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Antidiabetic effect of iron-rich fortified amla (*Emblica officinalis*) Ready to Serve (RTS) health drink

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Abstract
Many pathological illnesses, including diabetes, have been treated using *Emblica officinalis*, also known as Amla in Indian Medicine. The main aim of this experimental study is to assess the effects of using Apple Cider Vinegar (ACV) as a preservative in Amla Salt-based Ready to Serve (RTS) beverage in order to preserve blood glucose levels and to investigate the various concentrations of adding ACV to extend the shelf life of the product. The Amla RTS beverages were prepared by adding ACV at four different concentrations (7%, 8%, 9%, and 10%) and subjected to sensory evaluation test at 30 days, 60 days and 180 days interval. At the ICAR-Krishi Vigyan Kendra in Tirunelveli, a study on diabetic Amla ready-to-serve (RTS) beverage was carried out from January to August 2022 with the goals of establishing a standard method of preparation, researching the shelf-life, and assessing the outcomes using sensory evaluation scores. Additionally, evaluation scores for sensory qualities like appearance, colour, flavour, taste, texture, and overall acceptability negligibly decreased from their maximum value, and beverage compositions exhibited no microbial development after six months of storage. It was found that the formulation of Amla RTS beverage prepared with 10% ACV ingredient was more acceptable, safer, and better at maintaining its quality attributes. It will be commercialized as a healthy diet beverage that's suitable for diabetics. This project could increase farmer income by developing a salt-based, ready-to-drink Amla beverage that will help diabetes patients maintain normal blood sugar levels.

Keywords: Indian mustard, path coefficient analysis

Introduction
Diabetes mellitus (DM) is a metabolic disorder that is on the rise and poses a severe danger to global public health. It affects around 300 million people worldwide (Radheshyam *et al.*, 2022) [18]. Although there are chemicals and biochemical agents that can assist regulate diabetes, there is no long-term treatment that will help a person recover fully from this condition (Verma *et al.*, 2018) [27]. The globe is looking for alternative medical treatments since using allopathic medicine might have negative side effects (Ikram *et al.*, 2021; Radheshyam *et al.*, 2022) [9, 18].

In India, herbal medicine and plants are used to treat diabetes as well as many other diseases. This gives rise to the novel idea of researching herbal treatments for diabetes as an alternative medication (Goel, B., & Nanda, 2022) [5]. Since ancient times, Amla (*Emblica officinalis*) fruits have been used to treat diseases, and laboratory research based on *in vivo* and *in vitro* tests has shown its function in the control of different pathologies. Its anti-diabetic, antibacterial, antioxidant, anti-inflammatory, hepatoprotective, neuroprotective, cardio protective, gastro protective, and immunomodulatory action has demonstrated its function in the management of health (Kulkarni & Ghurghure, 2018; Almatroodi *et al.*, 2020; Gul *et al.*, 2022) [13, 2, 7]. The composition of Amla fruit is listed in Table 1 (Hussain *et al.*, 2021) [8].

The Amla fruit in particular, which is used in Ayurveda as a potent rasayana and in medicine to treat diarrhea, jaundice, inflammation, and many other conditions, has medicinal effects in all of its components. Its high polyphenol content gives it anticancer effects (Shrivastava *et al.*, 2022) [19]. It can help mankind live longer, have better digestion, alleviate constipation, lower temperature, clear out blood, get rid of a cancer, cough, ulcers, anemia or asthma attack, strengthen heart, take care of eyes, increase hair growth, and sharpen mind (Kapoor *et al.*, 2020; Tewari *et al.*, 2021) [11, 23].

