Formulation and development of a neutraceutical herbal drink by using "Gotukola"

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Declaration

I carried out the work described in this thesis at the Ceylon Cold Stores (Pvt) Ltd and at the Faculty of Applied Sciences under the supervision of Mr. D.A.M Arsecularathna and Mrs.Somawathi. A report on this has not been submitted to any other university for another degree.

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Dedicated
To
My Family Members
And To All of My Teachers

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Abstract

Gotukola (*Centella asiatica*) is an ancient ayurvedic remedy, which is used in the present as a useful tonic and cleaning herb for skin problems and digestive disorders. It is used to treat a variety of disorders, including leprosy, but it is valued chiefly as a revitalizing herb that strengthens nervous function and memory. Gotukola, which has a bitter, sweet and an acrid taste also be used as a leafy vegetable.

In the present era, where people are more interested in natural food products, herbal drink have a high demand in the drink market. Therefore this study was carried out with the main objective of to develop an herbal drink with maximum consumer satisfaction by using Gotukola (*Centella asiatica*). In addition, it was also aimed to select the best extraction method for Gotukola (extractor).

During this study the Gotukola juice was extracted using three different solvents. Hot water, alcohol and a true mixer of hot water and alcohol were used for this purpose. Most suitable time-temperature requirement and herbal content for preparation of drink in hot water extraction were determined using sensory evaluation. Absolute alcohol was used in the alcohol extraction and 30% alcohol was used in the extraction by solvent mixture. The results of both the sensory evaluation and TLC analysis were considered in determining the best extraction method. In the shelf life evaluation of the drink, both the physicochemical (Acidity, pH, SMS, Brix value) and microbiological (TCC, Yeast and moulds count, Coliform and E-coli count) characteristics were taken in to consideration.

According to the results it was revealed that the best temperature-time combination for the hot water extraction was 100^0C for 10 minutes and suitable herbal content that can be used in preparation was 10% v/v basis. Sensory evaluation results indicated the hot water extraction as the best method where as TLC results indicated the alcohol extraction as the best. The physicochemical and microbiological characteristics of the drink remained unchanged during the period of a storage life of eight weeks.

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Abbreviations

Et al	: And others
TSS	: Total Soluble Solid
CMC	: Carboxy Methyl Cellulose
SLS	: Sri Lanka Stranded
Kg	: Kilogram
G	: Gram
EC	: E.coli / Coliform
TLC	: Thin Layer Chromatography
Ex	: Example

Chapter 01

1. Introduction

Gotukola (*Centella asiatica*) is a slender stemmed, delicate, perennial creeping herb, belonging to the Umbelliferae. It is used in Ayurveda dates from prehistoric times. It has been found to be useful in diseases of skin and leading anti bacterial, anti fungal, anti cancer, anti-inflammatory and wound healing properties. It increases appetite, settle worm troubles and also it's good for diarrhea and catarrh. The various studies have shown that *Centella* is rich in triterpenoids, alkoloids, volatile oil and flavonoids.

Centella asiatica is commonly used as an ayurvedhic medicine as well as a leafy vegetable (Rajapaksha, 1998). Consumption of Gotukola as a leafy vegetable can only be done at main meals and also it takes time for the preparation. Hence instead leafy Gotukola products with similar nutraceutical properties are beneficial for consumers.

Gotukola products are available in the local market, such as Gotukola capsules, Tonic, Gotukola herbal Tea and Gotukola Kandha etc: As people are nowadays looking for natural food products in a convenient form to consume herbal soft drink, carbonated or non-carbonated can become popular in this changing world.

There is a possibility to substitute the real herbal for synthetic, artificial beverages. In Sri Lanka the production of herbal drinks is limited and if mostly happen in small-scale Ayurveda. Therefore preparation of a ready to serve herbal drink using "Gotukola" may be a good invention for the development of herbal beverage industry.

1.2. Overall Objective

Development of a nutraceutical herbal drink with maximum consumer satisfaction by using Gotukola (*Centella acsiatica*).

1.2.1. Specific Objectives

i. To develop a better extraction method with suitable organoleptic properties and minimum loss of medicinal value.

ii. To reduce the bitterness and raw taste of the drink

iii. Sensory evaluation to select the best combination of extraction process & other ingredients

iv. Shelf life evaluation of the prepared drink

Chapter 02

Review of literature

2.1. Taxonomy

Genus *Centella* was separated from hydrocotyle on the basis of their leaf, flower and fruit characters. In hydrocotyle, the two mericarps have 3 ridges each, flowers are white, and pericarp of seed is thin and the leaves are lobed while in *centella* the two mericarps possess 7-9 ridges each, thick and are red or purple, pericarp is thick and leaves are unlobed.

Family: Umbelliferae

Genus : *Centella*

Spices : *Asiatica*

2.1.1. Botanical name

Centella asiatica, Hydrocotyle asiatica (Linn), *Hydrocotyle coightiana* (wall), *Hydrocotyle lusida* (Honce), *Hydrocotyle nummulariods* (rich), *Hydrocotyle pallida.*

2.1.2. Vernacular names

Sinhala : Hin-Gotukola

Tamil : Babassa, Vallarai, Vallari, Orila tamari

English : Indian pennywort

Hindi : Vallari

Sonskrit : Mandoo kaparni

2.2. Plant description

A prostrate perennial herb with a short vertical rootstock and glabrous axillaries stems with long internodes.

Leaves: Simple, alternate, stipulate, several from the rootstock and 1 to 2 from each node of the runners, petioles 7.5-15cm long, erect, glabrous.

Flowers: Irregular, bisexual, dark pink, nearly sessile, usually there together at ends of short, erect, pubescent peduncles 1-3 from the nodes opposite the leaves, bracts 2,close beneath the umbel, orate, obtuse.

Fig. 2.1. Gotukola (*Centella asiatica*) leaves

Fruits: About 0.3-0.4cm long ovoid hard (flowers from May to October) (Rajapaksha, 1998).

2.3. Distribution

Grows in India, Sri Lanka and other tropical countries. It is a very common weed in Sri Lanka growing in waste grassy places from sea level to the highest elevations.

2.4. Food uses:

The leaves and young stem. It is eaten as a green vegetable either cooked or as a salad. It is boiled and drunk as a beverage. Extract of whole plant is used for preparation of porridge.

2.5. Storage

Harvested leaves can be kept for 4-6 days in a shady cool place (Rajapaksha, 1998).

2.6. Agronomy

2.6.1. Soil and climate

This plant is commonly found in the tropics. The plant grows well on sandy or clayey soils near water or marshy, with sufficient sunlight. The bush (large leave type) is usually cultivated in beds and pots. A shady moist location is essential to induce profuse leaf growth.

2.6.2. Propagation

The plant is propagated through the seed and the stolen. The stolen with nodes and roots is placed in the sand or wet soil for 1-2 weeks. Young plants will appear and will be ready for planting in one week. The plants can be harvested 6 months after planting by digging a clump of plant. The whole plant is collected, washed thoroughly and dried in the mild sun (Purnima, 1998).

2.6.3. Diseases

Bacterial wilt

The wilting of the leaves manifests the disease, which gives the appearance of the water supply to the plant having being suddenly cut off. The leaves remain drooping and do not recover, so that ultimately the plant dies. The disease is due to the bacteria *Pseudomanas solanacearum.* There is no chemical control measure for this disease and the only method is to destroy the diseased plants as and when they appear.

Leaf spot diseases

The first indication of the diseases is the appearance of small, water-soaked spots between the veins on the underside of the leaves. These spots become visible on the upper surface and resemble 'oil-spots'. As the disease progresses the spots coalesce to form large irregular dead areas, which eventually fall off, leaving a hole in the leaf. In wet weather, the leaves become slimy on the undersides. This slime is composed of bacteria, which have developed in the leaf tissue.

Since the bacteria are easily carried on the hands of the pickers, it is essential that strict hygienic measures should be adopted to prevent the spread of diseases. A diseased leaf should be picked first and burnt. The bacteria *Cochliobolus geniculatus* cause leaf spot disease. Pesticide such as Profenofos, Endosulfan, Chlopyrifos, Quinalphos, and Carbofuran are used in Gotukola.

2.7. Chemistry of plant

2.7.1. Chemical constituents of plant

Centella asiatica is commonly used in the Ayurvedic system of medicine as well as being a leafy vegetable. Various studies have shown that is in rich in triterpenoids, volatile oils and flavonoids.

The major triterpenoids compounds of *Centella asiatca* are asiatoside, asatic acid, madecassic acid, asiaticoside and madcassoside.Asiaticoside is the active agent in the treatment of leprosy. Other terpenoids, such as madecassic acid, Asiatic acid and asiaticoside also show bioactivity.

Centella contains, strongly volatile oil that contains unidentified terpene acetate which accounts for 35% of the total oil.Vallerine is the most active compound in *Centella* oil.

Considering bitter principle vallarine, pectic acid and resins are present in the leaves and roots of the plant. The plant also contains ascorbic acid in a concentration of 13.8mg per100g.

Centella also contains glyceried of fatty acids, various plant sterols and polyacetaline compounds.*Centella* has net digestible protein calorie percentage values above the minimum of five required for maintenance of nitrogen balance. Amino acid studies of the plant indicated that in the leaf, petiole and stolon, percentage of glutamate and serine is more compared to other amino acids. Roots are rich in amino acids, aspartate, glutamate, serine, threonine, alanine, lysine, histadine (Purnima, 1998).

2.7.2. Chemical composition

Table 2.1 Compositions of leaves

Compounds	Amount / 100g
Moisture	87.2g
Protein	1.7g
Fat	0.7g
Carbohydrate	4.8g
Crude fiber	3.4g
Ash	2.3g
Ca	176.0g
P	72.0g
Fe	12.0g
Vitamin C	42.0g
Niacin	0.8g
Carotene	2400g
Energy	320kcal

(Source: Purnima, 1998)

2.7.3. Key constituents of Gotukola extract

- Triterpenoids saponins (asiaticocide,brahmoside,thankuniside)
- Alkaloids(hydrocotaline)
- Bitter principle(vallarine)

(Andrew, 1996).

2.7.3.1. Main chemical components in *Centella aciatica*

Triterpenoids

Tritepenods have a carbon skelton based on six isoprene units. They are colorless crystalline, often high melting optically active compounds. The cyclic compounds are the tetra cyclic or pentacyclic.

Chemical test

To a chloroform solution of the drug, few drops of acetic anhydride and 1ml of concentrated sulfuric acid are added. Blue or blood red color is produced (Ali and Ahamad, 2003).

Flavonoid

Flavonoids contains fifteen carbon atoms in there basic nucleus and these are arranged in a C_6- C_3- C_6 configuration, that is ,two aromatic rings like by a three carbon unit which may or may not form a third ring They occur both in the free state and as glycosides and are the largest group naturally occurring phenols. The flavonoids are generally soluble in water and alcohol, but insoluble in organic solvents; the genins are only sparingly soluble in water but are soluble in ether. Flavonoids dissolve in alkalines, giving yellow solution, which on the addition of acid become colorless.

