EFFECT OF DRYING AND BLANCHING (STEAM AND LYE) ON THE PHYTOCHEMICALS COMPOSITION OF SITALCHINI (*Moringa oleifera*) LEAVES AND ITS SENSORY ATTRIBUTES

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A dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment for the degree of B.Tech. in Food Technology

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Approval Letter

This *dissertation* entitled *Effect of Drying and Blanching (Steam and Lye) on the Phytochemicals Composition of Sitalchini (Moringa oleifera) Leaves and its Sensory Attributes* presented by Anuradha Yadav has been accepted as the partial fulfillment of the requirement for the B. Tech. degree in Food Technology

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Anuradha yadav

Abstract

The antioxidant activity of plant materials is affected by post-harvest treatments. The present study was undertaken to evaluate the effects of blanching (steam and lye) and cabinet drying methods on phytochemicals composition of *Moringa oleifera* leaves. The fresh sitalchini (*Moringa oleifera*) leaves were collected from Sunsari district Dharan, Nepal. Initially adequate time- temperature for blanching was optimized and was found to be 3 min at 98°C for steam blanching and 2 min at 98°C for lye (sodium bicarbonate) blanching.

Proximate composition of fresh *Moringa oleifera* leaves were analyzed and was found to be for moisture, protein, crude fiber, fat and ash content was 73.25±0.89%, 21.65±0.57%,8.19±0.05%,5.52±0.26% and 8.72±0.10% respectively on dry basis except for moisture. Crude extract of sample were prepared using 80% methanol through maceration technique. Phytochemicals screening of the methanol extract of the plant showed the presence of total phenol content, flavonoid, tannin, glycosides and steroid. Total phenol, flavonoid, tannin content, antioxidant activity and chlorophyll content of fresh sitalchini leaves was found to be 165.54±0.62 mg/g, 558.63±55.59 mg/g, 14.008±1.73 mg/g,79.88±0.63% and 19.61±0.14 mg/g respectively. Result showed that drying and blanching decreased the levels of phytochemicals content and antioxidant activity significantly ($p<0.05$) as compared to fresh leaves. Drying slightly effect chlorophyll content. Chlorophyll content was affected by steam blanching and was not affected by lye blanching. Fresh leaves is superior than that of others in terms of both phytochemicals and sensory analysis which is suitable for fortification of foods and their use as nutritional supplements is highly promising.

Contents

List of Tables

List of Figures

List of Plates

List of Abbreviations

Abbreviation	Full Form
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical chemist
CCT	Central Campus of Technology
DPPH	2,2 –Diphenyl 1,1 picrylhydrazyl
GAE	Gallic acid equivalent
LSD	Least Significance Difference
NSA	Non-starch polysaccharides
ROS	Reactive oxygen species
SFE	Supercritical fluid extraction
TPC	Total phenol content
TFC	Total flavonoid content

Part I

Introduction

1.1 General introduction

Green leafy vegetables occupy an important place among the food crops as these provide adequate amounts of many vitamins and minerals for humans. They are rich source of vitamin like β-carotene, ascorbic acid, riboflavin, folic acid and minerals like calcium, iron and phosphorous. In nature, there are many underutilized greens of promising nutritive value, which can nourish the ever increasing human population. Many of them are adaptive and tolerant to adverse climatic conditions. Although, they can be raised at lower management costs even on poor marginal lands, they have remained underutilized due to lack of awareness and popularization of technologies for utilization. Nowadays, underutilized foods are gaining importance as a means to increase the percapita availability of foods (Mathur and Joshi, 2010).

Moringa, "The Queen of Green", is a medium-sized (about 10 meters high) tree belonging to the Moringaceae family. Moringaceae is a single genus family with 13 known species. *Moringa* trees are commonly known by names such as 'horse-radish' tree or 'drum stick tree'. *Moringa* is native to parts of Africa and Asia. It is called sitalchini in Nepali. They are available in the mid-hills and Terai of Nepal. *Moringa* is considered one of the world's most useful trees, as almost every part of the tree can be used for food or has some other beneficial property. The roots, leaves, bark and pods are said to have medicinal properties. It can provide rural families with income, food, nutritious vegetables, animal feed, etc**.** It is considered as one of the world's most useful plant and thus all the plant parts is widely used in curing various ailments like antibiotic, anti-hypertensive, antispasmodic, antiulcer, anti-inflammatory, anti-asthmatic, hypocholesterolemic and hypoglycemic (Singh and Prasad, 2013).

Presence of various types of antioxidant compounds make this plant leaves a valuable source of natural antioxidants and a good source of nutraceuticals and functional components as well (Siddhuraju and Becke, 2003). Phytochemicals such as vanillin, omega fatty acids, carotenoids, ascorbates, tocopherols, beta-sitosterol, moringine, kaempferol, and quercetin have been reported in its flowers, roots, fruits, and seeds. The leaves, in

particular, have been found to contain phenolic and flavonoids . these compounds have various biological activities, including antioxidant, anti-carcinogenic, immunomodulatory, anti-diabetic, anti-atherogenic, and hepatoprotective functions and the regulation of thyroid status. Moreover, leaves contain trace elements that are essential to human health.

It has been used as a traditional medicine around the world, for anemia, skin infections, blackheads, anxiety, bronchitis, catarrh, chest congestion, asthma, blood impurities, cholera, glandular, swelling, headaches, conjunctivitis, cough, diarrhea, eye and ear infections, fever, abnormal blood pressure, hysteria, pain in joints, pimples, psoriasis, respiratory disorders, scurvy, semen deficiency, sore throat, sprain, tuberculosis, for intestinal worms, lactation, diabetes and pregnancy. The healing properties of *Moringa* oil have been documented by ancient cultures (Ramachandran and Gopalakrishnan, 1980). It contains (seven times the vitamin C of oranges); (four times the vitamin A of carrots), (four times the calcium of milk), (three times the potassium of banana) and (two times the protein of yoghurt). The micro-nutrient content is even more in dried leaves; (ten times the vitamin A of carrots), (17 times the calcium of milk), (15 times the potassium of bananas), (25 times the iron of spinach) and (nine times the protein of yogurt) (Manzoor *et al.*, 2007). Many companies across the world manufacturing various products of drumstick leaves such as *Moringa* Tea, *Moringa* Tablets, *Moringa* capsules, *Moringa* leaf Powder, *Moringa* Soaps and *Moringa* Face wash. Some beverages are also available in market prepared by *Moringa* leaves. So it is necessary to hygienically drying and processing of *Moringa* leaves for further uses.

1.2 Statement of the problem

An excess of free radicals in the body leads to oxidative stress which plays an important role in the pathogenesis of several human diseases, such as cardiovascular diseases, neurodegenerative diseases, cancer, rheumatoid arthritis, and diabetes (Nobosse *et al.*, 2012). Natural antioxidants present in foods protect against these free radicals and are therefore important in maintaining and preserving good health (Nimse and Pal, 2015). Antioxidant-rich plants are the focus of intense interest since recent reports have expressed safety concerns over the use of synthetic antioxidants (Taghvaei and Jafari, 2015). *Moringa oleifera* leaves are considered a significant source of phytochemicals (carotenoids, phenolic compounds, vitamin C) and act as a good source of natural antioxidants. In developing countries where *Moringa* leaves are increasingly being used to

resolve malnutrition problems, these leaves are either cooked directly after harvesting or are sun or shade dried and stored for future use. There is a need to look inward to search for herbal medicinal plants with an aim to validate the use of sitalchini as medicine and subsequently, the isolation and characterization of compounds which will then be added to the potential list of drugs.

1.3 Objectives

1.3.1 General objective

To study the effect of drying and blanching (steam and lye) on phytochemical composition of *Moringa oleifera* leaves and its sensory attributes.

1.3.2 Specific objectives

1. To determine the proximate composition of fresh sitalchini leaves.
2. To prepare the methanol extract of fresh, dried and blanched dried sitalchini leaves.
3. To study the effect of blanching and drying on phytochemical composition of leaves.
4. To evaluate the sensory analysis of *Moringa* powder.

1.4 Significance of the study

Food based strategy' is used as a tool for combating micronutrient deficiencies. It is also referred as dietary modification, which encompasses a wide variety of intervention that aim at increasing the production, availability and consumption of food products, which are rich in micronutrients. One such food products are green leafy vegetables. There are many varieties of green leafy vegetables which are though rich in micronutrients, but are usually discarded or are not used for human consumption. One such leaf, a rich source of micronutrients but still under exploited is drumstick leaf (Joshi and Mehta, 2010). The leaves possess remarkable nutritional and medicinal qualities .Therefore it is necessary to increase the utilization of *Moringa* leaves consumption by the different communities. It should be consumed either fresh or dry. Dried leaves can be stored for a long time and can be used regularly.

The present study will be done with the objective to assess the effect of blanching and drying on the phytochemicals content of the drumstick leaves and helps to enhance the

consumption of this leaves. Since, it constitutes a large group of chemicals with high potential to treat various diseases, phytochemical analysis is the best way to bring forward *Moringa oleifera* as medicinally important plant. Several reports on post-harvest treatment of this leaves only consider the effect of either blanching or drying alone on macronutrients and vitamins , phenolic content and antioxidant activity (Mutiara *et al.*, 2012). Effects of different drying methods on nutritional, phytochemical and antioxidant activity of *Moringa* leaves has been reported. But in practice at the household-level, blanching is a common preliminary step prior to drying under different conditions. This research help to investigate the effect of blanching and drying on phytochemical content of *Moringa oleifera* leaves (Nobosse *et al.*, 2012). This study also helps to remove the misconception among the people that *Moringa* is a food item which is consumed not only by the grass level people but can equally be included in the cookery of affluent people. This dissertation will prove to be beneficial in contributing to the socioeconomic development of Nepal and the people.

1.5 Limitation of the study

1. Only one variety of *Moringa* was used for research work.
2. Single extraction technique was used to prepare the extract.

Part II

Literature review

2.1 Origin and distribution

Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae (Pandey *et al.*, 2012). It is a small native tree of the sub-Himalayan regions of North West India, which is now indigenous to many regions in Africa, Arabia, South East Asia, the pacific and Caribbean Islands, and South America (Mouminah, 2015). It is a type of vegetable plant shrubs with 5-12 m height and sometimes it reaches 15 m with a diameter of about 30 cm. Tree grows in areas 800-1200 m above sea level with rainfall of 400 mm per year . *Moringa* leaves are compound, pinnate double, and small rounded oval at the fingertips. Flowering plants all year round yellowish white. The fruit, called "drumstick", is long and angular, its sides form a triangle, at the length of 15-45 cm, with the number of seeds about 20 soft and brittle stems (Titi and Teti, 2013). It comprises 13 species from tropical and subtropical climates, ranging in size from tiny herbs to massive trees. The most widely cultivated species is *Moringa Oleifera* (Padayachee and Baijnath, 2012)

2.2 Consumption pattern of *Moringa oleifera*

The leaves of *Moringa oleifera* are collected, cooked and eaten like other vegetables. It is utilized in fortifying sauces, juices, spices, milk, bread, and most importantly, instant noodles. Many commercial products like Zija soft drink, tea, and neutroceuticals are available all over the globe Leaves have been used successfully in its dried state or powdered form to make delicious meals and porridge diets for pregnant expectant mothers, nursing mothers, infants and young children, as well as adults of all age groups. In Africa, nursing mothers have been shown statistically to produce far more milk when they add (Mahmood and Mugal, 2010). *Moringa oleifera* leaves to their daily diets and malnourished children have made significant weight gains when nursing mothers and care-givers add them to their diets as well (Kucha *et al.*, 2015). For pregnant and breast feeding-nursing women, the leaves can do much to preserve the mothers' health and pass on strength to the fetus or nursed child. 100 g portion of the leaves could provide a woman with over one-third of her daily requirement of calcium and gives her important quantities

of iron, protein, copper, sulphur, B-vitamin (Alakali *et al.*, 2015). It has also been consumed by incorporated in biscuits, porridges and other food products. *Moringa* tea is usually prepared by brewing dried leaf powder in hot water for some time and consumed (Tetteh, 2009).

2.3 Nutritional aspect of *Moringa* leaves

It has long been cultivated and all its parts been consumed and used for a variety of purposes across the tropics. *Moringa oleifera*, a plant with a wide range of medicinal, nutritional and economic benefits (Unuigbe *et al.*, 2014). This leaves are good source of micronutrients and are concentrated with protein (Moyo *et al.*, 2011; Olson *et al.*, 2016). The leaves are exceptionally excellent source of β-carotene, vitamin C. Allirani and Arumugam (2017) reported the presence the following minerals in the leaves: – sodium, potassium, calcium, magnesium, zinc, iron, manganese and nutrients such as carbohydrate, protein, fat, crude fibre, and ash.

It has also been reported that the amino acid profile of *Moringa* leaves meets the standards of the World Health Organization. (El Sohaimy *et al.*, 2015), confirmed that, *Moringa* leaves has high amount of essential amino acids; methionine, leucine, isoleucine, histidine, phenylalanine, valine, threonine, arginine and lysine compared to cereals and legumes. In addition to essential amino acids, the proteins in this leaves have different biological and functional properties, including, trypsin inhibitors, lectins, chitin binding proteins and proteases (Santos *et al.*, 2015).

2.4 Prospect of *Moringa* in Nepal

Moringa cultivation can help to increase rural incomes by generating employment, self-sustainability and alleviate poverty among poor, small holder farmers and marginalized communities. As it can be grown intercropping with many others vegetables and plants it has high potentiality to uplift the economic condition of poor farmers. Since *Moringa* requires minimum irrigation, and only small amount of manure and fertilizer which is not labor intensive and family members can take out time for *Moringa* cultivation this is a viable option. *Moringa* can help improve the livelihood of the farmers. The crop proves more profitable with minimum investment even within a small land in a short span of time. So cultivation of *Moringa* by smallholder farmers who have marginal land can be promoted. This crop diversification strategy will provide vegetables, medicinal values,

nutritional sufficiency and a raw material for oil extraction in the rural areas. Therefore, this crop can be a boon to the marginal community of rural areas (Pokhrel *et al.*, 2016).

2.5 Uses of *Moringa:* a multipurpose tree

Moringa is grown for its nutritious pods, edible leaves and flowers and can be utilized as food, medicine, cosmetic oil or forage for livestock (Padayachee and Baijnath, 2012). The many uses of *Moringa oleifera* include: alley cropping (biomass production), animal forage (leaves and treated seed-cake), biogas (from leaves), domestic cleaning agent (crushed leaves), blue dye (wood), fencing (living trees), fertilizer (seed-cake), foliar nutrient (juice expressed from the leaves), green manure (from leaves), gum (from tree trunks), honey- and sugar cane juice-clarifier (powdered seeds), honey (flower nectar), medicine (all plant parts), ornamental plantings, bio-pesticide (soil incorporation of leaves to prevent seedling damping off), pulp (wood), rope (bark), tannin for tanning hides (bark and gum), water purification (powdered seeds) (Mutiara *et al.*, 2012). *Moringa oleifera* seed oil (yield 30-40% by weight), also known as Ben oil, is a sweet non-sticking, non-drying oil that resists rancidity. It has been used in salads, for fine machine lubrication, and in the manufacture of perfume and hair care products (Tsaknis *et al.*, 1999).

2.6 Medicinal importance of *Moringa oleifera*

A number of medicinal properties have been ascribed to various parts of this highly esteemed tree as shown in Table 2.1. Almost all the parts of this plant: root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia.

