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Formulation development of a Herbal Candy for Altitude Health Problems

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Research Article

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ABSTRACT

People, who visit to high altitudes, do experience certain type of health problems as they transits to different altitude ranges. With ascend to high altitude, various kinds of acute and chronic physiological changes occur which influence all system of human body. These problems although have temporary manifestations, arising due to maladjustment of body to abrupt altitudinal changes in oxygen, atmospheric pressure and gravity but sometimes can be life threatening. Often it has been found that these symptoms are confused with other complications of body. Although various formulations are available for the treatment of altitude health problems but they associated with certain problems in their use such as freezing of liquid formulation, solid dosage forms need water which is again a problem at high altitude, difficulty in handling, taste problem etc. This work is done to formulate a suitable formulation (candy) for these altitude health diseases and to overcome the problems related to the dosage forms which are already available as mentioned above. Present work targeted not only a particular disease but on the main problem i.e. High Altitude Pulmonary Edema which is fatal in most of the cases and on the symptoms of other altitude problems also.

INTRODUCTION

Altitude

High altitude areas can be defined as areas at altitudes equal to or greater than 1500 m above mean sea level [1]. It ranges from 1500 to 5500 m. Each year millions of people ascend to altitudes between 2000 m and 4000 m.

Altitude Illness

Millions of people travel to high altitudes every year mostly above 1500 m, especially in the Himalayas in Asia, Alps in Europe, Rockies in the United States and Andes in South America. [2] Altitude sickness is a number of symptoms that can occur from ascending to high altitudes more quickly than the body can adjust. Atmospheric pressure is reduced at high altitudes. The reduced atmospheric pressure means that the air is less rich with oxygen and therefore less oxygen is available for body cells to use. The severity of the symptoms depends on the altitude reached, the rate of ascent, the time spent at the high altitude, and the person's overall health.

Why Does Altitude Sickness Occur?

The concentration of oxygen at sea level, where the barometric pressure averages 760 mmHg is about 21%. With an increase in altitude, barometric pressure decreases so there are less oxygen molecules taken up per breath. For e.g. at 12,000 feet, the barometric pressure is about 483 mmHg, resulting in about 40% fewer oxygen molecules per breath [3].

Effects of High Altitude on Human Body

a. Reduced physical performance: People cannot maintain the same physical performance at altitude as they can sea level, regardless of the fitness level.

b. Physiological effects: The physiological effects may result changes in senses (vision and taste), mood and personality. These defects are directly related to altitude and are common at over 3048 m.

Various Altitude Health Problems and Their Treatment

Altitude health problems are acute mountain sickness, high altitude pulmonary edema, high altitude cerebral edema, frostbite, chilblain, sleep disturbances.

Acute mountain sickness (AMS): AMS is a syndrome of non-specific symptoms and is therefore subjective [4]. AMS is defined as the presence of headache in an unacclimatized person who has recently arrived at an altitude above 2500 m. AMS is characterized by following symptoms, gastrointestinal problems (anorexia, nausea, vomiting etc), insomnia, dizziness and fatigue [5,6].

High altitude cerebral edema (HACE): HACE is the severe form of acute mountain sickness. It results due to swelling in the brain tissues because of low pressure. In this problem the blood circulation to the brain becomes very low. The mechanism of HACE is when human body is exposes to high, very high altitude, it leads to hypoxia (a state when low oxygen is present in the body) and low oxygen supply to the brain and hence high altitude cerebral edema. The symptoms of HACE are severe headache, vomiting, ataxia, confusion, localized, paralysis, coma and death._

High altitude pulmonary edema (HAPE): HAPE is a life-threatening non-cardiogenic form of pulmonary edema that afflicts vulnerable individuals following rapid ascent to high altitude above 2500 m. With usual ascent rates, the incidence is about 1 to 2% but as many as 10% of people ascending rapidly to 4500 m may develop the conditions [7]. It develops due to oxygen deficiency as well as low atmospheric pressure. Reduced clearance of fluid from the alveoli may also contribute to HAPE. The various symptoms of HAPE are external dyspnea, cough, and reduced exercise performance, breathlessness at rest, gurgling in the rest, chest pain, and fluid excess in the lungs [8].

Frostbite: Frostbite is a condition caused by the action of cold on the body. Appearance of frostbite not only depends on temperature but also on the duration of exposure to cold, humidity, airflow, pre-existing disorders such as arterial circulatory disorders, chronic alcoholism. The most frequent symptoms along with headache were lightheadedness and vertigo, but sleep disturbance was most common one.

Chilblains: It is an inflammatory skin condition presenting after exposure to cold as pruritic and/or painful erythematous to violaceous acral lesions. It may be idiopathic or secondary to an underlying disease [9]. Chilblains are most seen in young and middle aged women and in children, and in terms of sex ratio, women are affected more frequently than men. The direct cause of chilblain is cold exposure, but exposure to both mild non freezing cold and humidity seem to be required. People who exercise or work outdoors in wet and cold rooms, women and people who have acrocyanosis or erythrrocyanosis are prone to develop chilblain. The various symptoms of chilblain are inflammation, low body weight, hormonal changes, bluish red skin, pain, itchiness, Patients may experience burning sensation, ulcerating blister.

