Manufacture of the LOVATSARA

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Abstract:
LOVATSARA is made from the peels of various fruits and vegetables, combined with water and sugar to create a concentrated liquid. Fruit and vegetable peels often contain a large number of vitamins, minerals, and other valuable elements for human health. Experiments conducted on individuals over the years have demonstrated the potential benefits of this product. Additionally, LOVATSARA can help reduce food waste by using parts of fruits and vegetables that are often wrongly discarded. Therefore, its use could offer both medical benefits and environmental sustainability advantages.
The preparation of LOVATSARA relies on a simple yet effective method. The peels, rich in often overlooked nutrients, are carefully cleaned and mixed with water and sugar. This mixture is then left to ferment, which allows the extraction and concentration of the nutrients contained in the peels. Fermentation, in addition to preserving the vitamins and minerals, can also produce additional beneficial compounds through the action of microorganisms. Fruit and vegetable peels are an abundant source of fiber, vitamins (such as vitamin C and vitamin A), minerals (such as potassium and calcium), and antioxidants. These compounds play a crucial role in maintaining health by helping to strengthen the immune system, improve digestion, and reduce the risk of chronic diseases such as heart disease and certain types of cancer. Preliminary studies on LOVATSARA have shown that regular consumption could improve vitamin and mineral levels in individuals, thus contributing to better overall health. Additionally, the antioxidants present in the peels can help combat oxidative stress, a factor in aging and many degenerative diseases. The production of LOVATSARA is also an example of a circular economy applied to food.
By reusing fruit and vegetable peels, which are often discarded, this practice helps reduce food waste. Reducing waste is essential for decreasing the carbon footprint of the food supply chain, conserving resources, and promoting a more sustainable use of food. Additionally, by transforming potential waste into a useful and nutritious product, LOVATSARA supports more sustainable food practices and raises awareness about the importance of valuing food waste.

Keywords : **LOVATSARA**

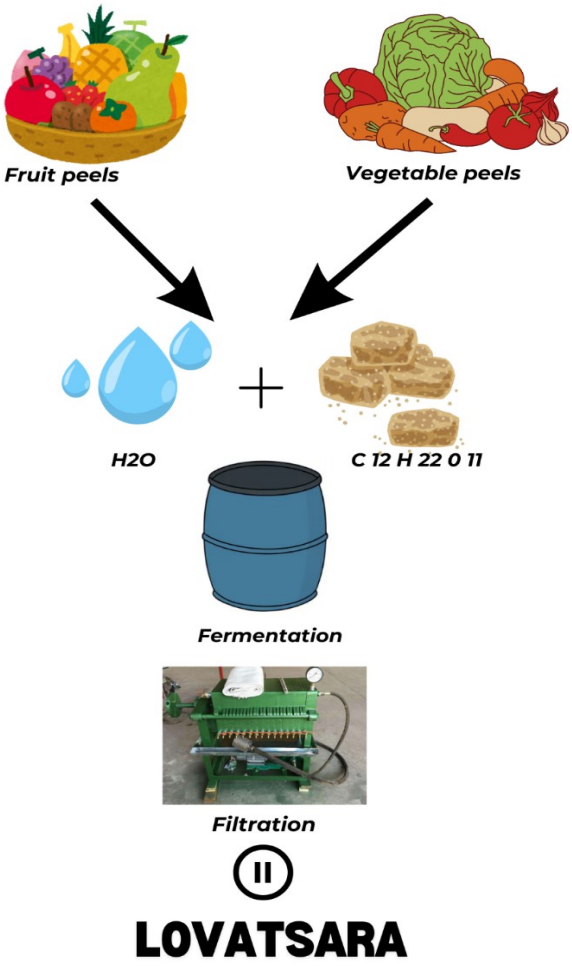
1. INTRODUCTION:

After decades of environmental degradation in Madagascar, the idea of rebuilding the ecosystem has gained traction. The adage "if you don't make a mess, you are already clean" illustrates this commitment. However, many fruit and vegetable peels are thrown away from households, even though they are the main components of LOVATSARA. According to current statistics, 30% of household waste in Madagascar consists of fruit and vegetable peels. Madagascar boasts a rich variety of fruits and vegetables spread across the island, offering a diverse palette of colors. For example, Madagascar has 36 varieties of bananas. The production of these goods comes from organic farming, which minimizes environmental risks. Moreover, Madagascar continuously produces using nature-friendly methods. Sugar, a basic ingredient, is also pure and environmentally friendly, derived from red sugar. This approach to sustainable and environmentally friendly production offers promising prospects for the preservation of Madagascar's natural resources.