Chemical test

Ammonia test: A filter paper dipped in an alcoholic solution of the flavonoids is exposed to vapors of ammonia solution. Yellow color is formed.

Volatile oil

Volatile oils have characteristic pleasant odor. A spot of the volatile oil on a filter paper pieces is evaporated with 24 hours. Volatile or essential oil are mono- and sesquiterpenes obtained from certain plant parts. They are colorless liquid and are lighter than water. They posses distinct odors, have high refractive indices, generally are optically active and immiscible with water. Volatile oils are freely soluble in ether, alcohol, chloroform, acetone etc (Ali and Ahamad, 2003).

Alkaloids

Alkaloids are complex compounds generally produced from plant. They are basic in nature and contain one or more nitrogen atom and have pronounced pharmacological action on man or animals.Generally alkaloids are colorless they are soluble in organic solvents but insoluble in water. An alkoloidal salts are soluble in water and insoluble in organic solvents. A number of alkaloids are degraded by exposure to light and air (Singh , 2003).

Table 2.2 shows constitute of *centella asiatica* essential oil

Table 2.2. Constituents of *Centella asiatica* essential oil

Compound	Kovats indices	Area percentage
Pentanal	980	0.3
2-Methyl-2-butanol	1000	0.8
α-Pinene	1030	t
Toluene	1042	t
Camphene	1068	t
Hexanal	1088	1.1
β-Pinene	1120	0.1
m-Xyiene	1142	0.2
Myrcene	1156	0.4
Pyridine	1180	0.3
Heptana	1186	0.2
Limonene	1188	0.1
3-Hexen-2-one	1211	0.1
γ-Terpinene	1231	0.3
(E)-2-Heptenal	1329	0.1
(E)-2-Octenal	1425	0.2
5-Nonnen-2-one	1447	0.3
2-Furancarboxaldehyde	1486	0.6
3-Nonen-2-one	1522	1.5
Linalool	1554	2.1
β-Caryophyllene	1598	26.8
Widdrene^{+}	1620	1.2
(E)-β-Farnesene	1673	t
α-Humulene	1675	33.7
β-Acoradiene^{+}	1688	t
β-Chamigrene	1710	0.2
α-Terpineol	1718	1.0
Germacrene-D	1727	10.0
β-Bisabolene	1745	0.9
α-Chamigrene	1760	1.9
δ-Cadinene	1765	0.7
δ-Cadinene	1776	0.9
β-Sesquiphellandrene	1817	0.5
1-Methyl-4-(1,2,2-tri-metylcylopentyl)benzene^{+}	1837 1856	0.1 1.9
Geraniol	1994	1.9
Caryophyllene oxide	2011	0.1
Epi-globulol	2053	1.2
Nerolidol*	2155	1.7
p-Vinylguaicol	2300	0.2
Farnesol	2314	0.2
2,3-Dihydrobenzofuran		

+ = tentative identification
* = correct isomer not identified

(Source: Jayaweera, 1994)

2.8. Biology health activity

Cetella asiatica has been used as a medicine since prehistoric times. In different parts of the world it is used for various illnesses. In India, it is widely used for Leprosy, other skin diseases such as lupus and skin disorders caused by syphilis and also as a diuretic. Chinese use it for colds, sore throat, fevers, sun-stroke, tonsillitis, urinary tract infection, hepatitis, liver diseases such as jaundice and cirrhosis and dysentery. France, West Indies and Brazil use it to cure uterine cancer, leprosy and elephantiasis and Central and East Africa, the leaf is for fevers, bowel complaints and syphilitics and scrofulous conditions.

In published literature, *Centella asiatica* shows, anti-bacterial anti-fungal, anti-cancer, anti-inflammatory, wound healing, tranquilizing anti-allergic, hypotensive, antipyretic, and peptic ulcer cleaning properties.

Also asiaticoside is the active component of treatment of leprosy. It has the capability of dissolving the waxy covering of the bacteria, *Bacillus leproe*, so that becomes fragile and may easily be destroyed by the tissues or by some other drug. Asiaticoside also improves general mental ability of mentally retarded children. Brahmic acid has a therapeutic value in ulceration, extensive wounds, eczymas etc. It also has an inhibitory effect on the biosynthetic activity of fibroblast.

2.8.2. Toxicology

Subcutaneous injection of 0.04 - 0.05 g/kg proved toxic to the mouse and rabbit while 0.2- 0.25 g/kg results in increasing bleeding time and hemorrhagic accidents. An oral dose of 1g/kg is shown to be tolerated by both mouse and the rabbit. Asiaticoside is carcinogenic. Yet it is said to stimulate wound healing. It has been reported to contain the poisonous hydrocyanic acid. Two saponin glycosides, brahminoside show sedative activity.

2.8.3. Pharmacology

Wound healing

The triterpenes in *Centella asiatica* have remarkable wound healing properties. Studies have shown that asiaticoside given to animals such as rats, guinea, pigs, and rabbits, stimulates hair and nail growth. It also increases vascularisation of connective tissue, and also increases the tensile integrity of the dermis, increases keratinization of the epidermis through stimulation of the stratum germinativum and possessa entrophic or balancing effect on connective tissue.

Burns

Centella asiatica accelerates healing of burns and minimizes scaring and has been thus used in treatment of second and third degree burns. It can prevent or limit the shrinkage and swelling of the skin caused by infection and inhibits scar formation. Studies have also shown that can accelerate healing of skin grafts (Purnima, 1998).

Nervous system

The triterpens in *Centella asiatica* have mild tranquilizing, anti-stress and anti anxiety action, by enhancing cholinergic mechanisms, which probably account for the effect in improving mental function. It possesses special tonic properties for the brain and nervous system and promotes mental calm and clarity. Studies have shown that *centella* contains an abundance of B vitamins and it has traquilizing, sedative and antispasmodic actions (Purnima, 1998).

2.8.4. Ayurvedic preparation

Centella asiatica had been used in Ayurveda medicine for a very long time in Sri Lanka and India.

Table.2.3 Native Ayurvedic Preparation

Preparation	Uses
Siddhajeewa murthaya	Tonic
Gotukola Beatha	Catarrh, nervous diseases, epilepsy
Gotukola Paniya	Catarrh, nervous diseases, piles
Gotukola Kwatha	Catarrh

(Source: Purnima, 1998)

2.8.5. Surgical uses

An injectable extract of *Centella asiatica* has been used to accelerate the healing of post-surgical wounds, to inhibit hypertropic formation of scar tissue in the treatment of second and third degree burns as general use in wounds, ulcers and scleroduma.

The leaf extract is shown to have a successful affect in clinical patients with soiled wounds and chronic atony, resistant to treatment.

The benefits been shown to act on chronic lesions such as cutaneous ulcers, surgical wounds, fistulas and gynecological wounds. It has also been used with success to improve the blood circulation in the lower limbs where the stimulation of collagen synthesis in the vein wall resulted in an increase in vein tonicity and a reduction in the capacity of the vein to distend, as in vericose veins and phlebitis (Purnima, 1998).

2.8.6. Cosmetic uses

Centella asiatica with astringent tannins and soothing essential oils, which are excellent ingredient for toning and stimulating the skin, is ideal for skin care formulations and also offers protective care.

The flavonoids are also used in hair care products where it stimulates the peripheral circulation of the scalp and will promote healthy scalp condition and prevent hair loss.

It is also reported to aid capillary growth in psychomatic alopecia in the case that the piliferous papillac are not atrophied. It is also responsible for accelerate growth of hair and nails (Purnima, 1998).

2.9. New Food Product Development

A simple definition for a new product might be a 'product not previously marketed or manufactured by a company'. However, this breaks down if one includes new packaging (shape or size) or if one enters a product into a new market niche – the food service sector, for example. The definition of new product development and introduction of a product not previously manufactured by a company into the market place or the presentation of an old product in to a new product into a new market not previously explored by a company (Fuller, 1994).

New food products are the major avenues open to a food company to be profitable and to service. The need for new food product development can be seen to be driven by five dominant forces.

1. All products have life cycles. That is, they enter their market place, flourish for an indeterminate time, then die and must be replaced.
2. A company is management they adopt a policy that requires an aggressive growth programmed to satisfy long-range business goals.
3. The market place may change, requiring new products more suited to respond to the changes.
4. New technology may take new food products available and new knowledge may tailor new food products more suited to the life cycles of today's consumers.
5. Changes in government legislation, health programme, agricultural policy, or agricultural support programmed dictate that development of new food products must be pursued (Fuller, 1994).

2.10. Basic ingredients of Herbal drink

2.10.1. Sugar

Sucrose is widely distributed in the plant kingdom although sugar cane or sugar beets are the commercial sources of most sugar. Sucrose is common disaccharides and they are anhydrides of two monosaccharide. Sucrose is particularly important in food processing and is commercially in many crystal sizes from extremely fine to very coarse. The sucrose inverted to glucose and fructose (Mayer, 1987).

2.10.2. Preservatives

Soft drink and ready to serve drink (RTS) preserves sometimes require addition of chemical preservatives to improve their storage stability. These additives should be used judiciously and happen to increase shelf life, prevent spoilage, or minimize the food-poisoning risk (Chapman, 2000).

Many chemicals will kill or inhibit the growth of microorganisms, but most of these are not permitted in foods. A few that are permitted in prescribed low level in certain foods include sodium benzoate, sorbic acid, Sulfur dioxide, Calcium propionate and ethyl formate. The use of chemicals to control microorganisms and for any other purpose in foods is part of the broader subject of food additives.

Sodium metabisulfite ($Na_2S_2O_5$) is a white to slightly yellowish, crystalline powder with a odor of Sulfur dioxide.

Sulfur dioxide is able to stabilize the color of fresh and processed fruits and vegetables. Sulfur dioxide also inhibits the activity of common oxidizing enzymes and has antioxidant properties. Sulfur dioxide reduces non-enzymatic Mallard type browning by reacting with aldehyde group of sugars. It also interferes with microbial growth.

Sulfur dioxide dips have been used to prolong the fresh appearance of cut vegetables and lettuce and other uncooked vegetables (Potter and Joseph, 1996).

2.10.3. Stabilizer

These substances include gums, starch, dextrin, protein derivatives, and other additives that stabilize and thicken foods by combining with water to increase viscosity and form gels (Potter and Joseph, 1996). Carboxyl Methyl Cellulose (CMC) is a thickening and stabilizing agent and prevents sugar from crystallization. It dissolves in hot or cold water and is fairly stable over a pH range of 5.0-10.0 but acidification below pH 5.0 will reduce the viscosity and stability except which differ in viscosity and degree of substitution. It functions as a thickener, stabilizer, binder, film former and suspending agent .The usage the usage range is from 0.05 to 0.5 % (Robert, 1998).

2.10.4. Salt (NaCl)

Chemical composition of salt is about 40% Na and 60% Cl by weight. In food containing salt as a preservative, the salt has been ionized. Collecting water molecule about each ion hydration. In describing the preservative action of salt, one must consider the dehydration effect; the direct affect the Cl ion, reduced oxygen tension, and interference with action of enzymes (Mayer, 1987).

