STUDY ON PHYSICOCHEMICAL QUALITY OF SUGARCANE JUICE INCORPORATED WITH CARDAMOM AND TEJPAT ESSENTIAL OIL

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Study on physicochemical quality of sugarcane juice incorporated with cardamom and tejpat essential oil

A dissertation submitted to the Food Technology Instruction Committee in Tribhuvan University in partial fulfillment of the requirements for the degree of B. Tech. in Food Technology

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Approval Letter

This ***dissertation*** entitled ***Study on physicochemical quality of sugarcane juice incorporated with cardamom and tejpat essential oil*** presented by **Rijan Rai** has been accepted as the partial fulfillment of the requirement for the **B. Tech. degree in Food Technology.**

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Abstract

Sugarcane is one of the most important crops grown in Nepal and is mainly grown for its rapid accumulation of sucrose but, in the countries of Southeast Asia, South Asia and Latin America, sugarcane juice is also very popular as a beverage. Due to the high concentration of nutrients in sugarcane juice, it degrades quickly due to microbial activity. The purpose of the study was to investigate and find out how essential oils from spices affected the quality and shelf life of sugarcane juice. Cardamom(C) and tejpat(T) essential oils were extracted using hydro-distillation method and incorporated in the sugarcane juice (10 µL essential oil+90 µL ethanol+50mL juice). The prepared essential oil samples along with control I (Juice only) and control II (50 mL juice+100 µL ethanol) were analyzed for their physico-chemical and microbiological changes. The notations C1, C2, C and T stand for sample with no additives, sample with ethanol, cardamom oil and tejpat oil respectively. The samples were stored under refrigerated condition (4±1°C) and studied for the period of 28 days while observation being carried out every 7 days. Using GenStat 12th Edition, analysis of variance (ANOVA) and Tukey's test were run to determine whether there was a significant relationship at $p<0.05$.

The physico-chemical properties like Total soluble solids (TSS), % total sugar, % reducing sugar, pH and titratable acidity were analyzed along with microbiological test i.e., Total Plate Count and Yeast & mold counts. At the end of 28 day, statistically the most significant difference was found in % total sugar content among the samples. The samples C1, C2, C and T had a percentage decrease of 35.65%, 32.44%, 23.94% and 27.93%, respectively which shows that sample containing cardamom oil resists the fall in the total sugar content by withstanding the hydrolysis and inhibiting microbes. Over the course of the 28-day observation period, each sample's parameters gradually changed. The essential oil-containing sample produced noteworthy outcomes when compared to the control sample. The most significant impact on the entire observation was demonstrated by cardamom essential oil. Thus, these results suggest that adding essential oils to sugarcane juice could help slow down its deterioration rate in the refrigerated storage conditions.

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List of Abbreviations

Abbreviation	Full form
ºBx	Degree Brix (measure of % soluble solids, m/m)
ANOVA	Analysis of variance
ASTA	American Spice Trade Association
CCT	Central Campus of Technology
CFU	Colony-forming unit
DFTQC	Department of Food Technology and Quality Control
FAO	Food and Agriculture Organization
GRAS	Generally recognized as safe
HD	Hydro Distillation
ISO	International Organization for Standardization
LSD	Least significant difference
MOALD	Ministry of Agriculture and Livestock Development
MSL	Mean Sea Level
NARC	Nepal Agricultural Research Council
SAARC	South Asian Association for Regional Cooperation
SD	Steam Distillation
SE	Solvent Extraction
SFE	Supercritical Fluid Extraction
TA	Titratable acidity
TPC	Total plate count
TSS	Total soluble solids
USDA	United States Department of Agriculture

Part I

Introduction

1.1 General Introduction

Nepal is an agricultural country. Various crops are cultivated all around the nation. Horticulture crops, among other agricultural products, are crucial to the nation's economic development. The two main categories of agricultural crops cultivated in Nepal are considered to be food crops and cash crops. Paddy, maize, wheat, millets, and barley are some of Nepal's most significant food crops. Contrarily, top cash crops include sugarcane, jute, tobacco, tea, cotton, cardamom, fruits, etc. (Chaudhary, 2013).

Among several crops grown in Nepal, Sugarcane is the most important one. Sugarcane (Saccharum officinarum), the principal crop in Nepal's tropical and subtropical regions, is grown for its rapid accumulation of sucrose. It is one the largest industrial crop that plays a pivotal role in national economy (Shrestha and Thapa, 2021). It pertains to the Poaceae family, has been harvested worldwide for its economical and medicinal valued products such as refined sugar, drinking cane juice, paper, pulp, alcohol, xylitol, chemicals, feed, electricity, and bio-manure (Xiao *et al.*, 2017). After cereals, sugarcane is the second-largest source of dietary carbohydrates for humans (10–12%). There are several applications for by-products of the sugar milling process from sugarcane such bagasse, molasses, furfural, furfuryl alcohol, dextran, and diacetyl. For example, molasses are used in syrups and animal feed and as a substrate for ethanol production (Brumbley *et al.*, 2009). In sugarcane, millions of plant cells must be burst in order to produce the juice, which is where the sugar content of cane is dissolved (S. Singh *et al.*, 2014).

A popular beverage in Southeast Asia, South Asia, Latin America, as well as other nations where sugarcane is farmed commercially, is sugarcane juice. Although sugarcane juice is an extremely popular beverage in countries like Nepal and India, it is still infrequently sold in bottled form on the commercial market. The sugarcane is crushed between roller drums to extract juice, and is consumed with or without ice (Karmakar *et al.*, 2011). Juice made from crushed sugarcane is typically consumed in the summer to quench one's thirst. Sugarcane juice is an excellent source of natural sugars, minerals, and organic acids. It also offers an array of medicinal advantages (Sankhla *et al.*, 2012).

Due to the high concentration of nutrients in sugarcane juice (mostly sugar), it degrades quickly due to microbial (yeast and bacteria like *Leuconostoc* species) activity that start the fermentation and other degradation processes right away and make long-term storage highly challenging (Abhilasha and Pal, 2018; A. Khare *et al.*, 2012). According to earlier research, heat pasteurization of sugarcane juice might hasten unfavorable changes in the beverage's nutritional value and sensory qualities while also extending the juice's shelf life and confirming its microbiological safety (Chew *et al.*, 2018). Additionally shown to have detrimental impacts on health are chemical preservatives (Lima Tribst *et al.*, 2009). Consumer desire for higher quality, less processed, preservative-free goods with longer shelf lives has grown recently. In order to replace the synthetic chemical compounds already in use, this tendency has boosted the alternative use of natural products, notably preservatives derived from herbs and plant products (Harvey, 2008).

Since ancient times, herbs and spices have been used by humans as a means of reducing food degradation and food-borne illnesses. The antibacterial and anti-browning properties of herbs and spices had already been studied by the turn of the 20th century, and it was recognized that their oils might delay microbiological spoilage and prevent enzymatic browning in food items. Utilizing natural antibacterial and anti-browning substances, particularly those derived from plants, for the preservation of food goods is gaining popularity (Dorantes *et al.*, 2000).

1.2 Statement of problems

Although sugarcane juice is an extremely popular beverage in countries like Nepal and India, it is still not frequently sold in bottled form on the commercial market. This is because of the fact that it is a highly perishable commodity. However, Sugarcane juice might be more extensively sold and its quality and safety would improve if appropriate treatments or processes were developed to maintain its freshness (S. Singh *et al.*, 2014).

Sugarcane juice ferments quickly after being extracted because of its high sugar content, and it also becomes dark as a result of polyphenol oxidase activity. Within a few hours of extraction, microbial fermentation changes the juice's flavor from sweet to tart. These undesirable modifications have a detrimental impact on sugarcane juice processing and marketing (Mishra *et al.*, 2011). The quality of sugarcane juice is also affected by chemical

(acid) and enzymatic inversion (I. Singh *et al.*, 2006). To overcome this spoilage problem, various process i.e., physical as well as chemical methods have been used. Physical method like pasteurization helps extend the shelf life of sugarcane juice but hasten unfavorable changes in the beverage's nutritional value and sensory qualities (Chew *et al.*, 2018). Similarly, juices of different fruits have been preserved using different chemical preservatives which may be allergic to certain people and may also result in various health effects. Due to this health issues and hazards, consumer have recently grown their desire for less processed, synthetic preservative free products (Harvey, 2008). Thus, the majority of recent studies have focused on technology for food products quality enhancement without introducing chemicals, without reducing their nutritional content, and without compromising product safety (Eissa *et al.*, 2003). For doing this, utilizing essential oils from spices will be one strategy.

1.3 Objectives

1.3.1 General objective

The general objective was to study the effect of spices essential oils on physico-chemical properties of sugarcane juice.

1.3.2 Specific objectives

- To carry out the extraction of essential oil from the spices.
- To study the effect of essential oils on sugarcane juice during storage.
- To analyze the physico-chemical properties of the sugarcane juice.
- To study the storage stability of the sugarcane juice incorporated with essential oils.
- To compare among cardamom and tejpat as the most effective natural preservative for sugarcane juice.

1.4 Significance of study

Preservation of juice with chemical preservatives has long raised the possibility of adverse human health impacts. In this regard, it is an effort to develop a natural preservative that can replace such artificial preservatives. For many years, many food items have employed spices as preservatives. For preservation purposes, spices from that nation will be somewhat cost-

effective than synthetic chemical preservatives. The use of essential oils as a food preservative for grains, cereals, pulses, fruits, and vegetables is possible. They include a number of active ingredients with significant roles in the food business, including as terpenes, terpenoids, carotenoids, coumarins, and curcumins, giving them strong antibacterial and food preservation capabilities. Even farmers will be encouraged to cultivate spices, which will afterwards enhance both the economic standing of the farmers and the nation.

1.5 Limitation of study

- Preservative properties of oleoresins of respective spices were not carried out.
- The experiment was carried out only under refrigeration.
- Sensory analysis of the product was not done.

Part II

Literature review

2.1 The Sugarcane

2.1.1 General description of the Sugarcane

Sugarcane is a large grass of the genus *Saccharum*, tribe Andropogoneae, family Poaceae. Modern sugarcane cultivars (*Saccharum* spp.) are interspecific hybrids created by the cross-pollination of *Saccharum officinarum* (or "noble cane") and *Saccharum spontaneum* (wild cane), followed by a number of backcrosses to the noble parent. (Brumbley *et al.*, 2009). It is considered as a crop of economic importance. It is mainly cultivated in tropical and subtropical regions of the world. Due to its therapeutic usefulness in addition to its economic advantages, it is a significant agro-industrial crop (Panigrahi *et al.*, 2021).

