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STUDY OF THE NUTRITINAL VALUE AND ANTIMICROBIAL ACTIVEITY OF JUICE EXTRACTED FROM WATERMELLON WASTE

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ABSTRACT: Watermelon (Citrullus lanatus (Thunb.), family Cucurbitaceae) can be both the fruit and the plant of a vine-like (scrambler and trailer) plant originally from southern Africa, and is one of the most common types of melon. This flowering plant produces a special type of fruit known by botanists as a pepo, a berry which has a thick rind (exocarp) and fleshy center (mesocarp and endocarp); pepos are derived from an inferior ovary. We have prepared the juice from watermelon rind (white part) which we generally throw it instead of eating it because its taste, somewhat bitter than the red portions while the juice which is prepared from white portion is also very tasty and healthy, as this drink was tasted by many persons and they found it tasty.
Large content of Vitamin C, Riboflavin, Calcium, Moisture, Ash and many other nutrients are present in the juice. White part of watermelon has higher amount of ash, Calcium and vitamin C, so in some aspects the juice of white portion of Watermelon is considered better than Red portion.

KEY WORDS: Watermelon, Indole Test, Photoflurometer, Carbohydrate and Energy test, watermelon juices.

1. INTRODUCTION

Watermelon rind, both the green and the white parts, are loaded with nutrients including pro-vitamin A, potassium and zinc. For commercial plantings, one beehive per acre (4,000 m^2 per hive) is the minimum recommendation by the US Department of Agriculture for pollination of conventional, seeded varieties. Because seedless hybrids have sterile pollen, pollinizer rows of varieties with viable pollen must also be planted. Since the supply of viable pollen is reduced and pollination is much more critical in producing the seedless variety, the recommended number of hives per acre, or pollinator density, increases to three hives per acre (1,300 m^2 per hive).

A watermelon contains about 6% sugar and 92% water by weight. As with many other fruits, it is a source of Vitamin C. Notable is the inner rind of the watermelon, which is usually a light green or white color. This area is edible and contains many hidden nutrients that most people avoid eating due to its unappealing flavour. The amino acid citrulline was first extracted from watermelon and analysed.

Watermelons contain a significant amount of citrulline and after consumption of several kg, an elevated concentration is measured in the blood plasma; this could be mistaken for citrullinaemia or other urea cycle disorders.

MATERIAL AND METHODS

Procurement of samples and bacterial cultures

Watermelon:-
A fresh watermelon is brought from Saharaganj, Hazratganj, Lucknow.
Bacterialcultures: - E. coli, S. aureus
Verification of bacterial cultures
Reference: IS: 5887 (Part 1): 1976
Culture Name: Escherichia coli
Media:
1. Nutrient Agar
2. Eosin Methylene Blue Lactose Agar/ Tergitol-7 Agar/ Mac Conkey Agar
3. Gram's Stain kit and media for biochemical tests.

Procedure:

Microscopic Examination:

Perform the steps of gram staining and observe under 100X oil immersion lens. E. coli will appear as gram-negative rods.

Cultural Characteristics:

Observe the colonies on NA and EMB or MacConkey agar or Tergitol-7 Agar. Presence of colonies with typical green metallic sheen on EMB or yellow colonies with yellow zone on Tergitol-7 agar or pink colonies on Mac Conkey agar is a positive presumptive test.

Biochemical Characteristics:

For confirmation, perform following biochemical tests:

Indole Test:

Inoculate the suspected colonies in peptone water, incubate at 37°C for 24 h. Add 0.5ml of Kovac's reagent. E. coli being positive for indole test forms red ring on the upper surface.

Methyl red test:

Inoculate and incubate the specific medium at 37°C for 48 h. Add 2 drops of methyl red solution and observe for red colour formation. E. coli shows positive MR test.

Voges-Proskauer test:

Inoculate and incubate the suspected colonies in VP medium at 37°C for 24 hr. Add two drops of creatine solution, three drops of ethanol solution of 1-napthol and two drops of KOH & Shake it gently to mix. E. coli are negative towards VP test.

Simmon's Citrate test:

Inoculate the strain onto Simmon's citrate agar with young nutrient agar slant culture using straight wire. Incubate at 37°C for 4 days for growth of the organism. Change in the colour of the medium from green to blue confirms positive test. E. coli gives negative test.

