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Potent Application of the Lyophilized Aqueous Leaf Extract of *Euphorbia hirta* (Tawa-Tawa) in the Development of a Naturally Flavored Ice Cream

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ABSTRACT

Euphorbia hirta (Tawa-tawa), also known as “gatas-gatas”, is a hairy herb grown in open grasslands, roadsides and pathways. This indigenous plant is considered as one of the most popular folkloric treatment for dengue in the Philippines. When researched, Tawa-tawa was found to be able to promote cell production, and to prevent platelet destruction (Philippine Council for Health Research and Development, 2013). In this study, a new application of the plant was evaluated. This was as a natural supplement for ice cream production without the degradation of its essential compounds. Fresh leaves of *E. hirta* were oven dried and macerated to water for 48 hours. The obtained extracts were subjected to lyophilization to prevent potential degradation of plant's essential compounds. Ice cream formulation was added with the obtained powdered extract of *E. hirta*. Results revealed that the lyophilized extracts from *E. hirta* leaves contained flavonoids, alkaloids, phenols and saponins. Promising amounts of Vitamin B₂ (5.02 mg/kg), Beta-carotene (9.8 mg/kg), Vitamin E (23.8 mg/kg) and Vitamin C (80.2 mg/kg) were also obtained. Meanwhile, the *E. hirta* supplemented ice cream was negative to *Salmonella* and a 16 CFU/g for Total Coliform Count was found. What is more, organoleptic testing for both taste and texture revealed excellent responses and very good aroma. The findings of the current study affirmed the feasibility of developing a naturally-based ice cream product supplemented with a medicinal plant like *E. hirta*. The results indicate that the developed ice cream is safer and healthier, hence could encourage everyone to enjoy the delights of eating ice cream without worry of gastronomic distress.

Keywords: *Euphorbia hirta*, Healthy ice cream, Antioxidant vitamins, Phytochemicals, Folkloric plant, Indigenous medicinal plant

1. INTRODUCTION

Ice cream is one of the most favourite treats in the Philippines. But then, continued consumption of it may cause serious health problems. It is a smooth, sweet, cold dessert food prepared from a frozen mixture of milk products and flavorings, containing a minimum of 10% milk fat (Karaman et al., 2014). The mixture is homogenized after pasteurization and aged to improve the physical properties before the freezing process. Ice cream is a representative frozen dairy product enjoyed by people of all ages due to its cooling effect in the mouth. Nowadays, manufacturers continue to develop formulations of ice cream mixtures according to consumer demands, resulting in the creation of various brand.

In our time, commercially produced regular ice cream has become a brew of chemicals and synthetic ingredients, it also contains sweeteners like high fructose corn syrup which is linked to diabetes, neurobehavioral disorders like ADHD in children, and decreased mental acuity in adults (Goraya & Bajwa, 2013). They cannot afford to eat such product because it will be too risky for their health. Naturally claimed flavored ice cream like mango, banana, jackfruit, peanut, avocado ice cream etc. had undergone many processes with chemicals and artificial additives as preservatives. This shows no assurance of a safer ice cream product.

Additionally, people suffering from hypertension cannot eat ice cream because it can bump up the risk of heart-related diseases because it has about 14.5 grams of total fat and 9 grams of saturated fat in each cup. Furthermore, research published in the April 2010 edition of "The Journal of the American Medical Association" found that a diet rich in sugar promotes increased cholesterol levels. Too much cholesterol in your blood leads to high blood pressure, increasing your risk for cardiovascular disease (Verma, Ansari, & Broadway, 2018).

Euphorbia hirta (Tawa-tawa), also known as "gatas-gatas", is a hairy herb grown in open grasslands, roadsides and pathways. This indigenous plant is considered as one of the most popular folkloric treatment for dengue in the Philippines. Also, several studies claimed that the local herb was able to promote cell production, and prevents platelet destruction.

This study aimed to develop an ice cream product which is safer and healthier supplemented with *Euphorbia hirta* (Tawa-tawa) leaf extract. Selected vitamin contents, and phytochemicals of the used plant were also evaluated in this study to assure the health benefits that can be obtained from the produced ice cream product. In addition, the *Total Coliform* and *Salmonella Counts* were also evaluated and tested to assure safety for intake of the product. Also, physical and chemical properties of the produced ice cream product were also tested.