The leaf, stalk, root system, and flower are the four main components of the sugarcane plant. The stalk has several sections, or internodes, and is roughly cylindrical (King *et al.*, 1953). Millions of plant cells must be burst in order to produce the juice, which is where the sugar content of cane is dissolved (S. Singh *et al.*, 2014). Sugarcane is a long perennial tropical grass that tillers at the base to create unbranched stems that can reach heights of 2 to 4 m or more with a diameter of around 5 cm. It is grown for the thick stems, stalks, or canes that are used to make sugar (James and Tate, 2004). The length of sugarcane increases by around 12 feet each year, and it produces two to four joints with an average length of 4 or 5 inches per month. Each joint has two parts: a root band from which "cutting roots" emerge when the joint is planted as a cutting and an eye that grows into the principal shoot. After the shoots have developed their own permanent shoot roots, these temporary roots disintegrate. Typically, stalk diameters fall between one and two inches (Burr *et al.*, 1957).

One of the oldest cultivated plants in the world, sugarcane is produced for commercial purposes in the tropics and subtropics. However, it's crucial to keep in mind that the variations that thrive in the subtropics are considerably different from those that do well in the tropics. The best growth conditions for sugarcane are those with evenly distributed rainfall (or irrigation) during the growing season, but rather dry preharvest ripening conditions, and a lot of sunlight hours during the whole season. The stalks contain sugar, but

for sugar production to be profitable, ripening conditions must be favorable. Ripening often occurs when the weather is colder and drier. Where the crop is produced under irrigation, this can be induced by a reduction in the water supply. Not only do varieties differ in cane production, but also in the caliber of their juice. The amount of time needed for them to mature also varies (James and Tate, 2004).

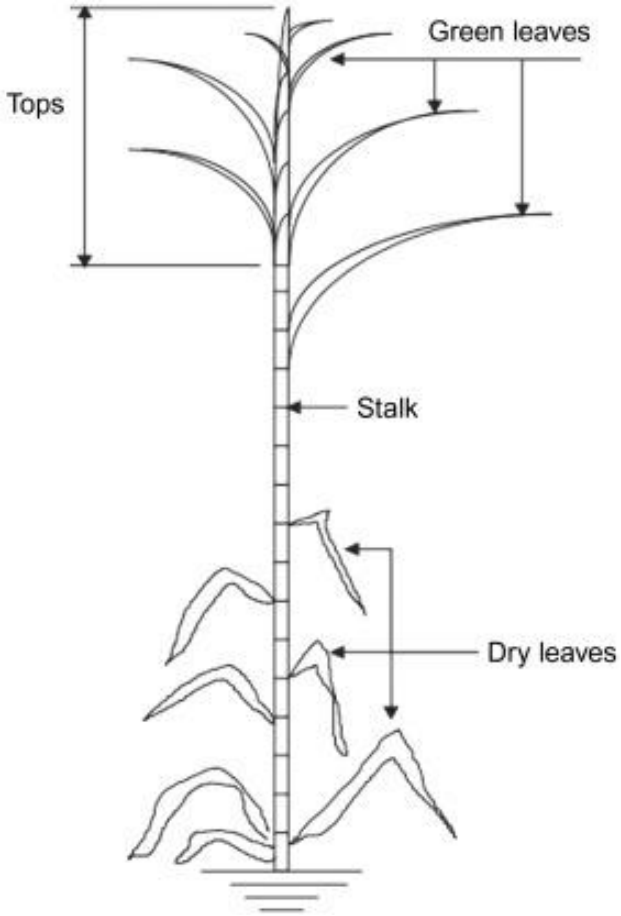

Fig 2.1: The sugarcane plant

2.1.2 Taxonomic Classification

Kingdom : Plantae

Sub-kingdom : Tracheobionta

Super-division : Spermatophyta

Division : Magnoliophyta

Class : Liliopsida

Subclass : Commelinidae

Order : Cyperales

Family : Poaceae

Genus : Saccharum L.

Species : Saccharum officinarum L.

Nepali Name : Ukhu

Source: United State Department of Agriculture (2023)

2.1.3 Historical background

The oldest historical accounts of sugarcane and sugar come from literature produced in India between 3000 and 3400 years ago. The generic name for sugarcane, Saccharum, comes from the Sanskrit word "sharkara" that describes the raw sweet substance made from honey reeds in India. During the first millennium BC, Indian sugarcane appears to have spread westward. It is believed that in 325 BC, soldiers of Alexander the Great transported it from India to Europe. Later, the idea of the Indian honey reed and its "honey" (sugar) output was known to Greek and Roman authors (Brumbley *et al.*, 2009). Following that, sugarcane was grown in West African islands such as So Tomé and Madeira, the Canary Islands, and Spain. Although there is no concrete proof that the plant was intentionally sent to those nations,

Columbus brought sugarcane to the New World in 1493 on his second journey from Spain. The first attempts to cultivate the crop in Hispaniola (now Haiti and the Dominican Republic) were unsuccessful, but success was eventually found in 1506 in the western region of the island. It was brought from Haiti to Puerto Rico in 1515, and in 1520 it was introduced into Mexico. This marked the beginning of Mexico's contemporary sugar industry (Barnes, 1974).

Brandes (1929), documented and provided illustrations of the swidden agriculture or garden culture of primitive tribes in Papua New Guinea who grew sugarcane for chewing along with a variety of other products, including bananas, betel nuts, breadfruit, sago, sweet potatoes, and yams. Additionally, there were several sorts or varieties of sugarcane that varied greatly in shape and colour. According to Artschwager and Brandes (1958), there were two phases of sugarcane movement. The oldest time dates back to the early Cretaceous, when the vast Asiatic-Australian continent created a continuous land bridge over which prehistoric Asiatic canes may have travelled to the region now known as Melanesia without the assistance of humans. They proposed the idea of a single wild progenitor who inhabited the entire area. Later, when flood conditions first appeared throughout the Asiatic-Australian continent in the late Cretaceous epoch, the mobility of flora was severely constrained. Many plant species benefited from the fact that they were able to withstand lengthy periods of time drifting in ocean currents as they developed for development in and near brackish water tracts.

Early *Saccharum* forms produced seeds, but they couldn't withstand extended exposure to salt water. As they do now, wild *Saccharum* species favoured watery environments near flood plains and river banks. Additionally, the seed or fuzz was not designed for wind or bird dispersal across large bodies of water. Therefore, it seems sense to assume that unique wild varieties of sugarcane arose in remote regions, such as Asia, New Guinea, and other Melanesian islands. Natural selection and hybridization must have resulted in significant modification (James and Tate, 2004).

It is preferable to explore the origin of sugarcane in regard to its taxonomy and geographic distribution in Southeast Asia, the Indonesian Archipelago, and New Guinea. With *S. officinarum* and *Saccharum robustum* in New Guinea, *Saccharum barberi* in India, and

Saccharum sinense in China, different species probably arose in different places. *S. officinarum* is said to have spread over a period of thousands of years into the Pacific Ocean region as well as through the island chain into Asia, whereas the thinner Indian canes were produced and grown in the North India/South China region (Brumbley *et al.*, 2009).

2.1.4 Composition of Sugarcane

Without the top, leaves, stubble, or roots, the cane stalk is primarily made up of water i.e., approximately up to 75%, with the balance being made up of fiber and soluble substances (Legendre, 1988). According to Irvine *et al.* (1977), varietal variations are widely documented, and the amount of each of these three ingredients is genetically determined. The noble varieties (*Saccharum officinarum*) have a higher water content and a lower level of sugar and fiber. The interspecific hybrids contain more sugars and fiber. Numerous internal and external elements, including variety, cane age, growing circumstances, soil type, fertilizers, water, pests, and diseases, all have an impact on the chemical makeup of the cane plant (Van Dillewijn, 1952). The most abundant soluble solids in cane stalk juice are sucrose, glucose, and fructose, followed by minerals, waxes, lipids, and phosphatides, and other minor components (Legendre, 1988).

The sugarcane plant usually contains from 6 to 14% fiber. With lesser levels of Beta and Gamma cellulose, this fiber mostly consists of Alpha cellulose (34.7 to 37.7%), pentosans and other sugars (21.4 to 29.1%), and lignin (18.2 to 26.6%). Lipids in sugarcane, commonly called the “cane wax” accounts for 0.05 to 0.5% of the cane which can be extracted by extracting residues after processing with organic solvents. The amount of fertiliser, age, and variety all have a significant impact on the cane plant's total nitrogen content. All of the nitrogen in sugarcane is either as ammonia or in organic form. Some of the amino acids found in the sugarcane are aspartic acid, glutamic acid, alanine, valine, lysine, leucine, glycine, serine, glutamine, phenylalanine, etc. (Burr *et al.*, 1957).

Table 2.1 Chemical composition of sugarcane and it's juice

Component	Amount (%)
Cane constituents:	
Water	73-76
Solids	24 - 27
Soluble solids	10 - 16
Fiber (dry)	11 – 16
Juice constituents:	
Sugars	75 – 92
Sucrose	70 – 88
Glucose	2 – 4
Fructose	2 – 4
Salts	3.0 - 4.5
Organic acids	1.5 - 4.5
Carboxylic acids	1.1 - 3.0
Amino acids	0.5 – 2.5
Other organic non-sugars	
Proteins	0.5 – 0.6
Starch	0.001 – 0.050
Gums	0.3 – 0.6
Waxes, fats, phosphatides	0.05 – 0.15
Others	3 - 5

Source: Irvine *et al.* (1977)

2.1.5 Present scenario of sugarcane production in Nepal

In Nepal, sugarcane has been farmed for more than a century, and at least three generations of farmers have contributed to its production. In terms of sugarcane output on a worldwide scale, Nepal comes in at 41st position (Neupane *et al.*, 2017). In addition, Nepal ranked in fourth place and contributed 0.83% of the SAARC nations' production, with Bangladesh and Pakistan producing the next-highest amounts of sugarcane after India. India and Pakistan are the SAARC nations that export sugar on the world market. Nepal imports a large amount of sugar and sugar-related goods, mostly from Pakistan and India (FAOSTAT, 2018; A Pandey and Devkota, 2020b).

Although sugarcane growing has been a practice since antiquity, commercial production only began in 1947 with the founding of Morang Sugar Mill Limited (Rathi, 2018). In 41 of Nepal's districts, sugarcane is grown, although only 14 of those are commercially farmed (MoALD, 2020). According to MoALD (2020), Nepal has 0.1 million commercial sugarcane producers. Province 2 produces more sugarcane than the other provinces combined. The enormous area expansion of sugarcane in province 2 is mostly due to the favorable tropical climatic features, concentration of sugarcane factories, and existence of the National Sugarcane Research Programme (MoALMC, 2018a, 2018b).