1. Materials:

LOVATSARA: "LegiOma sy Voankazo Alona TSARA,"

which means well-fermented vegetables and fruits



2. METHODS :

2.1 Screening:

(FONG H. H., TIN-WA M., FARNSWORTH N. R., Phytochemical Screening Plants, Documents of Department of Pharmacognosy and Pharmacology; University of Illinois, 1977)

- Hydroalcoholic Extraction 80%

  10 to 20g of powder is suspended in 80% ethanol for one hour at a temperature of 70°C. The mixture is then filtered, and the filtrate is used for the analysis.

- Alkaloid test

  2 to 5g of plant powder are mixed with 10 to 15ml of 5% HCl, and the mixture is agitated for 5 minutes in a water bath. The mixture is then filtered through cotton. The filtrate is divided into four equal parts and placed into four test tubes.

  - In the first tube, five drops of Wagner's reagent are added.
  - In the second tube, five drops of Mayer's reagent are added.
  - In the third tube, five drops of Dragendorff's reagent are added.
  - The fourth tube serves as a control.

  The presence of precipitation, cloudiness, or flocculation indicates a positive result.

- Polysaccharide test

  100mg of ground plant material is added to hot distilled water, filling half of the tube. The mixture is boiled for a few minutes, then filtered through cotton. The filtrate is mixed with 95% EtOH in a volume three times that of the filtrate and placed in the freezer for 15 minutes. The appearance of a precipitate indicates the presence of polysaccharides.

➢ Saponin test

100mg of ground plant material is added to cold distilled water, filling half of the tube, and then shaken vigorously for 30 seconds. The initial foam height (t0) is noted. After 30 minutes, the foam height (t30) is measured. If the foam height is ≥ 3cm, the result is positive.

➢ Cyanogenic Glycoside test

5g of plant powder is moistened in a jar with a sufficient amount of water. 1ml of CHCl3 is added to the powder. A strip of Whatman No. 1 filter paper is dipped into a freshly prepared sodium picrate solution and air-dried. This paper strip is placed inside the jar just above the powder, folded over the edge of the lid. The jar is sealed and placed on a hot plate at 75°C for three hours. The presence of cyanogenic glycosides is indicated by a color change from yellow to red.

➢ Unsaturated Sterols and Triterpenes test

5 grams of sample are evaporated using a water bath. The depigmented residue is dissolved with n-hexane or petroleum ether. 10 ml of CHCl3 is added, then the mixture is stirred and agitated for 5 to 10 minutes. The obtained solution is dried with anhydrous Na2SO4 and then filtered. The filtrate is distributed into three test tubes, with tube no. 1 serving as the control.

- Tube no. 2: Liebermann-Burchard test: Add three drops of acetic anhydride and stir gently. Then add one drop of concentrated H2SO4. Observe the color change for one hour: a blue-green color indicates the presence of steroids, while a red-violet to pink color denotes the presence of triterpenes.
- Tube no. 3: Salkowski test: Tilt the tube at a 45° angle and add 1 to 2 ml of H2SO4. Note the immediate color change. Stir the mixture gently and note the gradual color change: a red color indicates the presence of unsaturated sterols.

➢ Test for Flavonoids and Leucoanthocyanins

3 grams of sample are evaporated using a water bath and then cooled to room temperature. The residue is depigmented using 15 ml of petroleum ether or n-hexane, then filtered. This process is repeated until the pigments are eliminated. If the solution is clear, the filtrate is dissolved in 15 ml of 80° ethanol, then filtered using cotton. It is divided into 5 test tubes, with the fifth tube serving as the control.

- Tube no. 1: Willstatter test: Add concentrated HCl in the presence of three or four magnesium turnings. Observe the color change: a shift to red, red-purple, or violet-red indicates the presence of flavonoids.
- Tube no. 2: Modified Willstatter test: Follow the same experimental protocol, but add 1 ml of distilled water and 1 ml of isoamyl alcohol to the second tube. The color of the upper phase indicates the class of flavonoids (flavones, flavanols, flavanones, and flavanols or FF).
- Tube no. 3: Bate-Smith test: Add 0.5 ml of concentrated HCl to the third tube and heat in a water bath for thirty minutes. The appearance of a red color indicates the presence of leucoanthocyanins.