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Furthermore, it strengthens teeth and cleans the mouth. It also increases cell life by lowering the generation of free radicals and raising antioxidant levels (Grover *et al.*, 2015) [6]. Moreover, it drastically increases the amount of living cells and mitochondrial membrane potential, decreases apoptosis and oxidative stress, upregulated proliferator-activated receptor-gamma coactivator (PGC-1α), and downregulated vascular endothelial growth factor (VEGF) (Nashine *et al.*, 2019) [16]. The most significant antioxidant for avoiding many diseases is vitamin C. Compared to other popular Indian fruits, Amla has a higher proportion (252 mg per 100 g) of vitamin C (Srivasuki, 2012; Ismanto *et al.*, 2015; Tewari *et al.*, 2021) [21, 10, 23]. Table 2 depicts the dosage of vitamin C suggested for different age group by U.S. Recommended Dietary Allowance (RDA).

By combining the best compositions with the fresh Amla extracts, the present study focuses on preparing a natural fortified Amla Ready to Serve (RTS) health drink to treat diabetes and increase the product's shelf life.

Table 1: Composition of the fruit pulp of *Emblica officinalis*

Sl. No.	Nutrients	Amount per 100 g	Sl. No.	Nutrients	Amount per 100 g	Sl. No.	Nutrients	Amount per 100 g
1	Water	81%	7	Calcium	25 mg	13	Chloride	25 mg
2	Protein	1 g	8	Iron	1 mg	14	Vitamin C	720 mg
3	Total lipid	0.5 g	9	Magnesium	10 mg	15	Vitamin E	0.17 mg
4	Fibre	3.2 g	10	Phosphorus	21 mg	16	Vitamin A	290 IU
5	Carbohydrate	14 g	11	Potassium	198 mg	17	Vitamin B1	30 mg
6	Energy	170 kcal	12	Sodium	13 mg	18	Phosphorus	21 mg

Table 2: Recommended dosage of vitamin C

S. No.	Age Group	RDA/day	S. No.	Age Group	RDA/day
1	Infants - age below 1 year	30 to 35mg	4	Men - age over 18 years	90mg
2	Children - age 1 to 14 years	40 to 50mg	5	Women - age over 18 years	75mg
3	Adolescent - age 15 to 18 years	65 to 75mg			

Materials and Methods
Development of fortified Amla RTS with ACV: The fortified Amla RTS beverage was prepared in accordance with the FSSAI guidelines. The main ingredients in the prepared Amla RTS beverage were a combination of Amla juice, ACV at four different concentrations, curry leaves, ginger, salt, and water. The focus of this experimental analysis is to develop a fortified Amla RTS beverage using the aforementioned ingredients.

Experimental design and site: The formulation, standardization and acceptability experimental study of Amla RTS beverage was carried out at ICAR-Krishi Vigyan Kendra, Tirunelveli, Tamil Nadu of India in 2022. The most preferred fortified Amla RTS beverage formulation was selected based on sensory evaluation from Amla juice combined with curry leaves, ginger, salt, water and four varied percentages (7, 8, 9 & 10) of ACV. The methodology comprises the materials needed to prepare the Amla RTS beverage with varying concentrations of ACV and to examine the shelf life of the goods.

Amla RTS Beverage formulation and storage: With ingredients of 70ml, 80ml, 90ml, and 100ml/lit of ACV concentration, four distinct fortified Amla RTS beverage formulations were prepared. These formulations were then stored at room temperature (30 °C) and a relative humidity of 75-80% while sensory evaluation tests were conducted at predetermined intervals. The main ingredients of the fortified beverage are 1 kg of *Emblica officinalis* (Amla), 20 gm of curry leaves, 50 gm of ginger, 75 gm of salt, and 800 milliliters of water, in addition to the four different amounts of ACV. It is listed in Table 3. Two phases were involved in the formulation and standardization of fortified Amla RTS.

Phase I: Fresh *Emblica officinalis* (Amla) fruit weighing one kilogramme was purchased locally from Tenkasi area. It was thoroughly dried off with a towel after being washed with running tap water. After the seeds were removed, the Amla was crushed in a blender with the peeled ginger and water, and then the mixture was expressed to create juice. Then, the double boiling method was employed to boil the filtered juice for 50 minutes. ACV was added in four different concentrations-7%, 8%, 9% & 10% of each 100ml of Amla drink-after it had cooled to room temperature. Finally, four distinct containers with the names A, B, C, and D were filled with the prepared Amla RTS.