Table 2.1 Some common medicinal uses of different parts of *Moringa oleifera*

Plant part	Medicinal uses
Root	Antilithic, rubefacient, vesicant, carminative, antifertility, anti-inflammatory, stimulant in paralytic afflictions; act as a cardiac/circulatory tonic, used as a laxative, abortifacient, treating rheumatism, inflammations, articular pains, lower back or kidney pain and constipation

leave	Purgative, applied as poultice to sores, rubbed on the temples for headaches, used for piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrh; leaf juice is believed to control glucose levels, applied to reduce glandular swelling
Stem bark	Rubefacient, vesicant and used to cure eye diseases and for the treatment of delirious patients, prevent enlargement of the spleen and formation of tuberculous glands of the neck, to destroy tumors and to heal ulcers. The juice from the root bark is put into ears to relieve earaches and also placed in a tooth cavity as a pain killer, and has anti-tubercular activity
Gum	Used for dental caries, and is astringent and rubefacient; Gum, mixed with sesame oil, is used to relieve headaches, fevers, intestinal complaints, dysentery, asthma and sometimes used as an abortifacient, and to treat syphilis and rheumatism
flower	High medicinal value as a stimulant, aphrodisiac, abortifacient, cholagogue; used to cure inflammations, muscle diseases, hysteria, tumors, and enlargement of the spleen; lower the serum cholesterol, phospholipid, triglyceride, cholesterol to phospholipid ratio and atherogenic index; decrease lipid profile of liver, heart and aorta in hypercholesterolaemic rabbits and increased the excretion of faecal cholesterol
Seed	Seed extract exerts its protective effect by decreasing liver lipid peroxides, antihypertensive compounds thiocarbamate and isothiocyanate glycosids have been isolated from the acetate phase of the ethanolic extract of *Moringa* pods

Source: Anwar and Latif (2006)

2.7 Drying

Drying is an ancient technique for the preservation of fruits and vegetables. It stops the biochemical changes in perishable plant materials by reducing the moisture content of the material (Raja and Taip, 2017). It is one of the most cost-effective ways of preserving foods of all variety which involves removal of water by application of heat. A variety of food sub-types are preserved using drying, these include: marine products, meat products as well as all fruits and vegetables (Sikora *et al.*, 2008). Food products can have moisture content as high as 90% or more (e.g. water melon has moisture content as high as 93%) which needs to be reduced to an acceptable value so as to avoid microbial growth. In addition, each food product needs to be dried in a different way, using appropriate pre and post-processing steps and proper dryer type so as to add satisfactory value upon drying. The pre and/or post processing steps are very important to reduce the drying load as well as to make better quality products (Rakic *et al.*, 2007). Various pre-processing steps such as osmotic dehydration, blanching, salting, soaking are used depending on the food variety to be dried (Jangam and Mujumdar, 2010).

In food market dried foods play an important role in the food supply chain. There are various aspects that must be considered when drying small fruits and vegetables, whether for the food or nutraceutical and functional food industries. A system which minimizes exposure to light, oxidation and heat, (i.e. high heat 70^0C and shorter time duration) may help conserve critical bioactive compounds (Ahmed *et al.*, 2013).

2.7.1 Definition

Drying is a complex operation involving transient transfer of heat and mass along with several rate processes, such as physical or chemical transformations, which, in turn, may cause changes in product quality as well as the mechanisms of heat and mass transfer. Physical changes that may occur include: shrinkage, puffing, crystallization, glass transitions. In some cases, desirable or undesirable chemical or biochemical reactions may occur leading to changes in color, texture, odor or other properties of the solid product (Wangcharoen and Gomolmanee, 2013). Drying is highly energy consuming unit operation and competes with distillation as the most energy-intensive unit operation due to the high latent heat of vaporization of water and the inherent inefficiency of using hot air as the (most common) drying medium.

2.7.2 Mechanism of drying

When hot air is blown over a wet food, heat is transferred to the surface, and latent heat of vaporization causes water to evaporate. Water vapor diffuses through a boundary film of air and is carried away by the moving air. This creates a region of lower water vapor pressure at the surface of the food, and a water vapor pressure gradient is established from the moist interior of the food to the dry air. This gradient provides the driving force for water removal from the food (Ale, 2017).

Water moves to the surface by the following mechanisms:

- Liquid movement by capillary forces.
- Diffusion of liquids, caused by differences in the concentration of solutes in different regions of the food.
- Diffusion of liquids, which are adsorbed in layers at the surfaces of solid components of the food.
- Water vapor diffusion in air spaces within the food caused by vapor pressure gradients.

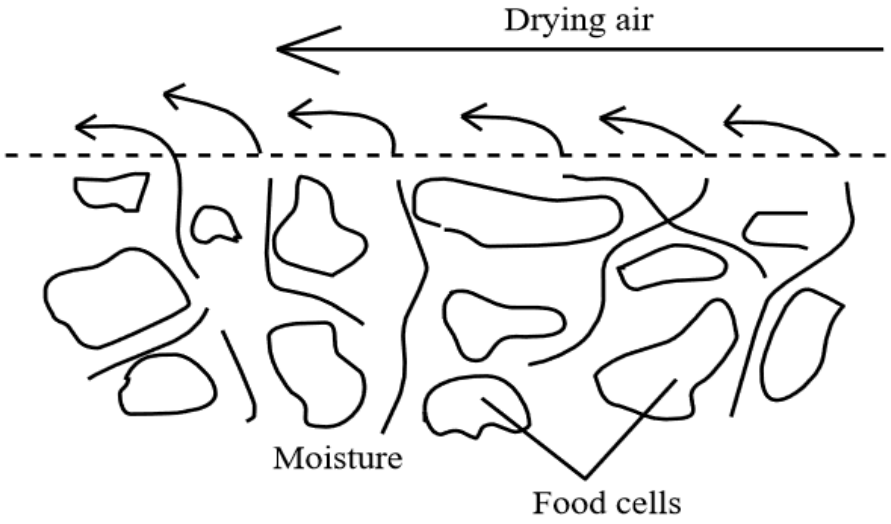

Fig. 2.1 Movement of moisture during drying (Kharel, 2004)

2.7.3 Different methods of drying

1. Sun drying

Sun drying is the most common method employed in tropical countries for the drying of agricultural products, food stuffs, etc. The method is simple, as it does not involve any costly equipment. The product to be dried is spread under sun, and the moisture evaporates from it over a course of time. Even though the process is simple, it suffers from disadvantages such as dust contamination, insect infestation, microbial contamination and spoilage due to rains. Product dried in this way is unhygienic and sometimes unfit for human consumption (Kumar, 2015).

2. Solar drying

Solar drying has been considered as one of the most promising areas for the utilization of solar energy, especially in the field of food preservation (Kumar, 2015). Solar dryer uses sun's energy for drying but excludes open air sun drying. It has a number of advantages as solar energy is non-polluting, free, abundant renewable energy source (Teng and Chen, 1999). A simple principle involved in solar dryer is that, air is drawn through the dryer by natural convection. The air is heated as it passes through the collector and then partially cooled as it picks up moisture from the produce. The produce is heated both by the hot air and sun. An alternative solution for traditional drying method and to overcome the problem of open sun drying, indirect type solar dryer is used (Lingayat, 2017). The main reasons are as follows:

- Solar drying maintains good product quality compared to open sun drying.
- Time for drying process can be significantly reduced as compared to open sun drying.
- Dried foods can be preserved for a long time period and the product becomes extremely lightweight hence easy for transportation.

3. Cabinet drying

Cabinet drier consist of an insulated cabinet fitted with shallow mesh or perforated trays, each of which carries a thin layer of food. Hot air is circulated through the cabinet tray. A system of duct and baffles is used to direct air, over and/ or through each tray, to promote

uniform air distribution either horizontally between the trays of food materials or vertically through the trays and food (Fellows, 2000). Air heaters may be direct gas burners, steam coil exchangers or electrical resistance heaters. The air is blown past the heaters and thus heated air is used for dying. It is relatively cheap to build and maintain, flexible in design, and produces variable product quality due to relatively poor control. It is used singly or in groups, mainly for small- scale production (1-20 ton/day) of dried fruits and vegetables (Fellows, 2000). Cabinet dryer is shown in Fig. 2.2

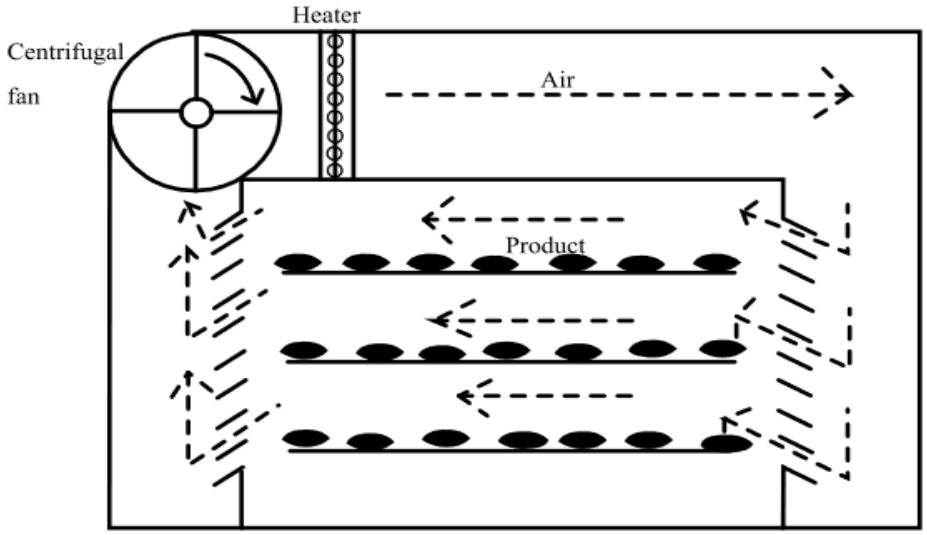

Fig. 2.2 Cabinet Dryer

2.8 Blanching

Blanching is a short heat treatment that is typically applied to vegetables prior to further processing with the aim of enhancing both safety and quality attributes (Stanley *et al.*, 2017). It imparts benefits such as the destruction of surface micro flora on vegetables and the enhancement of the color, texture and also the keeping quality of vegetable products (Abu-Ghannam *et al.*, 2011). Blanching is essential for vegetable products destined for further storage such as freezing or drying in order to inactivate certain enzymes including lipoxygenase, polyphenololoxidase, polygalacturonase, and chlorophyllase which are associated with losses in quality and nutritional properties. Apart from blanching, other processing methodologies of vegetables, including drying and freezing, are insufficient to inactivate these enzymes thus leading to deterioration in texture, color, and flavor during storage (Busari *et al.*, 2016).

The quality of blanched products depends significantly on the time–temperature combinations of blanching and also on the vegetable type. Under-blanching speeds up the activity of enzymes and is worse than no blanching (Abu-Ghannam *et al.*, 2011). Over-blanching causes losses in texture, color, phytochemicals and minerals (Saranya *et al.*, 2017).

Generally, blanching is carried out by the application of a wet medium such as steam or hot water in order to provide uniform heating and a high-heat transfer rate (Gupta *et al.*, 2012). Both in domestic and industrial processing, several blanching methods may be employed such as conventional water blanching, microwave, or steam blanching; the regime being dictated by the nature of the raw material and the desired properties of the final product (Francisco *et al.*, 2010). Traditionally, blanching is carried out either at a low temperature (55–75°C) for long-time, typically referred to as LTLT or high-temperature short-time (80–100°C) for less than 10 min, referred to as HTST depending upon the type of vegetable (Abu-Ghannam and Crowley, 2006).

2.8.1 Effect on foods by blanching

The heat received by the food during blanching inevitably causes some changes to sensory and nutritional qualities (Amin and Lee, 2005). The time-temperature combination used for blanching is a compromise which ensures adequate inactivation but prevents excessive softening and loss of flavor in the foods (Fellows, 2000). Some of the effects on foods of plant origin are discussed below.

2.8.1.1 Color

Blanching brightens the color of some foods by removing air and dust on the surface and thus altering the wave length of reflected light. Sodium carbonate or calcium oxide are often added to blancher water to protect chlorophyll and to retain the colour of green vegetables, although the increase in pH may increase losses of ascorbic acid (Fellows, 2000).

2.8.1.2 Texture

Blanching can result in undesirable softening of vegetable tissues. However, calcium can be added to reduce the softening (Seow and Lee, 1997). A combination of low-temperature blanching and calcium addition has also been shown to be effective in firming

canned vegetables (Bourne *et al.*, 1995). Texture assessment of the effects of blanching includes sensory characterization of firmness, crispness, and crunchiness (Corcuera and Cavalieri, 2015).

2.8.1.3 Flavor

Blanching indirectly and directly affects the flavor of many products by inactivation of enzymes responsible for off-flavor development. The most notable is lipoxygenase in several vegetables (Williams *et al.*, 1986). Sometimes blanching increases flavor retention, and sometimes it removes undesirable bitter flavors from the product (Corcuera and Cavalieri, 2015). In fruits and vegetables the change in flavor is due to complex reactions that involve the degradation, recombination and volatilization of aldehydes, ketones, sugars, lactones, acids, and organic acids.

2.8.1.4 Nutrients

Some minerals, water soluble vitamins and other water soluble components are lost during blanching. Losses of vitamins are mostly due to leaching, thermal destruction and to lesser extent oxidation (Fellows, 2000). The extent of vitamin loss depends upon on a number of factors including:

1. The maturity of food and variety.
2. Methods used in preparation of the food, particularly the extent of cutting, slicing or dicing.
3. The surface area to volume ratio of the pieces of food.
4. Method of blanching.
5. Time and temperature of blanching (lower vitamin losses at higher temperature for shorter time).
6. Method of cooling.

2.8.2 Methods of blanching

i. Hot water blanching

Hot water blanching is the most popular and commercially adopted blanching method, as it is simple to establish and easy to operate. In a typical hot water blanching, products are immersed in hot water (70 to 100°C) for several minutes (Schieber *et al.*, 2001). Then blanched samples are drained and cooled before being sent to the next processing operation. In general, after a certain amount of blanching time, the blanching water needs to be replenished as it becomes saturated with nutrients leached from the products. This step does not only consume high amounts of water and energy (Xiao *et al.*, 2017). In order to preserve the color of product and inactivate microbial activity, sodium sulfite and sodium metabisulfite are often added to the blanching water (Ferracane *et al.*, 2008). This makes it more difficult to deal with the wastewater generated from the blanching operation. Water blanching usually results in a more uniform treatment, allowing processing at lower temperatures. Prolonged water blanching results in considerable losses in phytochemicals and antioxidant properties (Jaiswal and Gupta, 2012). Limitations of Hot water blanching are as:

- Losses of nutrients during blanching

The loss of nutrients during hot water blanching is caused mainly by leaching or diffusion. All water-soluble nutrients, such as vitamins, flavors, minerals, carbohydrates, sugars, and proteins, can leach out from plant tissues to the blanching water. In addition, hot water blanching can also lead to degradation of some thermal sensitive substances such as ascorbic acid, aroma and flavor compounds (Xiao *et al.*, 2017)

- Wastewater from blanching

The discharged waste water from hot water blanching contain high concentrations of biochemical, soluble solids, and chemical oxygen demand due to leaching and dissolution of sugars, proteins, carbohydrates and water-soluble minerals. This wastewater can cause environmental pollution, e.g. eutrophication (Xiao *et al.*, 2017)

ii. Steam blanching

Steam blanching is generally carried out in a steam blancher where the vegetable product is exposed directly to a food-grade steam typically at a temperature close to 100°C (Nartnampong *et al.*, 2016). Superheated steam is commonly used as a heating media for blanching due to its high enthalpy contents. During the early stage of steam blanching, it condenses on the surface of the products and a large amount of latent heat transfers to the material because product temperature is lower than that of steam. The temperature of the products gradually increases until reaching the critical temperature of enzymes or organisms activity, after which they are inactivated. It is believed that the steam blanching is relatively inexpensive and retains most minerals and water-soluble components when compared with water blanching due to the negligible leaching effects (Xiao *et al.*, 2017).

Steam cooking may affect the antioxidant status of tropical green leafy vegetables due to the release of more phenolic compounds and destruction or creation of redox-active metabolites (Adefegha and Oboh, 2011). During the steam blanching process, softening of the tissue and undesirable quality changes often resulted a long heating time due to the lower heat transfer in steam blanching than hot water blanching, especially when the velocity of steam is very low (Juneja *et al.*, 2009). Steam blanching results in minimum losses in phytochemicals and antioxidant capacity (Faller and Fialho, 2009). It requires less time than conventional blanching because the heat transfer coefficient of condensing steam is greater than that of hot water and it is proven to be comparatively economical as it saves energy (Jaiswal, 2015). Steam blanching is more energy-efficient and produces lower BOD and hydraulic loads than water blanching. In addition, nutrient leaching is reduced compared to water blanching (Adefegha and Oboh, 2011).

iii. Microwave blanching

Microwave heating is three- to five-times faster than conventional heating and relies on the application of dielectric heating. This is accomplished by using microwave radiation to heat water and other polarized molecules within the food, leading to heat generation in the entire volume at the same rate due to internal thermal dissipation of water molecule vibrations in the food. It has advantages over conventional heating methods such as precision timing, speed, and energy saving (Jaiswal, 2015). Microwave technology has

been combined with water blanching to further reduce heating time (Xiao *et al.*, 2017). Limitations of microwave blanching are as:

- Loss of water during blanching
- Penetration depth of microwave is limited.
- Non uniform heating.
- Difficulities to precisely control blanching temperature.

iv. Lye blanching

Sodium bicarbonate is effective in maintaining green color of heat processed vegetable products but they cause changes in texture and loss of nutrients that are not desirable (Heaton and Marangoni, 1996). By displacing the phytyl and methyl groups, forming a bright green water soluble chlorophyllin sodium bicarbonate reacts with chlorophyll (Srilakshmi, 2003). In addition, improves chlorophyll stability is improves this may be due to sodium bicarbonate increases the medium pH.