Sugarcane is the sole source of sugar in Nepal and is also used to make other food items such syrups, molasses, jiggery, and fructose (Dotaniya *et al.*, 2016). 60% of the nation's sugar consumption is met by domestic sugarcane production (NSMA, 2018). With an average yield of 45.26 MT/ha, sugarcane is grown across an area of 78,609 hectares (ha) in Nepal (MoALD, 2019). Although a 12.7% rise in output was projected for 2018–19, actual growth was just 2.8% (MoALD, 2019). Since 2015, sugarcane yield and area have dropped. When compared to the real productivity of the varieties released or introduced, the productivity of sugarcane is limited to just 45 MT/ha (MoALD, 2018, 2020; Neupane *et al.*, 2017; Pokharel *et al.*, 2019). This decrease in the yield of sugarcane may be due to lower productivity, delay in payment by mills, disputes between farmers and sugar producers in relation to the Minimum Procurement Price, high cost of production and traditional cultivation practices(Amita Pandey and Devkota, 2020a).

2.1.6 Uses of sugarcane

Although sugar is the primary output of sugarcane processing, other important products including bagasse, brown sugar, molasses, syrup, and jaggery are also produced along with sugar. These sugarcane by-products, including molasses, brown sugar, and jaggery, can be found in their unprocessed state. These goods must include certain phenolic compounds since they are unprocessed, which increases their nutritional and therapeutic value (A. Singh *et al.*, 2015). Besides their industrial sugar production application, sugarcane is also used for fresh juice consumption and in the production of alcoholic beverages like ethanol, rum, etc. The anhydrous form of extracted ethanol can be used as an additional fuel for gasoline and hydrous ethanol can serve flex vehicles and a small market for non-energy uses (Santos *et al.*, 2020). The process of extracting sugarcane juice yields bagasse. Since it is a by-product, there are practically no costs associated with its production or transportation. As a result, it is highly valued, particularly for its ability to generate steam and electricity in place of fossil fuels and wood, enabling the energy independence of the producing units and, in some cases, the sale of excess electricity.

Additionally, bagasse is used to make cellulosic ethanol and furfural. Along with furfuryl alcohol, which is used as a raw material for furanic polymers, anti-corrosives, polymers of urea, modified formaldehyde, perfumes, and solvent of resins and dyes, furfural is used to refine lubricating oils, wood resins, and vegetable oils. In the process of making sugar, a byproduct known as residual molasses is created. Depending on the location, this substance is referred to as bad molasses, final molasses, or just molasses. It is a thick, viscous liquid with a dark brown color that is high in sugar and contains just a little amount of water. This waste product is frequently used to make ethanol in the distilleries attached to the mills, but it can also be used to grow biological yeast, fungi, and bacteria for use in other fermentation processes that produce chemicals and pharmaceuticals. Molasses is a common ingredient in the fermentation process used in East Asian nations to produce monosodium glutamate, acids (citric, formic), and amino acids (lysine). Molasses powder is a direct input for cattle ranchers and producers of feed and mineral salts. It is an organoleptic, energizing, and flavoring supplement for animal feed (Santos *et al.*, 2020).

2.1.7 Health benefits of sugarcane and sugarcane juice

Sugarcane contains flavonoids, phenolic acids, and a number of other phenolic components so, its syrup and juices have an antioxidant effect (Payet *et al.*, 2006). Although the majority of people are often unaware of it, sugarcane may provide health advantages. Sugarcane stem fragments or juice are both edible forms of the plant. Sugarcane juice is produced from the cane and is wholesome and cooling. It has around 15% natural sugar, which helps the body rehydrate and provides immediate energy. Minerals including phosphorus, potassium, calcium, iron, and magnesium, as well as vitamins like vitamin A, B1, B2, B3, B5, and B6, are all abundant in sugarcane juice. The energy content of 100 mL of sugarcane juice is 39 calories, while the carbohydrate content is 9 g (Chinnadurai, 2017). Since ancient times, the stem and roots of sugarcane have been employed in the Ayurvedic and Unani medical systems in India. It is advised to chew raw sugarcane for a sound and healthy physique (Chen *et al.*, 2008). Sugarcane chewing is known to give strong teeth and is advised for oral health (Chinnadurai, 2017).

Sugarcane juice produced by chewing the cane is very beneficial for teeth that are weak from lack of activity and an excessive diet of soft foods. It strengthens teeth and offers them the essential activity they need (Karthikeyan and Samipillai, 2010). It is used as a standalone treatment or in conjunction with other plant components in the ayurveda medical system to treat a variety of ailments. It is conceivable that the presence of phytochemicals such polyphenols accounts for the therapeutic benefits of sugarcane and its byproducts (Chen *et al.*, 2008; A. Singh *et al.*, 2015). These phenolic substances are primarily in charge of the antioxidant and cytoprotective properties (Maurício Duarte-Almeida *et al.*, 2006; Nayaka *et al.*, 2009). There are also significant amounts of other phyto-components such phytosterols, terpenoides, flavonoids, and glycosides (Colombo *et al.*, 2009; de Armas *et al.*, 1999; Deshmane and Dev, 1971; Duarte-Almeida *et al.*, 2007; McGhie, 1993; A. Singh *et al.*, 2015; Vila *et al.*, 2008). The sugarcane plant's roots and stems can be used as a remedy for constipation, jaundice, anaemia, blood pressure, bronchitis, cough, urinary tract infections, and many other health issues (Akber *et al.*, 2011). They are also used for the treatment of skin, eye diseases, loss of milk production as well as general debility (Kadam *et al.*, 2008).

In the tropics and subtropics, raw cane is a common consumer good used to treat a variety of illnesses and make a delightful beverage (Arif *et al.*, 2019). To make the cool sugarcane juice, fresh sugarcane culms are pulverised. It is very nutrient-dense, comprising starch, phosphatides, gums, numerous minerals, vitamins, amino acids, and organic acids in addition to natural sugars (Nishad *et al.*, 2017; Qudsieh *et al.*, 2001). When 100mL of Sugarcane juice are consumed, the body produces 40kcal of energy, 10mg of calcium, 1.1mg of iron, and 6g of beta-carotene. The juice has been said to help with the recovery from haemorrhage, dysuria, anuria, jaundice, cancer, cardiovascular, and urinary disorders in addition to its cooling properties (Cáceres *et al.*, 1987; Karthikeyan and Samipillai, 2010). Regular consumption of sugarcane juice aids in the kidneys' and the urine system's optimal performance (Arif *et al.*, 2019). Other advantages of sugarcane juice include its usage as a laxative (substances that increase bowel movements), an aphrodisiac (stimulating sexual desire), an antibacterial tonic, and a cooling agent (useful in fatigue, thirst and burning sensations) (C. P. Khare, 2008; A. Singh *et al.*, 2015).

It is said that tropical sugarcane is the sweetest and richest in juice. The juice is a good source of energy boost for the body because it includes iron and carbohydrates (Arif *et al.*, 2019). The juice, which is rich in minerals and organic acids, improves important organs like the kidney, stomach, brain, eyes, and genital organs. It is ingested while having a fever since it replenishes lost protein (A. Singh *et al.*, 2015). Sugarcane juice is used to treat the febrile condition in order to stop the body from losing protein and other nutrients. Juice consumption in large quantities also promotes urination. The kidney functions are improved, and the urine flow is clear. The juice is beneficial for treating acidity as well as complicated illnesses such gonorrhoea, cystitis, nephritis, and enlarged prostate (Arif *et al.*, 2019). Sugarcane juice with dry ginger helps to relieve hiccup (Agarwal, 1986). It is recommended for leprosy, intestinal troubles and for people suffering from hypotension (Karthikeyan and Samipillai, 2010; K. R. Kirtikar and Basu, 1918; Nadkarni and Nadkarni, 1976; Ross, 2005). The sugarcane juice also controls the host's natural defense against bacterial, viral, and protozoal illnesses (Lo *et al.*, 2005).

2.2 Spices

2.2.1 General definition

The American Spice Trade Association (ASTA) defines spices in very broad terms: dried plant products used primarily to season food.

The United States Department of Agriculture (USDA) defines spices as any aromatic vegetable substance in the whole, broken, or ground form, except for those substances which have been traditionally regarded as foods, such as onions, garlic and celery; whose significant function in food is seasoning rather than nutritional; that is true to name; and from which no portion of any volatile oil or other flavoring principle has been removed.

The Geneva-based International Organization for Standardization (ISO) also defines spices and condiments as: vegetable products or mixtures thereof, free from extraneous matter, used for flavoring, seasoning and imparting aroma to foods.

Spices come from many sections of plants, such as cardamom from the seed, bay leaf from the leaf, clove from the flower bud, pepper from the fruit (berries), cinnamon from the bark, ginger from the rhizome and saffron from the stigma of the flower. For the growth of several spices, tropical or subtropical conditions are necessary (Lewis, 1984; Tapsell *et al.*, 2006). Many of the fragrant seeds that are commonly referred to be spices are actually harvested from plants after they have stopped blooming. Coriander is a good example since the dried seeds are referred to as a spice and the leaves are considered a herb. Onions, garlic, and fennel bulbs, as well as the stem and roots of coriander, all of which are used in cooking tend to be categorized with herbs since they are frequently used fresh and applied similarly to herbs in cooking (Tapsell *et al.*, 2006). Since all the spices are coming from plants they have been generally recognized as safe (GRAS) (De La Torre Torres *et al.*, 2017).

2.2.2 History

Spice has a history that predates the development of human civilization. It is a history of new territories being discovered, empires being established and dismantled, battles being fought and lost, treaties being made and broken, tastes being sought for and provided, and the emergence and decline of various religious practices and beliefs. In both ancient and mediaeval eras, spices were among the most precious commodities traded. Different spices

were used by the ancient Egyptians to flavor meals, make cosmetics, and embalm their dead. Through the Middle East, the use of spices extended to the eastern Mediterranean and Europe. Historically, donkey or camel caravans were used to deliver spices from China, Indonesia, India, and Sri Lanka via land. Arab middlemen controlled the spice trade, until European explorers discovered a sea route to India and other spice producing countries in the East (Hadacek, 2002).