Phase II: The four separate containers are used to test four different ACV addition concentrations in an effort to find the best ACV addition for the fortified Amla RTS drink and develop a product that would be acceptable and taste nice for 180 days.

Table 3: Requirement of raw materials for Amla RTS drink preparation (4 replications)

Sl. No.	Ingredients	Quantities	Sl. No.	Ingredients	Quantities
1	Amla	1 kg (18nos.)	4	Curry leaves	20 gm
2	Water	800 ml	5	Apple Cider Vinegar	70ml, 80ml, 90ml and 100ml/lit of mixing formulation respectively
3	Ginger	50 gm	6	Salt	75gm

Fig 1: Steps in preparation of Amla RTS beverage

Table 4: Treatment with different level of Concentration (Apple Cider Vinegar)

Concentration of Apple Cider Vinegar	7 days	30 days	90 days	180 days
7%	Aroma & Taste is good	Fungal Formation	Fungal Formation	Fungal Formation
8%	Aroma & Taste is good	Fungal Formation	Fungal Formation	Fungal Formation
9%	Aroma & Taste is good	Discoloration	Fungal Formation	Fungal Formation
10%	Aroma & Taste is Excellent	Aroma & Taste is Excellent	Aroma & Taste is Excellent	Aroma & Taste is good

Table 2 shows the various ACV addition quantities needed to make a satisfactory Amla RTS beverage using four different ACV concentrations: 7%, 8%, 9%, and 10%. It has been found that 10% of ACV added over a 180-day period is the best amount to add to salted Amla drinks. The Amla RTS had a mildly sour taste, was a pale green color, and had a ginger scent.

Sensory Evaluation of Fortified Amla RTS Beverage
Sensory analysis is a process of identification, measurement, scientific analysis and interpretation of product attributes through the five human senses: sense of sight, smell, tasting, touch and hearing. An essential component of planning sensory analysis is the selection of skilled panellists. The quality characteristics of the prepared Amla RTS samples were evaluated in terms of appearance, colour, flavour, taste, texture, and overall acceptability by the trained panellists using a scorecard with a hedonic rating grade.

The samples placed in the four containers A, B, C, and D were scored using the grade descriptors in the table 5. The following discussion presents the findings of the assessment of panellists who indicated high value for the sensory analysis results related to the colour, taste, and flavour of Amla RTS beverage when various concentrations of ACV were added.

Table 5: Sensory evaluation score

I. Scale used		II. Sensory evaluation
5-point Hedonic scale		
Points	Attributes	Evaluated factors each of these
5	Excellent	1–Colour & Appearance
4	Good	2 –Aroma
3	Average	3 –Taste
2	Poor	4-Texture
1	Very Poor	5-Overall acceptability

The sensory evaluation score of Amla RTS beverage using the 5-point Hedonic is presented in Table 5 along with its attributes.

Results
Amla RTS beverage were prepared using a combination of Amla, ginger, curry leaves, salt, water and ACV at four different concentrations, and the prepared RTS attributes and shelf-life were assessed. The samples of Amla RTS beverage from four containers (A-D) were taken for this study. Semi-trained panel members from the product development team used a five-point hedonic scale to evaluate the product's sensory qualities (the Amla RTS beverage). Using a 10% concentration of Amla RTS beverage was the perfect ACV concentration needed to create a product that was acceptable. The mean sensory grades of overall acceptability of the four samples for diabetes are tabulated in (Table 6) using a five-point hedonic rating scale. Amla RTS beverage prepared as a fortified product had organoleptic scores for colour and appearance, aroma, taste, and overall acceptability of 5 during the evaluation periods of 0 days, 30 days, 90 days and 180 days respectively, according to the mean score reported in Table 6.