2.10.5. Citric acid

Acid used in processed food a variety of functions that enhance the flavour food. They are used as flavoring agents, preservatives in microbial control, chelating agents, and in many other ways. Considering citric acid, used as an accidulant in carbonated beverage and drink industry. The major reason for its wide employment is the fact that it combines well with fruity and light flavors. Citric acid is versatile, widely used, cheap and safe. It is practically odorless, colorless and solid forming either translucent crystals or white granules or powder. It has a pleasant sour taste and a Flavour reminiscent of lemon. It should be used in very small quantities as otherwise the syrup will taste sour (Jacobes, 1959).

The more important of these are

a) Acid assists in preserving the syrup and beverage by killing microorganisms and making the environment that is beverage, unsuitable for their growth.
b) Acid catalyzes the inversion of sucrose to invert sugar
c) Acid converts sodium benzoate to benzoic acid, which exerts its preservation action only on an acid environment.

2.11. Processing steps of herbal drink

2.11.1. Extraction

Extraction is the separation of the required constituents from plant material (which is generally in solid form), using solvent. A large number of analytical methods are for foods an extraction as a clean up procedure, as a concentration step, remove a slightly soluble material, or to aid in the identification of a compound. These various procedures involve liquid-liquid and liquid-solid extraction using batch continues and discontinuous counter current techniques (Kottegoda, 1964). In the case of medicinal plants, the extraction procedure falls into two categories.

A. Where extracts the drug it is sufficient to archive within set limits on equiblium of concentration between drug components and the solution

B. Where it is necessary to extract the drug to exhaustion until all solvent extractable are removed by the solvents.

To facilitate extraction, the solvent should diffuse inside the cell and the substance must be sufficiently soluble in the solvent. The speed with which an equiblium is attained between solute and solvent is dependent on it.

Soxhlet Extractor (Liquid- solid system)

Extracting a material from a solid generally requires considerable time because it's difficult to get the extracting solvent into direct contact with the solute. Because of this, the effective distribution ratios are low and large volumes of solvents are sometimes necessary.

An efficient device for liquid –solid extraction, which eliminates the use of large solvents volumes, is the soxhlet extractor. The soxhlet extractor works well with solids but not with liquids. Continuous extractors are excellent for using with materials having a low distribution ratio (Pomeranze and Melon, 1994).

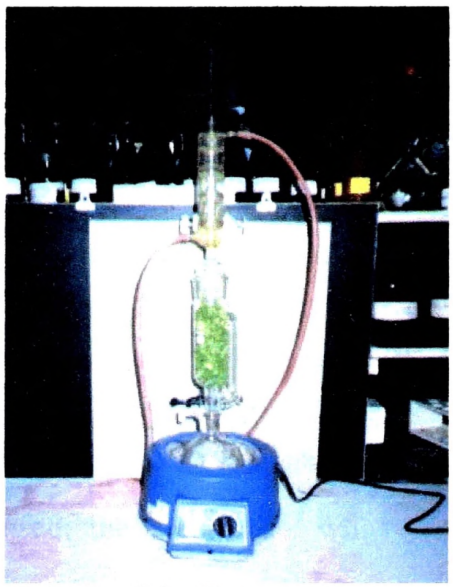

Fig. 2.2. Soxhlet Extractor

Choice of solvent

The ideal solvent for complete extraction is the most selective, has the best capacity for extraction, and is computable with the properties of the material to be extracted. These parameters must be pre-determined experimentally. In the industrial content, the factors of economy and availability come to the fore. Alcohol is widely used but because of its great extractive power, in that it extracts all soluble constituents. Alcohol in various ratios are used minimize selective, in that it extract s all soluble constituents. Alcohol is used in various ratio for woody or bark material is 75% for leafy material it is often less than 50% thus avoiding extraction of the chlorophylls which make purification problematical (Fototerapia, 1990).

Table 2.4. Ethanol

Compound	State	Boiling point
Ethyl alcohol (C_2H_5OH)	Liquid	70 ^0C

Test method

To the substance (extract solution), (2ml) in a test tube, add glacial ascetic acid (2ml) and concentrated sulfuric acid (1ml) on heating a fruity smell of ethyl acetate is produced.

Iodoform test

On addition of 3-4 drops of iodine solution and the sodium hydroxide solution drop by drop to the substance and warming the brown color of iodine disappears and yellow precipitate of characteristic smell is formed.

2.11.2. Distillation

It is process in which a liquid or vapor mixture of two or more substances is seperated into its component fractions of desired purity, by the application and removal of heat.

- Distillation is based on the fact that the vapor of a boiling mixture will be rich in the components that have lower boiling point.
- Therefore, when this vapor is cooled and condensed, the condensate will contain more volatile components at the same time.
- The original mixture will contain more of the less volatile material.
- Distillation column is designed to achieve this separation efficiency.

2.11.3. Filtration

Filtration is used to clarify liquids by the removal of small amounts of solid particles (For ex.wine, beer, oils, and syrup) or to separate liquids from the solid part of a food by cake filtration (ex. Fruit juice) (Fellows, 1997).

2.11.4. Pasteurization

Pasteurization is relatively mild heat treatment, usually performed below 100^0C, which is used to extend the shelf life of foods for several days or several months. It preserves food by inactivation of enzymes and destruction of relatively heat sensitive microorganisms. But cause minimal changes in the sensory characteristics or nutritive value of food (Fellows, 1997)

2.11.5. Packaging

The basic function of food packaging is to identify the product and ensure that it travels safety through the distribution system to the consumer (Paine and Paine, 1992).

Glass container:

Glass as a packaging material has the advantages of chemical internees, clarity, rigidity, and resistance to internal pressure, heat resistance and low cost. It s disadvantages are its fragility and heavy weight. Glass is also a complete barrier to vapor to gasses. However there still remains the possibility of loss or pick up of gasses or vapors via the bottle closure.

Fig. 2.4. Glass container with drink

2.12. Use of TLC in extraction method

Thin-Layer chromatography

Thin –Layer chromatography (TLC) is a chromatographic technique that is useful for separating organic compounds. Because of the simplicity and rapidity of TLC, it is often used to monitor the progress of organic reactions and to check the purity of products.

careful attention to details, it is possible to obtain quantitative data. An important characteristic used in paper and thin-layer chromatography for identification of compounds is the 'R_f value,

$$R_f = \frac{\text{Distance moved by the substance from origin}}{\text{Distanced moved by the solvent front from the origin}}$$

Thin layer chromatography applications have increased at a very rapid rate. This rapid growth has been prompted by the many advantages of TLC.

1. The method is quick.
2. Because inorganic layers are used as sorbent, more reactive reagent can be used to visualize spots than are possible in paper chromatography.
3. A wide range of sample sizes can be handled.
4. The equipment for TLC is simple and readily available.
5. No special manipulative are required.
6. Experimental parameters are easily varied to effect separation (Pomeranze and Melon, 1994).

Most TLC applications depend upon the adsorptive properties of the thin layer materials.

Table 2.5. Adsorbents for TLC

Solid	Used to separate
Silica gel	Amino acid, alkaloids, sugars, fatty acids, lipids, essential oils, inorganic anions and cations, steroids, terponoids,
Alumina	Alkaloids, fooddyes, phenoles, steroids, vitamins, caroteins, amino acids
Kieselguhr	Sugars, oligosaccharides, dibasic acids, fatty acids, triglycerides, amino acids, steroids.
Cellulose powder	Amino acid, food dyes,alkaloids,nucleotides
Ion exchange cellulose	Nucleotides, halide ions
Starch	Amino acid
Polymer powder	Anthocynins, aromatic acids, antioxidant, flavonoids, protein
Sephadex	Amino acids,protein

(Source: Pomeranze and Melon, 1994).

2.13. Sensory Evaluation

Scientific disciplines used to evoke measure, analyze and interpret people's reaction to products based on the senses.

2.13.1. Sensory parameters

Flavor: A complex group of sensation comprising olfactory, taste, and other chemical sensation such as irritation or chemical heat.

Odor: The characteristic smell of a substance.

Taste: Specialized sense organs on the tongue and soft palate contain the receptor for our sense of taste.

Aroma: The fragrance or odor of a product as perceived by the nose from sniffing through the external nares. In some cultures, aroma may also refer to retro nasal smell.

Color: Color is the perception that results from the detection of light after it has interacted with on object.

2.13.2. Sensory panel and panelist

Panel

A group of people that comprises a test population chosen for specific characteristic such as product usage, sensory quality or willingness to participate in repeated sensory test.

Panelist

Generally a participant in a sensory evaluation several related terms are commonly used "panelist" con notes a participant as a member of group that is often tested on more than one occasion (Lawles and Heyman, 1998).

Classification of test method

Table 2.6. Classification of test method in sensory evaluation

Class	Question of interest	Type of test	Panelist characteristic
Discrimination	Are products different in any way?	"Analytic"	Screened for sensory acuity, oriented to test method sometimes trained
Descriptive	How do products differ in specific sensory characteristics?	"Analytic"	Screened for sensory acuity and motivation, trained or highly trained.
Affective	How well are products liked or which products are preferred?	"Hedonic"	For product use, untrained.

(Source: Lawles and Heyman, 1998).

Hedonic Test

Hedonic test is used in the food industry to determine acceptance of food. The most common hedonic scale is the 9-point hedonic scale. This is also known as degree of liking scale.

The hedonic scale assumes that consumer preference exist on a continue and that preference can be categorized by responses based on likes and dislikes in other words, it was a scale with ruler like properties whose equal intervals would be amenable to statistical analysis (Lawles and Heyman, 1998)

2.14. Shelf life evaluation

Foods are perishable by nature. Numerous changes take place in foods during processing and storage. It is well known that conditions used to process and store foods may adversely influence the quality attributes in foods.

During storage and distribution, foods are exposed to a wide range of environmental conditions. Environmental factors such as temperature, humidity, oxygen and light can trigger several reaction mechanisms that may lead to food degrading. Chemical, physical and microbiological changes are the leading causes of food deterioration.

Shelf life determination of the new product often requires storage for significant periods and includes samples from early development stages as well as initial production runs. Through the evaluation of stored samples, potential storage problems can be identified and either eliminated or controlled before the food goes into production (Man and Jones, 1997)

2.14.1. Bacteria

*Gluconobacter (*Acetomanas) species are main spoilage bacteria because of there ability to grow at relatively law pH (3.0-3.7) and low nutrient levels. They are strict aerobes, and any development in packaging techniques and materials that increase oxygen tension in the will increase the incidence of spoilage by these bacteria (Gabutt, 1997).

Plate count

The use of plate counts to estimate the number of bacteria in a food is based on the fact that living bacterial cells or clumps of cells will grow and increase in number in or on the surface of a suitable agar medium to give visible colonies that can be counted. The first stage in carrying out a traditional plate counts on a food involves producing a homogenate of a sample and a series of dilution.

Production of the food homogenate and dilution series is followed by the inoculation of agar plates with samples from each dilution, incubating the plate and counting the numbers of colonies produced. A simple calculation can then be used to determine the number of colony forming units (CFUS) in the original sample (Gabutt, 1997).