Treatment of Altitude Health Problems

Various methods and drugs are used in the treatment of acute altitude sickness and are:

- **Gradual ascent:** Controlling the rate of ascent, in terms of the number of meters gained per day [10], is a highly effective means of preventing acute altitude illness.
- **Acetazolamide:** Multiple trials have established a role of acetazolamide in the prevention of AMS. The recommended adult dose for prophylaxis is 250 mg twice a day [11]. It is a carbonic anhydrase inhibitor and its mechanism of action is thought to be to acidify the blood, causing an increase in respiration centrally and an increase in oxygenation.
- **Gingko biloba:** It is also play an important role in AMS prevention; several negative trials have also been published [12]. This discrepancy may result from differences in the source and composition of gingko products.
- **Natural remedies:** *Zingiber officinalis*, kalium phosphate, ferrum phosphate, cocculum and *Pulsatilla vulgaris*. If symptoms don't go away, descend 300 m. These remedies in combination may help in improvement of breathing rate, disorientation and speed recovery.
- **Descent:** When HACE is feasible, descent remains the single best treatment for HACE. The symptoms typically resolves following descent of 300 to 4000 m, but the required descent will vary between persons.
- **Supplemental oxygen:** Oxygen delivered by nasal cannula at flow rates sufficient to raise arterial oxygen saturation to greater than 90% provides a suitable alternative to descent [13].
- **Portable hyperbaric chamber:** These devices are effective for treating HACE and other severe altitude illness, but required constant tending by care providers and are difficult to use with vomiting patients. Symptoms may recur when individuals are removed from the chamber.
- **Beta 2 receptor agonists:** Inhalation of beta-2-receptor agonists might be used in addition to nifedipine is commonly

used [14]. It is likely that sildenafil which attenuates hypoxic pulmonary vasoconstriction is effective for the treatment of HAPE but no clinical trials have yet been reported.

FORMULATION DEVELOPMENT OF HERBAL CANDY FOR TREATMENT OF ALTITUDE HEALTH PROBLEMS

Candy is a delicate, delicious, treat that we all love. The word "candy" comes from Arabic qandi, derived from Persian qand, meaning "sugar". It comes in many wonderful flavors including milk chocolate, caramel, peppermint, dark chocolate, butter scotch and various other fruit and mint flavors. It also comes in many consistencies such as chewing gum, hard candy, soft candy and all sorts of great delicious configurations and shapes. There are more than 2,000 kinds of candies are available. Candies are divided roughly into two main classes:

1) **Creamy or crystalline:** These candies contain small crystals in their structure, these are easily chewable and are creamy that melt in the mouth.
2) **Amorphous or non-crystalline:** These candies include hard candies, caramels and toffees. These are homogeneous and hard.

Creamy or amorphous candies are further of various types:

- **Hard candy:** It is a common type of sugar candy which contains water, sugar, color and flavor.
- **Candy bars:** These are the candy which available in the form of bars.
- **Chocolate candy:** These are chocolate containing candies and are of different types white chocolates, dark chocolates, unsweetened chocolates with no sugar and milk chocolates etc.
- **Soft candy:** These are soft in nature, manufactured by using soft ball stage according to candy thermometer.
- **Medium hard candy:** This candy is soft and flexible in nature, and easy to handle because of its durability.
- **Sugar free candy:** These are soft candies prepared without sugar especially for diabetic patients.
- **Chewing gum:** These are soft and chewy in nature.
- **Jelly beans:** These are soft sugar candies with high water quantity.
- **Peg line candy:** It is a kind of soft candy usually available in chocolate flavor in packed form.

Principle Ingredients of Candies

Different kinds of candies require different ingredients but there are certain basic ingredients which are required in almost every kind of candy. In most candies sugar is a fundamental ingredient, some candies are composed entirely of cooked sugar. Basic ingredients of candies and their role in candies are discussed here:

- **Sweeteners:** These are added to candies to make it good in taste. Various sweeteners include glucose, fructose, honey etc. Honey has a non-crystallization property, and can therefore be used in candies to maintain a soft, smooth consistency.
- **Acids:** Acids such as lemon juice or cream of tartar added because it causes conversion of sucrose into its two simpler components, fructose and glucose.
- **Fats:** Fatty ingredients such as butter help in interfering with crystallization again, by getting in the way of the sucrose molecules that are trying to lock together into crystals. A large amount of butter is added to maintain smooth texture and easy breakability of a candy.
- **Gelatin:** If you add a gelatin, starch, pectin or gum to the boiling mixture, the sugar will become gel and products look like jelly beans.
- **Flavors**: Various flavors are used such as melted chocolates, vanilla, mint etc. Commonly candy oils can be used and are preferred flavors as they are oil based, it makes them strong and less likely to evaporate when subjected to heat or added to a hot mixture.
- **Colors:** For sugar based candy, food coloring is best to use. It is a liquid, which comes in little bottles available at any supermarket or from a cake decorating store. It is particularly suitable for mixtures which do not combine readily with liquid, such as chocolate or to be used in baking recipes.
- **Salt and vegetable oil:** These are added to improve the taste of candy

Procedure for Making Candy

Various steps for making candy at industry level are:

- **Mixing and cooking:** Cooking vats are used for mixing and cooking of candy. These are sophisticated machines integrated with mixers and cookers. They have hydraulic lifts, agitators, temperature controllers with alarms.
- **Aerating:** Aerators are used for candy aeration; these devices also facilitate accurate temperature control during the aeration process.
- **Molding:** Two most common types of mold used for making candy are silicon rubber molds and thermoformed spinning molds. These types of molds are often used in the manufacture of chocolate candy.
- **Drawing:** The drawing of a candy is almost same as the drawing of metal or plastic. Dies are used to reduce its size or forming its perimeter to a shape.
- **Cooling:** There are various methods used for cooling, most common are using cold water or by refrigeration.
- **Coating:** Coating equipment's are used for coating candy, they are made up of stainless steel, contain pump units and high end ones are packed with advance electronics to optimize candy consumption minimizing waste.
- **Quality control analysis:** Finally the analysis of candy has been done to check its quality and effect.