2.9 Chlorophyll

Chlorophyll is the pigment that gives plants their characteristic green color (Rodoni *et al.*, 1997). Chlorophyll is a green photosynthetic pigment which helps plants to get energy from light. The plants use the energy to combine carbon dioxide and water into carbohydrate to sustain their life process (Rodoni *et al.*, 1997). In the cell chlorophyll is sandwiched between protein and lipid layers of the chloroplast lamellae (Adebooye and Singh, 2006). They occur in several distinct forms: chlorophylls a and b are the major types found in higher plants and green algae; chlorophylls c and d are found, often with a, in different algae; chlorophyll e is a rare type found in some golden algae; and bacterio-chlorophyll occurs in certain bacteria. In green plants chlorophyll occurs in chloroplasts, approximately in the ratio 3a:1b (Rai, 2006).

Chemically, chlorophylls are tetrapyrrole pigments (pyrrole rings held together by methane carbons, -CH=) in which the porphyrin ring is in the dihydro form and the central metal atom is magnesium. Magnesium atom is held by nitrogen on two of the rings by ordinary covalent bonds. The other two nitrogen share two electrons with the magnesium

to form a coordinate covalent bond (indicated by broken lines). The magnesium is removed very easily by acids, giving pheophytins a and b. The action of acid is especially important for fruits that are naturally high in acid. However, it appears that the chlorophyll in plant tissues is bound to lipoproteins and is protected from the effect of acid. Heating coagulates the protein and lowers the protective effect. The color of the pheophytins is olive-brown. Chlorophyll is stable in alkaline medium. The phytol chain confers insolubility in water on the chlorophyll molecule. Upon hydrolysis of the phytol group, the water-soluble methyl chlorophyllides (green-colored) are formed. This reaction can be catalyzed by the enzyme chlorophyllase. In the presence of copper, nickel, cobalt, iron or zinc ions, it is possible to replace the magnesium to give a stable derivative. Removal of the phytol group and the magnesium results in pheophorbides (gray-brown colored) (Rai, 2006). All of these reactions are summarized in the scheme presented in Fig.2.3

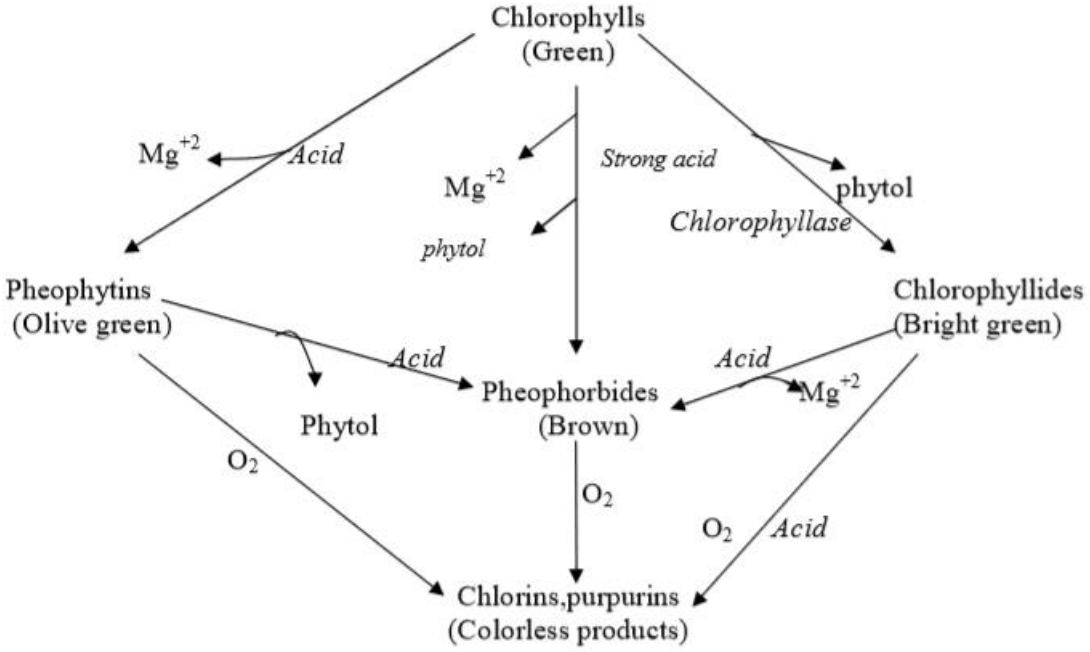

Fig. 2.3 Pathways for the degradation of chlorophyll

In food technology, chlorophyll is related to esthetic quality of fruits and vegetables. It is also extensively used as a parameter in studying maturation and ripening of fruits and vegetables (Rai, 2006).

2.10 Phytochemicals

Phytochemicals, which are non-nutritive plant chemicals, constitute a heterogeneous group of substances (Altiok, 2010). The plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Unuigbe *et al.*, 2014). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant (Abdulkadir *et al.*, 2015). These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property (Bholah *et al.*, 2015). There are more than thousand known and many unknown phytochemicals. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases. Because of this property, many researches have been performed to reveal the beneficial health effects of phytochemicals (Isitua and Jaramillo, 2015). Phytochemicals are not essential nutrients and are not required by the human body for sustaining life, but have important properties to prevent or to fight some common diseases (Saxena *et al.*, 2013). Plant phenolics and flavonoids play a great role in scavenging free radicals in the body and act as antioxidants (Agbo *et al.*, 2015). Bioactive and Disease preventing phytochemicals present in plant are shown in Table 2.2

Table 2.2 Bioactive phytochemicals in medicinal plants

Classification	Main Group of Elements	Biological function
Non-starch polysaccharides(NSA)	Cellulose,hemicellulose, gums,mucilages,pectins, lignins	Water holding capacity, delay in nutrient absorption, binding toxins and bile acids.
Antibacterial & Antifungal	Terpenoids, alkaloids, phenolics	Inhibitors of micro-organisms, reduce the risk of

		fungal infection
Antioxidants	Polyphenolic compounds, flavonoids,carotenoids, tocopherols, ascorbic acid	Oxygen free radical quenching, inhibition of lipid peroxidation
Anticancer	Carotenoids,polyphenols, curcumine, flavonoids	Inhibitors of tumor, inhibited development of lung cancer, anti-metastatic activity
Detoxifying Agents	Reductive acids, tocopherols, phenols, indoles, aromatic isothiocyanates,coumarins, flavones,carotenoids, retinoids, cyanates, phytosterols	Inhibitors of procarcinogen activation, inducers of drug binding of carcinogens, inhibitors of tumourogenesis
Other	Alkaloids, terpenoids, volatile flavor compounds, biogenic amines	Neuropharmacological agents, anti- oxidants, cancer chemoprevention

Source: Saxena *et al.* (2013)

2.10.1 Classification of phytochemicals

The exact classification of phytochemicals could have not been performed so far, because of the wide variety of them. In recent year phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidine of nucleic acids, chlorophylls etc. Secondary constituents are the remaining plant chemicals such as

alkaloids, terpenes, flavonoids, steroids, curcumines, saponins, phenolic, flavonoids and glucoside (Saxena *et al.*, 2013).

2.10.1.1 Phenolic

Phenolic phytochemicals are the largest category of phytochemicals and the most widely distributed in the plant kingdom. Phenolic are hydroxyl group (-OH) containing class of chemical compounds where the (-OH) bonded directly to an aromatic hydrocarbon group (Altiok, 2010). Based on the numbers of carbon atoms present in its structure, phenolic are categorized as Table 2.3

Table 2.3 Major classes of phenolic compounds in plants

S.N.	No of carbon atom	Basic skeleton	Class
1	6	C_6	Simple phenols benzoquinones
2	7	C_6-C_1	Phenolic acids
3	8	C_6-C_2	Acetophenones tyrosine derivatives
4	9	C_6-C_3	Hydroxycinnamic acid, coumarins
5	10	C_6-C_4	Naphthoquinones
6	13	C_6-C_1-C_6	Xanthones
7	14	C_6-C_2-C_6	Stilbenes
8	15	C_6-C_3-C_6	Flavonoids
9	18	$(C_6$-$C_3)_2$	Lignans
10	30	$(C_6$-C_3-$C_6)_2$	Bioflavonoids
11	N	$(C_6$-C_3-$C_6)_n$	Condensed tannins

2.10.1.2 Flavonoids

Flavonoids are polyphenolic compounds that are ubiquitous in nature. These appear to have played a major role in successful medical treatments of ancient times, and their use

has persisted up to now. These are ubiquitous among vascular plants and occur as aglycones, glucosides and methylated derivatives (Jaiswal and Gupta, 2012). The flavonoid polymers are also known as proanthocyanidins. They occur as plant secondary metabolites that are involved in pigmentation, antioxidants, antimicrobials, antistressors, and UV irradiation protection (Vaya and Aviram, 2001). More than 5000 flavonoids have been described so far within the parts of plants normally consumed by humans and approximately 650 flavones and 1030 flavanols are known (Harborne and Baxter, 2001). They are the largest group of phenolic compounds and have the a basic skeleton composed of the three rings (C6-C3-C6) (Harborne and Baxter, 2001). Basic structure of flavonoid is shown in Fig. 2.4

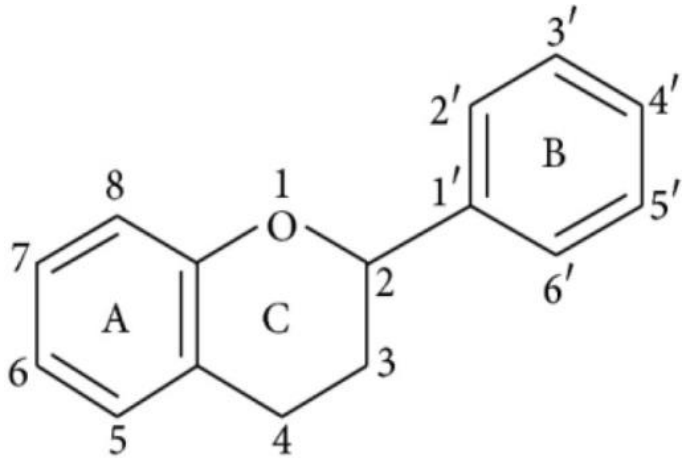

Fig. 2.4 Basic structure of flavonoid (Tapas *et al.*, 2008)

They have been classified into six subgroups (Ghasemzadeh and Ghasemzadeh, 2011):

1. Flavones (luteonin, apigenin, tangeritin).

2. Flavonols (quercetin, kaemferol, myricetin, isorhamnetin, pachypodol).

3. Flavanones (hesteretin, naringenin, eriodictyol).

4. Flavan-3-ols: catechins and epicatechins.

5. Isoflavones (genistein, daidzein, glycitein).

6. Anthocyanidins compounds (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin).

- **Activity of flavonoid**

Flavonoids have gained recent attention because of their broad biological and pharmacological activities. Flavonoids have been reported to exert multiple biological property including antimicrobial, cytotoxicity, anti-inflammatory as well as antitumor activities but the best-described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species. The capacity of flavonoids to act as antioxidants depends upon their molecular structure (Pakade *et al.*, 2013). The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. On the other hand flavonoids such as luteolin and cathechins, are better antioxidants than the nutrients antioxidants such as vitamin C, vitamin E and β-carotene (Agbo *et al.*, 2015). Flavonoids have been stated to possess many useful properties, containing anti-inflammatory activity, enzyme inhibition, antimicrobial activity, oestrogenic activity, anti-allergic activity, antioxidant activity, vascular activity and cytotoxic antitumor activity (Tapas *et al.*, 2008). Flavonoids constitute a wide range of substances that play important role in protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids and DNA (Atmani *et al.*, 2009).

2.10.1.3 Tannins

Tannins are widely distributed in plant flora. They are phenolic compounds of high molecular weight. They are soluble in water and alcohol and are found in the root, bark, stem and outer layers of plant tissue. Tannins have a characteristic feature to tan, i.e. to convert things into leather. They are acidic in reaction and the acidic reaction is attributed to the presence of phenolic or carboxylic group (Kar, 2007). They form complexes with proteins, carbohydrates, gelatin and alkaloids (Ale, 2017). Tannins are divided into hydrolysable tannins and condensed tannins. Hydrolysable tannins, upon hydrolysis, produce Gallic acid and ellagic acid and depending on the type of acid produced, the hydrolysable tannins are called Gallo tannins or egallitannins. On heating, they form pyrogallic acid. Tannins are used as antiseptic and this activity is due to presence of the phenolic group. Common examples of hydrolysable tannins include theaflavins (from tea), daidezein, genistein and glycitein (Doughari, 2012).

- **Activity of tannins**

The tannin-containing plant extracts are used as astringents, against diarrhea, as diuretics, against stomach and duodenal tumors (Bruyne *et al.*, 1999) and as anti-inflammatory, antiseptic, antioxidant and hemostatic pharmaceuticals (Dolara *et al.*, 2005). Tannins are used in the dyestuff industry as caustics for cationic dyes (tannin dyes), and also in the production of inks (iron gallate ink). In the food industry tannins are used to clarify wine, beer, and fruit juices. Other industrial uses of tannins include textile dyes, as antioxidants in the fruit juice, beer, and wine industries, and as coagulants in rubber Production (Gyamfi and Aniya, 2002). Recently the tannins have attracted scientific interest, especially due to the increased incidence of deadly illnesses such as AIDS and various cancers (Blytt *et al.*, 1988). The search for new lead compounds for the development of novel pharmaceuticals has become increasingly important, especially as the biological action of tannin-containing plant extracts has been well documented (Palavy and Priscilla, 2006).

2.10.2 Antioxidant and its activity

Antioxidants may be defined as substances that, when present in food, delay, control, or inhibit oxidation and deterioration of food quality. In the body, antioxidants reduce the risk of degenerative diseases arising from oxidative stress (Halliwell, 1999). Since ancient times, the medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities (Pari *et al.*, 2007). Antioxidants have been reported to prevent oxidative damage caused by free radical, it can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting as oxygen scavengers (Shahidi and Wanasundara, 1992). The potentially reactive derivatives of oxygen, attributed as reactive oxygen species (ROS), are continuously generated inside the human body. The generated ROS are detoxified by the antioxidants present in the body (Abdulkadir *et al.*, 2015).

Recently there has been a rise of attention in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Besides well identified and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices, some natural antioxidant (e.g. rosemary and sage) are already exploited commercially also as antioxidant additives or a nutritional supplements (Schuler, 1990).

Antioxidant activity is the total capacity of antioxidants for eliminating free radicals in the cell and in food (Otieno *et al.*, 2016). It has been mentioned the antioxidant activity of plants might be due to their phenolic compounds (Cook and Samman, 1996). Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski *et al.*, 1987).

Polyphenols are excellent antioxidants due to 3,4 dihydroxy group in their B ring and the galloyl ester in the C ring of flavonoids (Chen and Chu, 2006). Tocopherols have their own antioxidant activity, including hydrogen atom transfer at 6- hydroxyl group on the chroman ring and scavenging of singlet oxygen and other reactive species (Korus and Lisiewska, 2011). The antioxidant activity of ascorbic acid is based on hydrogen atom donation to lipid radicals, quenching of singlet oxygen and the removal of molecular oxygen (Lee *et al.*, 2004). (Chen and Chu, 2006) reported that flavonoids have the most potent antioxidant activity because their chemical structure contains an O-diphenolic group, a 2-3 double bond conjugated with 4-oxo function and hydroxyl groups in the position 3 and 5. Flavonoids effectively scavange hydroxyl and peroxyl radicals, form complexes with metals and inhibit metal initiating lipid oxidation. The antioxidant activity of phenolic acid also depend on the number of orientation of hydroxyl groups relative to the electron withdrawing CO_2H, CH_2CO_2H, or $(CH)_2CO_2CH$ functional groups (Rice-Evans and Miller, 1996).