2.14.2. *Escherichia coli*

Although *E-coli* is generally harmless part of the normal micro flora of the gut of humans and other warm blooded animals, a number of groups of E-coli are pathogenic for human and have been associated with food borne diseases. These are gram-negative rods 2 to 4 Mm by 0.4Mm commonly seen as colobacillary form rarely as air filamentous form (Gabutt, 1997).

3M Petrifilm

E-coli / Coliform count plate.

Petrifilm E.coli/ coliform count (EC) plates contain violet red bile (VRB) nutrients, a cold-water soluble gelling agent, an indicator of glucuronidase activity, and an indicator that facilitate colony enumeration. Most *E-coli* (about 97%) produce beta-glucuronidase, which produces a blue precipitate, associated with the colony. The top film traps gas produced by the lactose fermenting coliforms and *E.coli* produce gas, indicated by blue to red –blue colonies associated with entrapped gas on the petrifilm EC plate.

3M"Petrifilm"*E.coli/coliform* count plates are designed to identify both E.coli and other coliforms. With one easy test, will have confirmed results in just 24 to 48 hours. By eliminating the need for confirmation of presumtative colonies, will greatly increase lab efficiency and reduce overall costs. Petrifilm plates are simple-ready and provide the most cost-effective, convenient and reliable method for testing equipment, raw materials, food products and the manufacturing environment.

By using labor-saving petrifilm plates, will have time to monitor critical control points more frequently the end results is better process control and higher quality product.

3M Petrifilm is AOAC INTERNATIONAL validated method.

If E-coli present in the sample there will be blue colony with gases on petrifilm plates. Red and blue colonies with gases indicate that the coliforms present in the sample.

Fig. 2.5. Petrifilm plates

Fungi: Fungi are the principle microorganisms grow at the low pH and reduced water activity (a_W) drinks, juice and fruit preserves. Only lactic and acetic acid bacteria are able to grow.

Yeast: These predominant in spoilage of acid fruit products because of high acid tolerance and frequent ability to grow anaerobically.

Moulds: Most moulds are not nutritionally exacting but unlike yeasts, are with a few exceptions, strict aerobes. Mould cause spoilage by forming colonies and aerial mycelia on the surface of juice etc, or flocculation or floating mycelia within the product, clarification of the pectin cloud by break down.

Table 2.7. Microbiological tolerance limits for Herbal drink

Test	**Limit**	**Method of test**
Standard plate count,per,ml,max	50	SLS 516:Part 1
Coliform	Absent	SLS 516:Part 3
Yeast and moulds count, per ml	Absent	SLS 516:Part 3

(Source: SLS 729:1985)

2.15. Physicochemical properties assessment

Acidity

In evaluating the acidity of any beverage, two aspects must be considered. These are (1) the quantity aspect, that is, the total amount of available hydrogen ion present in a given volume of the beverage as estimated by a determination of the total titratable acidity and (2) the intensity aspect, that is, the apparent hydrogen-ion concentration usually expressed in terms of pH (Jacobs, 1959).

pH

The symbol pH has been adopted for the logarithm of the reciprocal of the hydrogen-ion concentration. If the hydrogen-ion concentration (represent by $[H^+]$) of a solution is known, the corresponding pH of the solution may be calculated from the formula:

$pH=\log 1/ [H^+]$

It is not really necessary to consider the meaning of pH in terms of the theory of solution. The pH numbers need only be accepted as a practical scale of acidity and alkalinity with a pH of 7.00 being the neutral point, that is, the point where the concentrations of hydrogen ion and hydroxyl ion are equal.

Solution in the hydroxyl-ion concentration, that is, acid solution, has lower pH values ranging down to 1.0 or lower. Solution in which the hydroxyl-ion concentration is greater than the hydrogen-ion concentration is alkaline, and the pH is expressed by higher value ranging up to 13 or 14 (Jacobs, 1959).

Total Soluble Solid (TSS)

Since the amount of sucrose dissolved in water is important industrially, a whole series of measuring indices were developed to indicate the relative proportion of the two materials. The most important of these is the Brix scale, which relates the percentage by weight of sucrose in a water solution. Therefore, 65° Brix would represent a solution that is 65 percent sucrose and 35percent water. The Brix scale is also used to measure solution other than pure sucrose and water (Knech, 1987).

2.16. Food spoilage and metal contamination

The browning reaction

The browning reactions are complex reaction that occurs when many foods are processed. In some of the brown flavor is highly desirable and intimately associated on our minds with a delicious high-grade product. Yet in other foods, browning during processing is undesirable and form off flavor and dulled or even objectionable color. The presence of carbohydrate in foods is intimately connected with the browning occurs (Mayer, 1987). Two types of browning reaction can be seen; Enzymatic and Non –Enzymatic.

Enzymatic browning

Enzymatic browning occurs in many tissues whenever they are injured. The injury can be the result of brushing, cutting, freezing or diseases. Oxidation of phenols or polyphenols by enzymes.

Prevention of enzymatic browning:

1. Heated sufficiently high to denature enzymes.
2. Use of concentrated sugar solution.
3. Use of antioxidant
4. Use Preservative such as SO_2 is an effective browning inhibitor

(Mayer, 1987).

Non-enzymatic browning

Three mechanisms can be seen,

1. The browning reaction that occurs between carbohydrates and amino acids.
2. Ascorbic acid undergoes oxidation with the formation of a compound, which produces brown pigments.
3. Carbohydrate or carbohydrate acids decompose to furfuraldehyde or related compounds which then polymers or react with nitrogen compounds to form brown pigments (Meyer, 1987).

Prevention of non-enzymatic browning:

1. SO_2 will reduce the non-enzymatic browning.

2.16.1. Limits for heavy metals

The cheap source of contamination with metals is the water, pipes, pans and other utensils used in various manufacturing process.

Table 2.8: Limits for heavy metals in drink

No	Characteristics	Limit	Method of test
1	Copper (as Cu), mg/kg	20.0	SLS 301
2	Lead (as Pb), mg/kg, max	2.0	SLS 311
3	Tin (as Sn), mg/kg, max	250	SLS 315
4	Arsenic (as As), mg/kg, max.	1.0	SLS 312

(Source: SLS 729:1985)

Table 2.9: SLS standard limits of Ready to serve drink (Compounds).

SI NO (1)	Characteristics (2)	Requirement (3)
(i)	Sugar content(as sucrose)per unit by mass,min	5
(ii)	Sulfur dioxide content,mg/kg max	70
(iii)	Benzoic acid content,mg/kg max	160
(iv)	Acidity(As anhydrous Citric acid)percent by mass,min	10

(Source: SLS 729:1985)

Chapter 03

Materials and Methodology

3.1. Extraction of Gotukola

Materials and Apparatus

Apparatus:

Stainless steel knife
Cutting board
Plastic tray
Stainless steel vessel
Thermometer (Z-Strengthened, England)
Gas cooker (Rinnai)
Muslin cloth
Suction pump (ASEA, 50/60Hz)
Blender ((KENWOOD)
Filter paper (Whatman, 110 Dia)
Soxhlet apparatus

Materials:

Fresh Gotukola
Filtered water
Sodium Meta bi Sulfite (SMS)
Alcohol

3.1.1. Extraction of Gotukola by using water

Method:

Two different treatments (Hot water and Portable water) were carried out in order to find out the most suitable water condition to extract Gotukola efficiently.

Treatment 01: Extraction of Gotukola using normal water

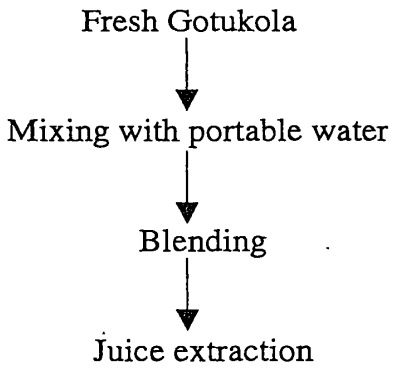

Fig. 3.1. Extraction of Gotukola using normal water

Treatment 02: Extraction of Gotukola using hot water

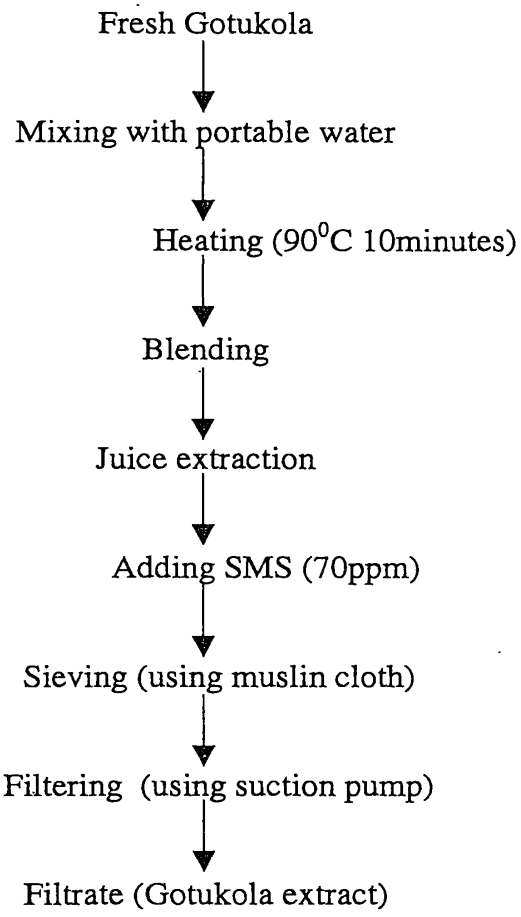

Fig. 3.2. Extraction of Gotukola using hot water

3.1.2. Selection of most suitable time and temperature for the hot water extraction

To find out the most suitable time temperature combination for the hot water treatment, hot water extractions were carried out as mentioned bellow in 4 different temperatures and 4 different times.

The table 3.1 Illustrates the different time temperature combinations.

Table 3.1. Different time - temperature combinations

Sample	Temperature (^{0}C)	Time (minutes)
A1	70	3
A2	80	3
A3	90	3
A3	100	3
B1	100	3
B2	100	5
B3	100	10
B4	100	15

3.1.2.1. Sensory evaluation to select the most suitable time temperature combination

Material and Methodology

Materials

Sensory evaluation ballet paper

Coded sample

White glasses

Serviettes

Glass of potable water

Methodology

Herbal drinks were prepared using the extracts acquired from the above different combinations. Then four glasses were taken and they were coded using three digit random numbers. 30 untrained panelists were selected to evaluate the taste, color, smell, thickness and overall acceptability of the products. They were given standard ballet papers to rank the attributes (App. I). The 9 - point hedonic scale was used to evaluate the degree of liking for particular sensory attributes. Finally the collected data was analyzed statistically.

3.1.3. Selection of suitable herbal content

Different percentages of herbal extracts were taken as shown in the table 3.2. and different herbal drinks were prepared by using above formula. Then sensory evaluation was conducted to find out the suitable herbal content of above prepared herbal drink by using below formula. Finally the collected data was analyzed statistically.