The first known usage of spices dates back to Egypt's Pyramid Age (2600 BC). During the construction of the pyramids, onions were served to the workers as a curative food. Anise, caraway, cassia, coriander, fennel, cardamom, onions, garlic, thyme, mustard, sesame, fenugreek, saffron, and poppy seed are just a few examples of the spices and herbs that are used as condiments today. They were also employed in medicine, cosmetics, cookery, and embalming. The Ch'u Ssu (Elegies of Chu), written in the fourth century BC, is the first credible mention of cassia usage in China. In his Analects, the renowned philosopher Confucius (551-479 BC) discussed the usage of ginger. Since the first millennium BC, spices and plants have been utilized, according to Indus Valley excavations. Herbs and spices were widely used in ancient Greece as culinary seasonings and in medicinal research. They employed herbs and spices from the Mediterranean region, such as anise, caraway, poppy seeds, parsley, and marjoram, as well as certain spices from the East, such as pepper, cassia, cinnamon, and ginger. The "Father of Medicine" Hippocrates (460–377 BC) produced several treatises on medicinal plants and their applications. Theophrastus, a Greek scientist and philosopher, is commonly referred to as the "Father of Botany" and is credited for compiling the botanical details of spices and herbs in his two writings “On Odours” and “An Enquiry into Plants” (Rosengarten Jr, 1969).

Ancient trade routes like the "Incense Route" and the "Silk Route" were established due to the movement of products like spices from East to West. The Europeans were pushed to discover new routes to the Orient's primary spice-growing regions by the high demand and price for spices in the Middle Ages. The forerunners who created new routes for the trade of spices included Marco Polo, Pedro Cabral, Vasco da Gama, Ferdinand Magellan, Christopher Columbus, and Hernando Cortes. Due to the vital role that spices played in the economy of the nation, new regions were discovered, conflicts broke out, and nations that grew spices were raided (Farrell, 1998; Parry, 1969).

2.2.3 Uses and benefits of spices

In contrast to vegetables, which include significant concentrations of protein, carbohydrates, fats, starch, fiber, minerals, and other vitamins, spices ingested in little amounts have little to no nutritional benefit (Farrell, 1998). Spices, however, offer secondary chemicals that have therapeutic, anti-inflammatory, and antibacterial properties. Variable concentrations of protein, fat, carbohydrate, vitamin (such as carotene, thiamine, riboflavin, and niacin) and inorganic (such as calcium, magnesium, manganese, phosphorus, potassium, chlorine, copper, iron, salt, and zinc) nutrients are found in spices. Additionally, certain spices include fatty acids, starches, sugars, cholesterol, and fiber (Farrell, 1998; Leung, 1980; Lewis, 1984).

Spices have been used in cuisine for a very long time, and not just for flavoring; they also contain medicinal and preservation characteristics (Davidson *et al.*, 1983). Additionally, spices increase salivation, have a carminative effect, and help preserve food through their antibacterial and antioxidant qualities (Lewis, 1984). Spices and herbs have been used to keep food from spoiling and degrading as well as to increase its shelf life since the dawn of civilization. Spices are added to meals to improve flavor and palatability (Ceylan and Fung, 2004). Dietary quantities of fat, sugar, and salt can be controlled by using herbs and spices (Rathore and Shekhawat, 2008). Due to consumer resistance to synthetic chemicals in the era of globalization, spices have gained even more significance as sources of natural colors, flavors, antimicrobials, and antioxidants for the food industry (Peter and Shylaja, 2012).

2.3 Essential oils

Essential oils, also known as aromatic plant essences, are volatile, fragrant, oil-like compounds that are primarily produced by plants. They come in a variety of colors, ranging from pale yellow to emerald green, from blue to dark brownish red, and can be liquid at room temperature, however few of them are solid or resinous (Balz, 1996). All plant organs, including buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood, and bark, produce them. They are then stored in secretory cells, cavities, canals, epidermic cells, or glandular trichomes (Bakkali *et al.*, 2008). Essential oils are very intriguing and have a variety of biological capabilities. The term "biological" refers to all effects that these concoctions of volatile chemicals (mostly mono- and sesquiterpenoids, benzenoids, phenylpropanoids, etc.) have on people, other animals, and other plants (Bas¸er and Buchbauer, 2010).

Essential oils (EOs) get their name because of their flammable characteristics. According to French Agency for Normalization: Agence Française de Normalisation (AFNOR), essential oils can be defined as: "The essential oil is the product obtained from a vegetable raw material, either by steam distillation or by mechanical processes from the epicarp of Citrus, or 'dry' distillation." EOs are insoluble in inorganic solvents (water) while soluble in organic solvents (ether, alcohol, fixed oils). They are volatile liquids, having a characteristic odor and density less than unity, except vetiver, sassafras, and cinnamon. A plant can govern and regulate its surroundings (play an ecological function) by using essential oils (EOs), which can operate as chemical messages to deter predators, draw pollinating insects, prevent seed germination, and communicate with other plants. Moreover, EOs have antifungal, deterring, and insecticidal properties. The presence of essential oils can be found in various parts of aromatic plants, including their flowers (pink, orange, lavender, flower buds in the case of clove and bracts in the case of ylang-ylang), leaves (mint, eucalyptus, bay leaf, thyme, sage, savory, pine needles), rhizomes (sweet flag and ginger), roots (vetiver), seeds (coriander and carvi), fruits (anise, fennel, and citrus epicarps), and wood and bark (in sandal-wood, cinnamon, and rosewood) (Hanif *et al.*, 2019).

Commercial antimicrobial agents have been used to control food degradation or contamination since the dawn of time. Nowadays, User worries about synthetic preservatives have led to a rise in interest in natural antimicrobials such essential oils. Essential oils from aromatic and medicinal plants and their constituent parts have antibacterial, antifungal, and food preservation properties against a variety of microbial diseases (Basim *et al.*, 2000; Gormez *et al.*, 2016; Iacobellis *et al.*, 2004; A. K. Pandey *et al.*, 2014; Sonker *et al.*, 2015; Tripathi and Kumar, 2007). However, precaution should be taken while using essential oils. They are 75 to 100 times more concentrated than fresh spices in terms of concentration. They are employed in recipes when a strong aromatic effect is sought even if they lack the full flavour profile of ground spices. In the finished product, essential oils are employed at a very low concentration of 0.01% to 0.05%. When consumed internally (by themselves), they can be hazardous to the neurological system, irritate the skin, and even result in miscarriages and allergic responses (Chaudhary, 2013).

2.3.1 Chemistry of Essential oils

The extraction of volatile compounds—primarily terpenes and oxygenated compounds—through a kind of distillation yields essential oils. The chemical components of essential oils are produced in plants via the melavonic and shikimic acid pathways as secondary metabolites, and they are then stored in glandular trichomes, oil cells, or ducts in plant tissue (Hunter, 2009). The majority of secondary metabolites produced by aromatic and medicinal plants are terpenoids, alcoholic compounds (such as geraniol, menthol, and linalool), acidic compounds (such as benzoic, cinnamic, and myristic acids), aldehydes (such as citral, benzaldehyde, cinnamaldehyde, and carvone camphor), ketonic bodies (such as eugenol, thymol) and phenols (e.g., ascaridole, anethole). The composition of different essential oils is discovered to be largely influenced by terpenes (such as pinene, myrcene, limonene, terpinene, and p-cymene), terpenoids (such as oxygen-containing hydrocarbons), and aromatic phenols (such as carvacrol, thymol, safrole, and eugenol) (Koul *et al.*, 2008)

Each essential oil has several chemical constituents, sometimes as many as fifteen, although the predominant scent often makes up between 60% and 80% of the whole oil. The components of essential oils include hydrocarbons (terpene derivatives) or terpenes (such as -terpinene, -pinene, camphene, limonene, phellandrene, and sabinene), oxygenated derivatives of hydrocarbons (such as linalool, citronellol, geraniol, carveol, menthol, borneol, fenchon, tumerone, and nerol), benzene compounds (alcohols, acids, phenols, esters, and lactones) and nitrogen- or sulfur-containing compounds (indole, hydrogen sulfide, methyl propyl disulfide, and sinapine hydrogensulfate) (Rosengarten Jr, 1969). Terpenes, which can be described as flowery, earthy, piney, sweet, or spicy, often contribute to the aromatic freshness of a spice. The main components of a spice's scent are its oxygenated derivatives, which also include alcohols, esters, acids, aldehydes, and ketones. While the sulfur- and nitrogen-containing compounds offer the distinctive characteristics to onion, garlic, mustard, citrus, and floral oils, the compounds with benzene structure produce sweet, creamy, and flowery notes (Guenther *et al.*, 1948).

2.3.2 Methods of extraction

Essential oils typically have a liquid state at normal temperature. In comparison to lipids, alcohols, organic solvents, and other hydrophobic solutions, their solubility in water is

minimal (Thormar, 2011). To retrieve these compounds from plant materials, a variety of extraction techniques have been devised. Although steam and hydrodistillation are the most widely used methods at a commercial scale for the extraction of EOs, numerous novel extraction technologies, also known as non-conventional methods, have been developed as alternatives to traditional methods in order to meet the green extraction concept for the recovery of natural products. Examples of these technologies include supercritical fluid extraction, ultrasound assisted extraction, and microwave assisted extraction (Hashemi *et al.*, 2017).

2.3.2.1 Hydrodistillation

The method that is employed the most frequently to extract EOs is hydrodistillation (HD) (Meyer-Warnod, 1984). In this method, the plant material is submerged in a water bath for this process, and the combination is then heated to boiling point at atmospheric pressure. An azeotropic mixture made up of odorous chemicals that are present in plant cells is released when it is heated. Despite the fact that the majority of the components have boiling temperatures greater than 100 °C, the water vapor acts mechanically to drive them. The separation of the combined water and EOs by decantation follows the cooling 9 by condensation.

According to the "Clevenger" approach promoted by the European Pharmacopoeia, the distillate's aqueous phase can be recycled in the boiler using a cohobage system (Clevenger, 1928). As a result, the separation between the water and the volatile molecules (EOs) is caused by their different densities. Depending on the plant matter, the HD lasts anywhere from three to six hours. This factor may have an impact on the chemical makeup and production of EO (Hashemi *et al.*, 2017). One benefit of HD is that there are no chemical solvents used, thus hazardous solvent residue in the EOs and losses of more volatile components during the solvent removal process may also be avoided (Filly *et al.*, 2016). However, The material is overheated in this process, which results in the burning of aromatic compounds and the formation of the desired product (essential oils) with a burned smell (Hanif *et al.*, 2019).