The development of the Tawa-tawa ice cream is a new leap among the ice cream development promoting purely naturally based-major components which resulted into a safer and healthier product. This could also help health conscious people particularly those with diabetes and hypertension to continuously enjoy the delights of eating ice cream without any hesitations and health risks. The development of Tawa-tawa ice cream promotes healthy way of living.

2. MATERIALS AND METHODS

Collection and Preparation of Materials

Fresh *Euphorbia hirta* (Tawa-tawa) leaves were gathered at Malitbog, Bongabong, Oriental, Mindoro. Sample of the *E. hirta* was identified at the Institute of Biology in the

University of the Philippines-Diliman. Other materials in the current study were all-purpose cream, condensed milk, pasteurized buffalo milk, peanuts, purple yam and cheese. These were purchased in the local supermarket in the area.



Figure A. The collected *Euphorbia hirta* (Tawa-Tawa) leaves

Extraction of the E. hirta (Tawa-Tawa) Plant

In extracting the plant, 50g of its leaves has been washed with distilled water to remove extra dirt. The leaves were oven dried, turned into powder and macerated to water for 48 hours (1:5 w/v). The obtained extracts were filtered to remove solidified particles. The extracts were put in a glass container, properly covered and set aside for future use. The aqueous extract was then subjected to lyophilization for 80 hours to obtain the powdered form of the extract and to avoid degradation of the plant compounds.

Phytochemical Testing of the Euphorbia hirta (Tawa-tawa)

Test for Alkaloids

A 2 ml test solution was mixed with little amount of dilute hydrochloric acid and Mayer's reagent. Formation of a white precipitate indicated the presence of alkaloids.

Test for Flavonoid (Shindo's Test)

A few magnesium turnings and a few drops of concentrate hydrochloric acid were added in a 2 ml test solution and were boiled for 5 minutes. Appearance of red or red orange color indicated the presence of flavonoid.

Test for Phenol

A few drops of ferric chloride solution were added in the 2 ml plant extract. Bluish green or red color indicated the presence of phenol.

Test for Saponin

The test solution was shook with water. Copious lather formation indicated the presence of saponin.

Standardized Testing for Antioxidant Vitamins

The plant material has been sun dried and powderized using grinding mill obtaining 1000 grams of powder. From this, 500 grams was set aside as sample for standardized laboratory testing of *Euphorbia hirta* (Tawa-tawa) plant regarding its antioxidant vitamins like Vitamins C, and E, B₂ and β -carotene at the Department of Science and Technology (DOST) - Industrial Technology Development Institute (ITDI) standard and testing division in the Philippines.

Creating the Ice Cream

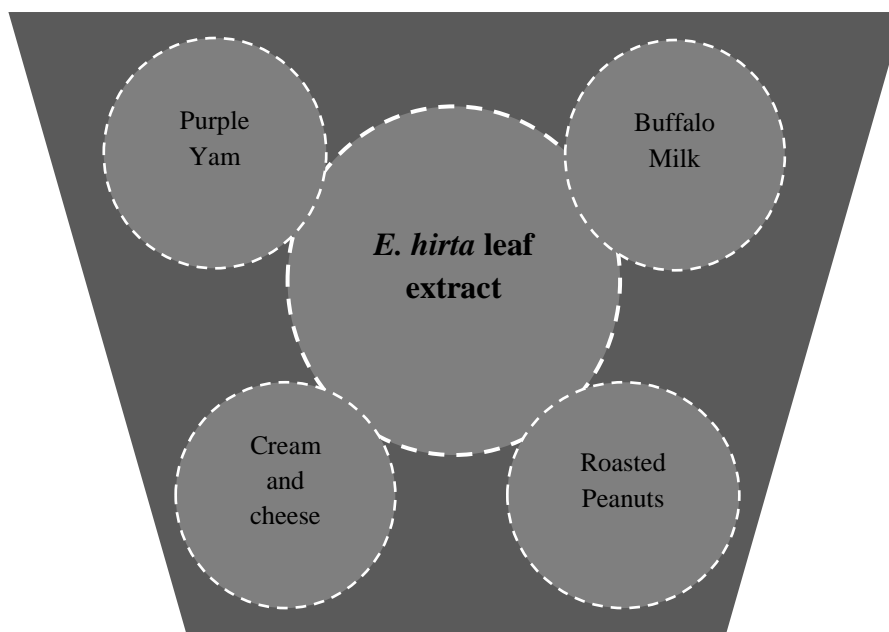


Figure B. Anatomy of the *E. hirta* (Tawa-tawa) Ice Cream

100 ml of condensed milk and 200 ml of heavy cream and buffalo milk were poured in a food mixer and mixed for about 10 to 15 seconds until totally combined.