Table 3.2. Different percentages of herbal extracts

Herbal content	percentages (%)
Q1	14
Q2	10
Q3	8

3.1.4. Extraction of Gotukola by using alcohol

Method: Two different treatments were carried out in order to extract Gotukola by alcohol. A mixture of water and alcohol was used fore the first treatment and for the second treatment Soxhlet extraction was used. Figure 3.1 shows flow diagram for the preparation of Gotukola extract using three different method.

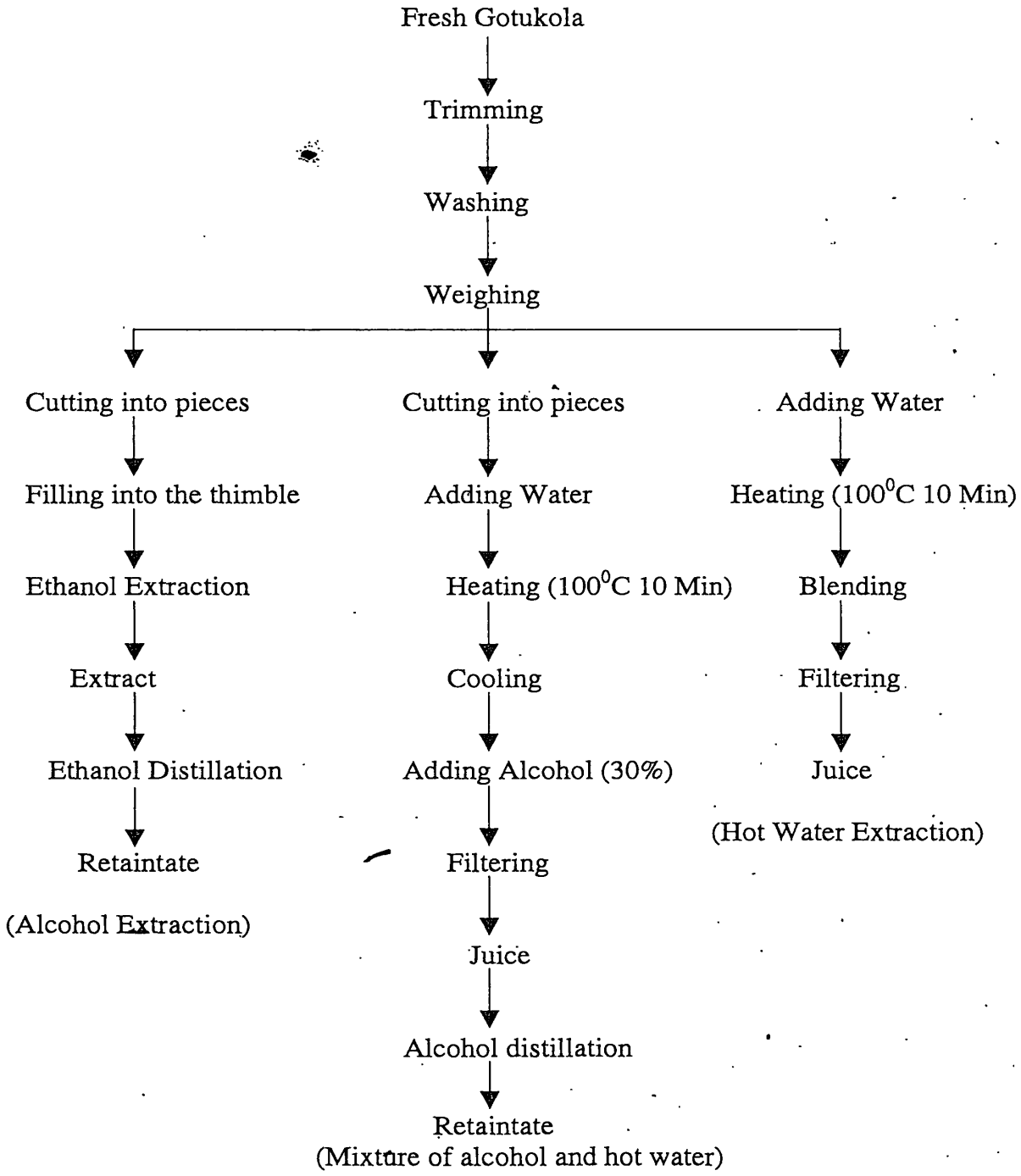

Fig. 3.3. Flow diagram for the preparation of Gotukola extracts using three different methods.

The acquired juices and retaintate from the above three different extraction methods were used separately for the formation the herbal Gotukola drink. Same amounts of sugar, salt, CMC, preservatives and citric acid were used in the three different preparations. Figure 3.4 shows the flow diagram for Preparation of Herbal drink.

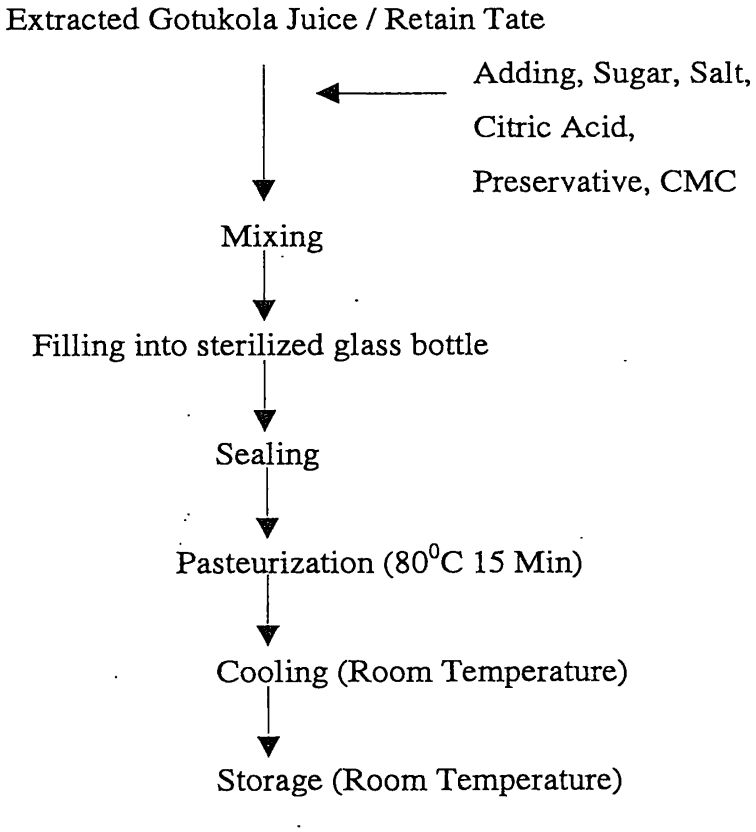

Fig. 3.4. Flow diagram of Preparation of Herbal drink.

3.2. Selection of a proper extraction method

By using the above (fig 3.4) formulae herbal drinks were prepared separately from the extracts gained from different extraction methods. Two different methods were done to find out the proper extraction method.

1. **Sensory Evaluation**
2. **TLC Techniques**

3.2.1. Sensory evaluation to find out proper extraction method

Herbal drinks were prepared separately from the extracts gained from different extraction methods. Then four glasses were taken and they were coded using three digit random numbers. 30 untrained panelists were selected to evaluate the taste, color, smell, thickness and overall acceptability of the products. They were given standard ballet papers to rank the attributes. The 9 - point hedonic scale was used to evaluate the degree of liking for particular sensory attributes. Finally the collected data was analyzed statistically.

3.2.2. TLC Techniques to find out proper extraction method

Materials and apparatus

Materials:	**Apparatus**
Silica gel	Glass TLC plate
Extract sample	Spreader
Alcohol	Filter paper
Diethyl ether	Capillary tube
Methanol	Reservoir
Acetic acid	
Proponol	

Methodology

The TLC technique was carried out in order to find out which extracted sample contains the many compounds.

Figure 3.5 shows selection of the best extraction method using TLC.

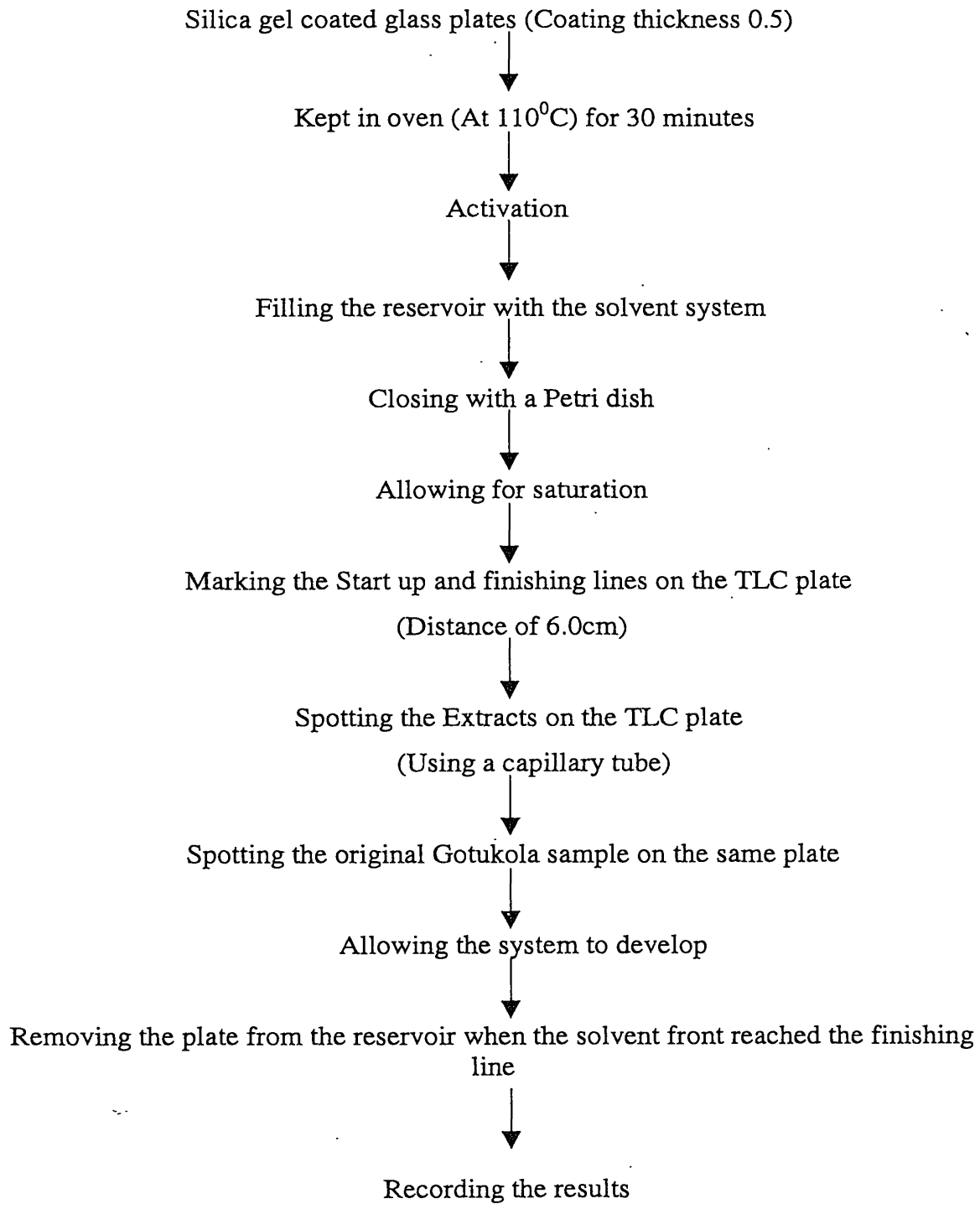

Fig. 3.5. Selection of the best extraction method using TLC.