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1- picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity (Chandra and and Goyal, 2014). The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free DPPH radical with an odd electron gives absorbance (purple color) at 517nm. When the antioxidants in plant extract react with DPPH, it is reduced to DPPH-H and results in decolorization to yellow color with respect to the number of electrons captured. The color absorbance corresponds inversely to the radical scavenging activity of the sample extract.

The scavenging of DPPH by radical scavengers can be summarized as:

$$DPPH\bullet + FE \longrightarrow DPPH - H + A\bullet \quad (1)$$

$$DPPH + A\bullet \longrightarrow DPPH - A \quad (2)$$

$$A\bullet + A\bullet \longrightarrow A - A \quad (3)$$

Where FE is a scavenger of the extract and A• is a radical. The newly formed radical (A•) can mainly follow radical-radical interaction to render stable molecules, via radical disproportionate, collision of radicals with abstraction of an atom by one radical from another equations (Chandra and and Goyal, 2014). Reaction of DPPH-free radical with an antioxidant is shown in Fig.2.5

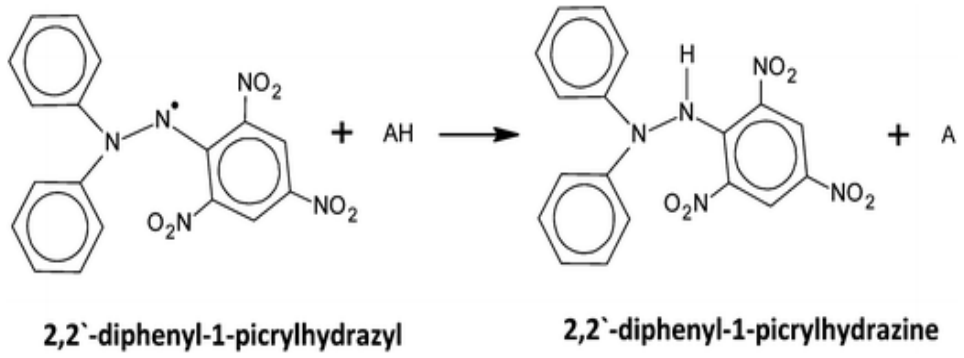

Fig. 2.5 Reaction of DPPH-free radical with an antioxidant (Nimse and Pal, 2015)

2.10.3 Extraction of phytochemicals

Extraction is the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use.The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contains complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans (Handa, 2008). The general techniques of medicinal plant extraction include

maceration, infusion, percolation, digestion decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, countercurrent extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction (Tiwari *et al.*, 2011).

- **Maceration**

Maceration is a technique used in wine making and has been adopted and widely used in medicinal plants research. It involves soaking plant materials (coarse or powdered) in a stoppered container with a polar solvent and allowed to stand at chilled temperature for a period of two to three days (Handa, 2008). This process is intended to soften and break the plant cell wall to release the soluble phytochemicals. After two to three days, the mixture is pressed or strained by filtration. The choice of solvents will determine the type of compound extracted from the samples (Azwanida, 2015). Maceration have been suggested by (Vongsak *et al.*, 2013) as more applicable, convenient and less costly method compared to other modern extraction methods since all these extraction methods resulted in crude extracts containing a mixture of metabolites having almost similar recovery of phytochemicals. This particular fact suggests that preparation of crude extract through modern technology, which is rather complex and time consuming is not necessary if proper preparation and extraction are done (Azwanida, 2015). Aqueous-methanol solvent system has better potential of extracting antioxidant components from leaves than that of water (Otieno *et al.*, 2016). 70% methanol extracted the maximum phenolic and flavonoid content occurred in the *Moringa* leaves compared to 70% ethanol and water (Siddhuraju and Becke, 2003).

- **Supercritical fluid extraction (SFE)**

Supercritical Fluid Extraction (SFE) involves use of gases, usually CO_2, and compressing them into a dense liquid. This liquid is then pumped through a cylinder containing the material to be extracted. From there, the extract-laden liquid is pumped into a separation chamber where the extract is separated from the gas and the gas is recovered for re-use. Solvent properties of CO_2 can be manipulated and adjusted by varying the pressure and temperature. The advantages of SFE are, no solvent residues left in it as CO_2 evaporates completely (Patil and Shettigar, 2010).

- **Infusion**

It is a dilute solution of the readily soluble components of the crude drugs. Fresh infusions are prepared by macerating the solids for a short period of time with either cold or boiling water (Cathrine and Banu, 2015)

- **Digestion**

This is a kind of maceration in which gentle heat is applied during the maceration extraction process. It is used when moderately elevated temperature is not objectionable and the solvent efficiency of the menstrum is thereby increased (Cathrine and Banu, 2015).

- **Soxhlet extraction**

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermolabile compounds as prolonged heating may lead to degradation of compounds (Nikhal *et al.*, 2010).

2.10.4 Factors affecting phytochemicals

Production of phytochemicals in plants is affected by many pre- and post-harvest factors including farming practices, environmental factors (microclimate, location, growing season, soil type and nutrients), plant maturity, post-harvest storage and processing, but genetics is the primary factor among all (Li *et al.*, 2012).

- **Genetic and environment**

Genetics has the greatest effect on the production of plant secondary metabolites (Tsao *et al.*, 2006). Mineral composition, soil type, temperature, light and water content are among the frequently reported factors that affect the total phytochemical contents in plants (Rao and Rao, 2007) (Hansen *et al.*, 2010).

- **Post-harvest storage and processing conditions**

Changes in both the quality and phytochemical composition of plants can occur rapidly depending on postharvest handling such as storage and processing conditions (Rodriguez-Amaya, 2003). The two major chemical changes causing the deterioration are lipid oxidation and non-enzymatic browning during storage and food processing, which can lead to altered color and flavor. Lipid oxidation is influenced by light, oxygen, temperature, the presence of catalysts, such as transition metals iron and copper, and water activity. Non-enzymatic browning occurs easily during the storage of dried and concentrated foods. Different phytochemicals are affected by these factors differently. Carotenoids are very sensitive to heat, and can incur significant losses during different vegetable processing steps (Zhang and Hamauzu, 2004).

The main cause of carotenoid degradation in foods is oxidation. Flavonoids and other phenolic compounds are relatively stable at high temperature and over long storage (Vallejo *et al.*, 2003). Phenolic in plants exist in both free and conjugated forms. Post-harvest loss of phenolic is mainly due to enzymatic oxidation by polyphenol oxidase and peroxidases (Jones, 2007).

- **Post -harvest storage conditions**

Post-harvest storage may affect the composition of some phytochemicals in plants; however, the degree of the effect depends largely on the storage conditions (Iqbal and Bhanger, 2006). Metabolism of the phytochemicals begins right after harvest, and it can involve complex biochemical reactions during transportation and post-harvest storage. These reactions can lead to significant changes in plant attributes (taste, smell, appearance and texture), and the health promoting phytochemicals, such as those with strong antioxidant activities (Michalczyk *et al.*, 2009). Storage temperature, atmosphere gas composition and use of chemicals are major factors that influence the quantity and quality of phytochemicals (Li *et al.*, 2012).

Lower temperature can slow the deterioration of phytochemicals (Devisetti *et al.*, 2016). Opposed to lower temperature, high temperature also brings a significant change in total phenolic, flavonoid, tannin content and antioxidant activity compared with its fresh form. However their concentration may vary according to the drying methods used and the

duration of exposure to hot air (Michalczyk *et al.*, 2009). Exposure to high temperature leads to discharge of phenolic compounds through disintegration of cellular constituents which then results in the migration of components, leading to losses by leakage or breakdown by various chemical reactions involving enzymes, light and oxygen as well as (Davey *et al.*, 2002). Season and agro-climatic locations have profound effect on the antioxidant activity of green leafy vegetables (Iqbal and Bhanger, 2006). Antioxidant activities and total phenolic content tend to decrease in the early stage of drying and then increase in the later stage, and that the dried leaves still have at least 60% of antioxidant activities compared to fresh leaves (Wangcharoen and Gomolmanee, 2013).

- **Food processing conditions**

Plant foods are processed to meet different consumer requirements. Generally, processed products have lower nutritional values than their respective fresh commodities mainly due to the loss of nutrients during processing (Muftugil, 1986). Industrial processing e.g., blanching, canning, sterilizing and freezing, as well as various cooking methods e.g., boiling, steaming and microwaving, of fruits and vegetables can affect the content and composition of nutrients including phytochemicals, normally leading to reduced availability of these compounds (Podsedek, 2007; Podsedek *et al.*, 2008). Studies have shown that blanching leads to reduced content of vitamins, carotenoids and phenolic compounds, which are relatively labile to heat treatments (Prochaska *et al.*, 2000). The contents of bioactive compounds in fresh-cut products can be affected by various processing steps (Isitua and Jaramillo, 2015). Green color losses in processed and minimally processed fruit and vegetable products are associated with decreases in the quality of such products (Heaton and Marangoni, 1996).

PART III

Materials and methods

3.1 Raw materials

The plant under study during the research was Sitalchini leaves (*Moringa oleifera*).

3.2 Identification of plant

The plant was identified as *Moringa oleifera* in botanical lab at Central Campus of Technology, Dharan.

3.3 Equipment and chemicals

The equipment and chemicals used were available in campus. The list of chemical and equipment's used for the analysis is shown in Table 3.1 and Table 3.2 respectively.

Table 3.1 List of chemicals used

Chemicals	Supplier/Manufacturer	Other specifications
Sodium hydroxide (NaOH)	Thermo fisher Scientific India Pvt. Ltd.	Pellets, AR grade, 98%
Hydrochloric acid (HCl)	Thermo Electron LLS India Pvt. Ltd.	36%, LR grade
Sulphuric acid (H_2SO_4)	Thermo fisher Scientific India Pvt. Ltd.	97%, LR grade
Boric acid	Merck (India) Limited	Amorphous
Oxalic acid	Merck (India) Limited	crystal
Petroleum ether	Merck life Pvt. Ltd.	B.P. 60℃-80℃
Sodium Carbonate (Na_2CO_3)	Qualigens fine chemicals	99.5%, LR grade
Sodium bicarbonate ($NaHCO_{3)}$	-	-

Methanol	Merck life science Pvt. Ltd	99% Liquid
Ethanol	Mt. Everest Industrial Works	
Guaicol	High media	99% liquid
H_2O_2 solution	Thermo electron LLS India Pvt. Ltd	30%
Sodium nitrate($NaNO_2$)	Thermo Fischer scientific India, Pvt. Ltd	98%
Aluminium chloride($AlCl_{3)}$	S.D fine-chem Ltd	98% hygroscopic
Ferric chloride($FeCl_{3)}$	Thermo Fischer scientific India, Pvt. Ltd	96% anhydrous
Chloroform	Merck life science Pvt. Ltd	99% Liquid
Folin-ciocalteu's reagent	Thermo Fischer scientific India, Pvt. Ltd	liquid
Acetic acid	Thermo Fischer scientific India, Pvt. Ltd	99% Liquid
Gallic acid	-	-
Ninhydrin solution	Central drug house Pvt. Ltd	powder
DPPH	Himedia laboratories(India) Pvt. Ltd	Amorphous

Table 3.2 List of equipment used

S.N	Physical apparatus	Specification
1.	Electric balance	Phoenix instrument, 620g
2.	Spectrophotometer	Labtronics, India
3.	Soxhlet apparatus	Y.P. Scientific industries
4.	Hot air oven	Victolab, India
5.	Incubator	Victolab, India
6.	Muffle furnace	Accumax, India

7. Knives: Stainless steel knives were used for the purpose of cutting

8. Micropipette, pipette

9. Desiccator

10.Thermometer

11. Measuring cylinder

12. Refrigerator

13. Motar and pestle, grinder, bamboo pinchers etc.

14. Steam cooker

15. Glasswares (beaker, volumetric flask, conical flask, burette, petridish, porcelain basin, crucible etc.)

16. Kjeldahl digestion and distillation set

3.4 Collection and preparation of sample

A common variety of sitalchini *(Moringa oleifera*) was collected from Sunsari district Dharan, Nepal. The leaf sample was divided into four portions:

i. Fresh Leaves: *Moringa* leaves selected and was washed with clean and portable to remove the adhered dirt and were stored at 4°C until analyzed.

ii. Cabinet dried leaves: Fresh leaves were dried in a cabinet dried at about 50°C for about 5 h.

iii. Steam blanched : Washed and drained leaves were steam blanched at 98°C for 3 minutes in a steam cooker. After that each blanched leaves were dried in cabinet drier at about 50°C. Samples were dried to a final moisture content of below 7 %.

iv. Lye blanched: Washed and drained leaves were lye blanched at 98°C for about 2 min (1 g/L). After that each blanched leaves were dried in cabinet drier at about 50°C. Samples were dried to a final moisture content of below 7 %.

From the collected fresh sample 10 g of plant leaf was grounded in mortar and pestle and then grounded plant materials were steeped in 80% methanol (100 ml) for 12 h at room temperature. They were then filtered through Whatman No. 41 filter paper. Finally, extracts were transferred to brown colored glass bottles, sealed by using bottle caps and stored at 4 ± 2°C until analysis. Similarly for dried sample, steam blanched dried sample and lye blanched dried sample methanolic extract were also prepared and stored. The crude extracts so obtained were analyzed for total phenol contents, flavonoids, tannins, chlorophylls and DPPH free radical scavenging activities. The basic flow diagram of methodology is shown in Fig. 3.1

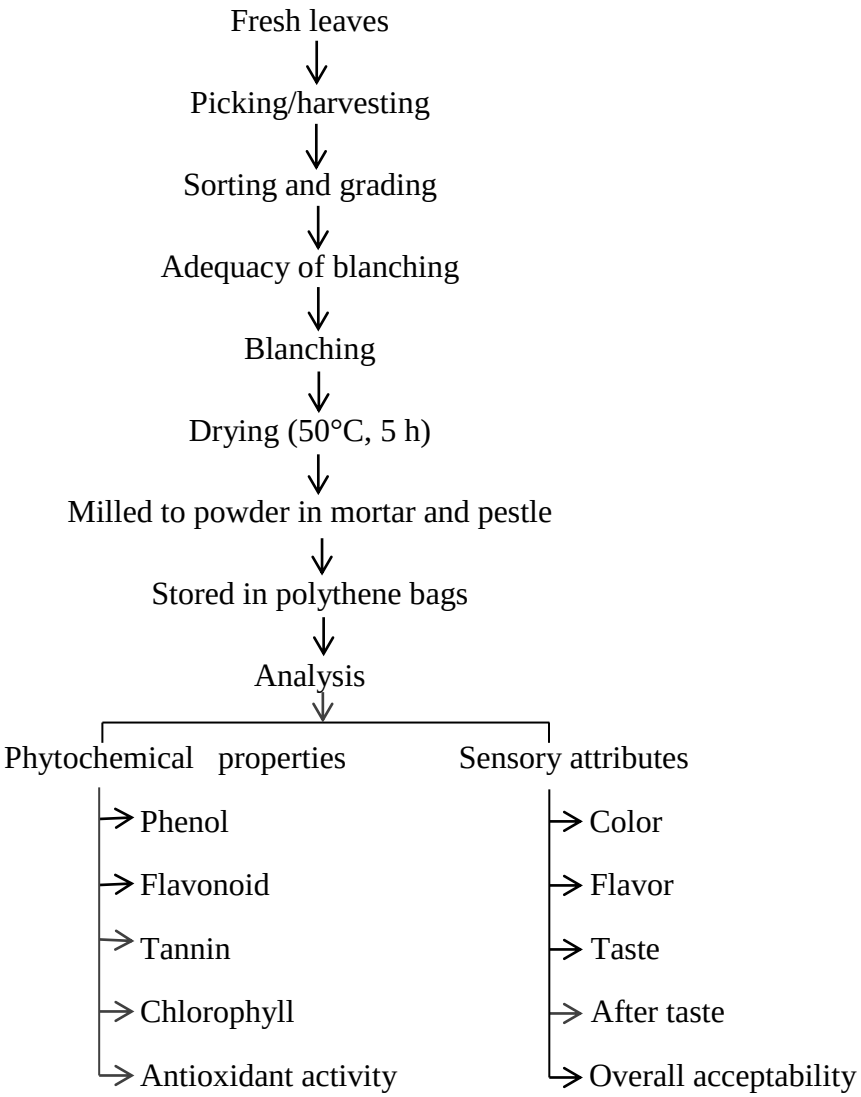

Fig 3.1 Flow diagram of methodology

3.5 Preparation of plant extracts

Plant materials were extracted as per (Ahmad *et al.*, 2014) with slight modifications. Briefly, 10 g of powdered plant materials were steeped in 80% methanol (100 ml) for 12 h at room temperature. They were then filtered through Whatman No. 41 filter paper. Finally, extracts were transferred to brown colored glass bottles, sealed by using bottle caps and stored at 4 ± 2°C until analysis. The extract concentration was determined by evaporating 5 ml of extract (at 80°C) to dryness and measuring the weight.

