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RESEARCH ARTICLE

Formulation and Development of Spirulina (*Athrospira plantasis*) Loaded Chocolates as Immunity Boosters

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Abstract

Adults as well as children have a great need for nutrients such Calcium, Iron, Zinc, Protein and Vitamin B12. Spirulina is very rich in protein, omega 3 and omega 6 oils, vitamin and mineral and its incorporation into chocolates will enrich their nutritional values. Spirulina satisfies the nutritional needs by providing all these nutrients, as well as all the essential amino acids. Spirulina is unique and 100% safe. In fact Spirulina is the only natural food that contains GLA (gamma linoleic acid) an essential fatty acid that is found only in mother's milk. This GLA plays a very vital in balancing the hormonal system in the body. So it is actually very good to give Spirulina to the children. Our Spirulina Chocolates were prepared by simple incorporation method by addition of 1gm Spirulina by dissolving in 10 ml NaCl as a solubility enhancer as well as to mask the taste and smell. This chocolate form is easier for every individual to chew and absorb. The chocolates were assessed for organoleptic properties, pH, blooming test, and hardness. This significant study revealed the efficacy of Spirulina Chocolate Formulation and it would definitely have wide scope in the future as a immunity booster and in treatments of malnutrition in children as well. Further study is required to reveal the quantification of constituents present in Spirulina Chocolate formulation by in vivo and invitro studies.

Keywords: Spirulina; Immunity booster; Chocolate; Athrospira plantasis; gamma linoleic acid

Introduction

Spirulina (*Athrospira plantasis*) is a type of blue-green algae that is rich in protein, vitamins, minerals, carotenoids, and antioxidants that can help protect cells from damage. It contains nutrients, including B complex vitamins, beta-carotene, vitamin E, manganese, zinc, copper, iron, selenium, and gamma linolenic acid (an essential fatty acid).

Spirulina, like many other plant-based super foods, is absolutely safe for kids and adults to eat. As food, spirulina represents a complete vegetarian protein source. When it is sourced safely and administered correctly, it can be a fantastic supplement for adults as well as children.

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The keys to safely giving your kids spirulina is to know that you are getting it from a safe, reputable source, and to know how much to give them. Spirulina is like the fuel that will boost overall development, growth and boost immunity [1].

Some of the benefits that Spirulina Possess are listed below:

GLA (Gamma Linolenic Acid)

It is an essential fatty acid that is found only in mother's milk, Gamma Linolenic Acid plays a very vital in balancing the hormonal system in the body. So it is actually very good to give Spirulina to the children. Because of this it is also believed to have anti-inflammatory properties and may help to relieve pain [2] .

Vitamins

- **Vitamin E:** Children need plenty of vitamin E for proper growth, and this need begins before a child is even born, ac-

cording to a 2006 article published in the "American Journal of Clinical Nutrition." Vitamin E also plays a role in gene expression and helps children convert the foods they eat into energy, according to the National Institutes of Health. Spirulina contains vitamin E that helps to supply the RDA for a child.

• **Vitamin C (Ascorbic Acid)**: It contains vitamin C, also known as ascorbic acid, this is one of the most essential elements of any diet, and is particularly beneficial to the immune system, in helping to prevent colds and flu which the children are quite prone to.

• **Beta Carotene (Vitamin A)**: Children require 3 to 6 mg of beta-carotene (the equivalent of 5,000 to 10,000 Units of vitamin A activity) per day. Spirulina contains beta carotene, that is pure vitamin A, this antioxidant aids vision in dim light [3] .

Minerals

• **Iron:** Infants ages 7-12 months need 11 milligrams of iron a day. Babies younger than 1 year should be given iron-fortified cereal in addition to breast milk or an infant formula supplemented with iron. Spirulina contains iron that can meet the daily requirement of iron in children. Iron helps carry oxygen throughout the body and is essential for healthy blood cells and keeps muscle healthy.