Table 6: Mean Sensory Evaluation results for 10% ACV - Amla RTS drink

Mean Sensory Evaluation results					
Evaluation Intervals	Colour & Appearance	Aroma	Taste	Texture	Overall acceptability
0 Day	5	5	5	5	5
30 Days	5	5	5	5	5
90 Days	4.9	4.9	4.9	5.0	4.9
180 Days	4.7	4.8	4.8	4.8	4.8
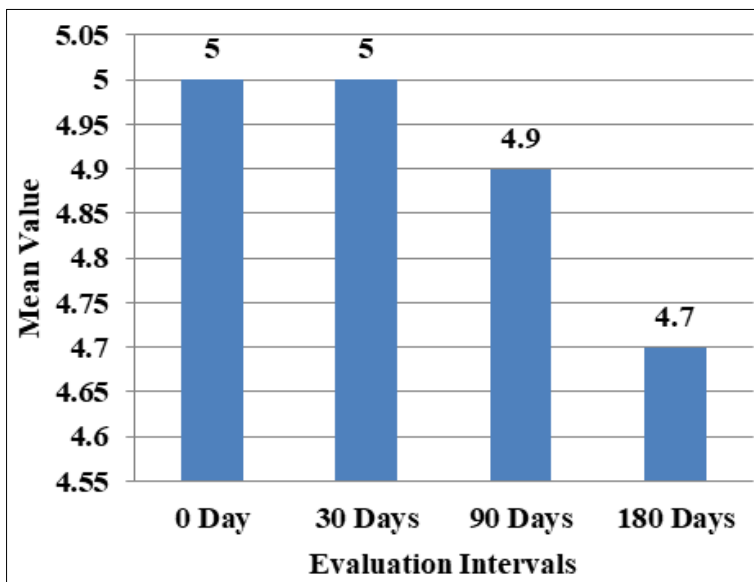
Fig 2: Sensory evaluation results - colour & appearance of Amla drink
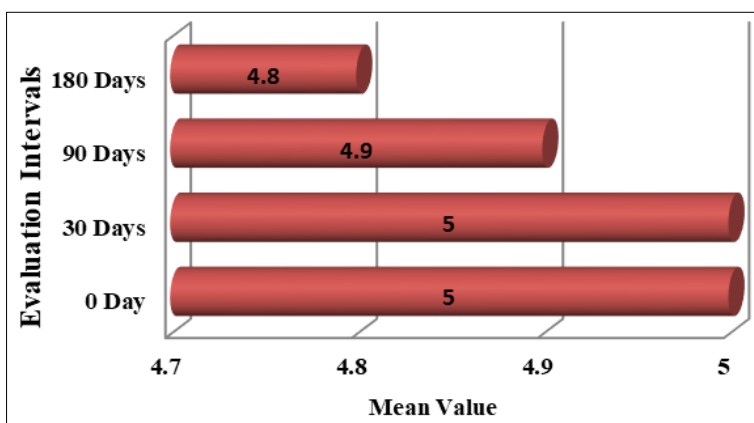

Fig 3: Sensory evaluation results – aroma of Amla drink
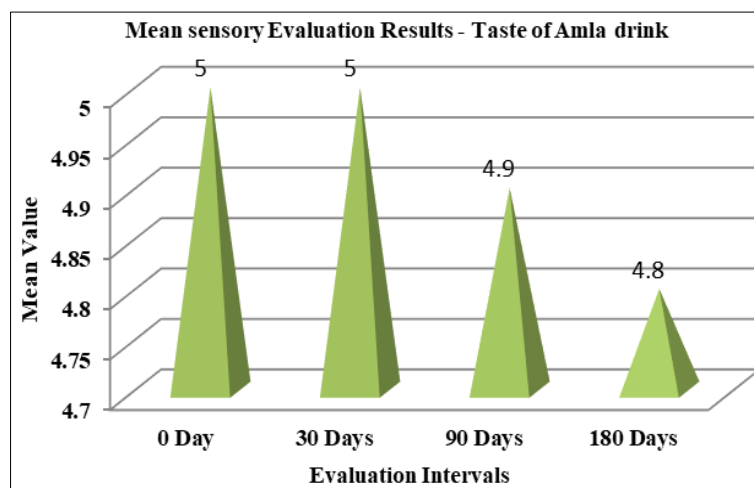