3.3. Chemical studies and shelf life evaluation of herbal drink

Store sample under normal conditions were evaluated physio-chemical and microbiological changes during two months.

Materials and apparatus

Materials:	**Apparatus:**
Distilled water	Brix meter (Atago, 0-60%)
Phenolphthalein	Burette
0.1M NaOH solution	Titration flask
Starch	Metal stand
N/50 Iodine	Porcelain dish
Yeast extract agar	Dropper
Nutrient agar	Plastic wash bottle
Brilliant green bile broth	pH meter
	Pipettes
	Petri dishes
	Test tubes
	Cotton wools
	Autoclave
	Incubator

3.3.1. Chemical analysis of herbal drink

1) Brix measurement

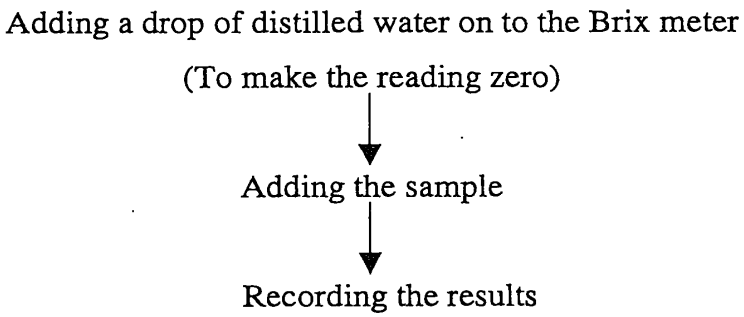

Fig. 3.6. Flow diagram of Brix measurement.

2) Acidity measurement

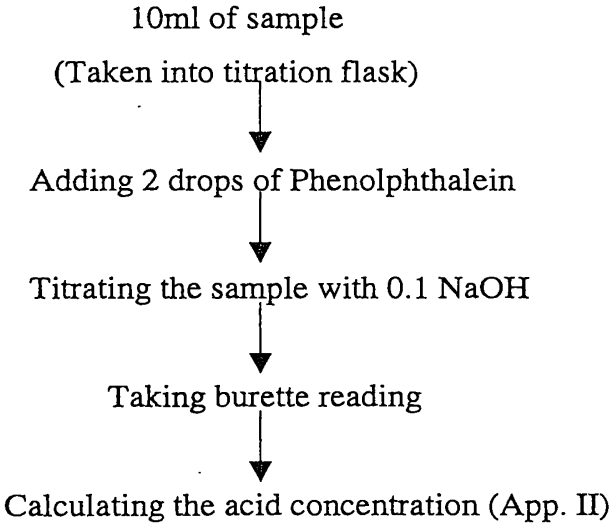

Fig. 3.7. Flow diagram of Acidity measurement.

3) Calculation of SMS amount

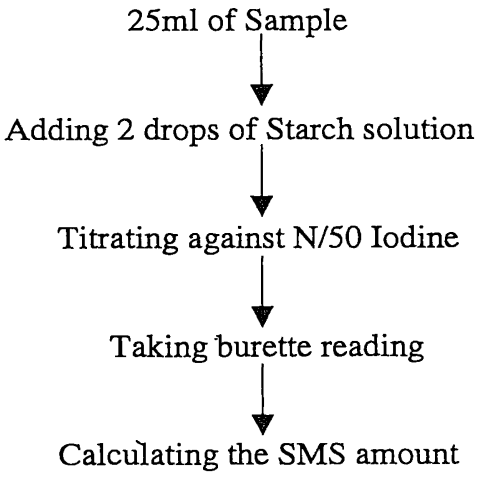

Fig. 3.8. Flow diagram of Calculation of SMS amount.

4) Calculation of pH value

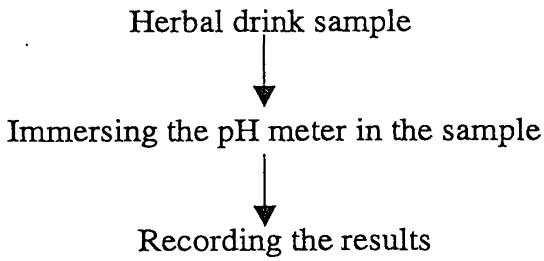

Fig. 3.9. Flow diagram of Calculation of pH value.

3.3.2. Microbiological testing

3.3.2.1. Media preparation

Nutrient agar

28g of sample was suspended in 1 liter of distilled water and it was boiled to dissolve completely. Then it was sterilized by autoclaving at 121^0C for 15 minutes.

Yeast Extract Agar

23g of sample was suspended in 1 liter of distilled water and boiled to dissolve completely. Then it was sterilized by autoclaving at 121^0C for 15 minutes.

Brillient Green (2%) broth

40 g of sample was added in to 1 liter of distilled water and mixed well. It was put into a container and then Durham's tubes were put into it. This whole unit was sterilized by autoclaving at 121^0Cfor 15 minutes.

Peptone water

15g of sample was added in to 1 liter of distilled water and mixed well and put into containers. Then it was sterilized by autoclaving at 121^0C for 15 minutes.

3.3.2.2. Preparation of the dilution

First dilution

10ml of sample was measured and added into the 90 ml of ringer solution and the mixture was mixed thoroughly.

Second dilution

1ml of sample was measured and added into the 99ml of ringer solution and the mixture was mixed thoroughly.

3.3.2.3. Total plate count

- 1ml of sample was measured by using sterilized pipette and introduced into aseptically sterilized plate.
- Then approximately 15-25ml of nutrient agar was poured, the temperature of agar was 45^0C.
- Then immediately lid was closed and shaken gently for even distribution of media in a plate. After that plates were kept few minutes to become solidified nutrient agar.
- After solidified plates were kept upside down in the incubator and temperature was set 30^0C and incubated for 48 hours.
- Colonies appearing in the media were counted by using the colony counter and results were recorded (Gunasekaran, 1995).

3.3.2.4. Pour plate techniques for yeast and moulds

- 1ml of sample was aseptically introduced into sterilized plate and approximately 15-25ml of yeast extract agar with antibiotic was poured (50ml of yeast extract agar was prepared by adding 1ml of antibiotic).
- The temperature of yeast extract agar was 45^0C.Then the lid was closed and shaken gently for even distribution of media in Petri dish.
- After that it was kept in few minutes at a room temperature for solidifying the yeast extract agar.
- The solidified plates were placed upside down in the sterilized plate in room temperature for 72 hours.
- Colonies appearing in the media were counted by using the colony counter and the results were recorded.

3.3.2.5. *Coliform* testing by using petrifilm

- The petrifilm plate was placed on level surface.
- 1ml of sample was placed on the center of bottom film, with pipette perpendicular to petrifilm plate.
- Top film was rolled down carefully to avoid entrapping air bubbles.
- Spreader was placed on top film over inoculum.
- Gently spreader was applied pressure on distribute inoculum over circular area before gel is formed (do not twist or slide the spreader).
- Spreader was left. Wait a minimum of one minute for gel to solidify.
- Plates were incubated with clear side up in stacks of no more than 20.

3.3.2.6. *Coliform* testing using tubes method

- 1ml of herbal drink sample was aseptically introduced into 3 sterilized tubes, which has Derham's tubes and prepared peptone tubes.
- 1ml each of one and two dilutions was also aseptically introduced into 6 sterilized tubes separately.
- Then it was closed using cotton wool, and it was shaken thoroughly.
- After that tubes were placed in an incubator, in 37 ^{0}C for 48 hours.
- Then the results were recorded.
- If the test is positive for *Coliform*, 1ml of Indol solution was added into peptone tubes.

Chapter 04
Results and discussion

4.1 Selection of suitable extraction

At the initial stage, hot water treatment and normal water treatment were given to the Gotukola sample in order to acquire Gotukola extraction. But the extraction gained from the normal water has following bad quality characteristics.

1. Raw odour
2. Raw flavor

Due to above reasons, hot water extraction was selected as the best method to extract Gotukola, which retains acceptable quality characteristics.

4.2. Results of the sensory evaluation for selection of the suitable temperature of preparing drink (Hot water extraction)

Results of the temperature affected to the Flavour of the drink

Analysis results of the sensory evaluation revealed that the temperature affected to the flavour of the drink. Table 4.1 shows result of the temperature affected to the flavour of the drink.

Table 4.1. Results of the temperature affected to the flavour of the drink.

Product	Average rank
1(A1)	33.7
2(A2)	40.2
3(A3)	76.5
4(A4)	91.5

P=0.000

According to the table p value for the test is less than 0.05. This implies that a significant difference exists among the four samples at 5 % significant level. When average rank value is considered, sample number 4 has the highest rank value. So the sample number 4 can be considered as the best when considering the flavour.

Results of the temperature affected to the color of the drink

According to the analysis results of the sensory evaluation, the temperature affected to the color of the drink. Table 4.2 shows result of the temperature affected to the color of the drink.

Table 4.2. Results of the temperature affected to color of the drink.

Product	Average rank
1(A1)	93.3
2(A2)	69.7
3(A3)	44.4
4(A4)	34.6

P=0.00

According to the table p value for the test is less than 0.05. This implies that a significant difference exists among the four samples at 5 % significant level. When average rank value is considered samples number 4 has highest rank value. So the sample number 1 can be considered as the best when considering the color (App. III).

Results of the temperature affected to the thickness of the drink

According to the analysis results of the sensory evaluation the temperature affected to the thickness of the drink. Table 4.3 shows result of the temperature affected to the color of the drink.

Table 4.3. Results of the temperature affected to the thickness of the drink.

Product	Average rank
1(A1)	60.5
2(A2)	58.6
3(A3)	62.5
4(A4)	60.5

P=0.724

According to the table p value for the test is higher than 0.05. There is no significant difference exists among the samples four at 5 % significant level. Then temperature has not affected the thickness of the drink (App. III).

Results of the temperature affected to the acid level of the drink

Table 4.4 shows result of the temperature affected to the color of the drink.

Table 4.4. Results of the temperature affected to the acid level of the drink.

Product	Average rank
1(A1)	60.5
2(A2)	58.6
3(A3)	62.5
4(A4)	60.5

P=0.724

According to the table the p value for the test is higher than 0.05. There is no significant difference exists among the four samples at 5 % significant level. That means there is no difference between the in four samples. That means the temperature not affected the acid level of the drink (App. III).