Importance of Herbal Candies

An herb is a plant or part of a plant valued for its medicinal, aromatic or savourly qualities. Nature produces several food items for every season. Their use in that particular season proves to be highly beneficial for the mankind which is packed with enormous medicinal advantages. Herbal drugs play a major role in systems of health in India; almost 70% of modern medicines in India are derived from natural products. In last few years there is an increment occur in the use of herbal medicines [15]. The herbs used in herbal candy are selected on the basis of their role in the treatment of altitude health problems with lesser side effects, also the selection based on their availability and their preferences. The herbal products are much better than the allopathic medicines. Herbal products have lesser side effects and more therapeutic effects.

Advantages of Herbs

- Safety
- Efficacy
- Lesser side effects
- Compatibility with the human body
- Cultural acceptability

Role of selected drugs in the treatment of altitude health problems

The plants will be selected on the basis of their role in the treatment of altitude health problems with lesser side effects, also the selection based on their availability and their preferences. Selected plants are discussed here:

- *Gingko biloba:* It has tonic effect on the brain, increases blood circulation and reduce oxygen requirement of the body **(Figure 1).**

Figure 1. *Gingko biloba* leaves.

- *Thea sinensis:* It provides mental alertness, helpful in headache and cold etc. **(Figure 2).**
- *Zinziber officinalis:* It has thermogenic properties and carminative **(Figure 3).**
- *Cinnamomum cassia:* It has thermogenic properties and also acts as circulation booster **(Figure 4 and Table 1).**

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Figure 2. Tea leaves.

Figure 3. Ginger rhizomes.

Figure 4. Cinnamon barks.

Table 1. Herbs and their parts used.

Herbs	Part used
Zinziber officinalis	Rhizomes
Cinnamomum cassia	Bark
Gingko biloba	Leaves
Thea sinensis	Leaves

Various Other Formulations Available in the Market for Altitude Health Problems

- **Tablets:** Oral formulation is available containing 80 mg of floating calcium used for treating high altitude gastric problems in defense forces at high altitudes.

- **Drinks:** Shining mountain herbs made a liquid formulation called as organic rocky mountain herbal bitters used for altitude health problems. Acli-Mate produced a drink which has vitamin C, *Gingko biloba* and Rhodiola for optimal acclimatization and performance at high altitude. It is a healthy alternative to coffee, soda and other caffeinated drinks.

- **Inhalers:** Nano-salbutamol Sulphate dry powder inhaler is a drug formulation. An effective therapy to treat acute mountain sickness and mild high altitude pulmonary edema.

MATERIALS AND METHODS

Selection of Herbs

An herb is plant or part of plant valued for its medicinal or aromatic qualities; various types of herbs are available in nature having different medicinal properties. Herbs selected for the preparation of herbal candy are represented in the **Table 2**. Selection of herbs is done on the basis of their importance in the treatment of altitude health problems.

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Table 2. Herbs selected for making herbal candy.

Ingredients	Biological name	Family
Maidenhair tree	*Gingko biloba*	Gingkoaceae
Ginger	*Zingiber officinalis*	Zingiberaceae
Cinnamon	*Cinnamomum cassia*	Lauraceae
Tea extract	*Thea sinensis*	Theaceae

Protocol for the Preparation of Herbal Candy

Preliminary trials for development of candy

Different concentrations of ingredients and taste enhancers were tried to prepare a suitable candy. The blending of various ingredients of candy is represented in flow chart shown below. The composition of extracts and other ingredients was made on the basis of taste and consistency of formulation. The most acceptable combination was selected and stored for further processing **(Figure 5).**

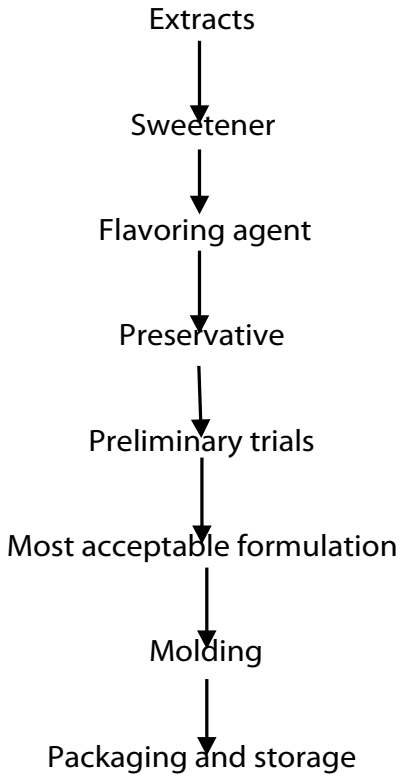

Figure 5. Flow chart for the preparation of herbal candy.