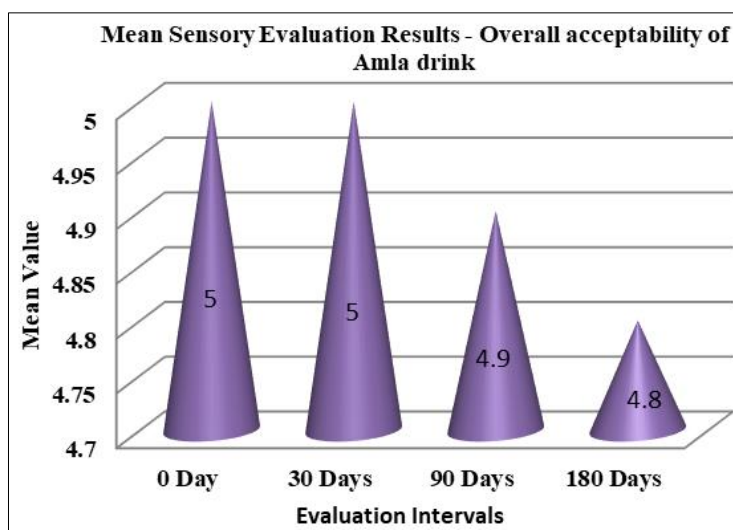**Fig 4:** Sensory evaluation results – taste of Amla drink

Fig 5: Sensory evaluation results – overall acceptability of Amla drink

Discussions

In today's wealthy cultures, diabetes mellitus (DM) has become a worldwide health hazard and a problem with lifestyle. Type 2 Diabetes Mellitus (T2DM), which accounts for more than 90% of all cases of diabetes and is characterized by impaired insulin action (Variya *et al.*, 2020) [25]. According to the International Diabetes Federation Atlas guideline report, 463 million individuals between the ages of 20 and 79 had a diabetes diagnosis in 2019, and that number is expected to rise to 700 million by the year 2045 (Majeed *et al.*, 2022) [14]. T2DM patients have hyperglycemia, often known as high blood sugar. People with diabetes who have hyperglycemia may be affected by a number of things. They consist of things like diet and exercise, health issues, and diabetes-unrelated drugs. Hyperglycemia can also result from skipping dosages, not using enough insulin, or using insufficient amounts of other blood sugar-lowering drugs. Treatment of hyperglycemia is crucial. It can worsen and lead to major health issues that need emergency treatment, including a diabetic coma, if it is left untreated. Even mild hyperglycemia that persists can cause issues with the heart, kidneys, eyes, nerves, and nervous system. The Amla's hypoglycemic (low blood sugar level) and antioxidant properties could be to responsibility for its effectiveness in protecting against induced hyperglycemia (Singh *et al.*, 2019) [20].

One of the most important risk factors for cardiovascular diseases (CVD) in diabetic patients is T2DM with dyslipidemia. The distinguishing characteristics of diabetic dyslipidemia, which is more atherogenic than general dyslipidemia, include increased triglycerides (TG), an excess of small, very-low-density lipoproteins (VLDL), remnant lipoproteins, postprandial hyperlipidemia, and decreased high-density lipoproteins (HDL). Due to insufficient glycemic control, patients with diabetic dyslipidemia are more likely to develop microvascular consequences as diabetic nephropathy, neuropathy, and retinopathy (Majeed *et al.*, 2022) [15]. On day 21, T2DM patients who had only received 3 g of Amla Fruit Exact (AFE) powder showed a substantial ($p<0.05$) reduction in total lipids. Normal and T2DM patients both had a substantial ($p<0.05$) improvement in HDL cholesterol and a reduction in low-density lipoprotein (LDL) cholesterol after taking 2 or 3 g of AFE powder (Akhtar *et al.*, 2011) [1]. It is

possible to treat both general and diabetic dyslipidemia with the AFE since it has demonstrated substantial potential in lowering total cholesterol (TC), triglycerides (TGs), lipid ratios, atherogenic index of plasma (AIP), and apoB/apo A-I levels in dyslipidemic individuals.

