Development of Green tea based herbal candy

Dissertation II Report

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CERTIFICATE

This is to that **Kajal Dhawan**(Registration No. 11713833) has personally completed M.Sc. dissertation II entitled **"Development of Green tea based herbal candy"**under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study. No part of dissertation has ever been submitted for any other purpose at any University.

The project report is appropriate for the submission and the partial fulfillment of the conditions for the evaluation leading to the award of Master of Nutriton and Dietetics.

Date: 11th May, 2018

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Chapter 1: INTRODUCTION

Confectionary products are very much famous in the people of all age groups. Starting from the preschool children to the elderly people, all are very fond of confectionary products. Confectionary is divided into three groups:Chocolate confectionary, Gum confectionary and Sugar confectinary.

The main aim of this research is to use this connection between the people and confections in the preparation of sugar based hard candies with green tea to deliver the bioactive compounds and phytochemicals, specially the antioxidants, it possesses which have various health benefits.

Since ancient times, spices and herbs have been used in many different ways such as for enhancing the flavour, as preservatives and also as medicine. This is because of their high antioxidant activity which have beneficial effect on human health. “Antioxidants from spices are a large group of bioactive compounds which consist of flavonoids, phenolic compounds, sulfur-containing compounds, tannins, alkaloids, phenolic diterpenes, and vitamins.”These antioxidants have many beneficial aspects such as in reducing oxidative stress (Yashin et al. 2017)

Herbs may be defined as the dried leaves of aromatic plants used to impart flavour and odour to foods with, sometimes, the addition of colour and Spices may be defined as the dried parts of aromatic plants with the exception of the leaves. (Thomas et al. 2001)Tulsi (Basil) comes under the category of herb. Cinnamon is an aromatic spice whereas garlic is a crop spice obtained from the rhizome of the plant (Thomas et al. 2001)

Tea, the second most consumed beverage in the world, is made from the leaves and buds of Camellia sinensis and is a shrub. Green tea is ‘non-fermented’ and is prepared by steaming the fresh leaves before drying which inactivates the polyphenol oxidase (PPO) and thus preserves the catechins (Mitscher et al. 1997)(Cabrera et al. 2006).

The human use of green tea is believed to have originated 4,000 to 6,000 years ago in China but now is cultivated across the world in tropical and subtropical regions of India, Japan, Sri lanka, Turkey, Kenya, Russia, Burma, Thailand, Viet Nam and Laos (Mitscher et al. 1997)(Parmar et al. 2012).

Catechins are the major flavonoids present in green tea that act as an antioxidant and is the active compound of green tea. These antioxidants help to reduce oxidative stress which is caused by high concentration of free radicals in cells and tissues. These catechins also have anti-aging process.Other spices and herbs are also ued due to their specific activity due to the presence of active compounds such as eugenol in ginger and tulsi and cinnamaldehyde in cinnamon.

Chapter 2: PROBLEM BACKGROUND

Green tea has by far been recognized for its antioxidant potential. However, its use has been limited to tea infusions so far. Most often due to disliking towards this product the benefits of green tea fail to reach to the masses. Thus the current product aims to deliever the benefits of green tea in form of a popular product such as hard candies. Hard candy products with various spice flavour will be attempted to increase the acceptability of green tea in the society.

Chapter 3: REVIEW OF LITERATURE

"Candy" means "good-good" in French (Manson et al. 2016). Candies come under the category of sugar confectionary. The sweetening agent used in the candy preparation is mainly sugar which is composed of sucrose (99.7% of the total weight). Some candies are made from honey which is a natural sweetener. Honey has high calorific value than sugar. 5 gram of sugar contains 49 calories and 5 gram of honey contains 68 calories (Koppico et al. 2008).

Candies are mainly classified into two types :

1. Crystalline candies in which the particle size of sugar does not matter.
2. Non-crystalline candies in which particle size of sugar is a parameter which is considered.

Candies are considered to be the instant sources of energy as they provide high calories and are also rich in flavour and palatability. They can be easily prepared, packed, transported and stored (Figiel et al. 2006). There are various types of candies such as hard candy/rock candy, soft candy/jelly candy, toffee, fondant and fudge candy. The main focus of this research is the preparation of hard candy/rock candy using green tea and other herbs and spices so as to increase the health potential of the green tea.

Hard candy was popular in india and Persia as mishri and nabat. The production of sugar candies started in the first half of the 9^{th} century (Gholamhosseinpur et al. 2008). Since the ancient times, candies are developed using different spices and condiments such as turmeric and saffron for their antioxidant and stimulant properties. That is why candies are regarded as the medicinal byproducts.

3.1 Maillard browning in confectionary

Maillard reaction is considered as the browning reactions of sugars which takes place between the carbonyl group of sugars and amino group of amino acids. The reaction was named after the scientist, Louis Camille Maillard. He heated the amino acids with high level of glucose present in sugar confectionary.

3.2 Hard candy

Hard candy is also known as rock candy. It is purely sugar based product. It is made by the combination of water and sugar only. The process of making hard candy is based on the principle of cyrstallsation. According to Le chatelier's principle, the process of crystallisation is defined as,' a system which is shifted away from equilibrium acts to restore the equilibrium by reacting in opposition to the shift'. Hard candies are a product of maillard browning. Maillard browning is defined as a non-enzymatic browning in which the carbonyl group of the free sugar reacts with the amino group of the amino acid leading to browning. Hard candies are different amongst all types due to its lowest moisture content in the final product (Srikanth et al. 2015). The hard candy can attain a number of sizes, shapes, flavours and colours.