3.6 Qualitative analysis for phytochemical

The plant methanolic extracts were screened for the presence of the phytochemical classes by using the standard following methods (Jaradat *et al.*, 2015)

a. Tests for proteins

- Ninhydrin test: Boil 2 ml of 0.2% Ninhydrin solution with the entire plant crude extract, appeared violet color indicate the presence of proteins and amino acids.

b. Tests for carbohydrates

- Fehling's solutions test: Boil a mixture of Fehling solutions A and B with equal volumes were added to crude plant extract. A red color precipitate indicated the presence of reducing sugars.
- Molisch's solution test: Shake 2 ml of Molisch's solution with crude plant extract then add 2 ml of H_2SO_4 concentrated and poured carefully along the side of the test tube a violet ring appeared at the inter phase of the test tube indicated the presence of carbohydrate.

c. Test for phenols and tannins :

- Two milliliter of 2% solution of $FeCl_3$ mixed with crude extract. Black or blue-green color indicated the presence of tannins and phenols.

d. Tests for flavonoids

- Shinoda test: A pieces of magnesium ribbon and HCl concentrated were mixed with crude plant extract after few minutes pink colored scarlet appeared that indicated the presence of flavonoids.
- Alkaline reagent test: 2 ml of 2% NaOH solution was mixed with plant crude extract, intensive yellow color was formed, which turned into colorless when added 2 drops of diluted acid to solution, this result indicated the presence of flavonoids.

e. Test for saponins

- Five milliliter of distilled water was added to crude plant extract in a test tube and it was shaken vigorously. The foam formation indicated the presence of saponins.

f. Tests for glycosides

- Liebermann's test: 2 ml of acetic acid and 2 ml of chloroform mixed with entire plant crude extract. The mixture was then cooled and added H_2SO_4 concentrated, green color indicated the entity of aglycone steroidal part of glycosides.
- Salkowski's test: H_2SO_4concentrated (about 2 ml) was added to the entire plant crude extract. A reddish brown color produced indicated the entity of steroidal aglycone part of the glycoside.
- Keller-kilani test A mixture of Acetic acid glacial (2 ml) with 2 drops of 2% $FeCl_3$ solution was added to the plant extract and H_2SO_4concentrated. A brown ring produced between the layers which indicated the entity of cardiac steroidal glycosides.

g. Test for steroid

- Two milliliter of chloroform and concentrated H_2SO_4 were mixed with the entire plant crude extract. In the lower chloroform layer produced red color that indicated the presence of steroids. Another test was performed by mixing 2 ml of each of acetic acid with H_2SO_4concentrated and crude extract with 2 ml of chloroform. Green color indicated the entity of steroids.

h. Test for terpenoids

- Two milliliter of chloroform was mixed with the plant extract and evaporated on the water path then boiled with 2 ml of H_2SO_4 concentrated. A grey color produced indicated the entity of terpenoids.

3.7 Quantitative analysis

3.7.1 Determination of moisture content

Moisture content of the sample was determined by using hot air oven method as standard method of (AOAC, 2002).

3.7.2 Determination of crude protein

The crude protein was determined by using Kjeldahl's method. 2 g fatless samples was digested, steam distillated after decomposing the former NaOH. Titration of entrapped NH3 boric acid was done with standard acid as standard method of Ranganna (2010).

3.7.3 Determination of crude fat

The fat content was determined by Soxhlet method. Solvent extraction of 10 g sample was done by recycling hot solvent for number of times until complete extraction and fat was recovered by evaporating away the solvent as standard method of Ranganna (2010).

3.7.4 Determination of ash content

Ash content was determined using muffle furnaces according to Ranganna (2010). 5 g of weighed sample in silica crucible was charred in hot plate till no smoke raise from it and finally, ashing was done in muffle furnace at 550c to the constant weight. The difference in weight was the total ash content remaining in crucible, under standardized condition Ranganna (2010).

3.7.5 Determination of crude fiber

Crude fiber was determined by using chemical process, the sample was treated with boiling dilute Sulphuric acid, boiling sodium hydroxide and then with alcohol as standard method of Ranganna (2010).

3.7.6 Determination of total phenol

Total phenolic content (TPC) in the plant methanolic and ethanolic extracts was determined using spectrophotometric method (Jaradat *et al.*, 2015) with some modifications. The reaction mixture was prepared by mixing 0.5 ml of plant extract solution, 2.5 ml of 10% Folin Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of Na_2CO_3 aqueous solution. The samples were thereafter incubated in a thermostat at 45ºC for 45 min. The absorbance was determined using spectrophotometer at wave length 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of gallic acid equivalent expressed in terms of (mg of GA/g of extract).

3.7.7 Determination of flavonoid

Total flavonoid content was determined using a modified aluminium chloride assay method as described by (Barek and Hasmadi, 2015). 2 ml of solution was pipette out in a test tube in which 0.2 ml of 5% Sodium Nitrate ($NaNO_3$) was mixed and stand for 5 min. 0.2 ml Aluminium Chloride ($AlCl_3$) was pipetted out, mixed in the tube and allowed to stand for 5 min. This followed addition of 2 ml of 1N Sodium Hydroxide (NaOH) in the tube and finally volume was made up to 5 ml. The absorbance was measured after 15 min at 510 nm against a reagent blank. The test result was correlated with standard curve of Quercetin (20, 40, 60, 80, 100 µg/ml) and the total flavonoid content is expressed as mg quercetin equivalents (QE) (Barek and Hasmadi, 2015).

3.7.8 Determination of tannins

Tannin was determined by Folin-ciocalteu method. About 0.1 ml of the sample extracts added to volumetric flask (10 ml) containing 7.5 ml distilled water and 0.5 ml folin-ciocalteu reagent,1 ml 35% Na_2CO_3 solution and dilute to 10 ml distilled water. The mixture is shaken well and kept at room temperature for 30 min. A set of reference standard solution of Gallic acid (20, 40, 60, 80, 100 µg/ml) are prepared in same manner as described earlier. Absorbance for test and standard solution are measured against blank at 725 nm with an UV/visible spectrophotometer. The tannin content is expressed in terms of mg of GAE/g of extract (Mythili *et al.,* 2014).

3.7.9 Determination of chlorophyll

Total chlorophyll content in *Moringa* sample is determined as per Rai (2006).

Chl a, mg/g tissue = $12.7(A_{663}) - 2.69(A_{645}) \times V/1000 \times W$

Chl b, mg/g tissue = $22.9(A_{663}) - 4.68(A_{645}) \times V/1000 \times W$

Total chlorophyll, mg/g tissue = Chl a + Chl b (calculated above)

Where A = absorbance at specific wavelengths

V = final volume of chlorophyll extract

W = fresh weight of tissue extracted

3.7.10 Determination of blanching time

100 g of *Moringa* leaves were tied up loosely and dipped in boiling water. The blanching time was ranged from 30 of interval from 1-5 min. Each sample was grounded in clean mortar and pestle with equal volume of water. The ground content was strained through filter paper in another clean test tube. About 5 ml of content was taken and to it 1 ml of 1% guaicol solution (in 95% alcohol) and 1 ml 0.5% of H_2O_2 added. The tube was allowed to stand for 5 min. The development of brown color in the content indicates the presence of peroxidase, the most heat resistant enzyme.

3.7.11 Determination of DPPH radical scavenging activity

DPPH free radical scavenging activities (antioxidant activities) of extracts were determined by method described by (Vignoli *et al.*, 2011) with slight variation. Different dilutions of the extracts were made using 80% methanol. Then 1 ml of the extract was mixed with 2 ml of 0.1 mM DPPH solution. The absorbance was read at 517 nm after 30 min incubation in the dark. Finally, percentage scavenging activity was determined using following equation

$$\% \text{ scavenging activity} = (Ac\text{-}As) \times 100 / Ac$$

Where Ac is the absorbance of control and As is the absorbance of test sample.

3.7.12 Sensory analysis

Sensory analysis of the moringa leaf powder were analysed as similar to tea testing procedure developed by TRA (Tea Research Association), Assam. 2.8 gm of tea samples were weighed and they were kept in a cup. 140 ml water was added and covered with lid. It was left for 5 min. The brew was then transferred to the bowl and the infusion was taken out in the lid. Brew was evaluated for sensory attributes (color, flavor, taste, after taste and overall acceptability).

3.8 Optimization of *Moringa oleifera* leaves

After the preparation of extract with 80% methanol, phytochemicals analysis for four samples: fresh leaves, dried leaves, steam blanched dried and lye blanched dried respectively were done. Sensory analysis for the prepared leaves powder was done as per 9 point hedonic ratings to optimize color, flavor, taste, after taste, and overall acceptability. Criteria of optimization for *Moringa oleifera* leaves are shown in Table 3.3 and Table 3.4

Table 3.3 Phytochemicals parameters

Parameters	Goals
TPC	Maximize
TFC	Maximize
Tannins	Minimize
Antioxidant activity	Maximize
Chlorophyll	Maximize

Table 3.4 Sensory parameters

Parameters	Goals
Color	Maximize
Flavor	Maximize
Taste	Maximize
After taste	Maximize
Overall acceptability	Maximize

3.9 Statistical analysis

Analysis were carried out in triplicate. Statical calculations were performed in Microsoft Office Excel 2010. All the data obtained in this experiment were analyzed for significance by Analysis of Variance (ANOVA) using statistical software Genstat Release 12.1 (Discovery Edition 12 developed by VSN International Limited). From this, means were compared using Fischer's protected LSD (Least Significant Difference) at 5% level of significance.

Part IV

Results and discussion

A common variety of the Sitalchini (*Moringa oleifera*) collected from sunsari district was used to test the presence of phytochemicals. Fresh sitalchini leaves were subjected to blanching and drying and their phytochemicals composition and antioxidant activity were analyzed.

4.1 Proximate composition

Table 4.1 Proximate composition of *Moringa oleifera* leaves

Parameter	Composition (%)
Moisture	73.25±0.89
Protein	21.65±0.57
Crude fiber	8.19±0.05
Fat	5.52±0.26
Ash	8.72±0.10

*Values are the means of triplicate determination ± S.D. (All values are expressed in dry basis expect for moisture)

The moisture, protein, crude fiber, fat and ash of fresh *Moringa* leaf was found from the analysis as shown in above table. In present study moisture content was found to be 73.45%. Similar to this study, Kucha *et al.* (2015) reported the value of moisture content of *Moringa oleifera* leaves as 80%. Crude protein value was obtained as 21.65%. Indriasari and Kumalaningsih (2016) reported higher value of crude protein 26.36% in *Moringa* leaves. Similar value for ash 9.15% and 8.33% was reported by Mandil *et al.* (2014) and Indriasari and Kumalaningsih (2016). Similar value of fat content of *Moringa oleifera* 8.53%was obtained by Indriasari and Kumalaningsih (2016). Crude fiber was found to be 8.19% in present study but Indriasari and Kumalaningsih (2016) and Mandil *et al.* (2014)

reported higher value of crude protein 12.72% and 23.09% respectively. This different might be due to geographical variations and development stage of *Moringa oleifera* plant.

4.2 Optimization of blanching

Proper combination of time and temperature during processing methods is important in order to minimize quality loss during processing. These methods cause undesirables changes on the physicochemical properties such as color, texture or bioactive compounds, on account of heat-induced diffusion or leaching losses. Thus, it is important to optimize the time and temperature of any processing method in order to achieve minimal loss of quality (Gupta *et al.*, 2012). Blanching time-temperature optimization for steam blanching is shown in Table 4.2 and Table 4.3

Table 4.2 Blanching time-temperature optimization

Blanching time(min)	1	1.5	2	2.5	3	3.5	4	4.5	5
Catalase test for sample at 90°C	+	+	+	+	+	+	+	-	-
Catalase test for sample at 91°C	+	+	+	+	+	+	+	-	-
Catalase test for sample at 92°C	+	+	+	+	+	+	-	-	-
Catalase test for sample at 93°C	+	+	+	+	+	-	-	-	-
Catalase test for sample at 94°C	+	+	+	+	-	-	-	-	-
Catalase test for sample at 95°C	+	+	+	-	-	-	-	-	-
Catalase test for sample at 96°C	+	+	-	-	-	-	-	-	-
Catalase test for sample at 97°C	+	-	-	-	-	-	-	-	-
Catalase test for sample at 98°C	-	-	-	-	-	-	-	-	-

Plus (+) =positive test; minus(-) =negative test

Table 4.3 Blanching time-temperature optimization

Blanching time(min)	1	1.5	2	2.5	3	3.5	4	4.5	5
Peroxidase test for sample at 90°C	+	+	+	+	+	+	+	+	+
Peroxidase test for sample at 91°C	+	+	+	+	+	+	+	+	+
Peroxidase test for sample at 92°C	+	+	+	+	+	+	+	+	+
Peroxidase test for sample at 93°C	+	+	+	+	+	+	+	+	-
Peroxidase test for sample at 94°C	+	+	+	+	+	+	+	+	-
Peroxidase test for sample at 95°C	+	+	+	+	+	+	+	-	-
Peroxidase test for sample at 96°C	+	+	+	+	+	+	-	-	-
Peroxidase test for sample at 97°C	+	+	+	+	+	-	-	-	-
Peroxidase test for sample at 98°C	+	+	+	+	-	-	-	-	-

Plus (+) =positive test; minus(-) =negative test

Blanching time-temperature optimization for steam blanching is shown in Table 4.4

Table 4.4 Blanching time-temperature for lye blanching

Blanching time(min)	1	1.5	2	2.5	3	3.5	4	4.5	5
Catalase test for sample at 80°C	+	+	+	+	+	+	+	+	+
Peroxidase test for sample at 80°C	+	+	+	+	+	+	+	+	+
Catalase test for sample at 95°C	+	+	+	-	-	-	-	-	-
Peroxidase test for sample at 95°C	+	+	+	+	+	+	+	-	-
Catalase taste for sample at 98°C	-	-	-	-	-	-	-	-	-
Peroxidase test for sample at 98°C	+	+	-	-	-	-	-	-	-

Plus (+) =positive test; minus(-) =negative test

Therefore, from the Table 2.3, optimum blanching time-temperature for *Moringa* leaves was found to be 3 min at 98°C. Similar results were observed for blanching black carrot (98°C for 3 and 6 min) reported by (Garba *et al.*, 2015). Optimum blanching time of *Moringa* leaves for sodium bicarbonate blanching was found to be 2 min at 98°C.

4.3 Qualitative analysis for phytochemical

During the experimental work, the methanol extract of *Moringa oleifera* leaves shown to have the total phenol, total flavonoid, tannin and antioxidant activity. Qualitative analysis for phytochemical which is shown in Table 4.5

Table 4.5 Qualitative analysis for phytochemical

Test	Result
Proteins	- ve
Carbohydrates	+ve
Phenols and tannins	+ve
Flavonoid	+ve
Saponins	+ve
Glycosides	+ve
Steroid	-ve
Terpenoids	-ve

Plus (+) =positive test; minus (-) =negative test

The phytochemicals screening of methanol extract of *Moringa oleifera* showed that the leaves were rich in carbohydrates ,phenol, tannins, flavonoids, saponins and glycosides but not in proteins, steroid and terpenoids. Similar result for phytochemicals screening in *Moringa oleifera* leaves was obtained (Otieno *et al.*, 2016; Unuigbe *et al.*, 2014) (Isitua and Jaramillo, 2015)

4.4 Quantitative analysis of phytochemicals in *Moringa oleifera* leaves

Treatment A, B, C, D refers to fresh leaves, dried leaves, steam blanched dried and lye blanched dried respectively. Quantitative analysis of phytochemicals in *Moringa oleifera* leaves is shown in Table 4.6

Table 4.6 Quantitative analysis of phytochemicals in *Moringa oleifera* leaves.