2.3.2.2 Solvent Extraction

In order to recover the EOs, solvent extraction (SE) entails dissolving the plant material that contains the EOs in a solvent and then evaporating it. After extraction, a filtering procedure and a subsequent distillation are performed on the liquid combination containing the EOs (and other compounds). Hexane is the most often used solvent due to its high volatility. Yet, due to their toxicity, benzene and dichloromethane have been outlawed despite being widely used. Solvent extraction has various uses in the world of agribusiness since it can extract a wide variety of chemical families from EOs (Jadhav *et al.*, 2009). The major disadvantage of this approach is that solvent residue may leave behind causing allergies and immune system effects (Hanif *et al.*, 2019).

2.3.2.3 Steam distillation

Steam Distillation (SD) is the method that is most frequently used to extract essential oils from plant material (Hanif *et al.*, 2019). It is a traditional technique used to recover EOs and is also one of the most effective ways to get high-quality EOs. SD is appropriate for extracting heat- 10 sensitive components (such volatiles) and purifying them with steam. The steam source for this method is distilled water. The way the extraction process works is that the plant material containing the desired chemicals is heated below their boiling point and then evaporated with steam at a temperature below 100°C and atmospheric pressure (Rojas and Buitrago, 2015). The steam destroys the structure of plant cells, releases the contained molecules and takes away most of the volatile components. When the steam has removed the oil from the column, it is condensed back into a liquid combination of water and oil in a cooling system (condenser). After that, this combination is gathered in a receiving receptacle.

During distillation, a water layer, known as a "hydrosol," must typically be removed from the distillate. After no more essential oils can be collected, the water and oil layers are then separated by decantation in a container commonly referred to as a "Florentine flask" or "separatory funnel" (Dugo and Di GA, 2002). Using SD, desirable chemical constituents of EOs can be preserved and also their degradation can be minimized. This process helps to create EOs for specific market users.

2.3.2.4 Supercritical fluid extraction

Supercritical fluid extraction (SFE) is one of the newest "green" technologies. It is beneficial to the environment. These days, this approach is used in industrial settings where valuable compounds need to be recovered from plant sources under controlled circumstances, as well as in analytical laboratories to quickly and effectively extract EOs from plant matrices. Generally speaking, carbon dioxide (CO_2) is the best supercritical solvent for the SFE technique for the following reasons: The critical pressure and temperature for CO_2 are also quite low, at 73 bar and 31 °C, respectively. Moreover, CO_2, a non-flammable, recyclable gas with high purity and cheap cost, may be easily isolated from the extract by depressurizing below the critical point (Koubaa *et al.*, 2015).

CO_2 is utilized at extremely high pressure during the CO_2 extraction process. Initially, CO_2 is refrigerated to a temperature between 35 and 55 °F before being pushed through plant material at a pressure of 1000 psi. In this situation, the carbon dioxide 11 condenses into a liquid. Also, Supercritical CO_2 are also used in this extraction process. In the supercritical CO_2 extraction (SCO_2) method, CO_2 is heated to 87 °F and fed through plant materials at a pressure of 8000 psi. CO_2 has been compared to thick vapor or fog under these circumstances. After the reaction media's pressure is released, carbon dioxide is removed in gaseous form while the essential oil is left behind. As a result, essential oils and CO_2 are separated. This method produces essential oils with an essence that is more similar to the essence of the original plant material (Reverchon, 1997).

2.3.2.5 Ultrasound-Assisted Extraction

In recent years, a laboratory scale ultrasound technology has become widely employed to quicken the extraction procedures, however scale up process of ultrasound assisted extraction process is taking much longer time to be widely accepted from food and pharmaceutical industries (Hashemi *et al.*, 2017). The fundamental method used by UAE to extract EOs from natural sources is producing sound waves (ultrasound frequency of 20 kHz), which cause cavitation bubbles to form in the solution (Roselló-Soto *et al.*, 2015) and have enough energy to shatter the structures holding the oil, so releasing it. UAE may also work as an emulsifier, dispersing lipophilic compounds in water and making it easier to separate and purify the EOs later on (Sereshti *et al.*, 2012).

2.3.3 Biological activities of Essential oils

Some of the biological activities (properties) of essential oils are discussed below.

2.3.3.1 Antioxidative property

Excellent antioxidant qualities may be found in essential oils. The composition of essential oils affects their capacity as antioxidants. Phenolic molecules and other secondary metabolites (those with conjugated double bonds) found in essential oils often have strong antioxidant effects (Koh *et al.*, 2002). The most important antioxidant qualities may be found in the essential oils extracted from nutmeg, thyme, cinnamon, mint, basil, clove, oregano, and parsley (Aruoma, 1998). Carvacrol and thymol are the most active chemicals that exhibit antioxidant effects. The phenolic structure of these chemicals influences their activity. The redox characteristics of phenolic compounds make them important for both the decomposition of peroxides and the neutralization of free radicals (Burt, 2004). Other substances found in essential oils such alcohols, ketones, aldehydes, ethers, and monoterpenes also contribute to the antioxidant action of EOs (Aruoma, 1998).

2.3.3.2 Antibacterial Property

Exceptional antibacterial qualities are displayed by essential oils. The main attribute of EOs is their hydrophobicity, which enables them to partition into the lipids of bacterial cell membranes, disrupting the bacterial structure and increasing permeability (Sikkema *et al.*, 1994). As a result, several biological molecules and various ions are released from the bacterial cell. While certain ions and other cellular components from the bacterial cells can be lost without any loss of viability, a higher loss of cellular components and ions can cause the death of bacterial cells (Denyer, 1991). The antibacterial properties of essential oils are typically attributed to phenolic chemicals found in them, such as eugenol, thymol, and carvacrol (Dorman and Deans, 2000). The cytoplasmic membrane, electron flow, proton driving force, and active transport can all be disrupted by these substances, which can also cause the cell contents to coagulate (Denyer, 1991; Pauli, 2001).

2.3.3.3 Anti-Inflammatory Activity

Using essential oils as anti-inflammatory medicines, inflammatory disorders including arthritis, allergies, and rheumatism can be treated (Maruyama *et al.*, 2005). Essential oils include active anti-inflammatory components that work as inhibitors of histamine release or as reducers of the generation of any inflammatory mediators. As one significant component of several essential oils, 1,8-cineole, works as a leukotriene (LTB4) and prostaglandin (PGE2) (Yoon *et al.*, 2000). Essential oils' anti-inflammatory properties are a result of their antioxidant properties as well as interactions with signaling cascades, including those involving cytokines and regulatory transcription factors, as well as the expression of genes that promote inflammation (Hanif *et al.*, 2019).

2.3.3.4 Anticancer Activity

Cancer treatment potential is demonstrated by essential oils. Natural anticancer substances found in essential oils are essential for both preventing and treating cancer. Certain foods, like turmeric and garlic, are regarded as good sources of anticancer agents (Edris, 2007). Garlic essential oil contains sulfur compounds such diallyl trisulfide, diallyl sulfide, and diallyl disulfide that have been shown to be cancer-preventive (J. Milner, 2001; J. A. Milner, 2006).

2.3.3.5 Repellent and Insecticidal Activity

Chemical compounds in essential oils vary in their structural complexity and have a variety of insecticidal and insect repellant actions. The marketing of essential oils is influenced by several variables. Among these are biological activity, the worth of intellectual property, product quality, legal requirements, and product functionality (Ahmed and Eapen, 1986). Both flying insects and granary insects are poisoned by the EOs (Mateeva and Karov, 1983). Gaultheria (Ericaceae) and eucalyptus (Myrtaceae) oils had extremely high levels of toxicity that killed insects. In general, insects can absorb EOs through their skin, their lungs, or both. EOs exhibit fumigant toxicity as well (Regnault-Roger and Hamraoui, 1995).

2.4 Cardamom

Cardamom is a bushy perennial herb that produces fruits in pods and belongs to the *Zingiberaceae* family. Each fruit is straw-colored, oval to oblong in form, three-sided, and ranges in length from 7 mm to 11 mm (5/16 to 9/16 inch), approximately the size of a cranberry. Each fruit contains 15 to 20 reddish brown, triangular, three-sided seeds that are 3 mm long and 2 mm broad. The seeds' flavor is sweet and somewhat camphoraceous, with an unusually strong and unique scent that is pungent and peppery. The seeds must be sent in their shells because, once removed, the volatile oil quickly degrades, losing up to 50% of its potency in only one week and nearly all of it in just three months. Just before the fruit turns yellow, it is picked. The fruit is chopped, sun-dried, and the calyxes and pedicels are taken off. For export, the seeds go through sorting and grading. About 3000 metric tons of crop are produced worldwide (Farrell, 1998).

Cardamom are broadly classified into two types (Rao *et al.*, 1993; Subba, 1984). They are:

- Small cardamom - *Elettaria cardamomum*. They are also known as “Queen of Spices”.
- Large cardamom - *Aframomum* and *Amomum* species.

Cardamom is a shade-loving plant that is grown at an elevation of 600–1200 m above mean sea level (MSL), where there is an average annual rainfall of 1500–4000 mm and a temperature range of 10–35 °C. Because of its therapeutic qualities and other attributes, it is employed in Ayurvedic medicinal formulations. In addition, it is utilized in many other products, including processed foods, oleoresins, and fragrances. The evergreen rainforests of South India and Sri Lanka are the origin of the genus Elettaria, one of the few compact natural plant groupings, , from where it spread to other tropical countries.(Purseglove *et al.*, 1981).

Fig 2.2: The Cardamom pods and plant

Cardamom is herbaceous perennial (2–5 m tall) with aerial pseudo-stems (tillers) formed of leaf sheaths and underground (subterranean) rhizomes. According to studies on vegetative development, suckers continue to grow for around 18 months after their emergence. When the suckers reach an age of approximately a year, in June and July, the rate of linear development is at its highest. In 89% of them, reproductive buds (panicles) could be observed developing, indicating that the suckers need between 10 and 12 months to mature. A vegetative bud takes about ten months to mature, and the panicle takes about a year to emerge from the freshly produced tillers. It took around 90–100 days for the emergence of the first flower from the panicles, irrespective of the variety. (Parthasarathy and Prasath, 2012).