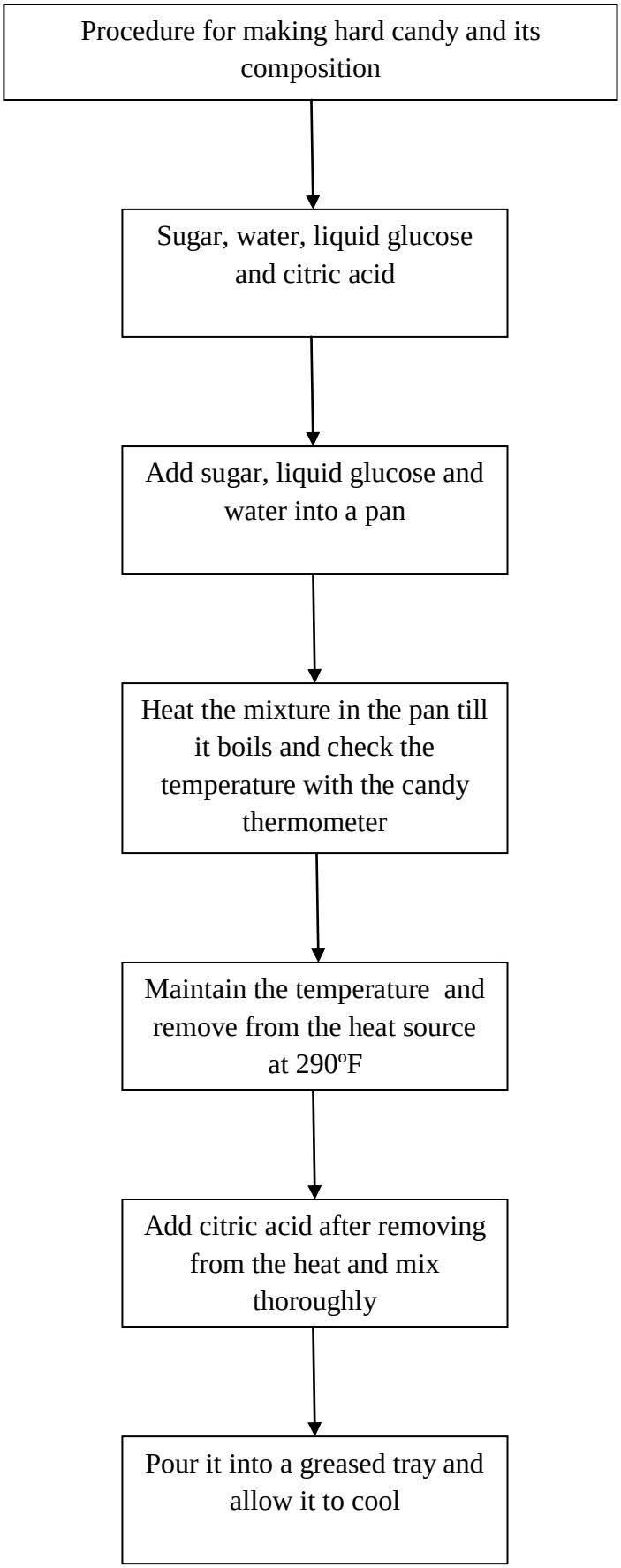


Fig. 3.3 PROCEDURE FOR HARD CANDY PREPARATION

3.4 Green tea

Since ancient times, plants have been used as medicines in the Indian history. Ayurveda and Indian literature shows the use of plants for the treatment of various human ailments. India is known for its rich repository of plants and has about 45,000 plant species. Out of this 45,000 species, several thousands have been claimed to possess medicinal properties (Parmar et al. 2012). One of them which is widely used for its antioxidant properties is green tea.

The palnt is an evergreen shrub, has a strong taproot and is two metres long when cultivated for the leaves. The leaves are 4-15 cm long and 2-5 cm broad.

Table 3.4.1 Scientific classification

SCIENTIFIC CLASSIFICATION	
Kingdom	Plantae
Order	Ericales
Family	Theaceae
Genus	Camellia
Species	C. Sinensis
Binomial name	*Camellia sinensis* (L.) Kuntze

Table 3.4.2 Chemical composition of green tea

CHEMICAL COMPOSITION OF GREEN TEA	
Proteins	15-20%
Amino acids	1-4%
Carbohydrates	5-7%
Flavonoids (Catechins)	0.5-1.5%
(-)epigallocatechin-3-gallate (EGCG)	59%
(-)-epigallocatechin (EGC)	19%
(-)-epicatechin-3-gallate (ECG)	13.6%
(-)-epicatechin (EC)	6.4%
Lipid	4.1 g
Minerals (mg)	
Calcium	390
Phosphorus	410
Iron	10.4
Sodium	11
Potassium	2800
Vitamins (mg)	
Niacin	6
Ascorbic acid	110
Caffeine	3.5%
Tannin	10%

(Cabrera et al. 2006)

