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Ruwena: Its Physicochemical and Functional Properties

Aboubakr Barkah[a], Rabiya Eshaewi[b], Sumaya Alghnaei[b], Mokhtar Mostapha[a], Abdulhakim Ilowefah[b], Muna Ilowefah[a]*

[a]Department of Food Science and Technology, Faculty of Food Sciences, Wadi Al-Shatii University. Brack Alshatii, Libya.
[b]Department of Medical Laboratory Science, Faculty of Medical Technology, Wadi Al-Shatii University. Brack Alshatii, Libya.

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ABSTRACT

Traditional foods are not only a reflection of culture, but they are rich in functional components. Besides, traditional diets have been connected with a lower risk of chronic diseases. Ruwena is one of the traditional foods that can be incorporated into modern foods and may provide a way to address health problems. Accordingly, this study aimed to identify the physicochemical and functional properties of a drinkable traditional food called ruwena and their impact on beneficial bacteria (Lactobacillus). In this study, ruwena was chosen as a typical example of traditional food. Ruwena was prepared in two ways based on the geographical location in the Wadi Al-Shatti areas, Libya. They were named Eastern area ruwena and Western area ruwena. They were prepared using some cereals, legumes, oil seeds, nuts and some aromatic herbs in different proportions based on the habits of the previously mentioned regions. The results of the study showed that ruwena was a good source of protein (13.80 and 12.67%) for Western and Eastern area ruwenas, respectively, and fibre [the original wording here is unclear and requires clarification]. The results for the studied minerals differed significantly between the two samples, which included K (0.67 and 0.89 ppm), Ca (0.52 and 0.36 ppm), P (0.27 and 0.29 ppm), Fe (0.094 and 0.066 ppm), Mn (0.022 and 0.023 ppm) and Zn (0.019 and 0.092 ppm) for Eastern and Western area ruwenas, respectively. The instant solubility of the Eastern area ruwena increased significantly compared to its counterpart. The study also indicated a significant increase in the solubility of the Western area ruwena after refrigerated soaking for 24 h compared to the instant solubility. The findings of the study presented that the selected food had a positive effect on the growth of beneficial bacteria.

الروينة: خصائصها الفيزيوكيميائية والوظيفية

ابوبكر بركه[a]، ربيعة اشعيوي[b]، سمية الغناي[b]، مختار مصطفى[a]، عبدالحكيم لويفة[b]، منى لويفة[a]*

[a]قسم علوم وتقنية الاغذية، كلية علوم الاغذية، جامعة وادي الشاطئ، براك الشاطئ، ليبيا.
[b]قسم المختبرات الطبية، كلية التقنية الطبية، جامعة وادي الشاطئ، براك الشاطئ، ليبيا.

الكلمات المفتاحية:
الاطعمة التقليدية.
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الخصائص الوظيفية.
البكتريا النافعة.

الملخص

لا تُعدّ الأطعمة التقليدية مجرد انعكاس للثقافة، بل هي غنية أيضاً بالمكونات الوظيفية. علاوة على ذلك، يرتبط اتباع نظام غذائي تقليدي بانخفاض خطر الإصابة بالأمراض المزمنة. يُعدّ مشروب الروينة أحد الأطعمة التقليدية التي يُمكن دمجها مع الأطعمة الحديثة، وهو وسيلة للتغلب على المشاكل الصحية. وعليه، هدفت هذه الدراسة إلى تحديد الخصائص الفيزيائية والكيميائية والوظيفية لمشروب الروينة، وهو مشروب تقليدي، وتأثيرها على البكتيريا النافعة (اللاكتوباسيلوس). في هذه الدراسة، تم اختيار الروينة كنموذج للأطعمة التقليدية. تم تحضير الروينا بطريقتين مختلفتين بناءً على طريقة التحضير في منطقة وادي الشاطئ بليبيا. سُمّيت روينة الشاطئ الشرقي وروينة الشاطئ الغربي. تم تحضيرهما باستخدام بعض الحبوب والبقوليات والبذور الزيتية والمكسرات وبعض الأعشاب الطبية بنسب مختلفة، وفقاً لعادات المناطق

