylolytic, proteolytic and lipolytic activity in comparison with the standard enzymes and commercial samples (HDG and ADU) for zero to 90 days and its stability study. The herbal digestive, Gasex is claimed to improve digestion, relieves gaseous distension, and renormalize the intestinal transit time. It has pre-biotic, anti-flatulent and antacid, antiulcer, anti-inflammatory, hepatoprotective, cholagogues and membrane modulating, antimicrobial, and antioxidant actions. Similarly, the allopathic digestive, Unienzyme claims to improve digestion, intestinal gas, flatulence, stomach gas, etc. The amylase activity of *R. chinensis* fruit and its value-added products was determined with maltose as standard. Amylase is one of the enzymes in the human body responsible for the breakdown of starch into more simple sugars[36]. Amylase enzyme in ADU showed the maximum amylolytic activity (40.03±1.19 mg/g) followed by RCF (11.90±0.14 mg/g), RCT (7.56±0.10 mg/g), RCC (5.11±0.07 mg/g), and HDG (7.34±0.08 mg/g) at zeroth day (Fig. 1).

Protease enzymes are the enzymes responsible for the breakdown of protein into building blocks, i.e. amino acids. Proteolytic activity was observed maximum in RCF followed by RCT, RCC, HDG and found least in ADU. Due to processing, a significant reduction was observed in the amylolytic and proteolytic activities of RCC and RCT in comparison to RCF. However, no significant changes were observed in all the samples over the storage period (Fig. 2). Although *R. chinensis* fruit and its value-

Table 2 — pH, TSS, Reducing sugar, and TTA of *R. chinensis* fruit (RCF), value-added products (RCC and RCT) and commercial samples (HDG and ADU) for a period of zero to 90 days of storage

Parameters	Storage period (days)	RCF	RCC	RCT	HDG	ADU
pH	Zero	3.19^{b}	3.32^{b}	3.50^{b}	7.64^{a}	7.02^{a}
	30	2.78^{b}	3.12^{b}	3.33^{b}	7.68^{a}	7.03^{a}
	60	2.78^{b}	3.05^{b}	3.30^{b}	7.68^{a}	7.04^{a}
	90	2.72^{b}	3.04^{b}	3.32^{b}	7.66^{a}	7.04^{a}
TSS (°Bix)	Zero	30.1±0.3^{c}	79.33±0.31^{a}	62.6±0.20^{b}	31.73±0.28^{c}	34.20±0.10^{c}
	30	30.11±0.15^{c}	79.70±0.46^{a}	63.13±0.31^{b}	33.15±1.19^{c}	34.57±0.32^{c}
	60	30.15±0.06^{c}	80.51±0.45^{a}	63.57±0.42^{b}	35.12±0.15^{c}	36.50±0.46^{c}
	90	30.55±0.4^{c}	80.60±0.63^{a}	64.01±0.10^{b}	35.4±0.56^{c}	38.40±0.36^{c}
Reducing sugar (%)	Zero	1.92±0.70^{c}	8.10±0.67^{a}	3.30±0.56^{b}	0.28±0.15^{d}	0.80±0.1^{d}
	30	1.95±0.70^{c}	8.41±0.74^{a}	3.81±0.43^{b}	0.29±0.15^{d}	0.81±0.1^{d}
	60	2.02±0.77^{c}	9.05±1.37^{a}	4.01±0.40^{b}	0.31±0.14^{d}	0.83±0.1^{d}
	90	2.07±0.80^{c}	9.19±1.41^{a}	4.10±0.35^{b}	0.33±0.14^{d}	0.82±0.1^{d}
TTA (%)	Zero	19.94±0.47^{a}	3.68±0.04^{b}	3.7±0.08^{b}	0.28±0.2^{c}	0.04±0.03^{c}
	30	20.08±0.50^{a}	3.76±0.06^{b}	4.01±0.24^{b}	0.35±0.14^{c}	0.05±0.01^{c}
	60	20.04±0.36^{a}	3.96±0.46^{b}	4.23±0.04^{b}	0.49±0.1^{c}	0.06±0.01^{c}
	90	20.64±0.41^{a}	4.01±0.44^{b}	4.32±0.15^{b}	0.51±0.1^{c}	0.06±0.01^{c}