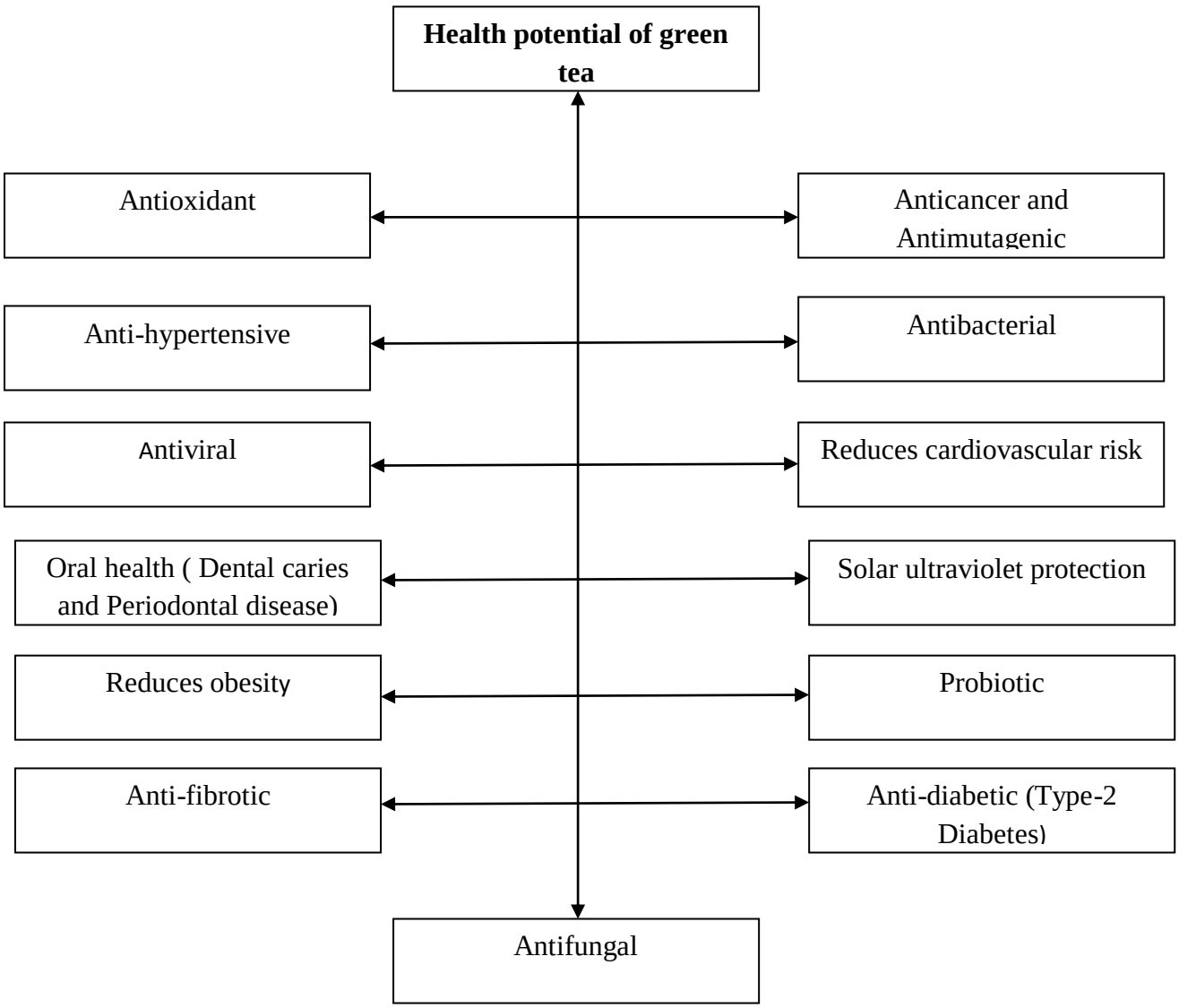


Fig. 3.4.3 Health potential of green tea

3.5 Ginger

The most commonly used condiment for foods and beverages ig ginger (*Zingiber officinale*). It is a rhizome and has a wide applications as a medicinal plant which dates back to 2500 years (Shukla et al. 2006)

It is a herbaceous perennial which grows annual stems about a meter tall bearing narrow green leaves and yellow flowers.

Table 3.5.1 Scientific classification

SCIENTIFIC CLASSIFICATION	
Kingdom	Plantae
Class	Spermatophyte
Order	Zingiberales
Family	Zingiberaceae
Genus	Zingiber
Species	Officinale
Binomial name	*Zingiber officinale*

Table 3.5.2 Chemical composition of ginger

CHEMICAL COMPOSITION OF GINGER	
Moisture	15.02 ± 0.04
Protein (g)	5.087 ± 0.09(5.98)
Fat (g)	3.72 ± 0.03 (4.37)
Insoluble fibre (%)	23.5 ± 0.06 (27.65)
Soluble fibre (%)	25.5 ± 0.04 (30.0)
Carbohydrates (g)	38.35 ± 0.1
Vitamin C (mg)	9.33 ± 0.08 (10.97
Total carotenoids (mg)	79 ± 0.2 (9296)
Ash (g)	3.85 ± 0.61 (4.53)
Calcium (mg)	88.4 ± 0.97 (104.02)
Phosphorous (mg)	174±1.2 (204.75)
Iron (mg)	8.0 ± 0.2 (9.41)
Zinc (mg)	0.92 ± 0 (1.08)
Copper (mg)	0.545 ± 0.002 (0.641)
Manganese (mg)	9.13 ± 001 (10.74)
Chromium (µg)	70 ± 0 (83.37)

(Adel et al. 2010)

3.5.3 Health potential of ginger

Ginger has long been used due to their potential antimicrobial activity properties against different microbial pathogens. It is widely used in the treatment of common cold, cough and flu (Indravati et al. 2017). It is also used as a remedy for throat pain. It has been traditionally used from time immemorial for the treatment of human ailments such as upset stomach, diarrhoea and nausea (Shukla et al. 2007).