*Corresponding author.
E-mail addresses: mona.melad2005@gmail.com, (A. Barkah) a.masuod@wau.edu.ly, (R. Eshaewi) ra.eshaewi@wau.edu.ly, (S. Alghnaei) s.alghnaei@wau.edu.ly, (M. Mostapha) m.mostapha@wau.edu.ly, (A. Ilowefah) elwafa_ly@yahoo.com.
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المذكورة. أظهرت نتائج الدراسة أن الروينة تُعدّ مصدرًا جيدًا للبروتين (13.80% و12.67% على التوالي) في عينتي روينة الشاطئ الغربي والشرقي، بينما سجلت كلتا العينتين نفس القيمة للألياف الغذائية (5.87%). وقد اختلفت نتائج المعادن المدروسة اختلافًا كبيرًا بين العينتين، حيث شملت البوتاسيوم (0.67 و0.89 جزء في المليون)، والكالسيوم (0.52 و0.36 جزء في المليون)، والفوسفور (0.27 و0.29 جزء في المليون)، والحديد (0.094 و0.066 جزء في المليون)، والمنغنيز (0.022 و0.023 جزء في المليون)، والزنك (0.019 و0.092 جزء في المليون) في عينتي روينة الشاطئ الشرقي والغربي على التوالي. كما لوحظ ارتفاع ملحوظ في ذوبانية الفورية لروينة الشاطئ الشرقي مقارنةً بروينة الشاطئ الغربي. أشارت الدراسة أيضًا إلى زيادة ملحوظة في ذوبان مشروب وروينة الشاطئ الغربي بعد نقعه في الثلاجة لمدة 24 ساعة مقارنةً بذوبانها الفوري. وأظهرت نتائج الدراسة أن هذا المشروب له تأثير إيجابي على نمو البكتيريا النافعة.

1.Introduction

Traditional foods hold a special place in the cultures of different societies. These foods are deeply embedded in the customs, rituals and history of societies. Traditional foods are often characterised by their diverse local ingredients and unique preparation methods. Traditional food refers to meals and cooking procedures, which have been passed down from one generation to another. The ingredients utilised in traditional foods indicate how people adapted to their environment. For instance, countries with long coastlines have rich traditional meals with seafood, whereas those with enormous farmlands depend on vegetables and grains. Today, processed meals and fast food are becoming more common, particularly amongst younger generations. Several people desire convenience over traditional cooking that can be time-consuming. Consequently, certain traditional foods are no longer prepared frequently. This underlines the importance of promoting this type of food particularly from a scientific perspective given its nutritional and health significance. They are not only a reflection of culture, but they are often a source of vital nutrients. These foods are rich in vitamins, minerals, antioxidants and dietary fibre, which contribute to promoting health. Furthermore, traditional diets have been associated with a lower risk of chronic diseases, such as heart disease, diabetes and obesity. Incorporating traditional foods into modern diets can promote healthy eating patterns and prevent diet-related health problems [1].

For example, Tshikororo et al. [2] studied a traditional food consumed in South Africa consisting of ten types of wild vegetables. The study found that these types of vegetables are rich in various essential minerals and have a good protein content, making them comparable to commercial vegetables. Another study indicated that Miwu, which is a traditional Chinese food, is rich in nutrients and lacks acute toxicity [3]. Another traditional food is freekeh, which is consumed in the Middle East and North Africa. It is made from early-harvested whole wheat grains, especially durum wheat. This food is also easy to process, has nutritional value and can be considered a functional food [4].

The spread of diseases in this era is a result of the tendency of most consumers to eat easy or fast foods, as well as what is known as hidden hunger due to the low nutritional value of many commonly consumed foods [5]. Therefore, nutritionists must pay attention to this situation and return to healthy foods, especially traditional ones and whole grain foods. Also, Islamic culture and the food practices of the Prophet Muhammad (may God bless him and grant him peace) emphasise the consumption of grains without refining, such as whole bread [6]. Also, a study indicated that cereal fibres were more effective in treating a number of chronic diseases and types of cancer compared with fibres from other sources such as fruits and vegetables [7].