Table 2.2 Composition of Cardamom edible part (per 100 gm)

Component	Amount
Water	8.3 gm
Energy	311 kcal
Protein	10.8 gm
Fat	6.7 gm
Total Carbohydrate	68.5 gm
Fiber	11.3 gm
Ash	5.8 gm
Calcium	383 mg
Iron	14 mg
Magnesium	229 mg
Phosphorus	178 mg
Potassium	1119 mg
Sodium	18 mg
Zinc	7 mg
Niacin	1 mg

Source: Farrell (1998)

Cardamom seed, decorticated, is the dried, ripe fruit of *Elletaria cardamomum* L. Maton. The hard, wrinkled, light reddish-brown to dark reddish-brown seed has a pleasant aromatic odor and a characteristic warm, slightly pungent taste. Depending on the source and growth

circumstances, cardamom seeds' essential oil concentration varies in quality and potency. The yield might range from 2% to 10%. Only seeds of extremely high grade will produce 10% essential oil. Even though the oil is quite flammable, when utilize as an emulsion, it can sustain baking temperatures. Cineole, methyl heptanone, β-terpineol, borneol, neryl acetate, geraniol, nerol, nerolidol, α-pinene, sabinene, myrcene, limonene, and p-cymene are the main organic flavouring components of cardamom oil. A-terpinyl acetate makes up roughly 28–34% of the essential oil's makeup, whereas cineole makes up 25–40%. The oil has a distinct musty odour after being exposed to air and is highly penetrating, irritating, cineolic, camphoraceous, warm, sweet, spicy, fragrant, and pleasant. Following 24 hours of exposure, the oil has lost all of its smell. The oleic, stearic, linoleic, palmitic, caprylic, and caproic acid glycerides make up the fixed oil content, which ranges from 1% to 2% on average (Farrell, 1998).

2.5 Tejpat *(Cinnamomum tamala)*

There are 270 species in the genus *Cinnamomum*, which is a member of the *Lauraceae* family and is native to Asia and Australia. The majority of these kinds of evergreen trees and shrubs are fragrant, and many are significant from an economic standpoint. There are about 20 species in India. The term's derivation comes from the Greek word "kinnamomon," which means "spices." The Phoenicians gave the Greeks the name, suggesting that they had been trading with the East from ancient times. The specific epithet "tamala" is modeled after an Indian plant's regional name (G. Sharma and Nautiyal, 2011).

A member of the *Lauraceae* family, *Cinnamomum tamala* is a little evergreen tree. It is frequently called Indian cassia. The tree may reach heights of 7.5 m and girths of around 1.4 m. It is widespread across India because to its tropical and subtropical distribution, but is more prevalent in the Himalayan area (3000–7000 ft) and the Sylhet and Khasia hills in North-East India (3000–4000 ft). The tree has slightly rough, dark brown or blackish bark. With white stripes pointing outward, Blaze is roughly 1.3 cm long and has a pinkish or reddish-brown tint.

Alternating, subopposite, or opposite leaf arrangements are possible. They have three nerves that run from just above the base almost to the apex and are ovate, lanceolate, or oblong, acuminate, coriaceous, faintly shiny above (V. Sharma and Rao, 2014). The

traditional names of the leaves include Tejpat, Tejpatta, Tamal-patra, among others. They are usually referred to as Indian bay leaves (K. Kirtikar and Basu, 1999). *Cinnamomum tamala* has monoecious (bisexual) blooms that grow on the same plant. White, many, tiny flowers with pedicels that are the same length as the calyx are found in terminal pubescent panicles and axillary cymes. Insects like honey bees are frequently used to pollinate the plant's blossoms, which bloom in the final week of March or the first week of April. The fruit is an ellipsoidal drupe, and the seeds take about a year to reach maturity. Fruits and flowers therefore cohabit from April until May. Dark purple fruits that have one seed are considered to be ripe (Gunjan *et al.*, 2009).

The leaves of *Cinnamomum tamala* (tejpat) are frequently used as spices and, after distillation, produce an essential oil. Tejpat oil, an essential oil extracted from the leaves, is used medically as a diuretic, carminative, and to treat heart diseases (Mir *et al.*, 2004). The usage of tejpatra leaves in "Ayurveda" is described for treating conditions including spermatorrhea, coryza, dry mouth, bladder diseases, and anorexia. It exhibits hypolipidemic and hypoglycemic characteristics (Kar *et al.*, 2003). According to the Yunani system, tejpat has benefits that include stimulating the brain, acting as an anti-helminthic, diuretic, being excellent for the liver and spleen, reducing inflammation, calming sore eyes, and decreasing salivation. They can also be used to treat lochia after delivery, rheumatism, colic discomfort, and diarrhea (V. Sharma and Rao, 2014). It is frequently utilized in the food industry due to its distinctive scent (Chang and Cheng, 2002). Tejpat are utilized in Nepal for both culinary and animal feed purposes. Along with myrobalan, it is also employed as a clarifier in dyeing and in the production of vinegar. Leaf essential oil is used in perfumery, confectionery, and other processed foods as a flavoring ingredient. They are used to flavor pickles, conserves and sweet preparations (V. Sharma and Rao, 2014).

The essential oil content obtained after hydrodistillation in a Clevenger type apparatus or steam distillation varies between 0.7–1.5% (Nath *et al.*, 1999). The composition of the essential oil is highly variable depending on many factors, major one being geographical region of growth. Many chemotypes of *Cinnamomum tamala* exist, e.g., eugenol type (eugenol- 66–70%), cinnamic aldehyde type ((E)-cinnamaldehyde- 79.4%), linalool type (linalool- 54.66%), trans sabinene hydrate-β-ocimene type (trans- sabinene hydrate- 28.8%, β-ocimene- 17.9%), etc., named after the main constituents present (Baruah *et al.*, 2008;

Joshi *et al.*, 2009; Kapoor *et al.*, 2009; Mir *et al.*, 2004; Sood *et al.*, 1979; Upadhaya *et al.*, 1994). α-pinene, camphene, myrcene, limonene, eugenol, p-cymene, methyl eugenol, eugenol acetate, and methyl ether of eugenol are the major components of tejpat (Siano *et al.*, 2003; Smith *et al.*, 2002).

Part III

Materials and methods

3.1 Materials

3.1.1 Raw materials collection

3.1.1.1 Sugarcane collection

Mature, firm and clean sugarcane stalk were purchased from the local market of Dharan sub-metropolitan city, Sunsari, Nepal.

3.1.1.2 Spice collection

Dry cardamom pods and tejpat were also purchased from the local market of Dharan sub-metropolitan city, Sunsari, Nepal.

3.1.2 Chemicals, apparatus and equipment

All of the chemicals, glassware, and equipment in the research were used of laboratory grade and were obtained from the CCT laboratory. The major apparatus and chemicals required are listed below.

a) Glassware and utensils: Petri-dish, Burette, Pipette, Test-tubes, Volumetric flask, Conical flask, Measuring cylinder
b) Chemicals: Buffer solution, Fehling's solution, Standard Dextrose, Carrez I and II, Citric acid, Ethanol, Sodium Hydroxide, lead acetate, Oxalic acid, phenolphthalein
c) Equipment: Hot air oven, Cleavenger apparatus, Desiccator, Electronic balance, Thermometer, Heating mantle (Burner), ph-meter, Refractometer, Incubator, Rotary shaker, Autoclave, Micropipette

3.2 Methods

3.2.1 Preparation of Sugarcane juice

1. Sorting

Matured, sound, physically free from extraneous matter and good quality sugarcane stalk were sorted from the bundle.

2. Washing

The selected stalks were washed with the water to remove any possible dirt, adhered dusts, impurities, soil residue, etc.

3. Extraction of juice

The cleaned stalks were then subjected to Mechanical Sugarcane juice extractor for extracting juice.

4. Filtration of juice

The extracted juice was filtered by passing it through multiple layers of muslin clothes. This process helps in removing any insoluble matters or bagasse that might enter during juice extraction.

3.2.2 Extraction of Essential oils

The Cardamom and Tejpat essential oils were extracted by Hydrodistillation process using Clevenger-type apparatus in accordance with procedure as mentioned in Clevenger (1928). The obtained essential oils were collected in opaque bottles and stored in a refrigerator in the dark at 4℃ until further use.

3.2.3 Preparation of samples

The extracted filtered sugarcane juice was divided into four equal batches of 50 ml each in the conical flasks. The control sample I (C1) was prepared without any addition of essential oils. The control sample II (C2) was prepared by adding 100 µL of ethanol in the sugarcane juice. For the preparation of essential oils incorporated juice sample, 10 µL of each essential

oils [Cardamom (C) and Tejpat (T)] pre-dissolved in 90 µL of ethanol were homogeneously mixed with two batches of sugarcane juice using rotary shaker. The research conducted by Kapoor *et al.* (2014) formed the basis for the usage of 10 µL of essential oil diluted in 90 µL of ethanol for mixing in the juice sample.

3.2.4 Sample storage

All four samples were stored at refrigerated temperature (4 ± 1°C) for 28 days. The effect of storage on shelf life and physicochemical properties such as Total soluble solids (TSS), pH, titratable acidity, ascorbic acid, reducing sugar, total sugar, and microbial analysis (total microbial count and yeast and mold count) were observed after an interval of 7 days for 28 days. Each analysis was carried out in triplicate on 0, 7th, 14th, 21st and 28th days (Kapoor *et al.*, 2014).

3.2.5 Physico-chemical analysis

3.2.5.1 Total soluble solids (TSS)

The total soluble solids of each sugarcane juice samples were measured using a hand refractometer (Ranganna, 1986).

3.2.5.2 pH

The pH values of each sugarcane juice samples were measured using a digital pH meter. The equipment was first calibrated with the buffer solution as mentioned in the analysis handbook of Ranganna (1986).

3.2.5.3 Titratable acidity

The acidity of all sugarcane juice samples were determined titrimetrically with 0.1N Sodium hydroxide solution and result were expressed as % Citric acid (Ranganna, 1986).

3.2.5.4 Reducing sugar

From the procedure described by Ranganna (1986), Lane and Enyon's method of determining reducing sugar was used to calculate the reducing sugar content of all juice samples.

3.2.5.5 Total sugar

For determining total sugar of the samples, first the clarified sample solution were subjected to complete sucrose inversion process as mentioned in Ranganna (1986) handbook. Then, employing same Lane and Enyon's method procedure for reducing sugar determination, the total sugar content of the samples were obtained.

3.2.5.6 Microbial Analysis

Total plate count and Yeast and Mold counts were determined as a microbial analysis for the prepared sugarcane juice samples. The procedure described by were followed for the total plate count and yeast and mold count analysis using the plate count agar and potato dextrose agar, respectively.

- **Total plate count**

  Total plate count of each samples were carried out using the plate count agar. For this test, 1 ml of each sample was serially diluted to 10^{-6} concentration. 0.1 ml of diluted sample was mixed thoroughly on agar in the petri-plate. They were then incubated at 37℃ (Aneja, 2003).