Furthermore, AIP is also related with estimation of T2DM (Upadya *et al.*, 2019) [24]. The main phytoconstituent in AFE, gallic acid, has been identified to be the biologically active component that gives AFE its ability to anti-hyperlipidemia (Variya *et al.*, 2018) [26]. The high fat diet (HFD)/Streptozotocin (STZ)-elevated blood glucose levels (BGL) and insulin levels were successfully reduced by AFE treatments, and Insulin Resistance (IR) was improved in all models used to assess IR (Panda *et al.*, 2021) [17]. The panel members found that the consumption of AFE cookies as a part of meal could control the T2DM level (Goel, B., & Nanda, 2022) [5]. It was found that Amla RTS beverages with a 0.5% dietary fibre fortification had the best sensory qualities and physical qualities (Surya *et al.* 2020) [22].

In this investigation, about 18 Amlas were used to make 1000 ml of Amla juice with the addition of ginger, curry leaves and water. Amla juice, which is high in vitamin C, is double-boiled for up to 50 minutes. Salt and ACV are then added in varying amounts. The appropriate dosage of the enriched Amla RTS drink was developed and standardized, helping to keep blood glucose levels in check and delaying human ageing. The approved fortified items were used in the sensory evaluation investigation. However, Amla RTS drink for 180 days has been proven to have the optimal amount of 10% ACV.

In present study, the mean value of sensory evaluation results from the responses of the 25-semi trained panellist for a period of 180 day with respect to the colour, appearance, aroma, taste and overall acceptability is illustrated as graph is depicted in figure 1, 2, 3, 4 and 5. On sensory evaluation of panellist the overall acceptability revealed excellent as per evaluation also the results of the final product were very encouraging. The shelf life of the products was of 180 days. Muhammad Shoaib Akhtari, 2015 [7], shared in his review article on "Effect of Amla fruit on blood glucose and lipid profile of normal subjects and type 2 diabetic patients", concluded that Amla fruit has both antihyperglycemic and lipid-lowering properties. It might be used as food supplement

in developing successful alternative therapies in the prevention and treatment of diabetes, dyslipidemia, obesity and cancers. The 25 semi-trained panellists who responded with the mean value of the sensory evaluation shown in Table 6 indicated that they approved of the product's colour and appearance. The research, which was conducted to assess the quality of the storage, yielded positive results. The chosen panellists participated in the sensory evaluation for 0 days, 7 days, 30 days, 90 days, and 180 days, and they approved of it. No effects of deterioration from storage at room temperature have also been recorded. The topic of "examining its role as an antioxidant" has been studied. Throughout the 180 days (6 months) of continuous study, no discernible seasonal influence of the product was seen. The panel lists unanimously agreed that the product's texture, flavour and taste were both pleasing. The results of this experimental study are presented in Figure 2-5.

Conclusion
To extend the shelf life of the product, Amla RTS beverages were made in the present study using a combination of Amla juice, ginger, curry leaves, salt, water, and ACV at four different concentrations. When choosing the optimal formulation to release the product for commercial purpose, maintaining quality parameter during storage is crucial. Based on the sensory characteristics and physical qualities of the four samples tested, the Amla RTS beverage fortified with 10% ACV was assessed to be highly preferable choice for the final product. The fortified Amla RTS beverage were found to have acceptable levels of formation, standardization, and good sensory features, a low microbiological load, and were stored under refrigeration settings for 180 days. Adults should use 80ml of Amla RTS every day, which is the recommended daily intake of concentrated Amla juice; anything more than this may have negative consequences on the health. The daily use of this fortified Amla RTS drink might be employed as a dietary supplement in the creation of effective alternative medicines for the treatment and prevention of cancer, diabetes, dyslipidemia, and obesity.

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