Mixing food ingredients to produce functional and therapeutic foods is a sound approach to obtain the greatest benefit from food. For instance, mixing grains with legumes balances amino acids, and addition of oilseeds to them in the same formulation may increase the levels of omegas in the meal. Additionally, nuts might enhance the value of minerals, vitamins and antioxidants. The mixture of the previously mentioned grains and seeds is an example of a traditional Libyan food known as “Ruwena”. The latter is a drinkable food made of a mixture of grains, legumes, oilseeds, nuts and spices that are mixed together in specific proportions after roasting and then grinding them to a fine flour.

It is worth noting that there were no studies found in the literature on this drinkable ruwena. Ruwena is a plant-based drink made from different grains to which aromatic herbs are added, including thyme, cumin, and rosemary. It is easy to prepare and is a fast food, but it is distinguished by its high nutritional value compared to other fast foods that have high energy content with poor nutritional value [8]. This food is notable for its content of whole grains, and therefore it is rich in fibre in its various forms provided by cereals, legumes, oilseeds, and herbs.

Ruwena may be one of the traditional foods that can be incorporated into modern foods and may provide a way to address health problems. The importance of this research supports the scientific system to be a starting point for developing traditional functional foods. From what has been mentioned, the current study will investigate two types of preparation methods based on the region in Wadi Alshatti city. The main objective of this study is to identify how different ingredients and their quantities might alter the nutritional value and functional properties of the ruwena. This will be verified through estimating moisture, protein, fibres and minerals of two types of ruwena. Secondly, measuring their solubility and abilities to absorb water and oil. Thirdly, studying the effects of ruwena extract on the beneficial bacteria (colon bacteria).

2. Materials and Methods

2.1. Materials

The raw materials used in this study were obtained from markets in Wadi Al-Shatii City, while the utilised chemicals were obtained from Sigma or Merck.

2.2. Methods

2.2.1. Ruwena Preparation

The study included two different methods of ruwena preparation based on the type of ingredients and their amounts according to their traditional preparation in two different areas in Wadi Alshatti city. They differ in terms of the type and quantity of the ingredients. The prepared samples were called Eastern area ruwena and Western area ruwena. The cereal grains (1 kg each) were roasted for 20 s; while, legumes and nuts (half a kilogram each) were roasted for 40 s and oilseeds (half a kilogram each) were roasted for 15 s following a traditional way by using sand and an aluminum pot over a direct fire. The roasted ingredients were left to cool down at room temperature. Then, the ingredients were mixed in the proportions mentioned (Tables 1 and 2), and then they were ground into fine flour without sifting using a local mill. The samples were kept in glass containers at 4 °C until laboratory tests were conducted.

Table 1: Ingredients of Western area ruwena

Cereals	(%)	Legumes	(%)	Aromatic plants	(%)
Wheat	24.84	Chickpea	6.21	Thyme	0.1
Barley	12.32	Bean	3.73	Rosemary	0.04
Millet	3.73	Lentil	3.73	Nigella sativa	0.15
		Pea	3.73	Coriander	0.09
		Bean	3.73	Turmeric	0.019
		Fenugreek	0.27		
Nuts	(%)	**Oil seed**	(%)		
Almond	6.21	Sesame	6.21		
Cashew	6.21	Flaxseed	6.21		
		Peanut	12.32		

Table 2: Ingredients of Eastern area ruwena

Cereals	(%)	Legumes	(%)	Aromatic plants	(%)
Wheat	21.21	Chickpea	7.07	Thyme	0.12
Barley	14.14	Fenugreek	0.31	Rosemary	0.04
Millet	28.28			Nigella sativa	0.17
				Coriander	0.11
				Turmeric	0.28
Nuts	**(%)**	**Oil seed**	**(%)**		
Almond	7.07	Sesame	7.07		
		Flaxseed	7.07		
		Peanut	7.07		

2.2.2. Moisture, Protein, and Total Fiber Determination

Moisture, crude protein, and total fiber contents of the studied samples were determined using NIRS™ DS2500 (Germany) [9]

2.2.3. Determination of Mineral Content

The quantities of a number of minerals in the two studied samples were estimated using an X-ray Fluorescence (XRF, Rigaku, Japan) device according to the method of Feng et al. [10].