Formulation development of herbal candy

The equipment's and procedure for making candy is described here:

Candy making equipments and tools

- A deep bottom saucepan
- A medium size spatula
- Wooden spoon to stir candy mixture
- A bowl filled with cold water (for cold water test)
- Candy thermometer
- Pastry brush to wash down sides of pan
- Candy mold
- Cooling racks
- Timer or Clock
- Rubber gloves
- Vegetable oil spray

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Procedure for making candy

- Mix water and sugar in a deep bottom saucepan and allowed to boil, add slight butter and salt. Stir the mixture with a wooden spoon.
- Now add all the four extracts one by one with the help of spatula with constant stirring and make temperature up to 300° F (Using cold water test which is mentioned below and candy thermometer) **(Figure 6).**

Figure 6. Candy thermometer dipped in candy mixture.

- Add flavoring agent and preservative to the mixture.
- Pour immediately into the candy mold (Sprayed with vegetable oil so that the mixture does not stick with the wall of candy mold) and allowed to cooled by placing in the cooling racks.
- After cooling store properly at a suitable temperature.

Cold water test

We can determine the temperature range or stage of our candy mixture by testing it in a small glass bowl filled with cold water **(Figure 7).**

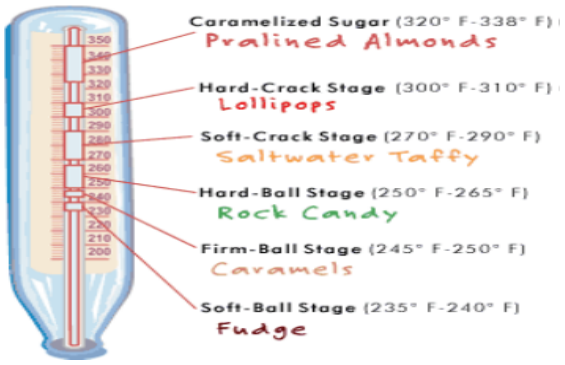

Figure 7. Candy thermometer showing different stages.

1) Thread stage: 230-233°

Dip a metal spoon into the hot candy mixture. Hold the spoon over the cold water. The mixture should fall off the spoon in a fine thread.

2) Soft-Ball Stage: 234-240°

Drop a small amount of the hot candy mixture into the cold water. When cooled and removed from the water, the ball will flatten immediately and run over your finger.

3) Firm-Ball Stage: 244-248°

Drop a small amount of the hot candy mixture into the cold water. When cooled and removed from the water, the ball will hold its shape and not flatten.

4) Hard-Ball Stage: 250-266°

Drop a small amount of the hot candy mixture into the cold water. When cooled and removed from the water, the candy will form a hard yet pliable ball.

5) Soft-Crack Stage: 270-290°

Drop a small amount of the hot candy mixture into the cold water. When cooled and removed from the water, the candy will separate into threads that are hard but not brittle.

6) Hard-Crack Stage: 300-310°

Drop a small amount of the hot candy mixture into the cold water. When cooled and removed from the water, the candy will appear as hard ball.

Adjustment in the procedure for making candy at high altitudes

At higher altitudes, pressure of air is less. The boiling point of water is 212°F at sea level. Most of the recipes for making candy are written for sea level. At high altitudes, the boiling point of water decreases. At 4500 feet above sea level water boils at 204°F. If candy is cooking until it reaches the hard ball stage according to cold water test, it will have cooked too long.

EVALUATION OF HERBAL CANDY

Quality Evaluation of Herbal Candy

Quality evaluation of prepared herbal candy is essential for the efficacy, safety determination. Both physicochemical and phytochemical evaluation was used to evaluate the formulation by comparing it with the standard parameters. Sensory evaluation was also performed and defined as a scientific discipline used to evoke, measure, analyze, and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing.

Sensory Evaluation of Herbal Candy

Consumer awareness concerning herbal candy depends upon various factors apart from therapeutic effect. However, no matter how effective the candy, the taste must be acceptable and appearance should be good for its consumption. Sensory analysis was performed by using nine points hedonic rating scale by a panel of eight people. The parameters for evaluation includes appearance, color, taste, flavor, consistency and overall acceptability of herbal candy.

Physicochemical Evaluation of Herbal Candy

Details of physicochemical parameters of food or any other medicinal formulation is very important and fundamental in analyzing the quality of formulation. The studies of these parameters is necessary because they influence the handling and treatment received during processing and also because these are the indicators of quality of formulation. Various physicochemical parameters of candies are pH, shape, hardness, friability, average weight, calories and disintegration time.

a) Average weight

Ten candies were accurately weighed using a suitable, previously calibrated balance and their average weight was recorded. The average weight was calculated by sum up the weight of all 10 candies and divide by ten.

b) Hardness testing

Solid formulations either tablets or candies requires a certain amount of strength, or hardness to withstand mechanical shocks of handling in manufacture, packaging and shipping. The hardness of a candy can be determined by using cold water test which was mentioned above. One more method to determine hardness is using Monsanto tablet hardness tester, in this test a candy is placed between two anvils, force is applied to the anvils, and the crushing strength that just causes the candy to break is recorded. Before starting the experiment zero reading is taken. The hardness of six candies in Kg/cm2 was tested and average hardness was recorded.

c) Friability testing

Another measure of a candy's strength is its friability. We can measure friability by using Roche friabilator, a plastic circular chamber that revolves at 25 rpm, dropping the candies at a distance of six inches with each revolution. The candies are then dusted and re-weighed. Tablets or candies that do not lose more than 0.5 to 1% of their weight are acceptable.

d) Disintegration time

Six candies were put in a suitable disintegration time machine along with sliding discs. The temperature of water was maintained at 25°c. The disintegration machine was switched on and the time required to disintegrate all the six candies, was recorded and average time was calculated.

e) Measurement of pH

The alkalinity or acidity of a product is indicated by using pH meter, a scale from 1.0 to 14.0. 1% W/V solution of candy was prepared by dissolving 1 g candy in 100 ml distilled water and its pH was recorded.

f) Shape

It is the important factor responsible for good appearance of formulation. The shape of candy depends upon the shape of mold in which it is placed. In this formulation development heart shaped candy mold is used, so the formulation is also heart shaped.