➢ Test for Tannins and Polyphenols

5 grams of sample are evaporated using a water bath. Then, 12 ml of hot distilled water is added and the solution is stirred. The solution is divided into four test tubes, with each of them receiving 4 drops of 10% NaCl. The first tube serves as the control.

- Tube no. 2: Add four to five drops of 1% gelatin.
- Tube no. 3: Add four to five drops of salted gelatin. The appearance of a precipitate in both tubes indicates the presence of polyphenols and tannins.
- Tube no. 4: Add four to five drops of FeCl3 in methanolic solution. The coloration observed indicates the type of tannins present:
  - Blue-green or green-black indicates catechol-type tannins.
  - Blackish-blue or blue-black indicates pyrogallol-type tannins.

A negative reaction with salted gelatin accompanied by a green or blue-black coloration with FeCl3 suggests the presence of other types of phenolic compounds.

2.2 Measurement of ANTIOXIDANT CAPACITY USING THE DPPH RADICAL:

(Sharififar et al., 2007; Kivrak et al., 2009; Houbairi, 2015)

The preliminary evaluation of the antioxidant activity of the samples was carried out using the method of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Antioxidants possess the property of donating a single electron to the DPPH radical. DPPH (2,2-diphenyl-1-picrylhydrazyl) is characterized by its ability to produce stable free radicals. The presence of these DPPH radicals gives a dark violet coloration to the solution, which absorbs around 517 nm. The reduction of DPPH radicals by an antioxidant agent results in a discoloration of the solution (yellow color). Two methods were used for antioxidant activity. Firstly, a qualitative method to verify if the samples indeed have antioxidant activity, and secondly, the UV-visible spectrophotometry method to quantify the antioxidant activity of each sample.

Procedure

Thin Layer Chromatography (TLC Method - Qualitative)

The extract to be tested is spotted onto a TLC plate (Thin Layer Chromatography). It is then sprayed with a methanolic solution of 0.004% DPPH. A pale yellow or light-yellow spot against a violet background indicates the presence of antioxidant activity.

Spectrophotometry Method (Quantitative Test)

Preparation of the DPPH

Solution 2 mg of DPPH are weighed using a precision balance and dissolved in 50 ml of methanol to obtain a final concentration of 0.004%. The optical density of this solution is then measured using a spectrophotometer (SECOMAM). The optical density of 0.004% DPPH should range between 0.221% and 0.640% at 517 nm.

Preparation of different concentrations of the extract

The tested extracts are diluted in methanol to obtain the desired final concentrations for the assay, ranging from 1 to 0.06 mg/ml. 20µl of the concentrated sample solution to be studied are mixed with 2ml of DPPH in methanolic solution. The mixture is then stirred and placed in the dark for 30 minutes before the absorbance readings at 517 nm are taken. For each concentration, two trials are performed.

Additionally, a similar method was adopted with ascorbic acid, which is chosen as a positive control. This means a control consisting of 20µl of methanol and 2ml of the DPPH solution.

Calculation method

Calculation of inhibition percentages

The results are expressed as antioxidant activity. This indicates the ability to scavenge the free radical, estimated by the percentage of DPPH discoloration. It is given by the following formula:

$$P(\%) = \frac{DOcontrol - DOtest}{DOcontrol} \times 100$$

With P (%): percentage of DPPH reduction or inhibition

DO control: optical density of the DPPH solution

DO test: optical density of the tested extract

Calculation of IC50

By definition, the IC50 value of ascorbic acid or the extract, which can reduce 50% of the DPPH, is determined graphically. The IC50 values are calculated graphically using the regression formula of the inhibition percentages as a function of the different concentrations of the tested extracts. P (%) = f (C)

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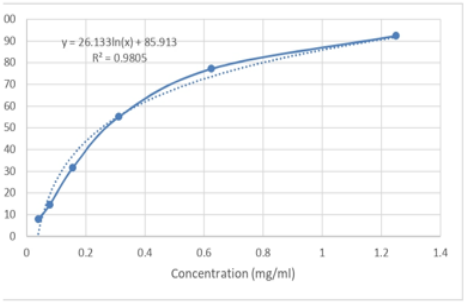
Acide ascorbique

C°	P%	DO	IC50	Doc
1.25	92.4	0.033		
0.625	77.19	0.099		
0.312	55.07	0.195	0.25	0.434
0.156	31.57	0.297		
0.078	14.52	0.371		
0.039	7.84	0.4		



2.3 Acute Toxicity Test: (Hodges et Sterner, 1943 ; Fontenelle *et al.,* 2007)

The toxicology test was conducted on SWISS mice (Mus musculus). They were housed in well-ventilated cages with ad libitum access to water and food. They were aged 6 to 8 weeks. This test was carried out in the animal facility of PBZT.