2.2.4. Determination of Functional Properties

2.2.4.1. Water and Oil Holding Capacities Measurement

Water holding capacity (WHC) or oil holding capacity (OHC) was measured by placing 1g of sample into a pre-weighed centrifuge tube. A 10 mL of either distilled water or corn oil was added and the mixture in the tube was vortexed for 2 min. The suspension was allowed to stand for 30 min at ambient temperature, then centrifuged at 3000 g for 15 min. After decanting the excess water or oil, the tube was inverted to drain following the method of Elkhalifa et al. [11]. The difference between the initial and final weights was calculated to determine the ability of sample to bind either water or oil.

2.2.4.2. Water Solubility Index Measurement

Two and a half grams of sample was placed in a centrifuge tube, mixed with 30 mL of distilled water and vortexed for 5 min. The mixture was heated at 90 ºC for 15 min, left to cool at room temperature (25 ºC). Then, it was centrifuged at 3000 g for 10 min. The supernatant was decanted into pre-weighed dish, which in turn was evaporated overnight at 110 ºC following the method of Elkhalifa et al. [11]. The water solubility index (WSI) was expressed as percentage of weight of dissolved solids in the supernatant to weight of the original sample.

2.2.5. Detection the Effects of Ruwena on Beneficial Bacteria

The sample with the highest solubility was selected to evaluate the effect of ruwena extract on the growth of isolated beneficial bacterial. The preparation of bacterial was obtained from a commercial probiotic product (Ledacatiol Fort/Lactobacillus LB, Egypt), purchased from a pharmacy in Brack Al-Shatii City. The prepared bacteria intended to represent beneficial intestinal microbiota. The experiment was conducted in the Department of Medical Laboratories, Faculty of Medical Technology, Wadi Al-Shatii University [12,13].

The bacterial preparation was first activated on nutrient agar medium. Following activation, bacterial isolates were obtained based on their colony morphology. The isolates were characterized using microscopic examination, colony characteristics, and appropriate biochemical tests (Such as catalase test, oxidase test, glucose fermentation (triple sugar iron test, TSI), indole, and urease test to establish their bacterial identity. The characterized bacterial isolates were then used for the subsequent growth experiment. A ten-fold serial dilution was prepared from the bacterial suspension, ranging from 10^{-1} to 10^{-4}. From the fourth dilution (10^{-4}), 1 mL was inoculated into each experimental plate. The experiment included a treatment group containing ruwena extract and a control group without ruwena extract. The ruwena extract (10%) was prepared from the sample with the highest solubility in a sterile water [11], and filtered through a 0.2-µm cellulose nitrate membrane filter (Germany) before use. Sterile water was used in the control condition. The culture medium was prepared according to the manufacturer's instructions. The same experimental conditions were maintained for both treatment and control groups. Each experimental condition was performed in duplicate. The inoculated plates were incubated aerobically at 37 °C for 24 h. After incubation, visible colonies were counted manually, and the colony counts obtained from the ruwena-treated plates were compared with those of the control plates to assess the effect of ruwena extract on bacterial growth.

2.2.6. Statistical Analysis

SPSS 19 program was used to analyze the results of the study at a significance level of $p \leq 0.05$ using Paired Samples t-test and L.D.S was used to find significant differences between the means.

3. Results and Discussion

3.1. Moister, Protein and Total Fibers

Table (3) shows that the Western area ruwena recorded higher values of crude protein and moisture (13.8% and 5.47% respectively). The statistical analysis showed significant differences in the levels of protein and moisture in favour of the Western area ruwena. The remarkable differences could be due to the type and quantity of the ingredients used in preparing ruwena. The reason for high protein content might be due to the high amount of the used legumes in preparing the Western area ruwena sample compared to the Eastern area ruwena. Legumes are a rich source of protein, and are also considered rich in lysine content, which is deficient in cereal proteins [14]. Accordingly, one of the most important health benefits of such a drink is the amino acids balance, due to the designed formulation and the utilized ingredients, which led to obtaining high quality of the consumed protein.