Carbohydrate fermentation test:

Inoculate the test organism into peptone water medium and add the carbohydrates, using 1% concentration for the lactose, and incubate at 37°C for 18 h. Record the presence of acid from pink colour and that of gas in the Durham's tube

Culture Name: Staphylococcus aureus

Reference: IS: 5887 (Part 2): 1976

Apparatus and Equipment:

Oven, Autoclave, Water bath (45°C ± 1), Incubator (37°C ± 1°C), Tubes, Durham's tube, Total delivery pipette (1ml), Inoculation loop, pH meter.

Media:

1. Nutrient Agar
2. Blood Agar
3. Baird Parker Agar medium
4. Gram's Stain kit and media for biochemical tests.

sProcedure:

Microscopic Examination:

Perform the steps of gram staining and observe under 100X oil immersion lense. S. aureus will appear as gram-positive cocci arranged in clusters.

Cultural Characteristics:

Observe the colonies on NA, Blood Agar and Baird Parker Agar medium. S. aureus produces golden yellow colonies on NA (not always), shiny black colonies with or without narrow grey- white margin on BPA and golden yellow colonies (not always) with beta- haemolysis on blood agar.

Biochemical Characteristics:

For confirmation, perform following biochemical test:

Coagulase test

This test may be carried by the following methods:

a) Slide method: Emulsify a portion of the suspect colony in normal saline or water. Mix this with a straight wire dipped in human or rabbit plasma. Coagulase positive staphylococci produce visible clumping immediately.

b)Tube method: Emulsify a single suspect colony from a 24 h growth of blood agar medium in 1 ml citrated rabbit plasma diluted 1 in 5 in .85 % saline. The test is usually carried out in narrow tubes. Place in an incubator or preferably in water bath at 37°C. Observe every hour to note clotting of plasma. Reading should be carried out for as long as possible, preferably avoiding overnight incubation. Positive control with a known Coagulase positive strain of Staphylococcus and a control of diluted plasma without inoculum should be included in the test. (Tube method shall be preferred).

S. aureus show positive Coagulase activity.

TOTAL PLATE COUNT PRINCIPLE:

This method is used for determining the total count of bacteria present in the product. A small quantity of the sample is mixed with a suitable Nutrient agar medium and rolled into a Petri-dish. After the agar has set, the plates are incubated at a specific temperature for a definite period of time and the bacterial colonies that develop on plates are counted. Each colony is presumed to have grown from bacterium or clump of bacterial cells present in the inoculums. The total number of colonies counted on the plates multiplied by the dilution factor is taken to represent number of viable organisms present in the sample. It should be borne in the mind that there is no medium, which is equally favorable to the growth of all species of bacteria present in the sample. Accordingly the agar colony count obtained under specified conditions is only approximate of the total number of organisms in the product.

MATERIAL REQUIRED:

Incubator (37^0C), Bacteriological delivery pipettes, Dilution blanks, Petri-dish, Plate count agar, sample etc.

PROCEDURE:

1. Perform serial dilution of sample.
2. Aseptically pipette 1ml sample dilutions into labeled Petri dishes.
3. Add melted agar media that has been cooled to approximately 44 - 45°C.
4. Mix well by slightly rotating plate with bacteria and agar mixture.
5. Allow the agar to solidify, trapping bacteria at separate discrete positions within the medium.
6. Incubate plates in a favorable environment.
7. Count the number of colonies and calculate the number of microorganisms in the original sample.

CALCULATION:

Total plate count = Number of colonies ×dilution factor

COLIFORM COUNT

MATERIALS REQUIRED: Incubator, VRBA (Voilet Red Bile agar), petriplates.

PROCEDURE:

1. Select the dilution according to the expected coliform.
2. Take sterile Petri dishes for the test to perform.
3. Select the dilutions to inoculate the sample.
4. Inoculate 1ml sample of water in each Petri dish through the pipette.
5. Do not inoculate control Petri dish with the sample.
6. Pour 15-18ml of sterile VRBA media in each test tube.
7. Mix the medium thoroughly with sample in Petri plates.
8. When media is solidified, invert the plate & keep for incubation at 37°C for 24-48 hours.

OBSERVATION:

Observe the characteristics colonies which are pink in colour in Petri plates nd count it.