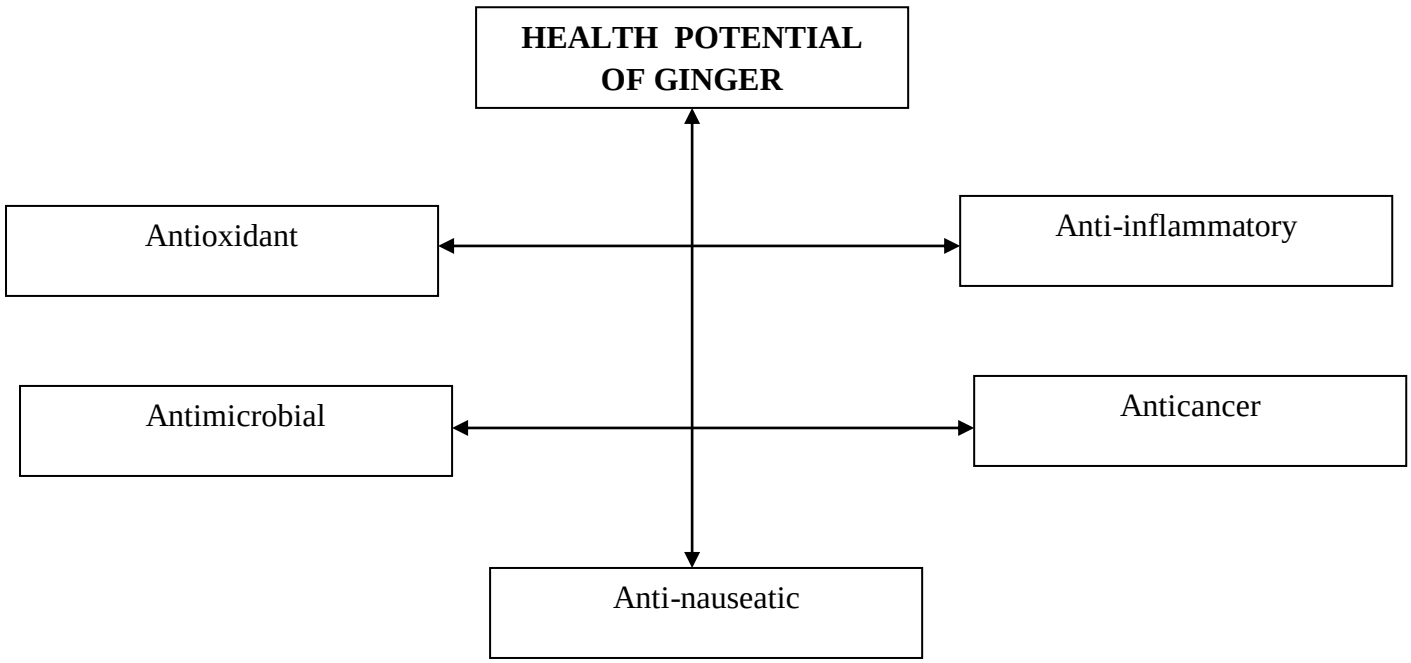


Fig. 3.5.3 Health potential of ginger

3.6 Tulsi

Holy basil also known as tulsi (*Ocimum sanctum)* is an erect, many-branched subshrub, 30–60 cm (12–24 in) tall with hairy stems. In Ayurveda, it has been documented for its therapeutic use and described as "Dashemani Shwasaharni (antiasthmatic) and antikaphic drugs (Kaphaghna)". Its medicinal use dates back to 4000-5000 B.C. (Prakash et al. 2004)

Table 3.6.1 Scientific classification

SCIENTIFIC CLASSIFICATION	
Kingdom	Plantae
Class	Magnoliopsida
Orderb	Lamianes
Family	Lamiaceae
Genus	Ocimum
Species	sanctum
Binomial name	*Ocimum sanctum*