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ANALYSIS OF HERBAL CANDY

Preliminary Phytochemical Analysis

Preliminary phytochemical analysis includes the tests for the presence of carbohydrates, proteins, glycosides, tannins, polyphenols and flavonoids in the prepared herbal candy by following standard procedures. The tests followed for the detection of compounds are explained below.

Tests for carbohydrates

- **Molisch test:** A candy was divided into few pieces, one piece was taken, crushed and placed in a test tube. The sample was treated with α naphthol dissolved in ethanol, and then sulphuric acid was added slowly along the sides of test tube. The purple color appears the interface between test sample and acid layers.

- **Fehling test:** One piece of candy was taken, crushed and treated with equal quantity of Fehling's solutions A and B, slightly heated. The red colored ppt. appeared.

Tests for terpenoids

- **Salkowski test:** Candy (sample) was crushed and 1 gm of crushed candy was added to 2 ml of chloroform. To this 3 ml of concentrated sulphuric acid was added carefully to form a layer. A reddish brown color appeared at interface.

- **Antimony trichloride test:** To 1 gm of sample, saturated solution of antimony trichloride in chloroform containing 20% acetic anhydride was added. Appearance of pinkish color on heating.

Tests for alkaloids

- **Mayer's reagent test:** The crushed candy was treated with Mayer's reagent (Prepared by dissolving a mixture of mercuric chloride and potassium iodide in water), cream colored ppt. appeared.

- **Hager's reagent test:** The sample (candy) was treated with freshly prepared Hager's reagent (saturated aqueous solution of picric acid). Yellow colored ppt. appeared.

- **Wagner's reagent test:** Sample was treated with wagner's reagent (Iodine in potassium iodide). Reddish brown ppt. appreared which indicates the presence of alkaloids.

Tests for glycosides

- **Borntrager's test:** The sample was treated with concentrated HCL for 2 hours on a water bath. Filtered and 10 ml of filterate was shaken with chloroform, the layer of chloroform was separated and added 10% ammonia, Pinkish color came out.

- **Legal's test:** 0.1 gm of sample was dissolved in pyridine, added sodium nitro-prusside reagent and sodium hydroxide solution to make the solution alkaline, reddish color appeared.

Tests for flavonoids

- **Aqueous sodium hydroxide test:** 5 gm NaOH dissolved in 50 ml water. 1 gm of sample was treated with this aqueous solution of NaOH, yellow color appeared.

- **Concentrated sulphuric acid test:** 50 mg of sample was treated with 2 ml of conc. Sulphuric acid, orange color came out.

Tests for polyphenols

- **Ferric chloride test:** 1 gm of sample was treated with 5% freshly prepared ferric chloride solution, deep blue color came out.

Test for saponins

- 50 mg of sample was mixed with 50 ml of distilled water and boling on a water bath for five min. and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for froth formation. 3 drops of olive oil were mixed with froth, shaken vigorously and observed for emulsion development. Emulsion not appeared which indicates the absence of saponins.

Determination of Total Phenolics Content

The total phenolic content of herbs was determined by Folin Ciocalteu's assay [16] by spectrophotometrically [17]. The following procedure was adopted to determine the phenolics content.

1) Preparation of Folin Ciocalteu's reagent:

2 ml of Folin's reagent was taken and diluted up to 20 ml with distilled water.

2) Preparation of 20% sodium carbonate solution:

20 g weighed sodium carbonate was dissolved in 100 ml distilled water.

3) Prepararion of gallic acid standard curve:

- Stock solution of 1 mg/ml Gallic acid was prepared by dissolving 100 mg of Gallic acid in 100 ml of distilled water.
- Various dilutions of Gallic acid i.e. 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml, 1 00 µg/ml, 200 µg/ml, 300 µg/ml and 400 µg/ml were prepared.
- To each dilution 1.5 ml of Folin's-Ciocalteu's reagent was added and kept in dark for half an hour.
- Absorbance of each dilution was measured at 765 nm with the help of UV Spectrophotometer. Standard curve was prepared between absorbance and concentration values.

4) Procedure:

- 10 mg of sample was taken and dissolved in 100 ml of distilled water.
- 1 ml (100 mcg/ml) of diluted sample was taken and mixed with 1.5 ml of Folin-Ciocalteu's reagent. Solution was kept aside for 5 minutes.
- 2 ml of 20% sodium carbonate solution was added to solution and kept in boiling water bath for 1-2 minutes.
- Absorbance was measured at 765 nm.
- The total phenolics content was determined from the standard curve of gallic acid.

The total phenolics content is expressed as gallic acid equivalents (GAE)/gm of candy mixture.

Amount of phenolics (GAE)=C × W_1/W2

Where:

C: Concentration obtained from standard curve of gallic acid (mg)

W_1: Weight used during procedure (mg)

W_2: Weight of total sample (mg)

Determination of Total Flavonoids Content

The following procedure was used for the determination of total flavonoids.