- **Yeast and mold count**

  For yeast and mold counts, all experimental procedure were the same as total plate count. The sole variation was that a Malt Extract medium was employed instead of plate count agar (Aneja, 2003).

3.2.6 Statistical analysis

The data were examined using a two-way ANOVA with no blocking at the 5% level of significance. The LSD method was used to compare the treatment means (Genstat 5 Version 12.1, Lawes Agricultural Trust, Rothamsted Experimental 35 Station, 2009). Tukey test was used for the post hoc test. All the referencing of citations was done using Endnote X9.

Part IV

Results and discussion

The study was carried out to find the physico-chemical changes of the sugarcane juice on incorporation of the essential oil extracted from cardamom and tejpat. For this research process, sugarcane juice was extracted from sugarcane stalk using mechanical juice extractor. The extracted juice was filtered using multiple layers of muslin cloth. Essential oils were obtained using hydro-distillation method and mixed with the sample juice. The samples were then kept in the refrigerator for 28 days, with 7-day intervals between analysis. The changes during the storage period are shown and discussed below.

4.1 Chemical composition of fresh sugarcane juice

Chemical analysis was carried out for Sugarcane juice and the result found are tabulated below.

Table 4.1 Chemical composition of Sugarcane juice

Constituents	**Value**
Moisture (%)	83.63±0.57
Total sugars (% db)	16.83±0.29
Reducing sugars (% db)	0.39±0.049
Acidity (% citric acid)	0.25±0.021
Ascorbic acid (mg/100gm)	2.99±0.13

*Values are means of triplicate with standard deviation.

The moisture content values obtained from the proximate analysis were slightly different from the result as per DFTQC (2017) which was moisture 90.2%. This variation in moisture content was may be due to difference in maturity stage of the sugarcane stalk or drying error. According to Chauhan *et al.* (2002), total sugars, reducing sugars, acidity and ascorbic acid content in sugarcane juice was found to be 17.6-19.0%, 0.20-0.65%, 0.24-0.39% and 1.33-1.98 mg/100gm respectively. The different results might be the consequence of a variety of sugarcane maturity and assessment methods.

4.2 Effect on Total Soluble Solids during storage

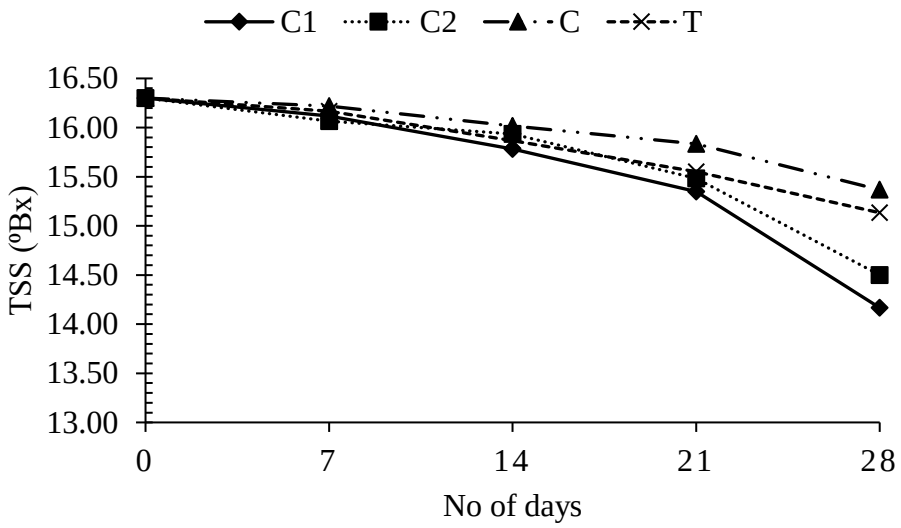

Fig 4.2: Effect of various spices essential oils on Total Soluble Solids of Sugarcane juice during storage

C1- Control I C2- Control II C- Cardamom oil sample T- Tejpat oil sample

The total soluble solid (TSS) of the samples were determined using the hand refractrometer. The initial TSS of the fresh sugarcane juice was found to be 16.30ºBx. This TSS value decreased with the time along all sample during storage as shown in Fig 4.2. The Analysis of Variance (ANOVA) showed no significant differences ($p>0.05$) between different samples. Least significance difference (LSD) at 5% level of significance also showed that there were no significant difference between the sample C1, C2, C and T.

On 7th, 14th and 21st days of sample storage, statistical analysis showed that there was no significant difference ($p>0.05$) between the sample C1, C2, C and T in terms of Total soluble solids. However, In 28th day, sample C1 and C2 were significantly different ($p<0.05$) from the sample C and T.

After 28 days of storage period of juice sample, there was a 13.07% drop in sample C1 for TSS value from day 0. Similarly, 11.04%, 5.71% and 7% drop were seen in the sample C2, C and T, respectively from the initial level of TSS during storage.

The decrease in the TSS content of the juice during storage maybe due to microbial activity present in the juice (Chauhan *et al.*, 2002). The observation on the decreasing TSS content align with the findings of Bhupinder *et al.* (1991). The fall in the TSS value of the juice samples could be the result of conversion of the sugars naturally present in the juice into acids during storage (Chauhan *et al.*, 2002). It is observed that decrease in TSS content of the sample containing cardamom essential oils i.e., C is less than all other samples. At the end of 28 days, the TSS of sample C was found to be 15.37. Comparatively, the highest value for TSS content was found in C followed by T, C2 and C1 as shown in Fig 4.2. The sample which had the least value of TSS was sample C1. The TSS content was found to be 14.17.

4.3 Effect on % Total sugar during storage

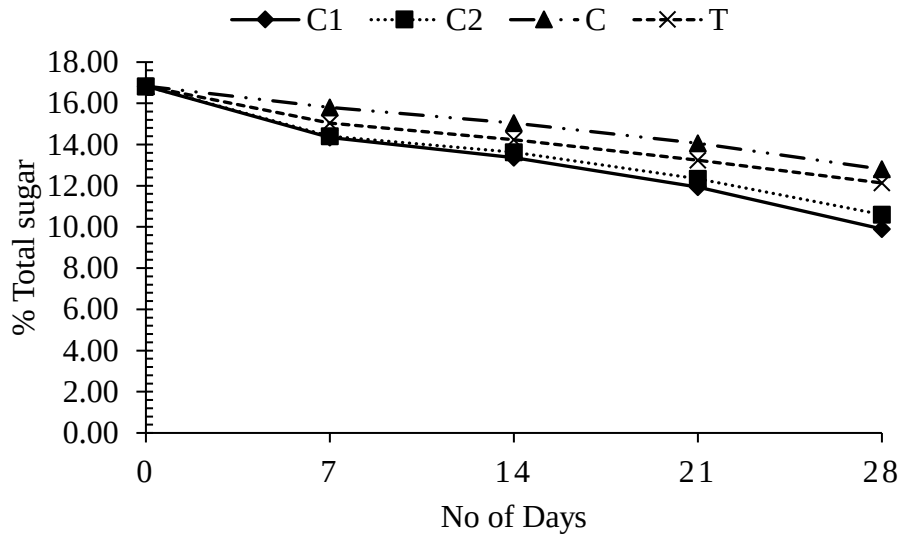

Fig 4.3: Effect of various spices essential oils on % Total sugar of Sugarcane juice during storage

C1- Control I C2- Control II C- Cardamom oil sample T- Tejpat oil sample

The total sugar content of the samples were calculated titrimetrically using the Lane and Enyon's method of determination. The % total sugar content of fresh sugarcane juice was found to be 16.83% initially. Throughout the whole sample's storage period, its value dropped with time. The Analysis of Variance (ANOVA) showed significant results ($p< 0.05$) among different sample and storage intervals as shown in Fig. 4.3. Least significance difference (LSD) at 5% level of significance indicated that there is a significant difference between C1 and T and C1 and C. However, there was no significant differences between C1 and C2, C2 and T and T and C.

In terms of % total sugars, on day 7^{th}, statistically there was no significant difference ($p>0.05$) between the sample C1, C2 and T. Also, there was no significant difference ($p>0.05$) between the sample C and T. But, there was a significant difference ($p<0.05$) between the sample C1 and C2 with C. Similarly, In day 14^{th}, 21^{st} and 28^{th} day, there was no significant difference ($p>0.05$) between the sample C1 and C2. There was also no significant difference ($p>0.05$) between the sample C and T. However, on these days, there was a significant difference ($p<0.05$) between the sample C1 and C2 with C.

After the end of 28 days storage time, sample C1 had the highest decrease in % total sugar i.e., 35.65% decrement from the level of day 0 while the sample C had the least decrease of 23.94%. Likewise, sample C2 and T had the percentage decrease of 32.44% and 27.93%, respectively from the initial level. This results shows that sample containing cardamom essential oils was best for resisting the drop in the % total sugar content in the sugarcane juice sample.

All samples showed a decreasing trend in percentage total sugars. The aforementioned graph makes it evident that, after 28 days, C had the highest total sugar content and C1 had the lowest. The sample C had the % total sugar value of 12.8% whereas the control sample C1 had the % total sugar value of 9.9% at the end of storage time. Up to 28 days, there was a steady fall in total sugar content during storage. The decrease in the total sugar content of the sugarcane juice samples might be due to the result of action of microorganisms present in the juice. Microorganisms fed on the available sugar present in the juice as an energy

source for their growth and metabolism resulting in declining total sugar content (Chauhan *et al.*, 2002).