CALCULATION:

Coliform count = Number of colonies ×dilution factor

Screening of antibacterial activity of watermelon juices

Agar-well diffusion technique (Iroegbu and Nkere, 2005) was used for determining antibacterial activity of aqueous extracts and crude juices of reference spices. The technique involved the following steps:

a) Freshly prepared Nutrient Agar media was poured in plates with 20 ml of appropriate media.

b) 24 hrs. Bacterial culture suspension was swabbed with sterile swab on solidified Nutrient Agar plates for uniform digging of wells.

c) Sterile cork borer (diameter, 5mm) was used to bore wells in the solified media plates previously seeded with bacterial inocula.

d) Subsequently, different volumes of test substances were introduced in the wells of agar plates.

e) The sterile distilled water, instead of test samples of spices served as negative control.

f) All petridishes were sealed with sterile laboratory parafilm tape to avoid eventual evaporation of the test samples.

g) These plates were allowed to stand at room temperature for atleast 1hr for the even diffusions of poured components and were incubated without inversion at their respective incubation temperatures in B.O.D. incubator for 24-48hr.

h) After the incubation, zones of inhibition formed around the wells measured in mm and the results were expressed as the net zone of inhibition (mm) which represented the substraction of the diameter of the well (5mm) from the measured zone.

Methods for nutritive analysis of watermelon samples:-

ASH

Requirements: - Muffle crucible, samples and gloves.

Note:-Preserve the dish containing this ash for the determination of acid insoluble ash.

Calculation:-Total ash (on dry basis) % = $100(W_2\text{-}W)/W_1\text{-}W$

Where, W_2=Mass in gram of the dish with the ash

W=Mass in gram of the empty dish and

W_1= Mass in gram of the dish with the Dried material taken for the test

MOISTURE

Requirements: - Petri plates, oven, sample, gloves.

Calculation:-Moisture % by mass =$100(W_1\text{-}W_2)/W_1\text{-}W$

Where, W_1=Mass in gram of the dish with material before drying

W_2=Mass in gram of the dish with the material after drying and

W=Mass in the gram of the empty dish.

CALCIUM

Reagents:-1.dilute HCL-1:1(50ml H_2O+50ml HCL)

2. Dilute HNO_3-1:1(50ml H_2O+50mlHNO_3)

3. Concentrated HCL

4. Ammonium Thiocyanate Solution:-50% (50gram in 100ml H_2O)

Calculation:-

Calcium content in gram/100gram=10X/VW

Where, X=volume in ml of 0.05N $KMnO_4$ (1ml 0.05N $KMnO_4$=0.001gram)

V=volume in ml of the solution A taken for the test

W=weight of the sample

IRON

Reagents:-

1. Concentrated H_2SO_4 (Iron free)

2. Saturated Potassium Persulphate ($K_2S_2O_8$)

Solution:-Take 7 to 8 gram of reagent grade iron free potassium per sulphate with 100ml of water in a glass stopper bottle. The undissolved excess settler to the bottom and compensates for loss by decomposition, shake briefly before using keep the reagent in refrigerator.

3. Potassium thiocyanate (KSCN) 3N Solution:-

Dissolve 146 gram of reagent grade potassium thiocyanate in water and dilute to 500ml, filter if turbid. Add 20ml of pure acetone to improve the keeping quality.

4 .Standard iron solution:-Dissolve 0.702 gram of reagent grade crystalline ferrous ammonium sulphate [$FeSO_4$ $(NH_2)SO_4..6H_2O$] in 100ml of distilled water. Add 5ml of concentrated H_2SO_4 warm slightly and add concentrated potassium permanganate solution drop by drop. Transfer it into a one liter volumetric flask, rinse with water and makeup volume. This solution contains 0.1mg of ferric iron per ml and is stable indefinitely.

Calculation:-

Iron (mg/100gm) = OD of sample*0.1*total volume of ash

Solution*100/OD of the standard*5*wt of Sample taken for ash

SUGAR

Reagents:-

Stock solution of dextrose, Fehling solution A & B (soxhlet modification).