1) Preparation of 10% $AlCl_3$:

10 g weighed AlCl3 dissolved in 100 ml distilled water.

2) Preparation of 1 M Potassium acetate solution:

The molecular weight of potassium acetate is 98.15 g. To prepare 1 M solution we should dissolve 98.15 g potassium acetate in 1000 ml distilled water calculated by using molarity formula. Both the quantities reduced by 10 times to avoid wastage of chemicals and reagents. Therefore dissolve accurately weighed 9.8 g potassium acetate in 100 ml distilled water to get 1 M potassium acetate solution.

3) Preparation of standard curve of Rutin:

- 10 mg of rutin was dissolved in 100 ml of 50% methanol to make 100 mcg/ml stock solution of rutin.
- Various dilutions containing 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml and 400 µg/ml were prepared.
- Standard curve was prepared by measuring absorbance of various dilutions at 415 nm.

4) Procedure: 10 mg of candy mixture was dissolved in 100 ml of 50% methanol, 1 ml of this diluted mixture was taken and mixed with 0.5 ml of 10% AlCl3 solution and 0.5 ml of 1 M potassium acetate solution. This should be kept for 30 minutes and absorbance was measured at 415 nm. The flavonoid content was calculated by using the same formula which was used for calculation of total phenolics content and expressed as rutin equivalent/gm.

Amount of flavonoids (RU)=C × W_1/W2

Where:

C: Concentration obtained from standard curve of rutin (mg)

W_1: Weight used during procedure (mg)

W_2: Weight of total sample (mg)

Determination of Antioxidant Activity

Antioxidants can be defined as the agents or substance that scavenge the free radicals and delays or inhibits oxidation caused by those free radicals. Antioxidants are divided into two main classes, primary and secondary antioxidants. The antioxidants that retard the rate of oxidation are called secondary antioxidants and those which inhibit the process of oxidation are called primary antioxidants. The antioxidant activity is found mainly due to the redox properties of a compound [18], which play an important role in neutralization of free radicals.The antioxidant activity was measured by using DPPH assay by a slightly modified method.

1) Preparation of DPPH solution: 1 mg DPPH (1,1-diphenyl-2-picryl-hydrazyl) was dissolved in 50 ml methanol and kept in a dark place. Fresh DPPH solution should be prepared at the time of experiment.

2) Preparation of sample: 10 mg of candy mixture was dissolved in 100 ml methanol.

3) Procedure:

- 1 ml of prepared sample mixed with 3 ml of DPPH solution and stored in dark place for half an hour.
- Absorbance was measured at 517 nm taking blank of methanol without DPPH.
- Percent inhibition of DPPH radical was calculated by using the below mentioned formula:

I%=[(Ablank-Asample)/Ablank] × 100

Where:

I%: Inhibition of DPPH

Ablank: Absorbance of control compound and

Asample: Absorbance of test compound.

Stability Studies

The formulated herbal candy was analyzed for organoleptic attributes such as color, odour and taste for any change at different temperatures.

RESULTS

Extractive Yield of Herbs

The extraction was done by using cold maceration method. 500 gm of each herb was taken and subjected to cold maceration process. After seven days, extracts were collected and yield of extracts of herbs shown in **Table 3** below.

Table 3. Extractive yield of herbs used in herbal candy.

Herb	Extractive yield/100 gm
Gingko biloba	5.4 gm
Zinziber officinalis	6.2 gm
Cinnamomum cassia	5.3 gm
Thea sinensis	8.5 gm

Preparation of Herbal Candy

The herbal candy was prepared by using cold water test. The final composition of formulation was developed as per the preliminary trials mentioned. Various formulations were prepared by varying concentrations of ingredients, formulation using honey as sweetener was not acceptable because of consistency (viscous) but formulation with sugar as sweetener was very good in taste and consistency both. Quantity of tea extracts is reduced and sugar is increased to get the most acceptable formulation. The final formula of herbal candy with sugar as sweetener was mentioned in **Tables 4-6** below.

Table 4. The final composition of herbal candy with sugar as sweetener.

Compound	Amount
Ginger	About 10% w/w
Cinnamon	About 5% w/w
Maidenhair tree	About 10% w/w
Tea extracts	About 10% w/w
Preservative	About 5% w/w
Sweetener	About 40% w/w
Flavoring agent	About 10%w/w

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Table 5. Preliminary trials for the development of herbal candy.

S. no	Cinnamon	Maidenhair tree	Tea extracts	Ginger	Flavor	Sweetener	Preservative
1.	3 gm	10 gm	15 gm	5 gm	2 ml	20 gm	2 gm
2.	3.5 gm	10 gm	15 gm	5 gm	2 ml	20 gm	3 gm
3.	3.5 gm	10 gm	15 gm	5 gm	4 ml	20 gm	4 gm
4.	4 gm	10 gm	15 gm	10 gm	5 ml	25 gm	4 gm
5.	4 gm	10 gm	10 gm	10 gm	7 ml	30 gm	5 gm
6.	5 gm	10 gm	10 gm	10 gm	8 ml	35 gm	5 gm
7.	5 gm	10 gm	10 gm	10 gm	10 ml	40 gm	5 gm

Table 6. Results of preliminary trials.