Treatments	TPC (mgGAE/g)	TFC (mgQE/g)	Tannins (mgGAE/g)	DPPH radical Scavenging activity (% inhibition)	Chlorophyll (mg/g)
A	165.54 ± 0.62^{d}	558.63 ± 55.29^{c}	14.008 ± 1.73^{c}	79.88 ± 0.63^{d}	19.61 ± 0.14^{c}
B	134.58 ± 1.25^{c}	432.63 ± 31.88^{b}	9.810 ± 0.99^{b}	57.96 ± 1.27^{b}	16.02 ± 0.04^{ab}
C	103.12 ± 1.12^{b}	305.07 ± 28.56^{a}	5.686 ± 0.89^{a}	61.86 ± 1.27^{c}	15.24 ± 0.12^{a}
D	97.47 ± 1.33^{a}	245.72 ± 34.04^{a}	4.313 ± 1.06^{a}	53.45 ± 1.27^{a}	18.61 ± 2.90^{bc}

*Values are the mean of triplicates results, figures in the parenthesis are the standard deviations. Figures with same superscript are not significantly different. Figures with different superscript are significantly different.

4.4.1 Effect of drying on total phenol content

The TPC of fresh *Moringa* leaves were found to be 165.54 mg/g. The statistical analysis showed there is significant difference in TPC of fresh and dry leaves. It was also observed that TPC content of *Moringa* was significantly decreased during drying. Similar value for phenol content of fresh *Moringa oleifera* was found to be 127.9 mg/g and 118±3 mg/g reported by Pakade *et al.* (2013) and Pari *et al.* (2007). Similarly phenol content of dried *Moringa* leaf was found to be 134.58 mg/g closer to the observation made by (Iqbal and Bhanger, 2006). Higher value of TPC in methanol extract of Mellilotus officinalis was found to be 289.5±5 mg/g.

The total phenolic content varied significantly among the plant extracts with higher levels in the methanol than aqueous extracts (Bholah *et al.*, 2015). It was noted that, extracts of dried leaves always showed lower concentration of total phenolic than those from fresh leaves. Present study showed the higher TPC this may be due to the fact phenolic content increases with increase in leaf age and is lower in early stage of leaf growth, gradually increasing with maturity of leaves (Iqbal and Bhanger, 2006). The loss of phenolic and flavonoids during drying might be due to the process conditions, in particular the temperatures and the duration used (Michalczyk *et al.*, 2009; Schieber *et al.*, 2001). The integrity of the cell structure is affected by thermal breakdown which then resulted in the migration of components, leading to losses by leakage or breakdown by various chemical reactions involving enzymes, light and oxygen. This means that the phytochemicals is affected by thermal processing as reported by Davey *et al.* (2002). Several factors such as the stage of maturity, variety, climatic conditions, part of the plant analyzed, post-harvest handling, processing, and storage varies widely the composition of antioxidants (Rodriguez-Amaya, 2003).

4.4.2 Effect of drying on falvonoid content

The flavonoid content for fresh *Moringa* leaves was found to be 558.63 mg/g respectively. The statistical analysis showed there is significant difference in TFC of fresh and dry leaves. It was also observed that TFC content of *Moringa* was significantly decreased during drying. Higher value of flavonoid content was revealed in *Platycerium bifurcatum* (648.67 ± 12.3 mg QE/g) in methanol extract as reported by Agbo *et al.* (2015). Flavonoid content of dried *Moringa* leaves was found to be 432.63 mg/g. Flavonoid content of dried *Moringa* leaves in Aqueous-methanol extract was found to be 366 ± 86.96 mg/CEg as reported by Otieno *et al.* (2016) which is higher value as compared to present study. Siddhuraju and Becke (2003) reported that total flavonoid content in methanolic extract of freeze dried powder leaf was 57-140 mgQE/g which was found to be lower than that of present study. The differencre in result might have occurred due to the same effects of agrocilmatic location as for TPC. Might be due to the process conditions, in particular the temperatures and the duration used (Michalczyk *et al.*, 2009; Schieber *et al.*, 2001).

4.4.3 Effect of drying on tannin

The tannin content in fresh *Moringa oleifera* was found to be 14.008 mg/g. During drying of leaves, the tannin content in decreased from 14.008 mg/g to 9.810 mg/g. The statistical analysis (one- way ANOVA) showed that the tannin content of *Moringa* was significantly decreased ($p<0.05$) during drying. It was noted that, dried *Moringa* leaves showed lower concentration of tannins than fresh leaves. Drying affects negatively phytochemicals was reported by (2017). The similar observation on decrease in tannin content was reported by Rakic *et al.* (2007) in oak plant and the decrease in tannin content may be due to the result of the thermal degradation of hydrolysable tannin (Rakic *et al.*, 2007).

4.4.4 Effect of drying on antioxidant activity

The antioxidant activity in fresh *Moringa* was found to be 79.88%. During drying of leaves the antioxidant activity decreased to 57.96%. Similar observation for antioxidant activity in fresh and dry leaves of *Moringa oleifera* was reported by Nobosse *et al.* (2017). These values are within the same range as reported by Amin and Lee (2005). Drying reduced AOA in both fresh and blanched samples. This reduction is in accordance with the observation of (Wangcharoen and Gomolmanee, 2013). The variations in AOA could be explained by the fact that heat treatments can either degrade or enhance the extractability of phytochemicals involved with AOA (Adefegha and Oboh, 2011). Structural modifications and/or polymerization of phytochemicals could cause by heat treatments leading to new-formed compounds with different AOA with respect to their parent compounds (Ferracane *et al.*, 2008).

4.4.4 Effect of drying on chlorophyll content

The chlorophyll content of fresh leaves was found to be 19.61 mg/g and that after cabinet drying was found to be 16.02 mg/g. The statistical analysis (one- way ANOVA) showed significant difference ($p<0.05$) in chlorophyll content. Similar value for chlorophyll content was reported by Nartnampong *et al.* (2016). Abdulkadir *et al.* (2015) reported an average of 35.40±1.2 mg/g as high chlorophyll content and average of 16.25±1.25 mg/g as low chlorophyll content in fresh *Moringa* leaf. Damaged tissue during blanching and other processing steps is initiated during Chlorophyll degradation (Heaton and Marangoni, 1996). Hence, low chlorophyll content result in present study might have occurred due to the processing effects that the leaf had undergone. Total chlorophyll content decreased

slightly during drying .This indicates a high potential for the acceptability of the dried leaves because they are desired by consumers in the green state. Chop and dry vegetables retained substantial amounts of chlorophylls as reported by Adebooye and Singh (2006).

4.4.5 Effect of blanching on total phenol content

The total phenol content in fresh *Moringa oleifera* was found to be 165.54 mg/g. Blanching decreased total phenol content to 103.12mg/g for steam blanched and 97.47 mg/g for lye blanched respectively. The statistical analysis (one- way ANOVA) showed that the phenol and flavonoid content was significantly decreased ($p<0.05$) during blanching as compared to fresh *Moringa* leaves.

Devisetti *et al.* (2016) reported that alkali pre-treatment decreases the phenolic acid content significantly ($p \leq 0.05$) compared to the fresh *Moringa* leaf flour. The total phenolic content determination in *Moringa* leaves revealed that the type of extraction solvent is a limiting factor in the extraction of phenolic and flavonoids. 70% methanol extracted the maximum phenolic and flavonoid content occurred in the *Moringa* leaves compared to 70% ethanol and water as reported by El Sohaimy *et al.* (2015). The decreasing trend in phenol content during blanching is also reported by Amin and Lee (2005) and Nobosse *et al.* (2017). Loss in the phenolic content of vegetables due to blanching has been reported by (Jaiswal *et al.,* 2012; Korus and Lisiewska, 2011; Sikora *et al.,* 2008) and this may be due to oxidation of the phenolic or their leaching into water during blanching (Roy *et al.,* 2009). It is known that phenolic compounds occur in soluble forms and in combination with cell wall components in plants (Francisco *et al.,* 2010). Thus, disruption of the cell walls and the breakdown of the phytochemicals occur due to high temperature of the blanching thereby leading to their leaching into the blanching water. The differential losses in the levels of the individual flavonoids and phenolic acids after blanching may reflect variations in their individual solubility and thermo- stability in hot water.

4.4.6 Effect of blanching on total flavonoid content

The total flavonoid content in fresh *Moringa oleifera* was found to be 558.63 mg/g. Blanching decreased TFP to 305.07 mg/g for steam blanched and 245.72 mg/g for lye blanched. The statistical analysis (one- way ANOVA) showed that the flavonoid content was significantly decreased ($p<0.05$) during blanching as compared to fresh *Moringa*

leaves. The decreasing trend in flavonoid content during blanching is also reported by Amin and Lee (2005) and Nobosse *et al.* (2017).

Similar to phenolic acids, significant ($p \leq 0.05$) reduction in flavonoid content occurs in the pre-treated leaf compared to the fresh *Moringa* leaf flour (Devisetti *et al.*, 2016). Loss in the phytochemicals content due to blanching has been reported by (Jaiswal *et al.*, 2012; Korus and Lisiewska, 2011; Sikora *et al.*, 2008).

4.4.7 Effect of blanching on tannin content

The tannin content in fresh *Moringa oleifera* leaves was found to be 14.008 mg/g and that after stem and lye blanching decrease to 5.686 mg/g and 4.313 mg/g. The statistical analysis (one- way ANOVA) showed that the tannin content was significantly decreased ($p<0.05$) during blanching. Similar value for tannin content in leafy vegetables was reported by Saranya *et al.* (2017).The decreasing trend in tannin content after blanching was reported by Nobosse *et al.* (2017).

4.4.8 Effect of blanching on antioxidant activity

Antioxidant activity of fresh leaves was found to be 79.88% and that after blanching was found to be 61.86 mg/g. The statistical analysis (one- way ANOVA) showed that antioxidant activity siginificantly decreased ($p<0.05$) during blanching. Similar value of antioxidant activity for cruciferous vegetables were obtained by (Amin and Lee, 2005). Yakubu *et al.* (2012) reported a significant decrease in the reducing power and the DPPH scavenging activity of blanched bitter leaves (*Vernonia amygdalina*) with respect to fresh leaves. Loss of activity of some antioxidant components during blanching might be reason behind low antioxidant activities of the blanched vegetables (Amin and Lee, 2005).

4.4.9 Effect of blanching on chlorophyll

Chlorophyll content of fresh leaf was found 19.61 mg/g and that after blanching was found to be 15.24 mg/g for steam blanched and 18.61 mg/g for lye blanched. The statistical analysis (one- way ANOVA) showed significantly difference ($p<0.05$) during steam blanching and not for lye blanching as compared to fresh leaves. Similar result for chlorophyll content in leafy vegetables were obtained by (Nartnampong *et al.*, 2016).

Steam blanching result highest degradation rate of chlorophyll in spinach as reported by (Teng and Chen, 1999). More losses of chlorophyll were detected in steam blanched samples than in lye blanched samples. Similar result of more chlorophyll loss in steam blanched sample than that of water blanched was reported by (Muftugil, 1986). More chlorophyll retention were found in lye (sodium bicarbonate) blanched sample. The reason behind this is that sodium bicarbonate is effective in maintaining green color of heat processed vegetable products. By displacing the phytyl and methyl groups, forming a bright green water soluble chlorophyllin sodium bicarbonate reacts with chlorophyll (Srilakshmi, 2003). In addition, improves chlorophyll stability is improves this may be due to sodium bicarbonate increases the medium pH.

4.5 Sensory evaluation

Sensory evaluation was carried out for color, flavor, taste, mouth feel and overall acceptability by semi trained panelists using 9 point hedonic scale. The statistical analysis (two way ANOVA no blocking) was done. ANOVA was carried out using LSD at 5% level of significance. There was significant difference for most of the sensory attributes viz., color, flavor, taste, mouth feel and overall acceptability at $P<0.05$.The result of the sensory evaluation and statistical analysis is given in Appendix B.

4.5.1 Color

Mean sensory score for color sample A, B, C and D were found to be 8.4, 7.9, 7.0 and 6.4 respectively. The color of sample was significantly different ($p<0.05$). LSD showed that sample A and B, C and D are not significantly different while other sample A and C, A and D,B and C,B and D are significantly different from each other at 5% level of significance. Sample D has lowest mean score and sample A has highest mean score but is not significantly different ($p<0.05$) from sample B. So, sample A and B were superior on the basis of color from statistical analysis. Mean sensory score for color is shown in Fig. 4.1

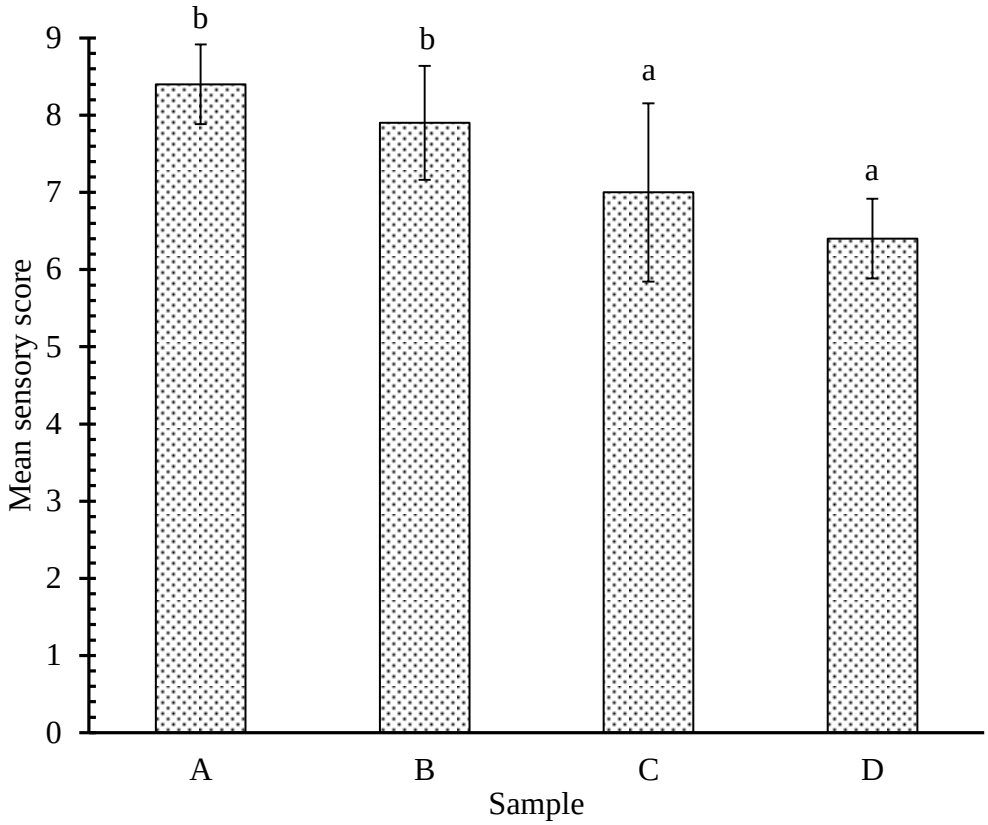

Fig. 4.1 Mean sensory score for color

The values in the figure are the mean sensory score for color. Values on the top of bar bearing similar alphabet are not significantly different at 5% level of significance. Vertical error bar represent standard deviation of scores.

Color is often used as an indicator to evaluate severity of the heat treatment and to predict the corresponding quality degradation caused by blanching process (Xiao *et al.*, 2017). The green color of leafy vegetable is a desirable quality parameter by consumers (Busari *et al.*, 2016) but processing interferes with color, either positively or negatively depending on the treatment (Stanley *et al.*, 2017). In this case processing has negatively affected color of stem blanched and lye blanched sample. The slight loss in color can be attributed to the loss in chlorophyll because of leaching and chlorophyll forming pheophytins being broken down (Rodoni *et al.*, 1997). Processing of any food whether through dehydration or blanching is expected to produce some changes in the sensory qualities of the product (Stanley *et al.*, 2017).