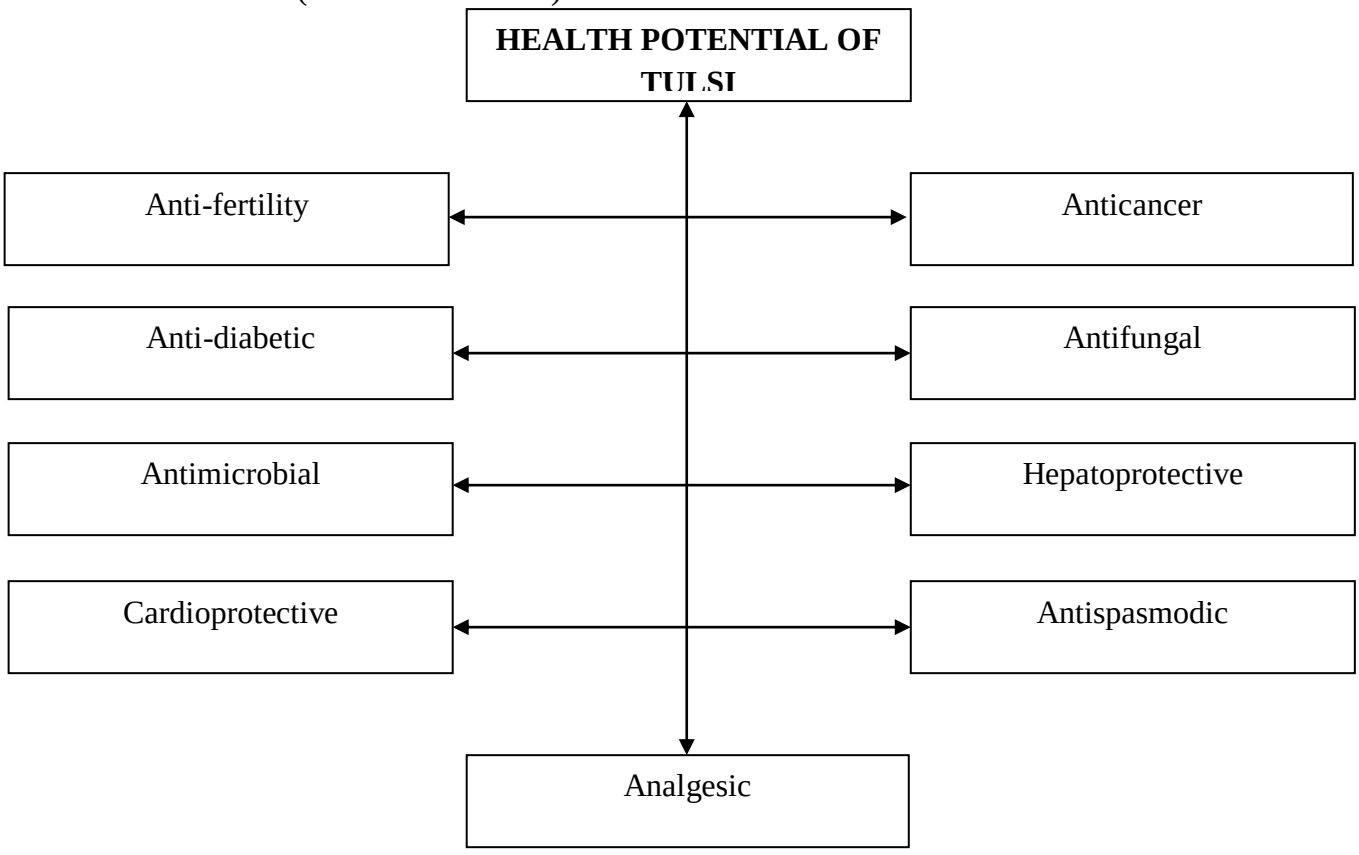
Table 3.6.2 Chemical composition of tulsi

CHEMICAL COMPOSITION OF TULSI	
Carbohydrates (g)	2.3 g
Protein (g)	4.2 g
Fat (g)	0.5 g
Minerals	
Calcium (mg)	25
Phosphorous (mg)	287
Iron (mg)	15.1
Vitamin C (mg)	25
Phytochemicals	0.7% total
Eugenol	71%
Methyl-eugenol	20%
Fatty acids	
Linoleic acid	52.23%
Linolenic acid	16.63%
Oleic acid	13.82%
Stearic acid	3.19%
Palmitic acid	11.69%

(Pattanayak et al. 2010) (Singh et al. 2012) (Mahajan et al. 2012)

3.6.3 Health potential of tulsi

It has been used in the treatment of fever and bronchitis. It also checks vomiting and antihelminthic . It is also used for treating malaria, diarrhea, dysentery, skin diseases, arthritis and chronic fever (Prakash et al. 2004).



3.7 Cinnamon

Cinnamon is obtained from the inner bark of the tree of cinnamon*(Cinnamomum verum).*It is one of the oldest herbal medicines and dates back to 4000 years ago (Quin et al. 2003).

Cinnamon is one of the most popular spices used by humankind and is the second most important spice next to black pepper. The term Cinnamomumis derived from the Greek root kinnamonor kinnamomon, meaning sweet wood. The Hindi name dalchini, meaning Chinese wood, refers originally to the Chinese cinnamon, which was popular in northern India before the Ceylon cinnamon became known (Ravindran et al. 2004).