Results of the temperature affected to the overall acceptability of the drink

Table 4.5 shows result of the temperature affected to the overall acceptability of the drink.

Table 4.5. Results of the temperature affected to the acceptability of the drink.

Product	Average rank
1(A1)	15.5
2(A2)	45.5
3(A3)	75.5
4(A4)	105.5

P=0.000

According to the table No.4.8 the p value for the test is less than 0.05.This implies that a significant difference exists among the four samples at 5 % significant level. When average rank value is considered, sample number 3 has highest rank value. So the sample number 3 can be considered as the best when considering the overall acceptability (App. III).

4.3. Results of the sensory evaluation for selection of the suitable time of preparing drink (Hot water extraction)

Results of the time affected to the flavor of the drink

Table 4.6 shows result of the time affected to the flavour of the drink.

Table 4.6. Results of the time affected to the flavour of the drink.

Product	Average rank
1(B1)	44.8
2(B2	32.7
3(B3)	92.2
4(B4)	72.4

P=0.000

According to the table the p value for the test is less than 0.05. This implies that a significant difference exists among the four samples at 5 % significant level. When average rank value is considered, sample number 3 has the highest rank value. So the sample number 3 can be considered as the best when considering the flavor (App. III).

Results of the time affected to the color of the drink.

Table 4.7 shows result of the time affected to the color of the drink.

Table 4.7. Results of the time affected to the color of the drink.

Product	Average rank
1(B1)	85.9
2(B2	77.6
3(B3)	48.8
4(B4)	29.7

P=0.000

According to the table p value for the test is less than 0.05. This implies that a significant difference exists among the four samples at 5 % significant level. When average rank value is considered samples number 1 has the highest rank value. So the sample number 1 can be considered as the best when considering the color (App. III).

Results of the time affected to the acid level of the drink.

Table 4.8 shows result of the time affected to the acid level of the drink.

Table 4.8 Results of the time affected to the acid level of the drink.

Product	Average rank
1(B1)	60.5
2(B2	58.6
3(B3)	62.5
4(B4)	60.5

P=0.724

According to the table p value for the test is less than 0.05. There is no significant difference exists among the four samples at 5 % significant level. So there is no significance difference between in four samples. Then temperature not affected the acid level of the drink (App. III).

Results of the time affected to the thickness of the drink

Table 4.9 shows results of the time affected to the thickness of the drink.

Table 4.9. Results of the time affected to the thickness of the drink.

Product	Average rank
1(B1)	60.5
2(B2	58.6
3(B3)	62.5
4(B4)	60.5

P=0.724

According to the table the p value for the test is higher than 0.05. There is no significant difference exists among the four samples at 5 % significant level. So there is no significance difference between in four samples. There no time affected the thickness of the drink (App. III).

Results of the time affected to the overall acceptability of the drink

Table 4.10 shows results of the time affected to the color of the drink.

Table 4.10. Results of the time affected to the overall acceptability of the drink.

Product	Average rank
1(A1)	44.0
2(A2)	31.9
3(A3)	92.1
4(A4)	74.0

P=0.000

According to the table p value for the test is less than 0.05. This implies that a significant difference exists among the four samples at 5 % significant level. When average rank value is considered the samples number 3 has the highest rank value. So the sample number 3 can be considered as the best when considering the overall acceptability (App. III).

4.4. Results of the sensory evaluation for selection of the suitable herbal content of preparing drink (Hot water extraction)

Results of the herbal content affected to the flavor of the drink

Table 4.11 shows results of the herbal content affected to the flavor of the drink.

Table 4.11. Results of the herbal content affected to the flavour of the drink.

Product	Average rank
1(Q1)	31.1
2(Q2)	64.9
3(Q3)	40.5

P=0.000

According to the table p value for the test is less than 0.05. This implies that a significant difference exists among the four samples at 5 % significant level. When average rank value is considered, sample number 2 has the highest rank value. So the sample number 2 can be considered as the best when considering the flavor (App. III).

Results of the herbal content affected to the color of the drink

Table 4.12 shows results of the herbal content affected to the color of the drink.

Table 4.12. Results of the herbal content affected to the color of the drink.

Product	Average rank
1(Q1)	65.9
2(Q2)	48.7
3(Q3)	21.9

P=0.000

According to the table p value for the test in less than 0.05. This implies that a significant difference exists among the four samples at 5 % significant level. When average rank value is considered, sample number 1 has the highest rank value. So the sample number 1 can be considered as the best when considering the color (App. III).

Results of the herbal content affected to the thickness of the drink

Table 4.13 shows results of the herbal content affected to the thickness of the drink.

Table 4.13. Results of the herbal content affected to the thickness of the drink.

Product	Average rank
1(Q1)	45.5
2(Q2	45.5
3(Q3)	45.5

P=1.000

According to the table p value for the test in less than 0.05. There no significant difference exists among the four samples at 5 % significant level. So there is no difference between the three samples. That mean herbal content not affected the thickness of the drink (App. III).

Results of the herbal content affected to the acid level of the drink

Table 4.14 shows results of the herbal content affected to the acid level of the drink.

Table 4.14. Results of the herbal content affected to the acid level of the drink.

Product	Average rank
1(Q1)	45.5
2(Q2	45.5
3(Q3)	45.5

P=1.000

According to the table p value for the test in less than 0.05. There was no significant difference exists among the four samples at 5 % significant level. So there is no difference between in three samples. That mean herbal content does not affected the acid level of the drink (App. III).

Results of the herbal content affected to the overall acceptability of the drink

Table 4.15 shows results of the herbal content affected to the thickness of the drink.

Table 4.15. Results of the herbal content affected to the thickness of the drink.

Product	Average rank
1(Q1)	30.5
2(Q2)	66.6
3(Q3)	39.4

P=0.000

According to the table the p value for the test in less than 0.05. This implies that a significant difference exists among the four samples at 5 % significant level. When average rank value is considered, sample number 2 has the highest rank value. So the sample number 2 can be considered as the best when considering the overall acceptability (App. III).

According to the sensory evaluation results the best time temperature combination for the extraction was 10 minutes and 100^0C

The suitable herbal content was selected 10% by sensory evaluation. But, the product that received the highest rank for good quality characteristics didn't get the highest rank for the color. The reason for this might be due to discoloration of pigments at 100^0C which is considerably a higher temperature and due to time in which it retain in 100^0C.

Though low time –temperature combinations achieved a more acceptable color the overall acceptability for the product was low. Another problem that was occurred when preparing the Gotukola herbal drink was the browning of the product. This happened mainly because of the sugar amount it contains.

Browning reaction may be caused by enzymatic oxidation of polyphenols and other susceptible compounds, if the oxidizing enzymes are not in active mainly caramalization of sugars and scorching of other materials if heat is excess. Highly important in food product development are non-enzymatic or Maillard browning from the reaction of aldehydes and amino groups of sugar and proteins. Mailard types browning, like other chemical reactions, are favored by temperature and by high concentration of reactive groups are concentrated (Potter, 1996).

To overcome this problem sugar solution was prepared seperately and it was added into the Gotukola extraction after the solution was cooled down. So from this way the browning was reduced for a better extent.

4.5. Alcohol extraction

Some Gotukola compounds are soluble only in alcohol, not soluble in water. So alkaloids insoluble in water, but soluble in organic solvents. Then volatile oils are freely soluble in ether, alcohol, chloroform, and acetone. The glycosides are generally soluble in water and alcohol, but insoluble in organic solvents. Above reason affected to the move to alcohol extraction (Alli and Ahmad, 2003).

Two treatments were carried out in order to gain an alcohol extraction. 100% pure alcohol was used for one treatment and for the other; a mixture of alcohol and hot water was used. In literature it was recommended to use alcohol less than 50% on preparation of drinks. Different treatments were carried out with different alcohol percentages. But alcohol percentages, which were below 30%, were sediment during distillation. So, 30% alcohol was selected as the most suitable percentage that can be

used in preparation of mixture of alcohol. 100% of pure alcohol was used in one extraction to find out whether more compounds can be acquired into the Gotukola extraction when the alcohol percentage increased.

4.6. Results of the sensory evaluation for selection of the suitable Extraction method

Results of selecting suitable extraction method

Table 4.16 shows results of the select suitable extraction method.

Table 4.16. Results of selecting suitable extraction method (overall acceptability).

Product	Average rank
1 (Hot water)	62.0
2 (Alcohol)	46.6
3 (Mixture)	27.9

P=0.000

According to the table p value for the test in less than 0.05.This implies that a significant difference exists among the four samples at 5 % significant level. When average rank value is considered, sample number 1 has the highest rank value. So the sample number 1 can be considered as the best when considering extraction hot water (App. III).

Thin layer chromatography method was used to determine the best extraction method, when developing herbal drink. Two solvent systems were selected according to the polarity differentce of two extracts. Die-ethyl ether: Methonol solvent system and acetic Acid: Butanol solvent systems were used for alcohol extraction and hot water extraction respectively. One spot can be observed according the 8:2 solvent ratio of Die-ethyl ether: Methonol system in alcohol extract and original sample. According to the ratio of 9:1, it can be observed two spots in both alcohol extract and original sample. It may be due to the polarity difference between two solvent systems.

TLC for hot water extraction was carried out by using Propanol and acetic acid solvent system and ratio of Propnol: Acetic acid was 1:1 in solvent systems.

After running TLC one spot can be identified on the TLC plate. Although ratios of solvent system were changed, there was no difference in the results. According to the results, there is possibility to obtain more compounds in the alcohol extract than hot water extract.

But it cannot be assured that, those extractions, which contain more alcohol percentages, are actually medicinal herbal drinks.

4.7. Formulation of Gotukola herbal drink

Herbal content – 10%
Sugar -14%
Citric acid -0.25%
Salt -0.02
SMS -0.1%
Table 4.17 shows preparation of 1000ml of Gotukola drink.

Table 4.17. Preparation of 1000ml of Gotukola drink.

Ingredients	Amount(g)
Herbal content (Gotukola amount)	100
Sugar	140
Citric acid	2.5
Salt	2.0
SMS	0.1
CMC	0.5

4.8. Chemical Analysis of herbal drink

When shelf life evaluation, were mainly considered physicochemical changes and microbiological activity. Table 4.18 shows result of chemical analysis of herbal drink.

Table 4.18. Chemical Analysis of herbal drink

	Hot water	Alcohol	Mixture
Initial			
Brix (^{0})	12.5	13.0	12.8
pH	2.6	2.8	2.9
Acidity (ml)	3.8	3.6	3.5
SMS (ppm)	71.2	70.0	70.5
After 2 weeks			
Brix (^{0})	12.5	13.0	12.8
pH	2.6	2.8	2.9
Acidity (ml)	3.8	3.6	3.5
SMS (ppm)	68.0	67.5	68
After 4 weeks			
Brix (^{0})	12.5	13.0	12.8
pH	2.6	2.8	2.9
Acidity (ml)	3.8	3.6	3.5
SMS (ppm)	65.0	64.0	64.0
After 6 weeks			
Brix (^{0})	12.5	13.0	12.8
pH	2.6	2.8	2.9
Acidity (ml)	3.8	3.6	3.5
SMS (ppm)	60.0	60.2	60.0
After 8 weeks			
Brix (^{0})	12.5	13.0	12.8
pH	2.6	2.8	2.9
Acidity (ml)	3.8	3.6	3.5
SMS (ppm)	60.0	60.2	60.0

According to the table no change in pH, acidity and total soluble solid has been observed in any of the sample during 8 weeks of storage life. Genarally in a beverage pH and acidity change after 4 months, mainly due to microbial action, which provide organic acids (Gurbutt, 1997). TSS change mainly due to degradation of starch into simple sugar.