4.5.2 Flavor

Mean sensory score for flavor sample A, B, C and D were found to be 7.7, 6.9, 6.8 and 6.3 respectively. The color of sample was significantly different ($p<0.05$). LSD showed that sample A and B, A and C, A and D, B and D are significantly different while other sample B and C, C and D are not significantly different from each other at 5% level of significance. Sample D has lowest mean score and A has highest mean score. So sample A were superior on the basis of flavor from statically analysis. Mean sensory score for flavor is shown in Fig. 4.2

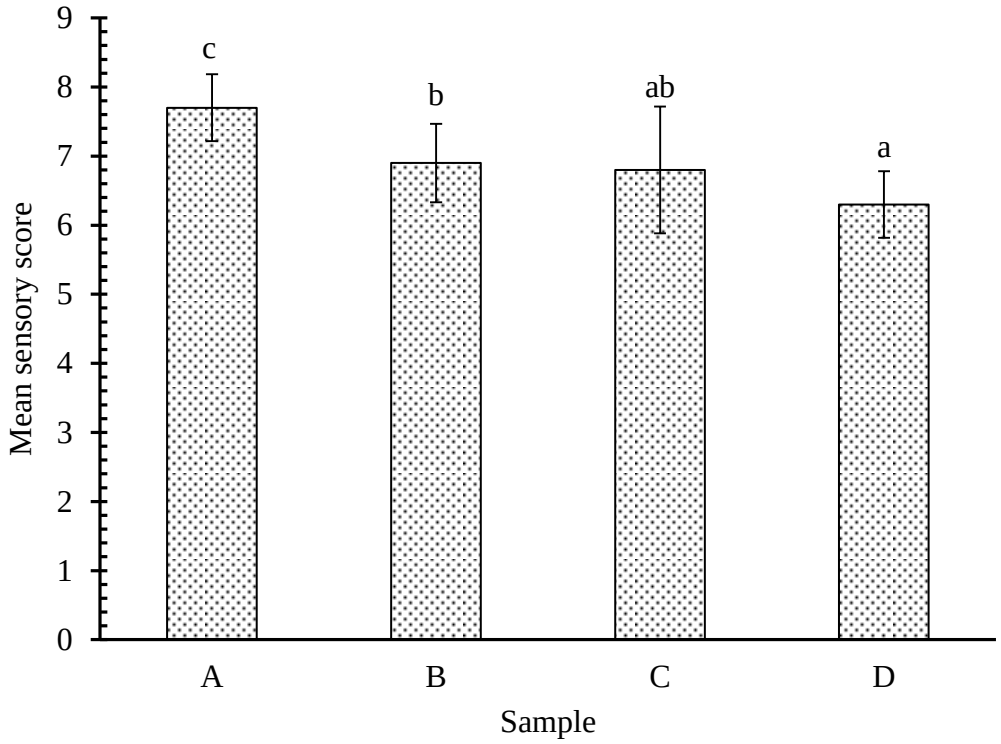

Fig. 4.2 Mean sensory score for flavor

The values in the figure are the mean sensory score for flavor. Values on the top of bar bearing similar alphabet are not significantly different at 5% level of significance. Vertical error bar represent standard deviation of scores.

Processing parameters (e.g., processing time, temperature) is responsible for change the quality of food by altering the sensory attributes which influence the consumer choices

(Liyun, 2016). High processing temperature might have decrease flavor retention that may be the reason that sample B, C, D were not liked by panelists.

4.5.3 Taste

Mean sensory score for taste sample A, B, C and D were found to be 6.0, 6.9, 7.7 and 5.2 respectively. The color of sample was significantly different ($p<0.05$). LSD showed that sample A and B, A and C, A and D, B and C,B and D are significantly different from each other at 5% level of significance. Sample D has lowest mean score and C has highest mean score. So sample C were superior on the basis of taste from statically analysis. Mean sensory score for taste is shown in Fig. 4.3

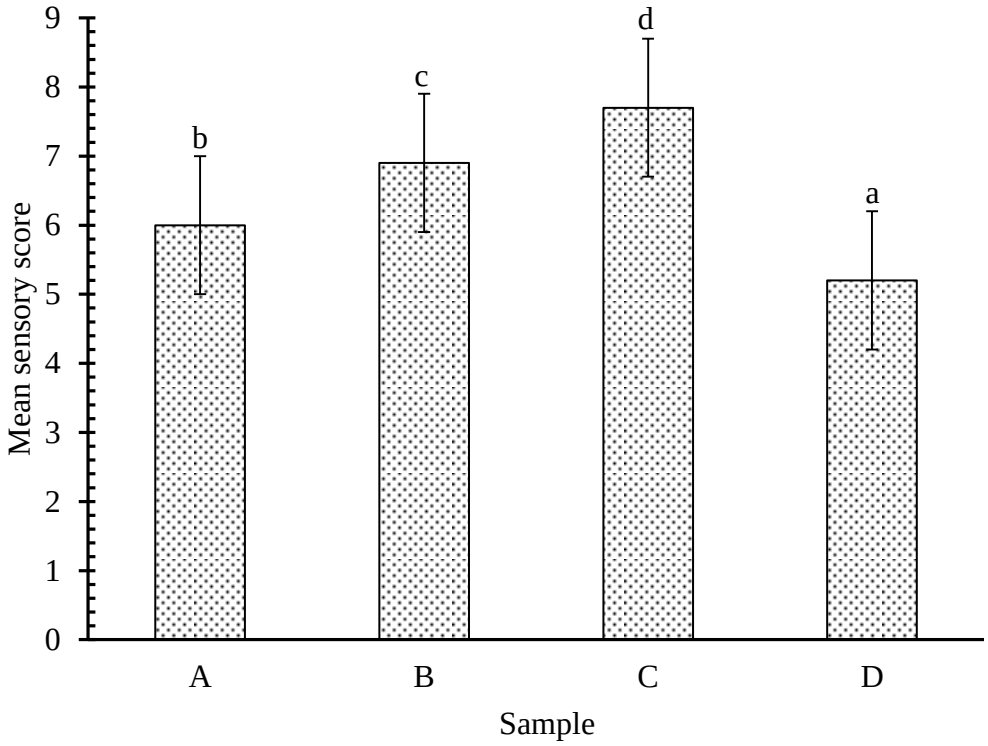

Fig. 4.3 Mean sensory score for taste

The values in the figure are the mean sensory score for taste. Values on the top of bar bearing similar alphabet are not significantly different at 5% level of significance. Vertical error bar represent standard deviation of scores.

Thermal processing has impact on the positive and negative taste attributes of foodstuffs (Liyun, 2016). Sample C were found to be superior in terms of taste this might be due to positive impact of thermal processing in *Moringa* powder.

4.5.4 After taste

Mean sensory score for after taste sample A, B, C and D were found to be 7.1, 7.2, 5.8 and 5.7 respectively. The color of sample was significantly different ($p<0.05$).LSD showed that sample A and C, A and D, B and C,B and D are significantly different while sample A and B are not significantly different from each other at 5%level of significance. Sample D has lowest mean score and B has highest mean score but is not significantly different ($p<0.05$) from sample A. So, sample A and B were superior on the basis of after taste from statistical analysis. Mean sensory score for after taste is shown in Fig. 4.4

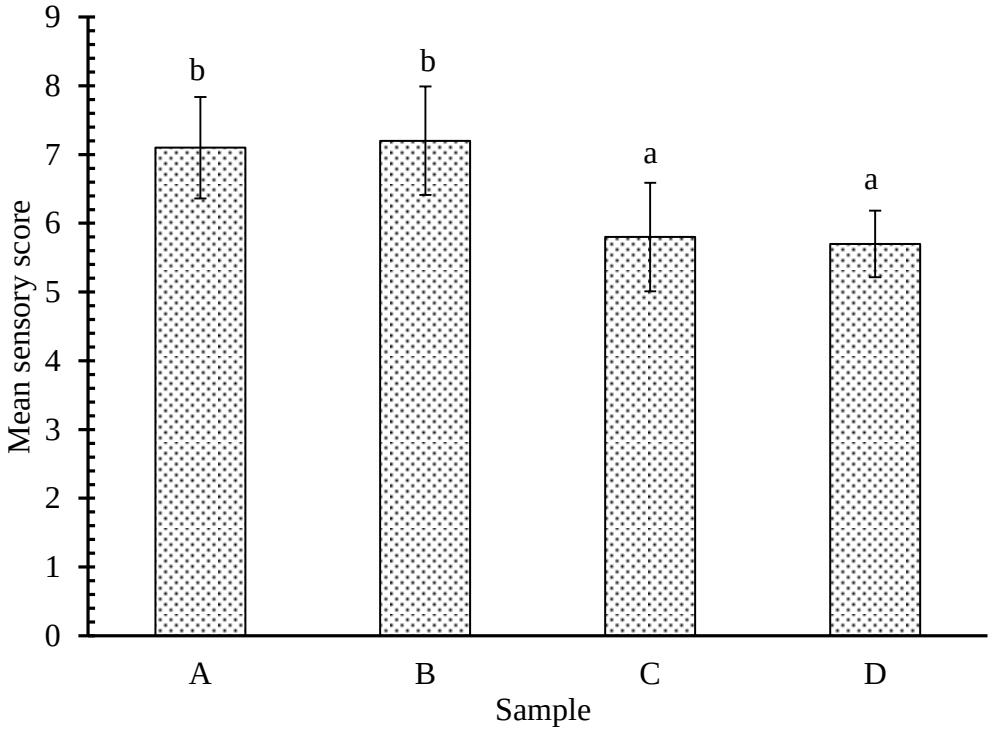

Fig. 4.4 Mean sensory score for after taste

The values in the figure are the mean sensory score for after taste. Values on the top of bar bearing similar alphabet are not significantly different at 5% level of significance. Vertical error bar represent standard deviation of scores.

4.5.5 Overall acceptability

Mean sensory score for overall acceptability sample A, B, C and D were found to be 7.8, 7.6, 6.5 and 5.9 respectively. The color of sample was significantly different ($p<0.05$). LSD showed that sample A and C, A and D, B and C, B and D are significantly different while sample A and B are not significantly different from each other at 5%level of significance. Sample D has lowest mean score and A has highest mean score but is not significantly different ($p<0.05$) from sample B. So, sample A and B were superior on the basis of overall acceptability from statistical analysis. Mean sensory score for overall acceptability is shown in Fig. 4.5

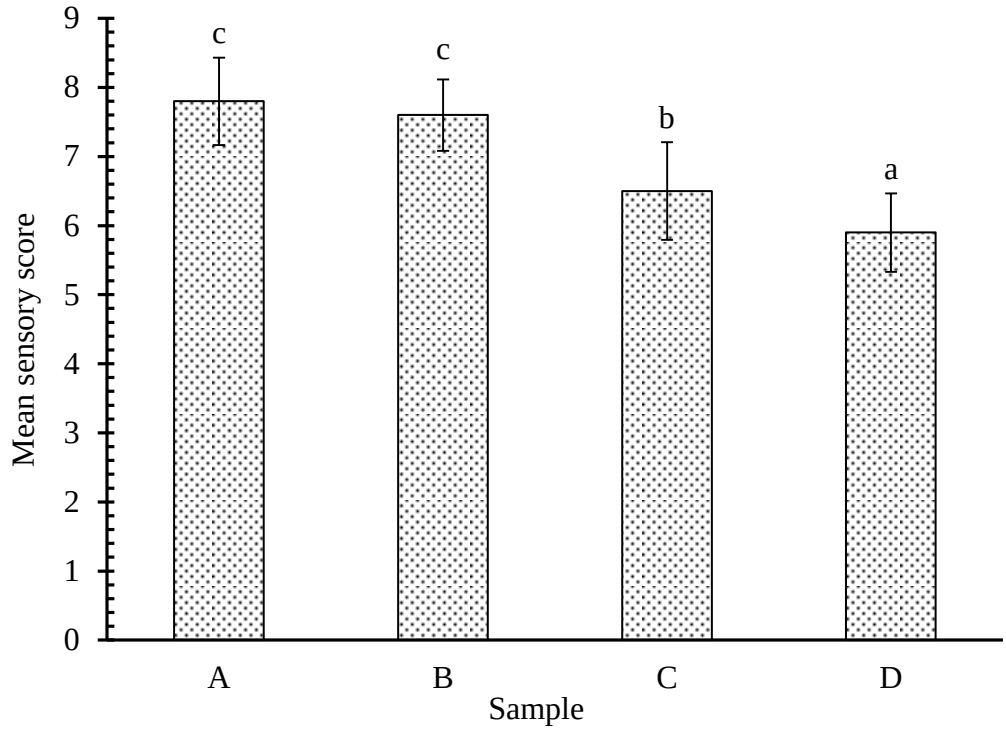

Fig. 4.5 Mean sensory score for overall acceptability

The values in the figure are the mean sensory score for overall acceptability. Values on the top of bar bearing similar alphabet are not significantly different at 5% level of significance. Vertical error bar represent standard deviation of scores.

Overall acceptability is the reflection of other sensory attributes. On the basis of color, flavor, taste, after taste sample A and B were most liked by panelists. Statically analysis showed higher acceptability for sample A and B.

4.5.6 Optimization study

As regard with the sensory scores and phytochemicals analysis, the optimization study is carried out as given below. Optimized goals for treatment of sensory analysis is shown in Table 4.7

Table 4.7 Optimized goals for treatment (sensory analysis)

Parameters	Optimized treatment
Color	A=B
Flavor	A
Taste	C
After taste	A=B
Overall acceptance	A=B

Regarding the sensory parameter, treatment A, i.e., fresh leaves was found to be superior.

Optimized goals for treatment of phytochemicals analysis is shown in Table 4.8

Table 4.8 Optimized goals for treatment (phytochemicals analysis)

Parameters	Optimized treatment
TPC	A
TFC	A
Tannins	A
Antioxidant activity	A
Chlorophyll	A=D

From the study of different parameter, treatment A had superior sensory characteristics. Similarly, in terms of phytochemicals analysis treatment A was found to be superior. It can be concluded that fresh leaves is superior than that of others in terms of both phytochemicals analysis and sensory analysis.

Part V

Conclusions and recommendations

5.1 Conclusion

Present work was carried out to study the effect of steam and lye blanching on the phytochemicals content and sensory attributes of *Moringa oleifera* leaves. Based on this research following conclusion can be drawn.

1. Proximate compositions of fresh leaves were determined that is moisture, protein, crude fiber, fat and ash content and found to be 73.25±0.89%, 21.65±0.57%,8.19±0.05%,5.52±0.26% and 8.72±0.10% respectively on dry basis except for moisture.
2. Blanching time-temperature optimization for *Moringa* leaves was found to be 98°C for 3 min and 98°C for 2 min.
3. Total Phenols, flavonoids, tannins, antioxidant activity and chlorophyll were detected in the fresh leaves of *Moringa* and was found to be 165.54±0.62, 558.63±55.29, 14.008±1.73 , 79.88±0.63, and 19.16±0.14 respectively.
4. Drying and blanching of *Moringa* leaves significantly decreased phytochemicals content.
5. Drying decreased slightly chlorophyll content where as more chlorophyll was retained in $NaHCO_3$ blanched and less in steam blanched.
6. Blanching and drying has significant effect of sensory attributes of *Moringa oleifera* leaves.
7. It can be concluded that fresh leaves is superior than that of others in terms of both phytochemicals analysis and sensory analysis. As fresh leaves content good source of antioxidants so consumption of fresh *Moringa* in daily diet either in raw or in cooked form can be a good source of natural antioxidants, beneficial for health.

5.2 Recommendations

The plant studied in this work is on high demand because of its nutritional value and traditional uses. Thus, following suggestions are recommended for future work in the field of *Moringa oleifera*.

1. Preparation and quality evaluation of *Moringa* incorporated product can be performed.
2. Effect of processing on the anti-nutritional content of *Moringa oleifera* leaves can be studied.

Summary

Moringa oleifera of the family Moringaceae, popularly called 'miracle tree' is a native of sub Himalayan tracts of Northern India and is widely cultivated in tropical and subtropical regions around the world. *Moringa* is considered one of the world's most useful trees, as almost every part of the tree can be used for food or has some other beneficial property. Presence of various types of antioxidant compounds make this plant leaves a valuable source of natural antioxidants and a good source of nutraceuticals and functional components as well. It comprises of 13 species and most widely cultivated species is *Moringa Oleifera*. It is called sitalchini in Nepali.