Table 3.7.1 Scientific classification

SCIENTIFIC CLASSIFICATION	
Kingdom	Plantae
Class	Magnoliopsida
Order	Lauranes
Family	Lauraceae
Genus	Cinnamomum
Species	Verum
Binomial name	*Cinnamomum verum*

Table 3.7.2 Chemical composition of cinnamon

CHEMICAL COMPOSITION OF CINNAMON	
Carbohydrates	59.55%
Protein	4.65%
Fat	2.2%
Moisture	9.9%
Fibre	20.3%
Total ash	3.55%
Calcium	1.6%
Phosphorous	0.05%
Iron	0.004%
Sodium	0.01%
Potassium	0.4%
Vitamins	
Niacin (mg)	0.14
Riboflavin (mg)	0.21
Vitamin C (mg)	39.8
Vitamin A (IU)	175
Volatile oils	4%
Cinnamaldehyde	80%-90%

(Thomas et al. 2001)

3.7.3 Health potential of cinnamon

Cinnamon improves blood circulation, stimulates appetite and digestion. useful for the treatment of anorexia, heart disease, piles and helminthic infections . It is effective against cold and associated fever and headache, muscular pain, arthritic pain and amenorrhea which is failure of menstruation. It is also useful in bronchitis and pneumonia (Vijayan et al. 2004)

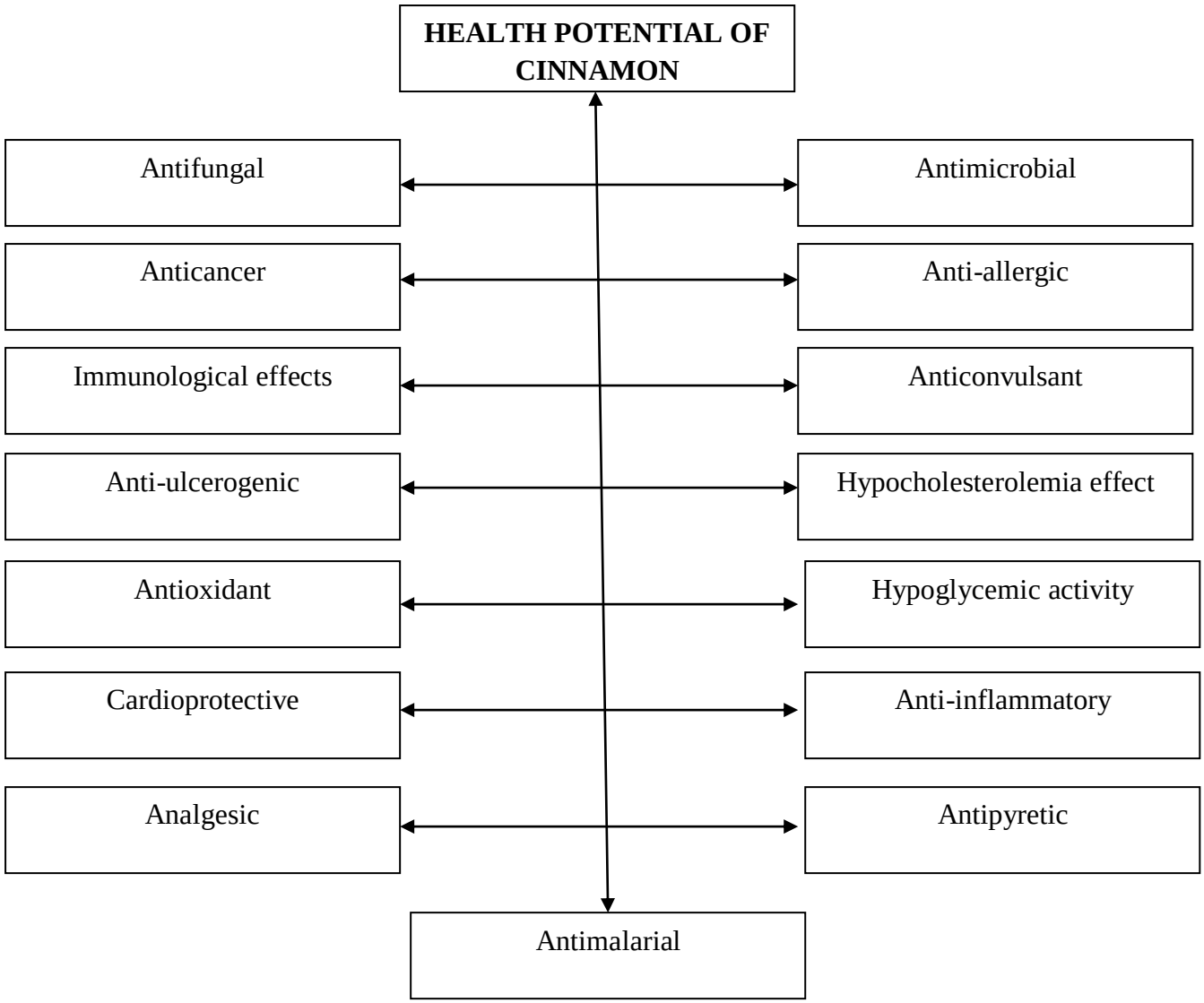


Fig. 3.7.3 Health potential of cinnamon

Chapter 4: PROPOSED RESEARCH OBJECTIVE

1. To standardize a process for green tea incorporated hard candy.
2. To optimize a process for hard candy incorporated with green tea and spice blends.
3. To evaluate the blends by fourier transform infrared (FTIR) spectroscopy.
4. To study the shelf life of the optimized products.

Chapter 5: PROPOSED RESEARCH METHODOLOGY

5.1 Detailed plan work

5.1.1 Objective 1:

To standardize a process for green tea incorporated hard candy.