The mixture has been added with 30g of graded purple yam (*ube*) and cheese and 10g of powdered peanuts as add-ons. The initial mixture has been mixed with 250 grams of Tawa-tawa extract and blended again until the mixture became smooth.

The blended mixtures have been spooned in containers and properly sealed. It was then placed inside the freezer at -10 to -20 °C at about 1-2 hours until almost firm.

In addition, an ice cream without Tawa-tawa extracts was also produced and used as negative control for the experimentations.

Testing for Total Coliform and Salmonella

300 grams of the produced ice cream and the negative control were prepared as sample for the standardized testing for *Total Coliform* and *Salmonella Counts* at Chempro Analytical Services Laboratory - FDA approved Laboratory.

Testing for pH of the product

In determining the pH of the experimental ice cream, 20 ml of it was put in a porcelain dish wherein the probe of a 3 in 1 multifunctional moisture, PH and light meter was placed to get the readings. There were ten replications for this test.

Melting Time Assessment

The 250 ml of the ice cream product was put in a porcelain dish and has been subjected to hot plate testing which was given at various temperatures such as 25 °C, 30 °C, and 40 °C. The time for total melting for the sample was recorded. Ten replications for the actual product sample were prepared and compared to that of negative control.

Testing the Texture, Taste and Aroma of the Product

Physical properties of the experimental ice cream like texture, taste and appearance were assessed by 30 respondents of ages 18-35 years old.

Data Analysis

A survey was also conducted in determining the acceptability of the produced ice cream. Mean/averages were used in testing the physical and chemical property of the product.

Table A. Rating scale for the texture of the experimental product

Rate	Description	Interpretation
4	Smoothness of the product is observed	Excellent
3	Slight smoothness of the product can be observed	Very Good
2	Slight roughness of the product can be observed	Fair
1	Roughness of the product is observed	Needs Improvement

Table B. Rating scale for the taste of the experimental product

Rate	Description	Interpretation
4	The ice cream is delicious.	Excellent
3	The ice cream is slightly delicious.	Very Good
2	The ice cream is not slightly delicious.	Fair
1	The ice cream is not delicious.	Needs Improvement

Table C. Rating scale for the appearance of the experimental product

Rate	Description	Interpretation
4	The product is very appealing, delightful, and looks like a real ice cream	Excellent
3	The product is slightly appealing and some parts are not properly polished but still look like ice cream.	Very Good
2	Majority of the parts of the product seem not properly mixed and resulted to not so appealing.	Fair
1	Expectations for an ice cream product were not achieved.	Needs Improvement

3. RESULTS

Table 1. Result of Phytochemical Constituent Testing for *E. hirta* (Tawa-Tawa)

Phytochemical	Result
Flavonoids	(+++)
Alkaloids	(+++)
Phenols	(+)
Saponins	(++)

Note: (+) Traces, (++) moderate, (+++) abundant (-) absence of constituents
 Reference: *Pharmacology, 15th edition, 2002, Trease & Evans*

Table 1 shows the presence of plant constituents such as flavonoids, alkaloids, saponins, and phenols in *Euphorbia hirta* (Tawa-tawa) leaves extracts.

Table 2. Result for Antioxidant Vitamins in *Euphorbia hirta* (Tawa-Tawa)

Parameter	Test Method	Result
Vitamin B ₂	HPLC	5.02 mg/kg
Beta-carotene	HPLC	9.8 mg/kg
Vitamin E	HPLC	23.8 mg/kg
Vitamin C	HPLC	80.2 mg/kg

Table 2 affirms the presence of Vitamin B₂ of 5.02 mg/kg, Beta-carotene of 9.8 mg/kg, Vitamin E of 23.8 mg/kg and Vitamin C of 80.2 mg/kg in *Euphorbia hirta* (Tawa-tawa) leaves extracts.