This drink is also rich in fiber, and therefore drinking a 250 ml cup of this drink daily provides approximately half of the dietary fiber requirements. It is also worth noting the diversity of fibers in this drink between soluble from barley and flaxseed and insoluble from other ingredients. The soluble fiber in barley (beta-glucan) is characterized by its many health effects against chronic diseases [15]. Flaxseed also contains a high amount of fiber, a large part of which is soluble (20%), in the form of gums and mucilage. It plays a major role as a laxative, and it has been noted that it has strong properties in lowering cholesterol, thus reducing the risk factors for cardiovascular diseases [16]. Traditional foods provide a sustainable, nutritious and culturally rich path to a future where everyone has access to the nutrition they need for a healthy life. The role of traditional foods in achieving food security cannot be overestimated, due to their nutritional value, health benefits, biodiversity and cultural identity, and thus the potential for studying innovative approaches and collaboration between producers and consumers to promote the integration of traditional foods into modern food systems.

Table 3: Moister, protein and fiber of ruwena samples

Component (%)	Western area ruwena	Eastern area ruwena
Protein	$13.80^{a} \pm 0.17$	$12.67^{b} \pm 0.12$
Total fiber	$5.87^{a} \pm 0.58$	$5.87^{a} \pm 0.58$
Moisture	$5.47^{a} \pm 0.12$	$4.33^{b} \pm 0.15$

The values are means ± standard deviations for three replicates, the values with the same letters in a raw have no significant differences ($p \leq 0.05$).

3.2. Minerals Content

Table (4) indicates significant differences in potassium in favour of the Western area ruwena, which recorded 0.89 ppm. The two samples did not record any significant differences in the values of the rest of minerals. From the results, it can be noted that ruwena is a good source for obtaining the necessary minerals for the body, such as potassium, calcium, phosphorus, iron, zinc and selenium. The reason is due to the used whole grains, whether it is cereals, legumes or oil seeds [17].

This drink is a good source of iron due to the presence of fenugreek [18], as well as the high value of calcium due to the presence of sesame [19]. While, high potassium content because of existence of cereals. Flax seeds are a good provenance of a number of minor minerals such as iron, manganese, zinc, and major minerals like potassium and chlorine [19, 20]. Herbs are mainly used to add flavor and aroma to food, and they also have a function in preserving food, and they have been used as medicines for a number of medical conditions, as many herbs have medicinal properties that possess beneficial effects on the digestion process and absorption of nutrients. For example, thyme contains different antioxidants, such as tannins (2%), vitamins C and A, beta-carotene and minerals [21]. A study found that rosemary contains a large amount of calcium, iron and potassium [22]. All these herbs were used in the studied drink.

Table 4: Mineral contents in ruwena samples

Mineral (ppm)	Type of ruwena: Eastern area ruwena	Western area ruwena
K	0.67±0.01[a]	0.89±0.02[b]
Ca	0.52±0.01[a]	0.36±0.01[a]
P	0.27±0.01[a]	0.29±0.01[a]
S	0.22±0.01[a]	0.22±0.01[a]
CL	0.18±0.00[a]	0.18±0.00[a]
Fe	0.094±0.01[a]	0.066±0.01[a]
Mn	0.022±0.002[a]	0.023±0.01[a]
Zn	0.019±0.002[a]	0.092±0.001[b]

The values are means ± standard deviations for three replicates, the values with the same letters in a raw have no significant differences (p≤0.05).

3.3. Functional Properties

The findings in Table (5) demonstrate the functional properties of the two studied samples. Instant solubility and solubility after 24 hours were measured. Eastern area sample documented a significantly higher level of instant solubility; whereas, after 24 hours of cooled soaking, the Western area sample had a higher solubility. The explanation could be referred to higher water-soluble components in the Eastern ruwena area compared to the other sample, specifically some fibers and proteins. However, after leaving the samples soaked for 24h other components in Western area sample were dissolved.

The results of the study exhibited equal values for both types of ruwena in terms of their abilities to ability to hold water and oil. Studying these properties from a chemical perspective may be useful in identifying the availability of hydrophilic and hydrophobic functional groups. Additionally, this kind of food consumed as a drink with addition of water. Moreover, it is possible to predict that the high ability of ruwena to bind water might be beneficial for human body in terms of increasing water content of the stool, as a result, bulky, soft and easy to pass stools could be provided to the body [23].