Taste	Color	Flavor	Consistency	Overall acceptability
Extreme bitter	Dark brown	Not good	Liquid	Not
Extreme bitter	Dark brown	Not good	Liquid	Not
Bitter	Dark brown	Acceptable	Semi-liquid	Not
Bitter	Dark brown	Acceptable	Viscous	Not
Slight bitter	Brown	Acceptable	Viscous	Not
Slight bitter	Brown	Good	Viscous	Not
Sweet	Brown	Good	Solid	Acceptable

Quality Evaluation of Herbal Candy

Various parameters were studied for the herbal candy for proving its safety and quality for human consumption.

Sensory evaluation

Sensory evaluation of herbal candy was done, following parameters were considered like color, taste, flavor, consistency and overall acceptability. On the basis of this evaluation following results came out.

Color: Brown; Taste: Sweet; Flavor: Pleasant; Consistency: Solid; Shape: Heart **(Table 7).**

Table 7. Sensory scores of herbal candy.

Parameters	Scores
Color	8.5
Taste	7.5
Flavor	8.5
Shape	09
Consistency	09
Overall acceptability	8.5

1: extremely dislike, 2: strongly dislike, 3: moderate dislike, 4: slight dislike, 5: neutral, 6: slight like, 7: moderate like, 8: strongly like, 9: extremely like

Physicochemical Analysis of Herbal Candy

Various physicochemical parameters were studied to evaluate the quality of formulation. Following parameters were studied.

Average weight

The average weight of herbal candies was found to be 6.5 gm.

Hardness testing

The herbal candy was prepared by using cold water test which was explained above, this test is enough to prove the hardness of candy. One another test was also done to check the hardness using Monsanto hardness tester. Solid dosage forms require a certain amount of strength or hardness to withstand mechanical shocks during handling. The hardness of six candies in kg/cm^2 was tested and average hardness was recorded which is equal to 8.56 kg/cm^2.

Friability testing

Friability testing is another factor which decides the strength of candies or tablets. This test was done by using Roche friabilator. The limit of weight loss during this test is between 0.5 to 1%. The weight loss occurred during this test was found to be 0.76% w/w.

%w/w=[(x-y) ×100]/x

Where, x=Initial weight of candies

y=Weight of candies after strokes

%w/w=[(26-25.8) ×100]/26=0.76% w/w

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pH of candy

The pH of 1.00% w/v solution of herbal candy in water was found to be 4.4.

Disintegration time

Candies were placed in disintegration time machine. The formulation passes the test if all the six candies were disintegrated within 15 minutes. The time required to disintegrate all the six candies were noted and average time was calculated. Average time for disintegration was found to be 3 min 17 seconds **(Tables 8 and 9).**

Table 8. Disintegration time of candies.

Candy no.	Disintegration time (min)
First	3.15
Second	3.20
Third	2.55
Fourth	3.05
Fifth	3.24
Sixth	3.40

Table 9. Phytochemical analysis of herbal candy.

Compound	Result
Carbohydrates	Present
Alkaloids	Present
Glycosides	Present
Flavonoids	Present
Tannins	Present
Terpenoids	Present
Saponins	Absent

Total Phenolics Content

The total phenolics content of herbal candy was found to be 85 mg/100 gm. High content of phenolics indicates the nutritional and physiological role as bioactive substances in human nutrition. The standard curve of gallic acid was shown in **Figure 8 and Table 10.**

Preparation of standard curve of Gallic acid: Standard curve of gallic acid was prepared to obtain the concentration of gallic acid equivalent to absorbance of sample **(Figure 8).**

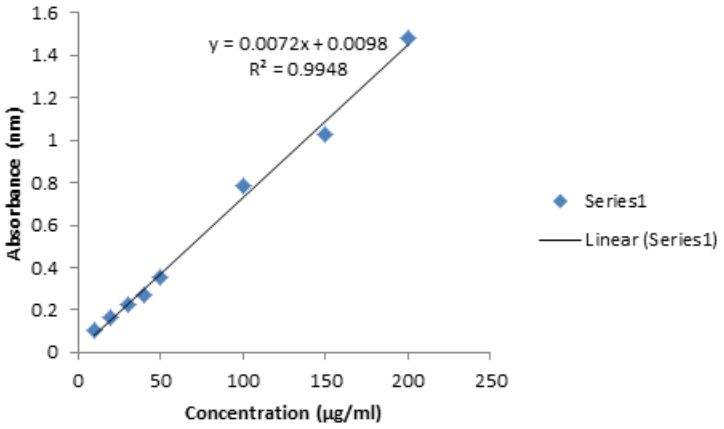

Figure 8. Standard curve of gallic acid at 765 nm.

Table 10. Absorbance of various dilutions of gallic acid at 765nm is shown here.

Dilutions (µg/ml)	Absorbance (nm)
10	0.1025
20	0.1636
30	0.2254
40	0.2689
50	0.3529
100	0.7818
150	1.0269
200	1.4829

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Total Flavonoid Content

Flavonoids show a strong antioxidant and radical scavenging activity and involved in reduction of various diseases such chronic, cardiovascular, and cancerous processes. The average daily intake of flavonoids lies between 70 to 170 mg (Gattuso et al., 2007). Total flavonoid content was determined following a method by Park et al (2008), and it was found to be 55 mg/100 gm **(Table 11).**

Table 11. Various dilutions of rutin and their absorbance.