Table 3. Result for Assessment of Microbiological Quality of the *E. hirta* (Tawa-tawa) Ice Cream

Parameter	Test Method	Result
<i>Tawa-tawa Ice Cream</i>		
Total Coliform Count (CFU/g)	Petrifilm	16
Salmonella	Conventional	Absent in 25g
<i>Negative Control (Without Tawa-tawa Extracts)</i>		
Total Coliform Count (CFU/g)	Petrifilm	TNTC
Salmonella	Conventional	Absent in 25g

Remarks: CFU/g = Colony Forming Units per gram;

TNTC = Too Numerous to Count

Reference(s): *Bacteriological Analytical Manual, Online. AOAC Official Method 991, 14/3M Petrifilm Interpretation Guide*

Table 3 depicts the result for assessment of microbiological quality of the Tawa-tawa Ice Cream. It simplifies that the produced ice cream product with *Euphorbia hirta* (Tawa-tawa) extracts has 16 (CFU/g) for the *Total Coliform Counts* and negative to the presence of *Salmonella*. Meanwhile the negative control appeared to be negative also for *Salmonella* but its *Total Coliform Count* was given as TNTC (*Too Numerous to Count*).

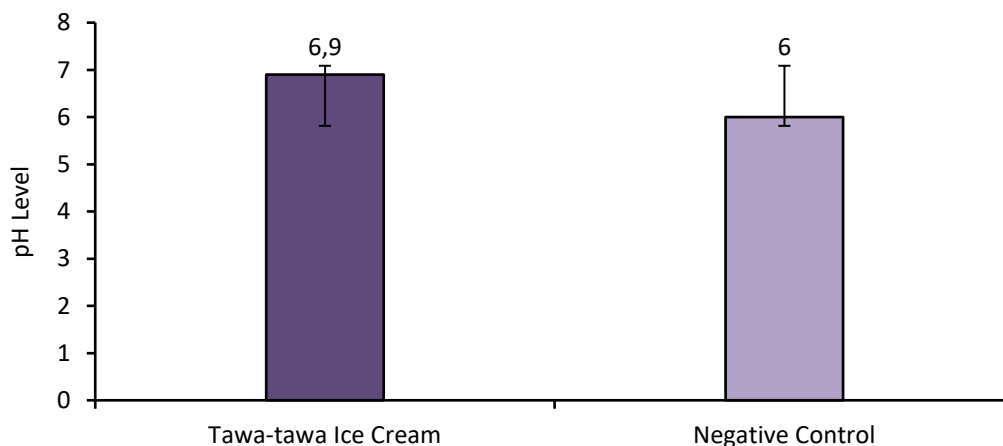


Figure 1. Result of the pH readings of the samples of the ice cream product with *E. hirta* (Tawa-Tawa) and its negative control.

Figure 1 shows the pH levels of the produced ice cream with Tawa-tawa and the one which was just plain ice cream. It can be gleaned in the figure that the Tawa-tawa ice cream and the negative control obtained 6.9 and 6 as averages for pH levels respectively given at ten replications for each prepared sample.

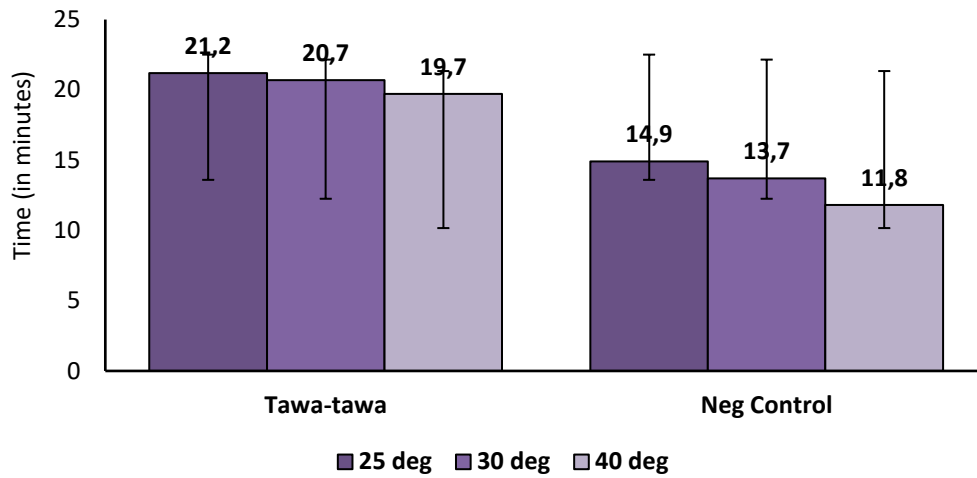


Figure 2. Result of the melting time assessment of the *E. hirta* (Tawa-Tawa) ice cream at various temperatures

Figure 2 shows the result of the melting time assessment for the experimental ice cream as compared to the negative control. The figure also affirms that the Tawa-tawa ice cream had longer time before totally melted as tested in different temperatures compared to the negative control.