4.9. Microbiological results

Table 4.19. Microbiological results

Method	Yeast and mold	Total colony count	*Coliform*
After 2 weeks			
Hot water extraction	Nil	30	Nil
Alcohol extraction	Nil	5	Nil
Mixture	Nil	10	Nil
After 4 weeks			
Hot water extraction	Nil	32	Nil
Alcohol extraction	Nil	8	Nil
Mixture	Nil	19	Nil
After 6 weeks			
Hot water extraction	Nil	41	Nil
Alcohol extraction	Nil	9	Nil
Mixture	Nil	22	Nil
After 8 weeks			
Hot water extraction	Nil	45	Nil
Alcohol extraction	Nil	12	Nil
Mixture	Nil	25	Nil

Then E.coli coliform was used to both Petri film and tube method. So Petri film method was greatly increase lab efficiency and reduced overall costs. Petrifilm plates are sample ready and provide the most cost effective, convenient and reliable method for testing equipment, raw materials, food products and the manufacturing environment.

Chapter 05

Conclusions and Recommendations

5.1. Conclusions

The best temperature for the hot water extraction was 100^0C and the best time was 10 minutes. The suitable herbal content that can be used in herbal drink preparation was 10% v/v basis. Hot water extraction and alcohol extraction were the best method according to the result of sensory evaluation and TLC techniques respectively.

The new herbal product can safely be kept for 8 weeks at storage without the changes either in microbiological or physicochemical characteristics.

5.2. Recommendations

The neutraceutical compounds extracted by both hot water and alcohol extraction should be identified and quantified in further studies.

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Appendix I

Sensory evaluation ballet paper

Name:
Date:
Sample codes:

Introduction:

- Please taste the sample given here.
- Give the score for each characteristics.

Like extremely.....9
Like very much.....8
Like moderately.....7
Like slightly.....6
Neutral.....5
Dislike slightly.....4
Dislike moderately.....3
Dislike very much.....2
Dislike extremely.....1

Character				
Flavour Color Suitable thickness Suitable acid level Overall acceptability				

Comments:

Thank you!

Appendix II

Calculation for acidity (as citric acid)

$$\text{Acidity, percent by mass} = \frac{6.404VC}{m}$$

Where,

V = Volume, in ml, of standard sodium hydroxide required for the titration.

C = Concentration, in mol/l, of the standard NaOH solution.

m = Mass, in g of the sample taken for test.

Appendix III

Statistically analysis data for finding suitable Temperature of herbal drink

Kruskal-Wallis Test: code versus sample
H_0: Temperature is not affected to the Flavour of the drink.
H_1: Temperature is not affected to the Flavour of the drink

Product	N	Median	Ave Rank	Z
1	30	5.000	33.7	-4.87
2	30	5.000	40.2	-3.70
3	30	7.500	76.5	2.90
4	30	8.000	91.7	5.67
Overall 120		60.5		

H = 58.47 DF = 3 P = 0.000
H = 60.88 DF = 3 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: code versus sample

H_0: Temperature is not affected the color of the drink.
H_1: Temperature is effected the color of the drink.

Sample	N	Median	Ave Rank	Z
1	30	5.000	33.7	-4.88
2	30	6.000	43.1	-3.16
3	30	8.000	73.5	2.36
4	30	8.000	91.7	5.67
Overall 120		60.5		

H = 53.62 DF = 3 P = 0.000
H = 55.72 DF = 3 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: code versus Sample

Kruskal-Wallis Test on code
H_0: Temperature is not affected the thickness of the drink.
H_1: Temperature of the effected the thickness of the drink.

Sample	N	Median	Ave Rank	Z
1	30	8.000	60.5	0.00
2	30	8.000	58.6	-0.35
3	30	8.000	62.5	0.35
4	30	8.000	60.5	0.00

Overall	120		60.5	

H = 0.19 DF = 3 P = 0.979
H = 1.32 DF = 3 P = 0.724 (adjusted for ties)

Kruskal-Wallis Test: code versus Sample

H_0: Temperature is not effected the acid of the drink.
H_1: Temperature is effected the acid of the drink

Sample	N	Median	Ave Rank	Z
1	30	8.000	60.5	0.00
2	30	8.000	58.6	-0.35
3	30	8.000	62.5	0.35
4	30	8.000	60.5	0.00
Overall	120		60.5	

H = 0.19 DF = 3 P = 0.979
H = 1.32 DF = 3 P = 0.724 (adjusted for ties)

Kruskal-Wallis Test: code versus Sample
H_0: Temperature is not affected the overall acceptability
H_1: Temperature is affected to the overall acceptability

Sample	N	Median	Ave Rank	Z
1	30	5.000	33.0	-5.01
2	30	6.000	40.4	-3.65
3	30	8.000	85.2	4.48
4	30	8.000	83.5	4.17
Overall	120		60.5	

H = 56.94 DF = 3 P = 0.000
H = 59.32 DF = 3 P = 0.000 (adjusted for ties)

Statistically analysis data for finding suitable Time of herbal drink

Kruskal-Wallis Test: code versus Sample

H_0: Time treatment not effect the color appearance
H_1: Time treatment effect the color appearance.

Sample	N	Median	Ave Rank	Z
1	30	8.000	85.9	4.61

2	30	8.000	77.6	3.11
3	30	6.500	48.8	-2.12
4	30	6.000	29.7	-5.59
Overall	120		60.5	

H = 50.04 DF = 3 P = 0.000
H = 53.52 DF = 3 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: code versus Sample

H_0: Time treatment not effect the acid level of drink
H_1: Time treatment effect the acid level of the drink.

Sample	N	Median	Ave Rank	Z
1	30	8.000	60.5	0.00
2	30	8.000	58.6	-0.35
3	30	8.000	62.5	0.35
4	30	8.000	60.5	0.00
Overall	120		60.5	

H = 0.19 DF = 3 P = 0.979
H = 1.32 DF = 3 P = 0.724 (adjusted for ties)

Kruskal-Wallis Test: Code versus Sample

H0: Time difference not affected the Flavour.
H1: Tme difference affected the Flavour.

Kruskal-Wallis Test on Code

Sample	N	Median	Ave Rank	Z
1	30	6.000	44.8	-2.86
2	30	5.000	32.7	-5.06
3	30	8.000	92.2	5.75
4	30	.000	72.4	2.16
Overall	120		60.5	

H = 53.68 DF = 3 P = 0.000
H = 56.66 DF = 3 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: Code versus Sample

Welcome to Minitab, press F1 for help.
H_0: Time difference not effected to overall acceptability.
H_1: Time difference effected to the overall acceptability.

Kruskal-Wallis Test on Code

Sample	N	Median	Ave Rank	Z
1	30	6.000	44.0	-3.01
2	30	5.000	31.9	-5.19
3	30	8.000	92.1	5.75
4	30	7.000	74.0	2.45
Overall	120	60.5		

H = 56.32 DF = 3 P = 0.000
H = 59.32 DF = 3 P = 0.000 (adjusted for ties)

Statistically analysis data for finding suitable Herbal of herbal drink

Kruskal-Wallis Test: Code versus Sample

H_0: Herbal content not efect the flavour of the drink.
H_1: Herbal content efect the flavour of the drink.

Sample	N	Median	Ave Rank	Z
1	30	6.000	31.1	-3.69
2	30	8.000	64.9	4.97
3	30	7.000	40.5	-1.28
Overall	90	45.5		

H = 26.67 DF = 2 P = 0.000
H = 28.76 DF = 2 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: Code versus Sample
H_0: Herbal content not effected the color of the drink
H_1: Herbal content effected to the color of the drink

Sample	N	Median	Ave Rank	Z
1	30	8.000	65.9	5.23
2	30	7.000	48.7	0.83
3	30	6.000	21.9	-6.06
Overall	90	45.5		

H = 43.14 DF = 2 P = 0.000
H = 46.32 DF = 2 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: Code versus Sample
H0: Herbal content not affect the Flavour of the drink.
H1: Herbal content Affect the Flavour of the drink.

Sample	N	Median	Ave Rank	Z
1	30	6.000	31.1	-3.69

2	30	8.000	64.9	4.97
3	30	7.000	40.5	-1.28
Overall	90	45.5		

H = 26.67 DF = 2 P = 0.000
H = 28.76 DF = 2 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: Code versus Sample

H_0: Herbal content not effected to overall acceptability of drink.
H_1: Herbal content effected to overall acceptability of the drink

Sample	N	Median	Ave Rank	Z
1	30	6.000	30.5	-3.85
2	30	8.000	66.6	5.41
3	30	7.000	39.4	-1.56
Overall	90	45.5		

H = 31.06 DF = 2 P = 0.000
H = 33.48 DF = 2 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: Code versus Sample

Ho: Herbal content not effected to overall acceptability of drink.
H1: Herbal content effected to overall acceptability of the drink

Sample	N	Median	Ave Rank	Z
1	30	6.000	30.5	-3.85
2	30	8.000	66.6	5.41
3	30	7.000	39.4	-1.56
Overall	90	45.5		

H = 31.06 DF = 2 P = 0.000
H = 33.48 DF = 2 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: code versus Sample
H_0: Herbal content not effected to acid level of the acid
H_1: Herbal content not effected to acid level of the acid

Sample	N	Median	Ave Rank	Z
1	30	7.000	45.5	0.00
2	30	7.000	45.5	0.00
3	30	7.000	45.5	0.00
Overall	90	45.5		

H = 0.00 DF = 2 P = 1.000
H = 0.00 DF = 2 P = 1.000 (adjusted for ties)

Kruskal-Wallis Test: code versus Sample

H_0: Herbal content not effected to thickness of the drink
H_1: Herbal content not effected to thickness of the drink

Sample	N	Median	Ave Rank	Z
1	30	7.000	45.5	0.00
2	30	7.000	45.5	0.00
3	30	7.000	45.5	0.00
Overall	90		45.5	

H = 0.00 DF = 2 P = 1.000
H = 0.00 DF = 2 P = 1.000 (adjusted for ties)

Statistically analysis data for finding suitable Extraction method of herbal drink

Kruskal-Wallis Test
H0: Extraction method not effect the herbal drink
H1: Extraction method effect the herbal drink.

Sample	N	Median	Ave Rank	Z
1	30	8.000	62.0	4.25
2	30	7.000	46.6	0.29
3	30	6.000	27.9	-4.53
Overall	90		45.5	

H = 25.76 DF = 2 P = 0.000
H = 26.91 DF = 2 P = 0.000 (adjusted for ties)

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