Calculation: -

1. Formula for invert sugar = Fehling factor*volume

makeup*100/T.V*sample weight

2. Sucrose = $(R_1\text{-}R)*0.95$

3. Total sugar% = Reducing sugar + sucrose

FAT

Reagents:-HCL (8N), Ethanol 95% (v/v), Petroleum ether, Glass beads

Calculation:-

Fat % = $(W_2\text{-}W_1)100/W$

Where W_2 = Dry weight of thimble,

W_1 = Blank weight of thimble,

W = Weight of sample taken

PROTEIN

Reagents:-

1. Potassium sulphate anhydrous (K_2SO_4) = 7gm
2. Selenium power (Se) = 5 mg
3. Concentrated sulphuric acid 96% (H_2SO_4) = 12ml
4. Hydrogen peroxide35% (H_2O_2) = 5ml

Calculation:-

Protein% = T.V*N of acid*2.809*factor * 100/sample wt
*0.2*1000

CARBOHYDRATE AND ENERGY

Calculation:-

Formula of carbohydrate % = 100-(ash +moisture +fat +protein).
Formula of energy (calorific value) % = 4(carbohydrate + protein)+ (9*Fat).

RIBOFLAVIN

Apparatus: -

Photoflurometer: - Use a flurometer suitable for accurately
Measuring fluroscence of solutions containing Riboflavin in concentration of 0.05 to 0.20ug/ml. A flurometer having the input filter of narrow transmittance with maximum of about 440mu and the output filter of narrow transmittance with the maximum of about 565mu has been found satisfactory.

Reagents:-

1. Standard HCL of 0.1N
2. NaOH 4% (W/V)
3. Dil. HCL 1:1 (V/V)
4. Riboflavin stock solution
Add 50 mg of USP riboflavin reference std. or equivalent IP standard previously dried and stored in dark in a dessicator over phosphorus pentaoxide to about 300ml. of 0.02N acetic acid and warm the mixture on a steam batch with constant stirring until the riboflavin is completely dissolved. Cool and then add 0.02Nacetic acid to make the volume to 500ml.
5. Riboflavin stock solution2:- 250 ml of the riboflavin stock solution 1, add 0.02N acetic acid soln. to make 500ml, store the solution under toluene in the cold in a dark bottle. 1ml of this solution is equivalent to 1ug of riboflavin.
6. Standard riboflavin solution:- dilute 10ml of the riboflavin stock solution 2 with water to make 100ml. 1ml of this solution is equivalent to 1ug of riboflavin. Prepare this solution freshly for each assay.
7. Glacial Acetic Acid
8. Potassium permanganate solution:- Dissolve 4gm of $KMnO_4$ crystals in 100 ml of water, keep for a few days, filter and store in a dark bottle.
9. 3% H_2O_2 Solution.
10. Na- diothionate of high purity, unexposed to light or air should be taken.

Calculation:-

Calculate the riboflavin content of the samples on the basis of aliquots taken as follows:
Mg of riboflavin/ml of the sample solution = B-C/A-B*1/10*1/1000

Note:-The value of B-C/A-B shall not be less than 0.66 and not more than 1.5. Express the results as riboflavin mg/100g.

VITAMIN C

Reagents: -

1. Trichloroacetic acid (TCA) reagent:-10% , dissolve 10g of TCA in 100ml of water.
2. Metaphosphoric acid = 5%
3. Standard ascorbic acid solution: - Weigh approx.100mg of USP ascorbic acid reference standard or equivalent IP standard accurately to 0.1mg.Transfer it to a 100ml glass Stoppard graduated flask, dissolve and dilute to the mark with the TCA or Metaphosphoric acid.
4. Standard indo-phenol solution:- Dissolve 50mg of sodium 2,6-dichlo-obenzenone indophenol, that has been stored in a dessicator over soda lime, in 50ml of water to which 42mg of Na_2CO_3 has been added, shake vigorously & when the indophenol has completely dissolved. Dilute it to 200ml with water. Filter the solution through a fluted filter paper into an amber glass Stoppard bottle. Keep the bottle Stoppard, out of direct sunlight & store in a refrigerator. Decomposition products that make the end point indistinct occur in some batches of dry indophenol & also develop with time in stock solution. Add 5ml of the TGA reagent or Metaphosphoric acid containing excess of ascorbic acid to15ml of the indophenol solution. If the reduced solution is not practically colorless, discard & prepare a new stock solution. If the dry indophenol dye is proved to be of a bad quality, obtain a new sample.

Calculation:-

Calculate the vitamin c content in the sample as follows:-

Vitamin c, mg/100g of the sample = A*B*1000/W

Where, A= vol. in ml of the indophenol solution used for titration.

B= weight in mg of the ascorbic acid equivalent to 1ml of the indophenol solution.

W= weight mg of the sample taken for the test.