*Values or parameters are taken from the water decoction of the sample (2 g/10 mL). Values are given as mean±standard deviation of three independent determinations. Values in the row followed by the same letter are not significantly different at $P \leq 0.05$ as measured by Tukey's test. TSS- total soluble solids; TTA- total titratable acidity.

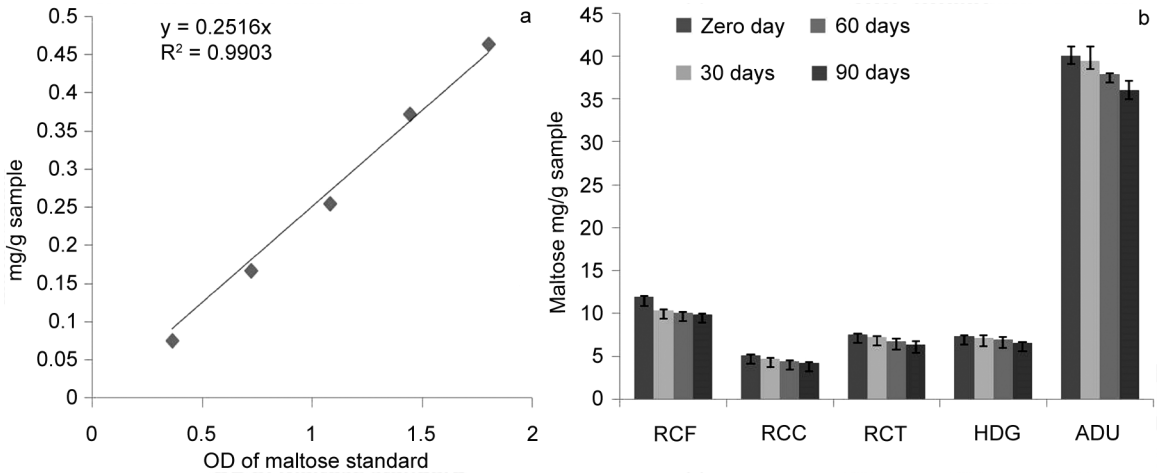

Fig. 1 — *In vitro* amylolytic property, a) Maltose standard, b) Amylolytic activity of samples, *R. chinensis* fruit (RCF), value-added products (RCC and RCT) and commercial samples (HDG and ADU).