Measuring solubility of a food sample is useful as an indicator of its biological value and its bioavailability for the body and the beneficial bacteria [24]. It can be observed that an increase in the solubility of the studied samples after cooled soaking in water for 24h, which could suggest that ruwena should be left soaked for a longer period of time before consuming to increase its availability. Foodstuffs are classified as insoluble when their solubility is less than 0.1 g per 100 ml of solvent. Therefore, this drink is considered highly soluble and thus highly available to the body [24].

Table 5: Functional properties of ruwena samples

Property	Western ruwena area	Eastern ruwena area
Instant solubility (%)	10.37[a]±0.35	14.24[b]±0.78
Solubility (24h) (%)	15.5[a]±0.58	14.78[a]±0.53
Water holding capacity (g/g)	3.00[a]±0.00	3.00[a]±0.00
Oil holding capacity (g/g)	2.00[a]±0.01	2.00[a]±0.01

The values are means ± standard deviations for three replicates, the values with the same letters in a raw have no significant differences (p≤0.05).

3.4. Effects of Ruwena of Colon Bacteria

The results presented in Table 6 showed a statistically significant difference in the growth of *Lactobacillus* between the nutrient agar medium and the medium containing ruwena extract ($p \leq 0.05$). The number of colonies increased from 753×10^4 in nutrient agar to 1583×10^4 in ruwena extract medium, indicating an approximately 2.1-fold increase in the growth of the tested bacterium. This finding suggests that ruwena extract was able to support the in vitro growth of the tested *Lactobacillus* strain under the experimental conditions used.

The present observation is consistent with the findings of Sliżewska and Chlebicz-Wójcik [25], who demonstrated that an alternative semi-solid fermentation medium based on cereal flours could effectively support the growth of probiotic *Lactobacillus* strains. In their study, bacterial cell densities obtained in the alternative medium were higher than those observed in conventional MRS medium, with an increase up to 1.5 log units. Furthermore, the maximum growth rate of the *Lactobacillus* strains was up to two times higher in the alternative medium, accompanied by a shorter generation time. These findings demonstrate that appropriately formulated alternative media can provide a favourable nutritional environment for *Lactobacillus* growth.

The enhanced growth observed with ruwena extract might similarly be associated with the availability of nutrients that can be utilized by *Lactobacillus*. Sliżewska and Chlebicz-Wójcik [25] reported that *Lactobacillus* species have relatively high nutritional requirements and require fermentable carbohydrates, amino acids, B-group vitamins, nucleic acids, and minerals for effective growth. Therefore, the increased bacterial count observed in ruwena extract medium could suggest that the extract contains one or more nutritional components capable of supporting bacterial proliferation. However, because the present study did not determine the specific nutritional or chemical composition of ruwena extract, the precise component(s) responsible for the observed growth-promoting effect cannot be established.

The present findings therefore suggest that ruwena extract has potential as a growth-supporting component for the tested *Lactobacillus* strain. Nevertheless, further investigation is required to determine whether the observed increase is attributable to specific carbohydrates, proteins, vitamins, minerals, or other bioactive constituents present in the extract. Evaluation of growth kinetics, pH changes, viable cell counts over time, and the chemical composition of the extract would provide additional evidence regarding the mechanism underlying the observed enhancement.

Ruwena is traditionally prepared from whole grains, seeds, and legumes, which contain dietary carbohydrates, proteins, fibers, vitamins, and minerals. Some soluble and fermentable components of plant-based foods can provide substrates that are utilized by microorganisms. However, the present experiment did not separate or quantify the individual components of Ruwena and therefore cannot determine which specific component was responsible for the observed increase in Lactobacillus growth. Thus, the nutritional composition of Ruwena should be considered a possible explanation rather than a demonstrated mechanism.

Dietary fibers are of particular interest because many non-digestible carbohydrates can escape digestion in the upper gastrointestinal tract and reach the colon, where they can be metabolized by intestinal microorganisms. Their fermentation can produce short-chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate, which contribute to intestinal and host physiology [26, 27, 28]. Previous studies have also demonstrated that dietary fiber can influence the composition and metabolic activity of the gut microbiota [27, 29]. Nevertheless, these established effects of dietary fiber cannot be directly attributed to ruwena in the present study, because the fiber composition of the extract and its fermentation products were not experimentally determined.