TDS/BRIX

Theory: - Degrees Brix (symbol °Bx) is a unit representative of the sugar content of an aqueous solution. One degree Brix corresponds to 1 gram of sucrose in 100 grams of solution and thus represents the strength of the solution as a percentage by weight (% w/w) (strictly speaking, by mass). If the solution contains dissolved solids other than pure sucrose, such as other sugars, minerals etc., then the °Bx only approximate the dissolved solid content. The °Bx has traditionally been used in the wine, sugar, fruit juice, honey and other industries. It is intended to represent exactly the same thing as the degree Plato (°P), widely used by the brewing industry, and the degree Balling which, while it is the oldest of the three, is still in use in some parts of the world and found in textbooks which are considered current today. While all three are intended to represent the same thing (the number of grams of sucrose in 100 grams of solution) in fact they do not, though the differences are small.

RESULTS

Results of bacterial identification

E.coli:-

S.No.	*Test*	*Result*
1	Microscopic Examination	Gram negative rods
2	Cultural Characteristics	
	• MacConkey agar	Red colonies with pinkish zone
	• EMB	Colonies with green metallic sheen
	• Tergitol-7 Agar	Yellow colony with yellow zone
3	Indole Test	+ ve
4	Methyl red test	+ ve
5	Voges-Proskauer test	- ve
6	Simmon's Citrate test	- ve
7	Carbohydrate fermentation test	+ ve

S. aureus

S.No.	*Test*	*Result*
1	Microscopic Examination	Gram positive cocci
2	Cultural Characteristics	
	• Nutrient Agar	golden yellow colonies
	• Baired parker Agar	shiny black colonies
3	Coagulase Test	+ ve

Results for total plate count

S.No.	*Test*	*Result*
1	Microscopic Examination	Gram positive cocci
2	Cultural Characteristics	
	• Nutrient Agar	golden yellow colonies
	• Baired parker Agar	shiny black colonies
3	Coagulase Test	+ ve

Results for Coliform count = absent /25ml

Result for Nutritive analysis of Samples:-

S.No.	*Samples of Watermelon juice*	*%age of Ash content*
1.	*Raw Sample*	*0.705*
2.	*Processed Sample*	*1.23*
3.	*Pudina Flavored*	*0.4332*
4.	*Ginger Flavored*	*0.990*

Results for Moisture test of different Samples:-

S.No.	*Samples of Watermelon juice*	*%age of Moisture content*
1.	*Raw Sample*	*97.02*
2.	*Processed Sample*	*97.32*
3.	*Pudina Flavored*	*92.24*
4.	*Ginger Flavored*	*90.528*

Results for Calcium test of different Samples:-

S.No.	*Samples of Watermelon juice*	*Calcium content in mg/100gm*
1.	*Raw Sample*	*34.78*
2.	*Processed Sample*	*35.78*
3.	*Pudina Flavored*	-
4.	*Ginger Flavored*	-

Results for Sugar test of different Samples:-

S.No.	Samples of Watermelon juice	%age of Sugar content			
		Invert sugar(R_1)	Reducing sugar(R)	Sucrose (S)	Total Sugar
1.	Raw Sample	8.71	8.32	0.37	8.68
2.	Processed Sample	8.71	8.31	0.38	8.69
3.	Pudina Flavored	8.57	8.32	0.37	8.68
4.	Ginger Flavored	8.67	8.31	0.38	8.69

Results for Fat test of different Samples:-

S.No.	Samples of Watermelon juice	%age of Fat content
1.	Raw Sample	0.0011
2.	Processed Sample	0.195
3.	Pudina Flavored	0.303
4.	Ginger Flavored	0.177

Results for Protein test of different Samples:-

S.No.	Samples of Watermelon juice	%age of Protein content
1.	Raw Sample	0.590
2.	Processed Sample	0.5092
3.	Pudina Flavored	–
4.	Ginger Flavored	–

Results for Carbohydrate and Energy test of different Samples

S.No.	Samples of Watermelon juice	%age of Carbohydrate content	Energy Content in Kcal/100gm
1.	Raw Sample	1.38	7.89
2.	Processed Sample	1.045	7.97
3.	Pudina Flavored	6.5146	30.822
4.	Ginger Flavored	7.7958	34.813