• **Magnesium**: Magnesium is literally used for hundreds of different functions in the human body. If your growing child is short on this important mineral, any one of those hundreds of functions may be effected, like his ability to sit still, relax his facial muscles, stop twitches, process insulin, and deal with loud noises. Spirulina is a good source of Magnesium.

• **Potassium**: Getting children in the habit of eating foods high in potassium may help them keep blood pressure in check as they age. Potassium helps produce new cells and enzymes and promotes the healing of wounds in children. Kids, just like adults, don't eat enough of the fresh fruits, vegetables, and whole grains that are richest in potassium sp Spirulina is a great way that can help to meet up their requirement for potassium.

• **Calcium:** For growing children and teens, getting enough calcium is crucial to building bone mass, which may help guard against osteoporosis and fractures later in life. But by age 12, fewer than 1 in 10 girls and 1 in 3 boys get adequate daily calcium: 700 mg for children ages 1 to 3; 1,000 mg for ages 4 to 8; and 1,300 mg equal to about 4 cups of milk for ages 9 to 18. Spirulina has enough calcium to fulfill this requirement of Calcium.

Builds Immunity

Human beings are more prone to flu, as they come in contact with different people while in school and working place. So it essential to to build their immunity. A number of animal and test tube studies suggest that spirulina increases production of antibodies, infection-fighting proteins, and other cells that improve immunity and help ward off infection and chronic illnesses [4] .

It has the ability to modulate immune functions and exhibits anti-inflammatory properties by inhibiting the release of histamine by mast cells. [5]

Supplements Protein Requirement

Proteins are building blocks of our body, children are in a growing phase of their life and need more of protein to perform all the bodily functions. Amino acids make up 62% of spirulina. Because it is a rich source of protein and other nutrients, spirulina can be used as a nutritional supplement for children [6].

Prevents Allergic Reactions

Animal and test tube studies suggest that spirulina may protect against allergic reactions by stopping the release of histamines, substances that contribute to allergy symptoms, such as a runny nose, watery eyes, hives, and soft-tissue swelling. All these symptoms are very common in children thus Spirulina can help the child fight these issues [7–9].

Antibiotic-Related Illness

Although antibiotics destroy unwanted organisms in the body, they may also kill "good" bacteria called probiotics, such as Lactobacillus acidophilus. This can cause diarrhea. In test tubes, spirulina has boosted the growth of L. Acidophilusand other probiotics. Hence Spirulina can be beneficial for kids having frequent diarrhea and stomach infections [10].

Materials and Methods

Materials

Spirulina powder was obtained from local vendor Satara, Milk powder, Caster sugar, Cocoa powder were purchased from local market, pune and 10% NaCl Solution prepared at Laboratory.

Methods

Preparation of Chocolate:

All the ingredients were weighed accurately. Evaluation of Spirulina powder and all dry contents were done by color, odor, taste, extractive value and Ash Value. Solubility of Spirulina was checked in different concentrations of NaCl as solubility enhancer with little warm condition and prepared Spirulina solution. Chocolates were prepared by mixing all the ingredients in required quantity along with Spirulina solution and was made into a dough. By using appropriate mould, dough transferred

into designed mould.tes were kept in refrigerator for better hardness [11, 12].

Evaluation Parameters

Determination of ash Place about 3 g of the ground material, accurately weighed, or the quantity specified in the monograph, in a suitable tarred dish (for example, of silica or platinum), previously ignited, cooled and weighed. Incinerate the material by gradually increasing the heat, not exceeding 450 °C, until free from carbon; cool, and weigh. If a carbon-free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ash less filter-paper, incinerate the residue and filter-paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450 °C. Calculate the content in mg of ash per g of air dried material [13–18].

Determination of acid-insoluble ash Boil the ash for 5 minutes with 25 mL of hydrochloric acid (~70 g/l) TS; collect the insoluble matter in a sintered crucible, or on an ashless filter-paper, wash with hot water, and ignite at about 500 °C to constant weight. Calculate the content in mg of acid insoluble ash per g of air-dried material.