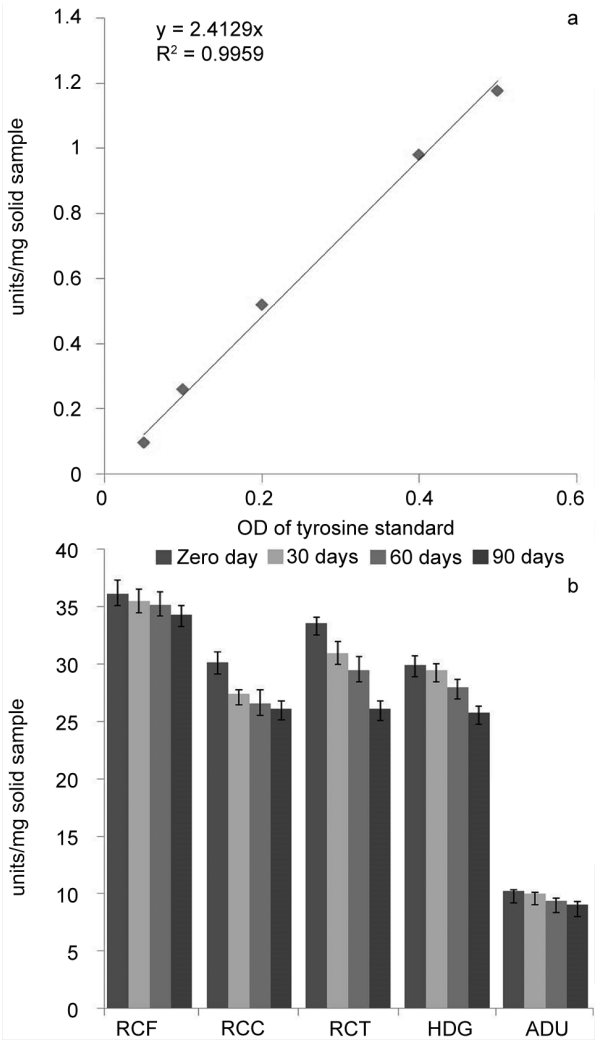

Fig. 2 — *In vitro* proteolytic activity, a) Tyrosine standard, b) Amylolytic activity of samples, *R. chinensis* fruit (RCF), value-added products (RCC and RCT) and commercial samples (HDG and ADU).

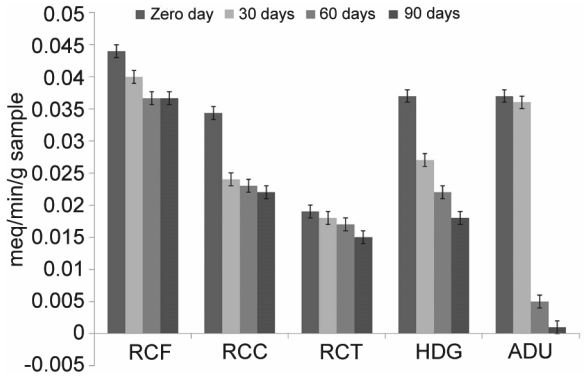

Fig. 3 — *In vitro* lipolytic activity of *R. chinensis* fruit (RCF), value-added products (RCC and RCT) and commercial samples (HDG and ADU).

added products showed lesser activity in terms of amylolytic activity in comparison to the Unienzyme, the proteolytic activity was significantly better compared to the Unienzyme and Gasex. Moreover, no significant changes were observed in the lipolytic activity in all the samples (Fig. 3). In light of the above results, *R. chinensis* fruit and its value-added products could be concluded to possess promising natural properties for breaking down starch, protein, and lipid into simpler units and help in digestion activities. Furthermore, *R. chinensis* fruit and its value-added products were also found to be stable over the storage period and hence it can be used as an alternative to the currently commercially available digestive products.

Conclusion

The medicinal use of *R. chinensis* fruit by local medical practitioners as a reliable natural medicine for multiple diseases *viz*. diarrhoea, gastrointestinal

problems, urinary complaints, stomach ulcer without any scientific validation for so many decades indicates the advanced knowledge in medicinal science in ancient times. However, the practice and knowledge of using *R. chinensis* fruit as a natural medicine for different diseases were limited geographically and population-wise. Hence, like any other medicinal fruits and plants, it becomes pertinent for the scientific community to authenticate and validate such age-old practices with a thorough scientific substantiation using the latest technology to make sure that such natural treatments are popularized and used more widely and effectively. This study is a simple answer for the above questions as it substantiates the age-old traditional practice of using *R. chinensis* as a popular and effective medicine by traditional medical practitioners. The fruit and its value-added products showed significant amylolytic, proteolytic and lipolytic activities in comparison with other commercial samples. They were found to possess the property of breaking down starch, protein, lipid into simpler units, hence, substantiating the traditional practice of digestive activity. Therefore, RCC and RCT could become reliable natural medicine in near future and hence, large scale production and commercialization of the products should be the next big step after due clinical investigation to ascertain the fruit's mechanism of action.

Conflict of interest

The authors have no conflict of interest to declare.

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