Sitalchini was collected in a polythene bags with the help of bamboo pinchers from Sunsari district Dharan, Nepal for the study. Initially adequate time- temperature for blanching is optimized and found to be 3 min at 98°C for steam blanching and 2 min at 98°C for lye (sodium bicarbonate) blanching and then subjected to blanching and cabinet drying at 50°C . Proximate composition of fresh *Moringa oleifera* leaves was analyzed. Crude extract of sample were prepared using 80% methanol through maceration technique. Phytochemicals screening of the methanol extract of the plant showed the presence of total phenol content, flavonoid, tannin and glycosides. Total phenol, flavonoid, tannin content, antioxidant activity and chlorophyll content for four samples: fresh sample, dried sample, steam blanched dried sample and lye blanched dried sample were used for performing phytochemicals content, antioxidant activity and chlorophyll content.

Drying and blanching decreased the levels of phytochemicals content and antioxidant activity significantly ($p \leq 0.05$) as compared to fresh leaves. Drying slightly effect chlorophyll content whereas steam blanching least affected chlorophyll content and not affected chlorophyll content by lye blanching. The composition of phytochemicals varies widely with several factors like the stage of maturity, variety, climatic conditions, part of the plant analyzed, post-harvest handling, processing, and storage.

Fresh leaves is superior than that of others in terms of both phytochemicals analysis and sensory analysis. This indicates a bright future scope for commercialization of fresh *Moringa oleifera* leaves. It constitutes a large group of chemicals with high potential to treat various diseases, so phytochemical analysis is the best way to bring forward *Moringa oleifera* as medicinally important plant. Consumption of nutrient and phytochemical-rich

vegetables, like *Moringa*, leads to a better immune response compared to consumption of vegetables that are rich in fiber but lower in nutrient content, like common cabbage. *Moringa* leaves should be promoted for greater consumption to improve nutrition and strengthen immune functions for fighting infectious diseases.

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Appendices

Appendix A

SPECIMEN CARD FOR SENSORY EVALUTION

Hedonic rating test

Name of the panalist …….

Date ……..

Name of the product : ***Moringa*** **powder**

Dear panelist, you have 4 sample of *Moringa* leaves. Please taste the sample and score how much you prefer the each one. Please give point for your degree of preference for each parameter as shown below using the scale given.

Parameter	A	B	C	D
Color				
Flavor				
Taste				
Mouthfeel				
Overall acceptability				

Give points as follows:

Like extremely **9**	Like slightly **6**	Dislike moderately **3**
Like very much **8**	Neither like nor dislike **5**	Dislike very much **2**
Like moderately **7**	Dislike slightly **4**	Dislike extremely **1**

Comment(if any)……

Signature……

Appendix B

Table B.1 Average sensory score

Sample	Color	Flavor	Taste	After taste	Overall acceptability
A	8.4 (0.51)^{b}	7.7 (0.48)^{c}	6.09 (0.66)^{b}	7.1 (0.73)^{b}	7.8 (0.63)^{c}
B	7.9 (0.73)^{b}	6.9 (0.56)^{b}	6.9 (0.73)^{c}	7.2 (0.78)^{b}	7.6 (0.51)^{c}
C	7.0 (1.15)^{a}	6.8 (0.91)^{ab}	7.7 (0.48)^{d}	5.8 (0.78)^{a}	6.5 (0.70)^{b}
D	6.4 (0.51)^{a}	6.3 (0.48)^{a}	5.2 (0.42)^{a}	5.7 (0.48)^{a}	5.9 (0.56)^{a}

Figures in the parenthesis are the standard deviations. Figures with same superscript are not significantly different. Figures with different superscript are significantly different.

Table B.2 Absorbance reading (100 fold dilution)

Sample	Abs for TPC	Abs for TFC	Abs for tannins
Fresh leaves	0.132, 0.131	0.005, 0.006	0.008, 0.007
Dried leaves	0.196, 0.194	0.007, 0.008	0.008, 0.009
Steam blanched dried	0.165, 0.161	0.006, 0.005	0.007, 0.006
Lye blanched dried	0.127, 0.125	0.004, 0.003	0.005, 0.006

Table B.3 Absorbance reading

Samples	Abs for AOA	Abs for chl at 645 nm	Abs for chl at 663 nm
Fresh leaves	0.022, 0.023	0.626, 0.621	0.442, 0.436
Dried leaves	0.046, 0.048	0.442, 0.445	0.992, 0.991
Steam blanched dried	0.043, 0.041	0.498, 0.491	0.355, 0.351
Lye blanched dried	0.053, 0.051	0.582, 0.587	0.418, 0.419

Appendix C

Standard curve of Gallic acid for phenol

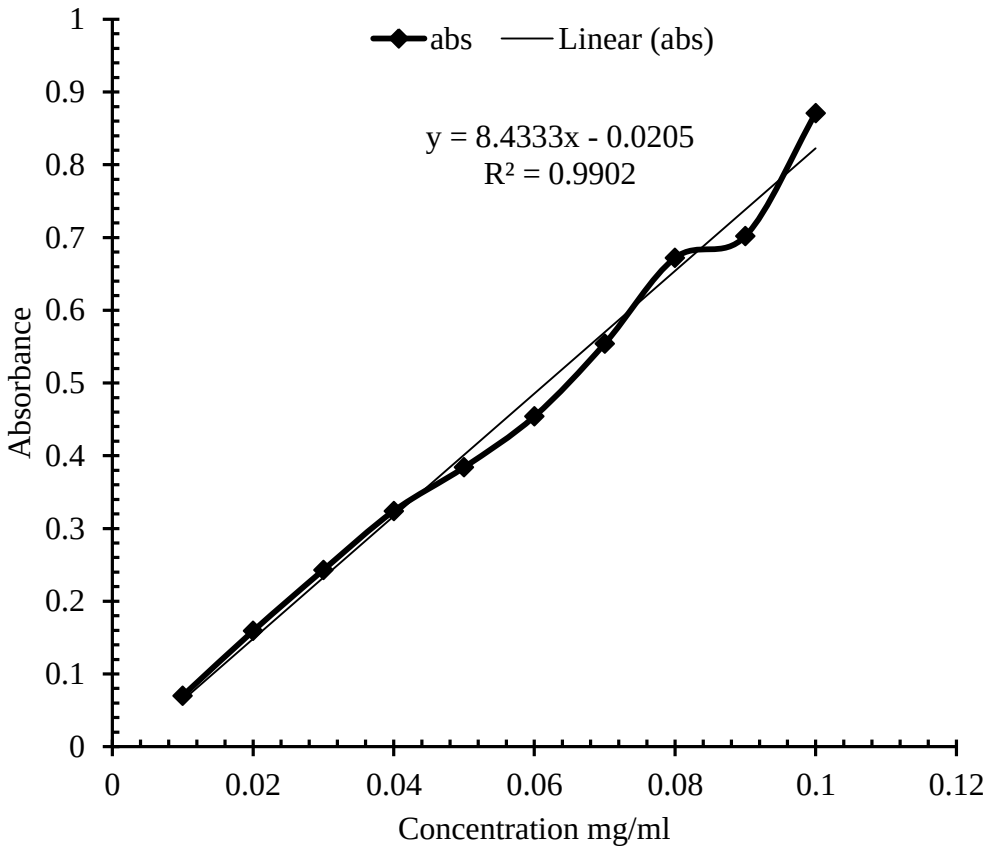


Fig. C.1 Gallic acid standard curve

Standard curve of Quercetin for flavonoid

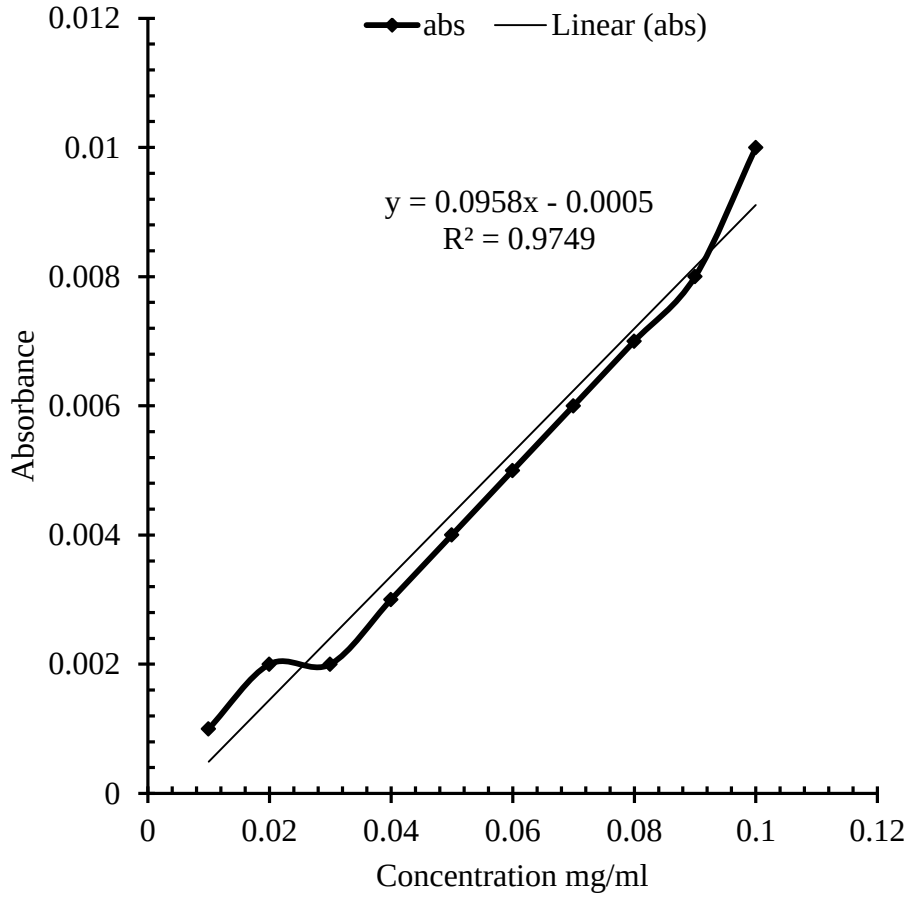


Fig. C.2 Quercetin standard curve

Standard curve of Gallic acid for tannin

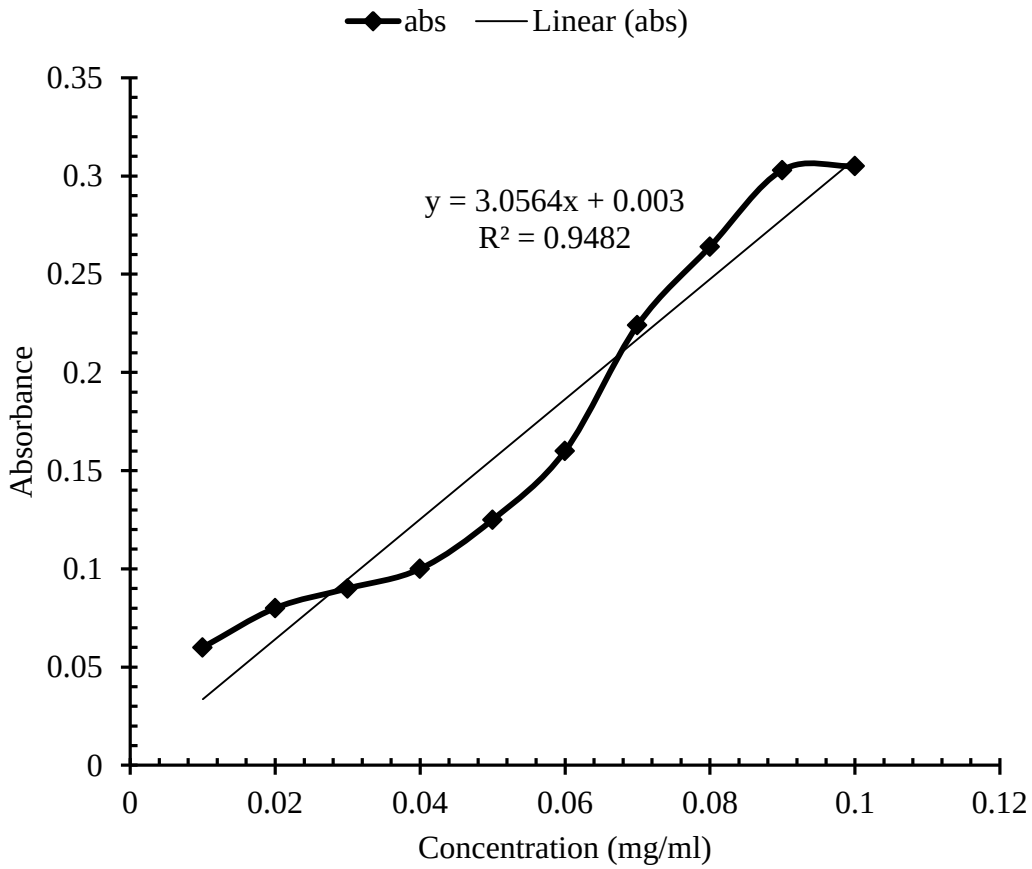


Fig. C.3 Tannin standard curve

Appendix D

Table D.1 One way ANOVA (no blocking) of Phenol content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	8913.85	2971.28	2363.86	<.001
Residual	8	10.056	1.257		
Total	11	8923.91			

Table D.2 One way ANOVA (no blocking) for Flavonoid content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	174604	58201	38.49	<.001
Residual	8	12098	1512		
Total	11	186702			

Table D.3 One way ANOVA (no blocking) for Tannin content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	172.48	57.493	38.7	<.001
Residual	8	11.885	1.486		
Total	11	184.365			

Table D.4 One way ANOVA (no blocking) for Antioxidant activity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
sample	3	1207.36	402.452	457.72	<.001
Residual	8	7.0341	0.8793		
Total	11	1214.39			

Table D.5 One way ANOVA (no blocking) for Chlorophyll

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
sample	3	38.690	12.897	6.09	0.018
Residual	8	16.935	2.117		
Total	11	55.626			

Table D.6 Two way ANOVA (no blocking) for Color

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	24.075	8.025	11.92	<.001
Panelist	9	3.525	0.3917	0.58	0.8
Residual	27	18.175	0.6731		
Total	39	45.775			

Table D.7 Two way ANOVA (no blocking) for Flavor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	10.075	3.3583	8.11	<.001
Panelist	9	3.525	0.3917	0.95	0.503
Residual	27	11.175	0.4139		
Total	39	24.775			

Table D.8 Two way ANOVA (no blocking) for Taste

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	26.5	8.8333	21.68	<.001
Panelist	9	2.4	0.2667	0.65	0.741
Residual	27	11	0.4074		
Total	39	39.9			

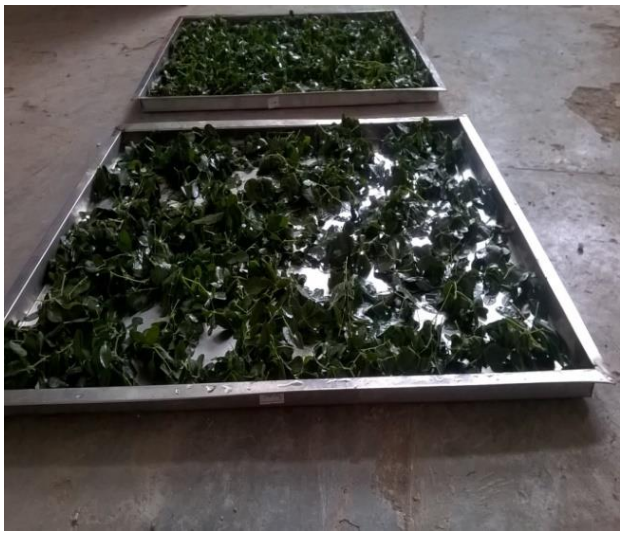
Table D.9 Two way ANOVA (no blocking) for After taste

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	19.7	6.5667	12.4	<.001
Panelist	9	3.9	0.4333	0.82	0.605
Residual	27	14.3	0.5296		
Total	39	37.9			

Table D.10 Two way ANOVA (no blocking) for Overall acceptability

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	24.5	8.1667	22.05	<.001
Panelist	9	3.4	0.3778	1.02	0.449
Residual	27	10	0.3704		
Total	39	37.9			

List of Plates



P1 Cabinet drying

P2 Absorbance reading

P3 Sample preparation

P4 Sensory analysis