Preparation of green tea extracts

Two types of extracts will be used: ethanolic extract and hot water extract. The third type used is matcha powder.

Ethanolic extract of green tea:

The green tea (1g) will be extracted with 70% ethanol (ethanol/water, v/v, 10ml) and will be sonicated for 1 hour. The resultant solution will be filtered using the filter paper (Ma et al. 2017)

Hot water extract of green tea:

The green tea (1g) will be extracted with 20 ml of water at temperature for 60ºC for 7 minutes. The mixture will be centrifuged at 4,000 rpm for 5 minutes. This will be done twice and both the supernatants will be concentrated using a vaccum rotatory evaporator at 55ºC and freezed (Shuyuan et al. 2017)

Preparation of hard candy

Hard candy:

Material required: Candy thermometer, measuring cups, utensils and heating source.

Hard candy with matcha powder

Ingredients	Blend (1%)	Blend (2%)	Blend (3%)	Blend (4%)	Blend (5%)
Water	22.2	22.2	22.2	22.2	22.2
Sugar	66.6	66.6	66.6	66.6	66.6
Liquid glucose	11.1	11.1	11.1	11.1	11.1
Green tea powder	2	4	6	8	10
Citric acid	0.2	0.2	0.2	0.2	0.2

Hard candy with green tea extract

Ingredients	Blend (1%)	Blend (2%)	Blend (3%)	Blend (4%)	Blend (5%)
Water	22.2	22.2	22.2	22.2	22.2
Sugar	66.6	66.6	66.6	66.6	66.6
Liquid glucose	11.1	11.1	11.1	11.1	11.1
Green tea extract	2	4	6	8	10
Citric acid	0.2	0.2	0.2	0.2	0.2

5.1.2 Objective 2:

To optimize a process for hard candy incorporated with green tea and spice blends.

The concentration of green tea extract and matcha powder standardised in the objective 1 will be used with other spices and herbs.

Preparation of tulsi extract:

Two types of extract will be used : ethanolic extract and hot water extract.

Ethanolic extract of tulsi:

The tulsi powder (1g) will be extracted with 70% ethanol (ethanol/water, v/v, 10ml) and will be sonicated for 1 hour. The resultant solution will be filtered using the filter paper (Ma et al. 2017)

Hot water extract of tulsi:

Fresh leaves of Tulsi (20g) will be washed thoroughly with double-distilled water and will then be cut into small pieces. The cut pieces will be mixed with 100mL doubled-distilled water, and this mixture will be kept for boiling for a period of 5 minutes. After cooling, it will be filtered through Whatman Filter paper no.1 (Ramteke et al. 2012)

Dry tulsi powder:

Tulsi leaves will be sun dried and will be grinded to make powder. Finally this powder will be sieved to get the finest powder for better texture of the product.

Preparation of hard candy

Hard candy:

Material required: Candy thermometer, measuring cups, utensils and heat source.

Hard candy with extract

Ingredients	Blend (1%)	Blend (2%)	Blend (3%)	Blend (4%)	Blend (5%)
Water	22.2	22.2	22.2	22.2	22.2
Sugar	66.6	66.6	66.6	66.6	66.6
Liquid glucose	11.1	11.1	11.1	11.1	11.1
Green tea extract	Concentration as standardized in objective 1				
Tulsi extract	2	4	6	8	10
Citric acid	0.2	0.2	0.2	0.2	0.2

Hard candy with matcha powder

Ingredients	Blend (1%)	Blend (2%)	Blend (3%)	Blend (4%)	Blend (5%)
Water	22.2	22.2	22.2	22.2	22.2
Sugar	66.6	66.6	66.6	66.6	66.6
Liquid glucose	11.1	11.1	11.1	11.1	11.1
Green tea powder	Concentration as standardized in objective 1				
Tulsi powder	2	4	6	8	10
Citric acid	0.2	0.2	0.2	0.2	0.2

Preparation of ginger extract:

Two types of extract will be used : ethanolic extract and hot water extract.

Ethanolic extract of ginger:

The ginger powder (1g) will be extracted with 5 ml of 96% ethanol (1:5) for 3 x 24 hours. Every 24 hours, the extractfrom each, maceration will be filtered. Afterward, all filtrates from the first, second and third days of maceration will be combined and then evaporated using a rotary vacuum evaporator (Indravati et al. 2017)

Hot water extract of ginger:

Fresh ginger rhizome will be sliced and will be extracted with hot water in a round bottom with a cooling condenser at 95ºC for 1 hour. The extract will be filtered using whatman filter paper (Oh et al. 2016)

Preparation of hard candy

Hard candy:

Material required: Candy thermometer, measuring cups, utensils and heat source.

Hard candy with matcha powder

Ingredients	Blend (1%)	Blend (2%)	Blend (3%)	Blend (4%)	Blend (5%)
Water	22.2	22.2	22.2	22.2	22.2
Sugar	66.6	66.6	66.6	66.6	66.6
Liquid glucose	11.1	11.1	11.1	11.1	11.1
Green tea powder	Concentration as standardized in objective 1				
ginger powder	2	4	6	8	10
Citric acid	0.2	0.2	0.2	0.2	0.2

Hard candy with extract

Ingredients	Blend (1%)	Blend (2%)	Blend (3%)	Blend (4%)	Blend (5%)
Water	22.2	22.2	22.2	22.2	22.2
Sugar	66.6	66.6	66.6	66.6	66.6
Liquid glucose	11.1	11.1	11.1	11.1	11.1
Green tea extract	Concentration as standardized in objective 1				
Ginger extract	2	4	6	8	10
Citric acid	0.2	0.2	0.2	0.2	0.2

Preparation of cinnamon extract:

Two types of extract will be used : ethanolic extract and hot water extract.