Loss on drying Procedure set forth here determines the amount of volatile matter (i.e., water drying off from the drug). For substances appearing to contain water as the only volatile constituent, the procedure given below, is appropriately used. Place about 10 g of drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 g) it in a tarred evaporating dish. For example, for underground or unpowered drug, prepare about 10 g of the sample by cutting shredding so that the parts are about 3 mm in thickness. Seeds and fruits, smaller than 3 mm should be cracked. Avoid the use of high speed mills in preparing the samples, and exercise care that no appreciable amount of moisture is lost during preparation and that the portion taken is representative of the official sample. After placing the above said amount of the drug in the tarred evaporating dish dry at 105º for 5 hours, and weigh. Continue the drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference.

Determination of Alcohol Soluble Extractive value Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of Alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 105º, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Phytoconstituents analysis

Preliminary analysis of extracts was carried out to identify the presence of various phytoconstituents by employing standard protocols [19, 20].

Tests for alkaloids

(a) Dragendorff"s 's test: By adding 1 mL of Dragendorff"s reagent to 2 mL of extract, an orange red precipitate was formed, indicating the presence of alkaloids.

(b) Mayer's test: Few drops of Mayer's reagent were added to 1 mL of extract. A yellowish or white precipitate was formed, indicating the presence of alkaloids.

(c) Hager's test: Two milliliters of extract were treated with few drops of Hager's reagent. A yellow precipitate was formed, indicating the presence of alkaloids.

Tests for flavonoids

(a) Alkaline reagent test: Two to three drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow color appeared but it gradually became colorless by adding few drops of dilute HCL, indicating that flavonoids were present.

(b) Shinod's test: Ten drops of dilute HCL and a piece of magnesium were added to 1 mL of extract, the resulting deep pink color indicating the presence of flavonoids.

Test for phenolic compounds and tannins

(a) Ferric chloride test: Two milliliters of 5% neutral ferric chloride solution were added to 1 mL of extract, the dark blue coloring indicating the presence of phenolic compounds and tannins.

(b) Lead tetra acetic acid test: One milliliter of lead tetra acetate solution was treated with 0.5 mL of extract, precipitate formation indicating the presence of phenolic compounds and tannins.

Tests for proteins

(a) Biuret test: Two drops of 3% copper sulphate and few drops of 10% sodium hydroxide were added to 1 mL of extract, violet or red color formation indicating that proteins are present.

(b) Ninhydrin test: Two drops of 0.2% freshly prepared ninhydrin solution added to 1 mL of extract. Production of purple colour shows the presence of proteins.

Test for carbohydrates

(a) Molish test: Few drops of alcoholic a-naphthol solution were added to 2 mL of extract. Later, few drops of concentrated H2SO4 were added along the walls of test tube. At the junction of two liquids, a violet colour ring appeared, indicating that carbohydrates were present.

(b) Benedict's test: To 5 mL of Benedict's reagent, 8-10 drops extract were added, then heated for five minutes; the resulting dark red precipitate indicated the presence of carbohydrates.

(c) Fehling's test: To 2 mL of extract, an equal volume of Fehling's (A & B) solution was added and heated for five minutes, the resulting red/dark red precipitate indicating the presence of carbohydrates.

Tests for glycosides

Keller Killiani test: A solution of 0.5 mL, containing glacial acetic acid and 2-3 drops of ferric chloride, was mixed with 2 mL of extract. Later, 1 mL of concentrated H2SO4, was added along the walls of the test tube. The appearance of deep blue colour at the junction of two liquids indicated the presence of cardiac glycosides.

Tests for saponins

A drop of Na2CO3 solution was added to 5 mL of extract in a test tube. After vigorous shaking, it was left to rest for five minutes. Foam formation indicated the presence of saponins.

Test for triterpenoids

Horizon test: Two milliliters of trichloroacetic acid was added to 1 mL of extract. The presence of terpenoids was confirmed by the formation of a red precipitate.

Test for steroids

Salkowski test: The test extract was shaken with chloroform and concentrated H2SO4 was added along the walls of a test tube; a red colour appeared, indicating the presence of steroids.