Fiber-rich foods from plant sources, such as whole grains, vegetables, fruits, and legumes, have been an important component of human diets throughout history [27]. Dietary fiber can influence the composition and metabolic activity of the gut microbiota, which contributes to several physiological functions, including the digestion of dietary components and the production of certain vitamins and metabolites [30]. Changes in dietary fiber intake may influence the composition and function of the gut microbiome, and alterations in the gut microbiota have been associated with several chronic diseases, including obesity, diabetes, cancer, and cardiovascular diseases [31, 30]. However, these associations provide general background on the potential importance of dietary fiber and the gut microbiota and cannot be directly interpreted as evidence of similar health effects of ruwena in the present study.

The mechanism of dietary fiber action in the digestive system involves the resistance of some non-digestible carbohydrates to digestion in the upper gastrointestinal tract, allowing them to reach the colon where they can be metabolized by intestinal microorganisms. This fermentation can produce several metabolites, including gases such as H_2, CH_4, and CO_2, lactate, succinate, and short-chain fatty acids (SCFAs) [32, 33]. The major SCFAs include acetate, propionate, and butyrate. These metabolites contribute to intestinal and host physiology, with butyrate serving as an important energy source for colonic epithelial cells [34]. Dietary fiber can also influence the composition and metabolic function of the gut microbiota [35, 36]. Nevertheless, the present study did not directly evaluate fiber fermentation, SCFA production, or changes in the intestinal microbiota; therefore, these mechanisms should be considered established background information rather than demonstrated

mechanisms underlying the observed growth of *Lactobacillus* in the present experiment.

The present findings therefore provide preliminary in vitro evidence that ruwena extract can promote the growth of the tested *Lactobacillus* strain. However, increased growth of a single bacterial strain in vitro does not by itself establish that Ruwena is a probiotic or definitively demonstrate a prebiotic effect. Probiotic effects require the administration of live microorganisms with demonstrated health benefits, whereas a prebiotic effect requires evidence that a substrate is selectively utilized by host microorganisms and provides a health benefit. The current experiment did not assess selective utilization, changes in the overall intestinal microbiota, production of SCFAs, or health outcomes. Future studies should characterize the carbohydrate and dietary fiber composition of ruwena, determine which components contribute to bacterial growth, compare its effects on different intestinal microorganisms, and investigate fermentation products such as SCFAs. Such studies would help clarify whether Ruwena contains components with genuine prebiotic potential and whether the in vitro findings have biological relevance in vivo.

Table 6: Effect of ruwena extract on colon bacteria

Type of media	Number of colony	Total number
Nutrient agar	753[a]	753×10^4
Ruwena extract	1583[b]	1583×10^4

The values with the same in column letters have no significant differences ($p \leq 0.05$).

4. Conclusions and Recommendations

Through this study, it can be concluded that ruwena is an example of a traditional food that contains whole cereal grains, seeds and legumes, which are a source of fibre, plant protein and minerals. The present study also demonstrated that ruwena extract significantly increased the in vitro growth of the tested *Lactobacillus* strain compared with nutrient agar alone. This finding suggests that ruwena contains nutritional components that might support the growth of the tested bacterium under the experimental conditions used. However, the specific component responsible for this effect was not identified, and the present findings do not establish ruwena as a probiotic or definitively demonstrate a prebiotic effect. Further studies are recommended to characterize the dietary fibre and carbohydrate composition of ruwena, identify the components responsible for the observed bacterial growth, evaluate its effects on a broader range of intestinal microorganisms, and investigate fermentation products such as short-chain fatty acids. In vivo studies are also needed to determine whether the observed in vitro effect is associated with measurable benefits to intestinal health. Ruwena represents a traditional food of potential nutritional and microbiota-related interest, and further investigation may help establish its biological properties and possible role within a healthy diet.

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Conflict of Interest: The authors declare that the work is original and has not been published before in any form. It is not under consideration for publication elsewhere. All authors have read and approved the work. The authors declare that they have no conflict of interest.

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