Ethanolic extract of cinnamon:

The cinnamon bark powderwill be weighed and placed into a flask. Ethanol solvent (96%) will be added to the flask until the plant materials will be submerged in the solvent. The extraction process will be done for 2 hours after boiling the solvent and this will be repeated 3 times. Then the extract will be evaporated using a rotatory evaporator (Julianti et al. 2017)

Hot water extract of cinnamon

The cinnamon powder (2.5) will be added to a 500 mL flask with 100 mL sterile distilled water and then boiled for 5 min. Then it will be filtered with whatman filter paper and extract is obtained (Kumar et al. 2009)

Preparation of hard candy

Hard candy:

Material required: Candy thermometer, measuring cups, utensils and heat source.

Hard candy with matcha powder

Ingredients	Blend (1%)	Blend (2%)	Blend (3%)	Blend (4%)	Blend (5%)
Water	22.2	22.2	22.2	22.2	22.2
Sugar	66.6	66.6	66.6	66.6	66.6
Liquid glucose	11.1	11.1	11.1	11.1	11.1
Green tea powder	Concentration as standardized in objective 1				
Cinnamon powder	2	4	6	8	10
Citric acid	0.2	0.2	0.2	0.2	0.2

Hard candy with extract

Ingredients	Blend (1%)	Blend (2%)	Blend (3%)	Blend (4%)	Blend (5%)
Water	22.2	22.2	22.2	22.2	22.2
Sugar	66.6	66.6	66.6	66.6	66.6
Liquid glucose	11.1	11.1	11.1	11.1	11.1
Green tea extract	Concentration as standardized in objective 1				
Cinnamon extract	2	4	6	8	10
Citric acid	0.2	0.2	0.2	0.2	0.2

Proximate analysis of the hard candies:

1. Proximate analysis
    i. Total sugars (Ranganna 2016)
    ii. Reducing sugars (Ranganna 2016)

iii. Proteins by Lowry method (Magomya et al. 2014)
iv. Total ash (Romelle et al. 2016)
v. Water solubility index and water absorption index (Wani et al.2013)

2. Chemical analysis
   i. Total phenolics acid content (Roby et al.2013)
   ii. Total flavonoid content (Baba et al. 2015)
   iii. Total carotenoids content (Ding et al. 2016)
   iv. Ascorbic acid content (Baba A et al. 2016)
   v. Catechin content (Ananingsih et al. 2013)
3. Antioxidant activity
   i. DPPH radical scavenging activity (Rasane et al. 2014)
   ii. ABTS free radical scavenging activity (Khan et al. 2012)
   iii. FRAP assay (Hassan et al. 2013)

5.1.3. Objective 3:

To evaluate the blends for catechin activity by Fourier transform infrared (FTIR) spectroscopy.

The blends will be evaluated for the catechin activity by Fourier transform infrared (FTIR) spectroscopy (Hamed et al. 2006) and UV/Vis spectroscopy (Ananingsih et al. 2013) for quantification.

5.1.4 Objective 4:

To study the shelf life of the optimized products.

1. A group of people who are a regular consumer of hard candy will be there for the sensory evaluation. This will be done after seven days to analyse the acceptability by 9-point Hedonic scale method.
2. The shelf life of the hard candies will also be determined by analysing the microbial changes taking place during the storage of the products. The following tests will be performed:
   i. Colic forms analysis (FSSAI 2012)
   ii. Estimation of yeast and mould (FSSAI 2012)
   iii. Total plate count (FSSAI 2012)
3. The chemical tests including the following:

i. Total phenolics acid content (Kamtekar et al. 2014)
ii. Total flavonoid content (Baba et al. 2015)
iii. Total carotenoids content (Ding et al. 2016)
iv. Ascorbic acid content (Baba A et al. 2016)
v. Catechin content (Ananingsih et al. 2013)

4. Antioxidant activity test includes:
    i. DPPH radical scavenging activity (Rasane et al. 2014)
    ii. ABTS free radical scavenging activity (Khan et al. 2012)
    iii. FRAP assay (Hassan et al. 2013)

The above study will be followed by a consumer acceptance study as below:

About 50 random subject from the premises of the Lovely Professional University will be used for sensory evaluation using a hedonic and descriptive analysis to access the acceptability of the developed green tea hard candy.

Chapter 6: EXPECTED RESEARCH OUTCOME

1. Standardization of a process for green tea incorporated hard candy will be done.
2. Optimization a process for hard candy incorporated with green tea and spice blends will be done.
3. Evaluation of the blends for catechin activity by Fourier transform infrared (ftir) spectroscopy will be done.
4. The hard candy acceptable on sensory, physical and chemical parameters will be developed.
5. The obtained optimized product will be evaluated for shelf life and an optimum shelf life will be predicted.

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