Test for starch

Iodine test: Two milliliters of iodine solution with potassium iodine were added to 2 mL of test extract, and the appearance of a blue colour indicated that presence of starch.

Preparation of the formulation

The various formulations were made as per the method described, and the composition of each formaulation is summerized in Table 1.

Table 1 Formulation table for composition of chocolate

Contents	F1	F2	F3
Spirulina Powder	1gm	2gm	3gm
NaCl	10 ml	10ml	15ml
Milk Powder	4gm	5gm	6gm
Caster sugar	2.5gm	3gm	3.5gm
Cocoa Powder	2.5gm	3gm	3.5gm

Result and Discussion

Qualitative analysis of Spirulina powder

Qualitative evaluation parameters of raw Spirulina powder such as ash value, extractive value, LOD etc. were done and all the results were satisfactory with compared to standard range. (Table 2)

Organoleptic evaluation of Spirulina powder

All the organoleptic evaluation parameters were done such as color, odor, taste, mouth feel, appearance etc were done and the results were satisfactory. (Table 3)

Preliminary Phytochemical screening of chocolate formulation

Chemical tests were performed for the presence of different chemical constituents in Spirulina loaded chocolate. In that alkaloids, glycosides, carbohydrates, proteins, energy were found to be present. (Table 4)

pH and blooming test

pH for F1 formulation was found to be 6.1 and no blooming was observed in any formulation.

Stability study

F1 batch was found to be optimized with all good and satisfactory results after evaluation. The stability study of batch F1 was performed for 3 months at 2-8^{0}c. In that Color, Odor, Taste, Mouth feel, Appearance were checked at the time of evaluation. Batch F1 of chocolates were found as Brown. Chocolaty, slightly salty, smooth, glossy. The color, taste, texture were retained up to 3 months. Hence the stability of Spirulina chocolates is found to be excellent. (Table 5)

Table 2 Qualitative analysis of Spirulina powder

Parameters	Observed value
Ash Value	**14.56%w/w**
Acid insoluble ash value	**8.2%w/w**
Water soluble ash value	**10.3%w/w**
Extractive value	**15%w/w**
Loss on drying	**12.66%w/w**

Table 3 Organoleptic properties

Parameters	F1	F2	F3
Color	Brown	Brown	Brown
Odor	Chocolaty	Chocolaty	Chocolaty
Taste	Sweet	Sweet	Sweet
Mouth Feel	Smooth and Pleasant	Smooth and Pleasant	Smooth and Pleasant
Appearance	Glossy	Glossy	Glossy

Table 4 Preliminary Phytochemical screening of chocolate formulation

Phytoconstituents	Chocolate formulation
Carbohydrate	+
Protein	+
Fats	+
Energy	+
Glycoside	+
Alkaloids	+

Note: + indicates presence of Phytoconstituents

Table 5 Stability study: F1 batch was selected for stability

Parameters	Storage condition	At the time of Preparation	After 1 month	After 3 months
Color, Odor, Taste, Mouth feel, Appearance	2-8^0 c	Brown. Chocolaty, slightly salty, smooth, glossy	No change	No Change

Figure 1 The actual photograph of formulated Spirulina Chocolate

Conclusion

From the above results, it can be concluded that the F1 batch was an optimized batch, provides sweetening property as compare to others, pH and Stability profile to be satisfactory. Wide scope is available for further in vivo and in vitro study by using cognition model or any suitable model. Spirulina powder with 10% NaCl solution was proved to be the perfect combination to formulate the chocolate. The organoleptic properties of chocolate were excellent for masking the unpleasant flavor associated with Spirulina powder. The chocolate formulation provides a palatable means for delivering medicaments through oral delivery. Each chocolate contains 1gm dose of Spirulina which can be consumed 3 times a day as the standard dose of Spirulina is 1 – 8 gm. per day. Spirulina solution along with the other excipients are used in the dose range are safe consumption and can be swallowed without any risk of systemic side effects.

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