Dilutions (µg/ml)	Absorbance (nm)
10	0.0467
20	0.0934
30	0.2489
40	0.4345
50	0.5532
100	1.1384
150	1.5845
200	2.0945
300	2.7209

Preparation of Standard Curve of Rutin

Standard curve of rutin at 415 nm is shown in **Figure 9.**

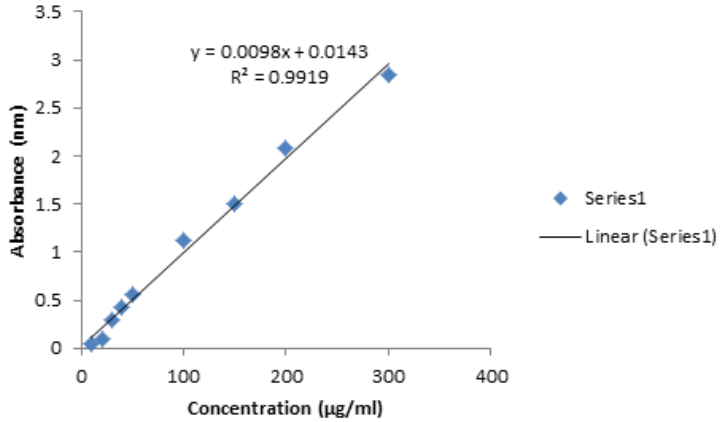

Figure 9. Standard curve of rutin at 415 nm.

Total Antioxidant Activity

The antioxidant activity means the free radical scavenging activity and due to the presence of flavones the antioxidant activity of the herbal candy should be high. DPPH was used to measure the free radical scavenging activity, and it was found to be 59.62%.

DISCUSSION

Herbal candy is prepared for the problems of high altitude especially for soldiers who stay there for long time. Four herbs selected for making candy on the basis of their role and importance in the treatment of altitude health problems. Different types of altitude health problems are found such as acute mountain sickness (AMS), high altitude cerebral edema (HACE), high altitude pulmonary edema (HAPE), frostbite, chilblains etc. Various methods and drugs are used in the treatment of acute altitude sickness and are: gradual ascent, acetazolamide, *Gingko biloba,* natural remedies such as *Zingiber officinalis*, kalium phosphate, ferrum phosphate, cocculum and *Pulsatilla vulgaris,* descent, supplemental oxygen, portable hyperbaric chamber, beta 2 receptor agonists etc. The four herbs selected for making candy are *Gingko biloba, Zingiber officinalis, Thea sinensis, Cinnamomum cassia. Gingko biloba* is the most effective herb in the treatment of altitude health problems especially for high altitude cerebral edema; it acts by reducing the effect of hypoxia, improving blood circulation. *Zingiber officinalis and Cinnamomum cassia* has thermogenic properties. *Thea sinensis* helpful in headache as well as in the symptoms of altitude sickness. Various methods are available for making candy either by using candy thermometer or by using cold water test to determine the exact temperature required for making candy. Sometimes both methods can be used to get better result; here I use both candy thermometer and cold water test for preparation of herbal candy. During preparation the major problem came out was regarding consistency of candy, even after proper heating it does not got enough consistency, to overcome this problem reduce the amount of water and increase the amount of sugar which acts as a solidifying agent. Candy mixture after heating should be properly poured into the mold so that it does not stick to the surface of mold which may not good for shape of candy. After molding it should be properly placed in cooling rack away from the reach of children. After 15 to 20 hours of molding candies can be removed from the mold with the help of hands (covered with gloves), than they packed in wax paper and placed in refrigerator for evaluation. Various types of evaluation done to check the quality of it. Sensory evaluation included following parameters color, taste, flavor, consistency and

overall acceptability. On the basis of the evaluation the overall acceptability was found to be 8.5 (rating on the scale of 9). Various physicochemical parameters were studied to evaluate the quality of formulation, although there are no separate physicochemical parameters for candies, parameters used for solid dosage forms are also applicable for it. Various parameters are average weight found to be 6.5 gm, optimum average weight of candy should be in between 4 to 7 gm. Disintegration time is also an important factor, six candies were subjected to disintegration and the average disintegration time was found to be 3.17 minutes, it should not more than 15 minutes. During handling of any product hardness should be enough to tolerate mechanical shocks. Although cold water test is enough to get good hardness but for further its hardness measured by using Monsanto hardness tester which was found to be 8.56 kg/cm^2. Formulation was subjected to friability testing using Roche friabilitor, it passes the test as it lost only 0.2 gm during the procedure which lies within the limit.

After sensory analysis candy was subjected to phytochemical analysis, it was analyzed for presence of carbohydrates, proteins, glycosides, tannins, saponins, polyphenol and flavonoids by using general identification tests. Test for saponins came out negative; all other compounds are present in the formulation. Free radicals are responsible for wide variety of pathological manifestations; antioxidants fight against these free radicals and protect us from these manifestations. They exert their action by two mechanisms either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. The antioxidant activity was measured by using DPPH assay, and it was found 59.62%. The total phenolic content was determined by Folin Ciocalteu's assay using spectrophotometric method. Folin Ciocalteu's reagent and 20% sodium carbonate solution was prepared. Various dilutions of gallic acid was prepared to make a standard curve, sample solution was made of concentration 100 mcg/ml and absorbance was measured at 765 nm with the help of UV Spectrophotometer. Standard curve was prepared between absorbance and concentration values. The total phenolic content was determined from the standard curve of gallic acid, and it was found to be 85 mg/100 gm. Similarly total flavonoid content was determined by using rutin assay which was found to be 55 mg/100 gm. Overall this is a suitable formulation for altitude health problems especially for soldiers, which has number of advantages such as easy to handle and store, effective and good in taste.

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