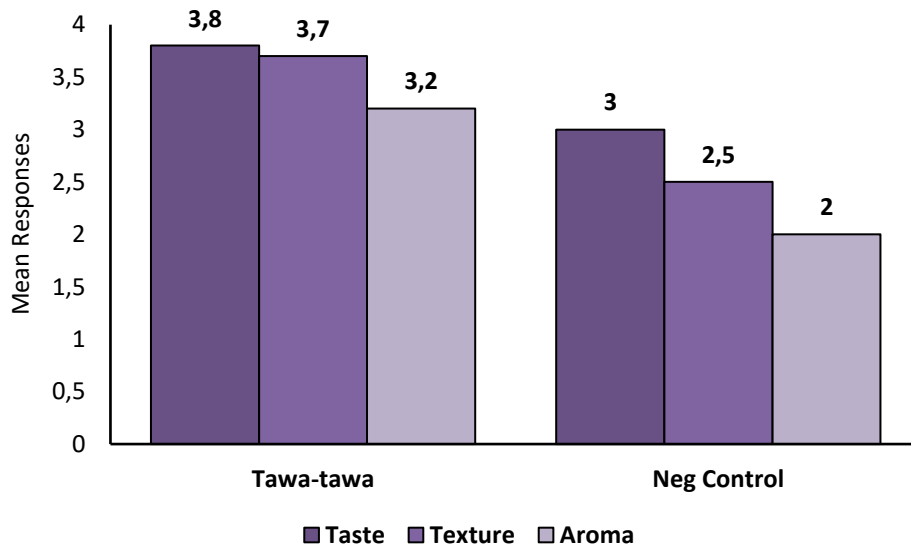


Figure 3. Result of the organoleptic test for the taste, texture and aroma of the *E. hirta* (Tawa-taw) a ice cream

Figure 3 shows the averages of the responses of thirty respondents towards the *E. hirta* (Tawa-Tawa) ice cream in terms of its taste, texture and aroma being compared to its negative

control. It can be gleaned that the formulated ice cream with *E. hirta* (Tawa-Tawa) obtained higher mean responses compared to the negative control.

4. DISCUSSION

According to Goraya and Bajwa (2013) flavonoids, polyphenolic compounds found in plants, have antioxidant powers that may provide important health benefits. Diets rich in flavonoids have been associated with reduced risk of a variety of diseases. This gives then implication that the Tawa-tawa ice cream is a one of a kind ice cream product with good impact to human health.

On the other hand, Tawa-tawa has also an abundant alkaloids, moderate saponins and trace occurrence for phenols. Alkaloids give strong radical scavenger power, so they can be used as natural and good sources of natural antioxidants (Mohd Nayeem Ali, et al., 2014).

All of these phytoconstituents found in the *E. hirta* help in maintaining good antioxidant activity. These results strengthen the claim for the produced ice cream to be exemplary ice cream product which could give antioxidants to the consumer.

According to Choo, Leong and Lu (2009), plant materials containing flavonoids could offer large health benefits since flavonoid is directly associated to better antioxidant properties.

In addition, results gave implications that the naturally-based ice cream product supplemented with *E. hirta* extracts is not an ordinary ice cream. It possesses significant health benefits. The product has a promising antioxidant activity due to its antioxidant vitamins.

According to several studies antioxidants, found in fruits, vegetables and tea, help your body fight free radicals and can protect you from a number of diseases. Antioxidants protect the heart as well as human DNA and cover the body from oxidative stress (Mohd Nayeem Ali et al., 2014).

On the other hand the added *E. hirta* on the ice cream product also affirmed the presence of Vitamins B₂, E, C, and Beta-carotene.

In the study of Verma, Ansari and Broadway (2018), the presence of Vitamins B₂, E, C, and Beta-carotene also confirmed the good antioxidant potential of the herbal plant, since these vitamins are considered as antioxidant vitamins which help the body to be protected from oxidative stress, infections, and diseases/illness.