Classification:

Kingdom: ANIMALIA

Phylum: CHORDATA

Subphylum: VERTEBRATA

Class: MAMMALIA

Order: RODENTIA

Suborder: MYOMORPHA

Family: MURIDAE

Subfamily: MURINAE

Genus: Mus

Species: Mus musculus

Procedure:

The tested mice were fasted for 24 hours before administration. The product, at a dose of 2400 mg/kg, was injected into a group of 3 mice, while another group served as controls and received only distilled water. These mice had an average weight of 22.66g. The administration route was oral gavage, with a dose of 0.3ml for a 25g mouse. Signs of toxicity were observed immediately after administration. The mice were then housed in their cages under favorable conditions (water, food, and temperature).

The lethal dose 50, or LD50, is a quantitative indicator of a substance's toxicity. This indicator measures the dose of a substance that causes the death of 50% of a given animal population under specific experimental conditions. The lower the concentration, the more toxic the substance.
Determining the LD50 allows for the classification of products according to their toxicity, based on the Hodge and Sterner scale (1943).

Toxicity Class	Term used	Toxicological Parameter(LD50)
1	Extremely toxic	$DL_{50} \leq$ 1mg/Kg
2	Highly toxic	1mg/Kg $\leq DL_{50} \leq$ 50mg/Kg
3	Moderate toxic	50mg/Kg $\leq DL_{50} \leq$ 500mg/kg
4	Slightly toxic	500mg/Kg $\leq DL_{50} \leq$ 5000mg/Kg
5	Almost toxic	5000mg/Kg $\leq DL_{50} \leq$ 15000mg/Kg
6	Relative harmless	$DL_{50} \geq$ 15000mg/Kg

Test Result:

No signs of toxicity were observed after gavage, and the mice appeared to be in a normal state. No mice died after 24 hours, indicating that the LD50 is higher than the administered dose according to Hodge and Sterner, classifying the product as slightly toxic. According to Ramade, the dose is well above the threshold concentration of 25 mg/kg, below which any substance is considered toxic. We can assume that the product Lovatsara is not toxic. It is evident that the results obtained in mice cannot be directly applied to humans; however, these results reassure the safety of the product.

Les maladies	Posologies	Durée du traitement
Condition	**Dosage**	**Duration of Treatment**
Stomachaches	Children (2-4 years): 1-0-1 tsp	3 weeks
	Adults: 2-0-2 tsp	3 weeks
Abdominal pain	Children (2-4 years): 1-0-1 tsp	3 weeks
	Adults: 2-0-2 tsp	3 weeks
Liver disease	Children (2-4 years): 1-0-1 tsp	3 weeks
	Adults: 2-0-2 tsp	3 weeks
Dandruff	Apply twice daily	3 weeks
Acne	Apply twice daily	3 weeks
Wounds	Apply twice daily	3 weeks

3. CONCLUSION:

Over the past thirteen years, significant strides have been made in the medical field through human trials, both in traditional medicine and modern treatments. These advancements have led to a better understanding and treatment of a wide range of diseases, thereby improving the quality of life for many people around the world.
Simultaneously, it is crucial to recognize the importance of the environmental and economic impact of proper management of organic waste, such as fruit and vegetable peels. By encouraging recycling and composting practices, cities and countries can reduce their carbon footprint while creating cleaner and healthier environments for their residents. By limiting the amount of organic waste dumped in streets and markets, we contribute to environmental preservation and the fight against pollution.

Furthermore, effective management of fruit and vegetable waste can also have a positive impact on household incomes. By allowing farmers and producers to sell these wastes for use in composting or other products, we promote a circular and sustainable economy that benefits all stakeholders involved. The additional income generated can help improve living conditions in local communities and reduce economic inequalities.
In summary, the medical discoveries of the past thirteen years and the responsible management of organic waste are not separate aspects but rather interconnected elements of the same ecosystem. By investing in medical research and adopting sustainable environmental practices, we can create a healthier and more prosperous future for generations to come.