Results for Vitamin C Content of different Samples:-

S.No	Samples of Watermelon juice	%age of Vitamin C content
1.	Raw Sample	5.675
2.	Processed Sample	5.675
3.	Pudina Flavored	-
4.	Ginger Flavored	-

Results for TDS/Degree Brix of different Samples:-

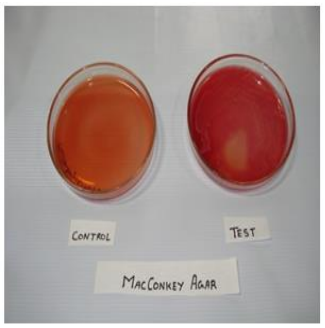
S.No.	Samples of Watermelon juice	%age of Degree Brix
1.	Raw Sample	3
2.	Processed Sample	11.7
3.	Pudina Flavored	12.01
4.	Ginger Flavored	12.7

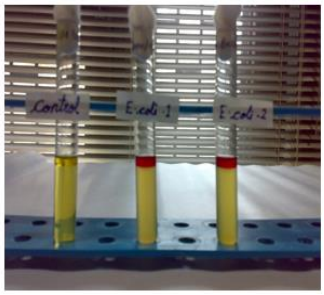
Results for Riboflavin of Different samples:-

S.No.	Samples of Watermelon juice	%age of Riboflavin
1.	Raw Sample	1.0
2.	Processed Sample	0.98
3.	Pudina Flavored	1.02
4.	Ginger Flavored	0.99

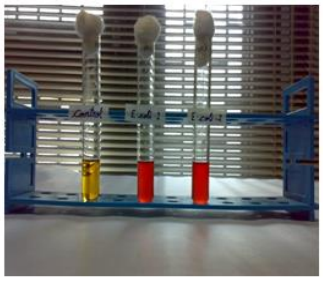
Antimicrobial activity of watermelon juices:-

Biochemical tests of E.coli





IndoleTest



MR Test

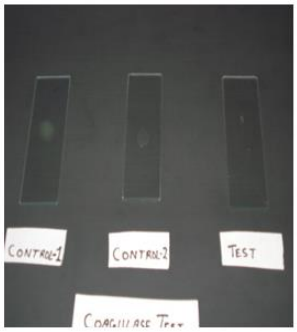
VP Test

Simmon citrate Test

Carbohydrate fermentation Test

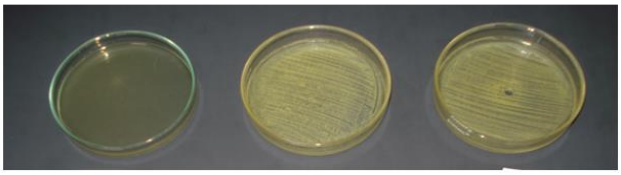
Biochemical tests for S.aureus

Test on NA



Coagulase Test

Figure for antimicrobial activity of watermelon juices:-



Control1 Control2 Control3

CONCLUSION

From the above results we finally came to the conclusion that waste of watermelon is also very useful and it is good for our health. We have prepared the juice from watermelon rind (white part) which we generally throw it instead of eating it because its taste, somewhat bitter than the red portions while the juice which is prepared from white portion is also very tasty and healthy, as this drink was tasted by many persons and they found it tasty.

Large content of Vitamin C, Riboflavin, Calcium, Moisture, Ash and many other nutrients are present in the juice. White part of watermelon has higher amount of ash, Calcium and vitamin C, so in some aspects the juice of white portion of Watermelon is considered better than Red portion.

So we should make this drink useful and must be launched in the market as instead of throwing the white portion. The juice with lemon flavored, pudina etc. will create a taste in it and will be benefited to reduce weight and it's a healthy diet.

As we observe from the results that the content of minerals, vitamins, protein and energy is more in the juice of white part , while the fat content is low or negligible as compared to the red portion. From microbiological point of view we observe that the total plate count (bacteria) of the white part juice is less than the red part juice and if preserved properly at high temperature then it could prove to be a healthy nutritional drink. Thus we conclude that the juice of white part of water melon is more beneficial and healthier as compared to the red portion. There is no zone of inhibition shown by bacteria in the sample because the extract which is taken is low in concentration, if concentration of extract taken in higher amount then zone of inhibition shown by the sample.

We prepare the sensory table of the prepared watermelon juice to perform the organoliptic test. So according to scoring of this test the pudina flavor of watermelon juice is more acceptable, but the test of other juice is also good and accepted by people.

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