Vitamin B₂ is needed for growth and overall good health. It helps the body break down carbohydrates, proteins and fats to produce energy, and it allows oxygen to be used by the body (Bradford, 2015). This vitamin also helps for good skin maintenance.

Meanwhile, Beta-carotene is a carotenoid and an antioxidant. Some studies have suggested that those who consume at least four daily servings of beta-carotene rich fruits and/or vegetables have a lower risk of developing cancer or heart disease (Nordqvist, 2016).

On the other hand, Vitamins E and C are also good indicators of antioxidant activities. Basically, if these vitamins are found in food, it means that the food promotes good effect to the body. According to NDTV Food (2017), Dr. Adarsh Kumar, Internal Medicine, National Heart Institute remarks, "If consumed in adequate amounts, Vitamins E and C aid in protecting against heart disease, cancer, liver damage, kidney damage and even macular degeneration i.e. age-related eye damage". Vitamin E helps in boosting your immunity, maintaining a youthful glow by warding off free radicals, and also offering sun-protection.

Based on the “Revised Guidelines for the Assessment of Microbiological Quality of Processed Food” of the Food and Drug Administration (FDA) under FDA Circular No. 2013-010, ice cream with added ingredients should not exceed 10^3 or 1000 CFU/g in terms of *Total Coliform Counts* because this indicates potential health hazard or imminent spoilage. In this case, the Tawa-tawa ice cream has only 16 CFU/g as TCC results while *Salmonella* is absent in 25g.

The presented results on the *Total Coliform Counts* and *Salmonella* of the Tawa-Tawa ice cream affirmed that safety for intake of the produced ice cream supplemented with *E.hirta* extracts.

According to Wangcharoen (2012), the application of herbal plants and other natural materials might also decrease the colony of some harmful microorganisms. These plants might have also antibacterial and antifungal capacities which could help in reducing or preventing the growth of some bacteria and other microorganisms which cause early spoilage. In the present study, *E. hirta* has revealed the presence of alkaloid, flavonoids and phenols. These phytochemicals are known to contribute to several mode of actions for antibacterial/antifungal activities.

Meanwhile, the results of the pH gave implication that the produced ice cream supplemented with *E. hirta* extracts is safe since it is in the acceptable pH level state for processed food with milk. Acceptable pH of 6.5-6.9 was determined for common ice cream products (Shelton, 2017). The result also proved that possible spoilage in the ice cream during preparation was avoided because too much acidity on the samples was not evident. According to Dentino (2012), she reported that other ordinary ice creams could have 5.4-6 as pH levels - these are the ice creams which are made with synthetic preservatives.

Also, the results for the melting time simply affirm that the naturally-based ice cream product with *E. hirta* is more sticky, stretchy and chewy. The extracts of the plant extracts gave effects to the pH (making an increase) which hold now to the stickiness of the particles of the ice cream. The pH of the mixture affects the binding of all the combined components. According to Guner, Aredic, Keles and Dogruer (2007), pH level of ice cream may have different acidity, but through the application of natural products, too much acidity may be addressed.

Meanwhile, based on the results of the conducted survey from 30 respondents, the *E. hirta* supplemented ice cream has an excellent taste and texture and very good in terms of its aroma. This is higher to the obtained responses for its negative control.

According to Khaliduzzaman et al., (2012), ice cream products supplemented with natural plant extracts may have better freezing point which could results better acceptability given by the consumers. The good aroma given by the natural peanuts also contributed a lot in the very good aroma of the product. This gave the implications that the produced ice cream is well-liked by the possible market. This also affirms the potential high marketability of the product.

5. CONCLUSIONS

Based on the results and findings of the current study, it is proven that the development of a naturally-based ice cream product supplemented with *E.hirta* (Tawa-tawa) leaves extracts is feasible. The produced ice cream is rich in Vitamins B₂, C, E, and Beta-carotene with the

abundant presence of essential flavonoids, alkaloids which are all anchored to its promising antioxidant potential.

The Tawa-tawa ice cream is safe since it has low *Total Coliform Counts (TCC)* and negative to *Salmonella*. The ice cream proved its health benefits with a promising market potential which could encourage everyone to enjoy the delights of eating ice cream without thinking any health risk.

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