4.4 Effect on % Reducing sugar during storage

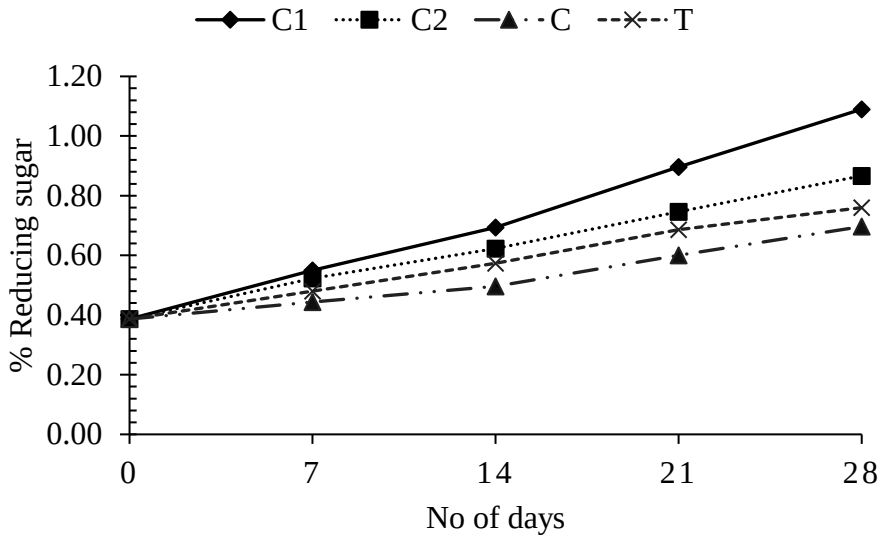

Fig 4.4: Effect of various spices essential oils on % Reducing sugar of Sugarcane juice during storage

C1- Control I C2- Control II C- Cardamom oil sample T- Tejpat oil sample

The reducing sugar content of the sugarcane juice samples were also find out titrimetrically using the Lane and Enyon's method of determining reducing sugar. At the beginning, the % reducing sugar of the fresh sugarcane juice was found to be 0.39%. During the storage period, there was an increase in the percentage of reducing sugar. As seen in the Fig 4.4, the Analysis of Variance (ANOVA) revealed significant differences ($p<0.05$) across various sample and storage intervals. Least significance difference (LSD) at 5% level of significance indicated that there was no significant difference in samples C2, C and T. Similarly, there was no significant difference between sample C1 and C2. But, the sample C and T were significantly different from the sample C1.

In day 7^{th}, there was no significant difference ($p>0.05$) between all the samples for % reducing sugars. In 14^{th} and 21^{st} day, statistically there was no significant difference ($p>0.05$) between the sample C1 and C2. Similarly, there was no significant difference ($p>0.05$)

between the sample C2 and C. However, there was a significant difference ($p<0.05$) between the sample C1 and C. In day 28th, there was a significant difference ($p<0.05$) between the sample C1, C2 and C and no significant difference ($p>0.05$) between the sample C and T.

After the 28 days storage period, the percentage increase in the % reducing sugar content of all the juice samples namely C1, C2, C and T were 179.49%, 123.08%, 79.49% and 94.87%, respectively. This results showed that the hydrolysis of the polysaccharides was highest in the sample C1 whereas, sample C had the lowest.

The hydrolysis of polysaccharides may be the cause of the steady rise in reducing sugars. The conversion of sugar like sucrose to glucose and fructose which is the reducing sugars was observed by Qudsieh *et al.* (2001) during the juice storage. The increase in reducing sugar was found to be linear and positively related to storage time, as reported by Ewaidah (1992). After comparing the samples, it was found that C1 had the highest value for % reducing sugar while C had the lowest value. At the end of 28 days, the control sample C1 had the reducing sugar content of 1.09%. While on the contrary, sample containing cardamom essential oils had the reducing sugar content of 0.7%. The slow inversion of acids and non-reducing sugars into reducing sugars may be the cause of an increase in reducing sugar with an increase in storage time.

4.5 Effect on pH during storage

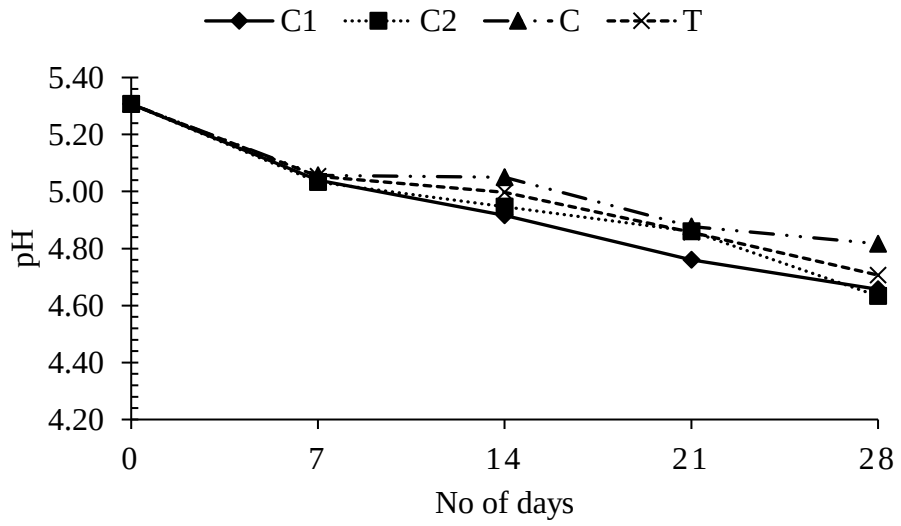

Fig 4.5: Effect of various spices essential oils on pH of Sugarcane juice during storage

C1- Control I C2- Control II C- Cardamom oil sample T- Tejpat oil sample

The pH of the sugarcane juice samples were measured using a calibrated digital pH meter. In the above Fig.4.5, it illustrates the pH variation of the sugarcane juice under storage conditions. It is evident from above graph that pH of all the samples of the sugarcane juices had fallen during the course of storage. The Analysis of Variance (ANOVA) showed significant difference ($p< 0.05$) among different sample and storage intervals. Least significance difference (LSD) at 5% level of significance indicated that there was a significant difference between C1 and C. However, the samples C1, C2, T and C2, T, C showed no significant difference between each other.

In 7^{th} day, statistical analysis showed that there was no significant difference ($p>0.05$) between all the samples. On day 14^{th}, there was no significant difference ($p>0.05$) between the sample C1 and C2 and C2 and T. But, sample C was found to be significantly different ($p<0.05$) from the sample C1, C2 and T. In 21^{st} day, there was no significant difference ($p>0.05$) between the sample C2, C and T. But, sample C1 was significantly different ($p<0.05$) from the sample C2, C and T. Similarly, on day 28^{th}, sample C1 and C2 were significantly different ($p<0.05$) from the sample C and T. The sample C and T were also significantly different ($p<0.05$) from each other. However, there was no significant difference ($p>0.05$) between the sample C1 and C2 and C1 and T.

At the end of 28 days storage, sample C2 had the highest fall in the pH value with a decrease of 12.81%. However, sample C1 also had also same level of decline as C2 i.e., 12.24%. Similarly, the percentage decrease in the sample C and T were 9.23% and 11.29%, respectively.

The decreasing trend in the pH can be observed among the samples from the graph. Initially, the pH of the fresh sugarcane juice was found to be 5.31. At the end of 28^{th} day storage, sample C had the maximum pH while sample C2 had the minimum although sample C1 also had the pH in the same range as C2. The sample C had the pH of 4.82 while the pH of sample C1 and C2 were 4.66 and 4.63, respectively. The fall in the pH was due to increasing titratable acidity of the sample as a result of sugar conversion into acids overtime during storage. However, samples treated with essential oils tends to resists the fall of the pH than the control samples.

4.6 Effect on Titratable Acidity during storage

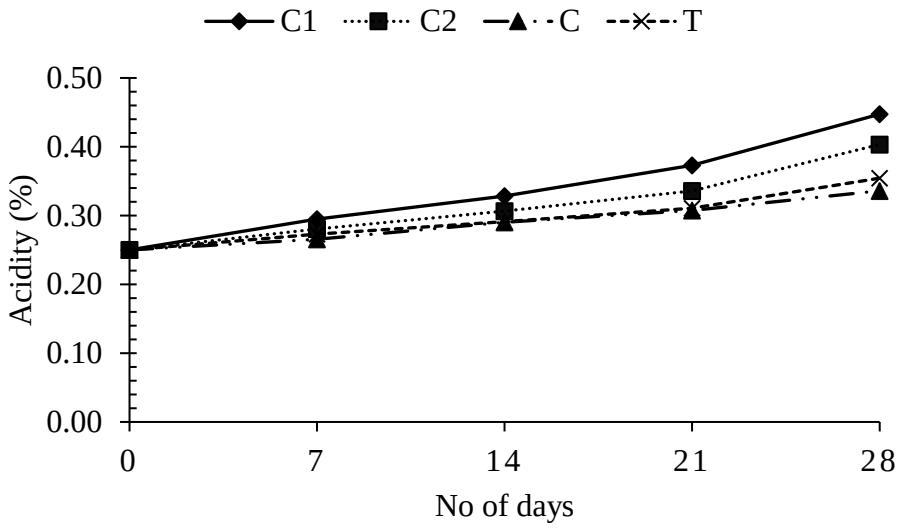

Fig 4.6: Effect of various spices essential oils on titratable acidity of Sugarcane juice during storage

C1- Control I C2- Control II C- Cardamom oil sample T- Tejpat oil sample

The % acidity of the sugarcane juice samples were calculated titrimetically using sodium hydroxide. Above Fig.4.6, illustrates the change in the titratable acidity of sugarcane juice's after various samples were stored for 28 days. The graph shows a gradual increase in acidity across the samples. Significant results ($p<0.05$) were obtained from the Analysis of Variance (ANOVA) for different sample and storage intervals. Least significance difference (LSD) at 5% level of significance showed that the sample C and sample T were significantly different from the sample C1. However, there was no significant difference among samples C, T, C2 and samples C2, C1.

In 7^{th} and 14^{th} day, statistical analysis showed that there was no significant difference ($p>0.05$) between the sample C1, C2, C and T. On day 21^{st}, there was no significant difference ($p>0.05$) between the sample C2, C and T. The sample C1 and C2 also had no significant difference ($p>0.05$). But, the sample C1 was significantly different ($p<0.05$) from the sample C and T. In 28^{th} day, there was a significant difference ($p<0.05$) between the sample C1 and C2. Similarly, sample C and T was significantly different ($p<0.05$) from the

sample C1 and C2. However, no significant difference ($p>0.05$) was seen between the sample C and T.

After the span of 28 days storage, control sample C1 had the highest rise in the titratable acidity of 80% from the level of day 0. The sample C2, C and T had the increment of 60%, 36% and 40%, respectively from the initial level of titratable acidity.

The increment in the titratable acidity coupled with the decrease in the pH value of the samples during the storage period might be due to the acetic acid and lactic acid production (Yusof *et al.*, 2000). The conversion of the sugar present in the sugarcane juice naturally into acids by degradation and by the microbial contamination which produces lactic acids from the hexose sugars could be the reason. Similar findings were observed for the pineapple juice by Kapoor *et al.* (2008). From the above graph, after 28 days of storage period, sample C1 had the highest acidity value followed by samples C2, T and C. The titratable acidity (expressed as % Citric acid) of the control sample C1 and C2 were 0.45% and 0.40%, respectively. However, the sample containing cardamom and tejpat essential oil C and T had the acidity of 0.34% and 0.35%, respectively.

4.7 Effect on Total Microbial Count during storage

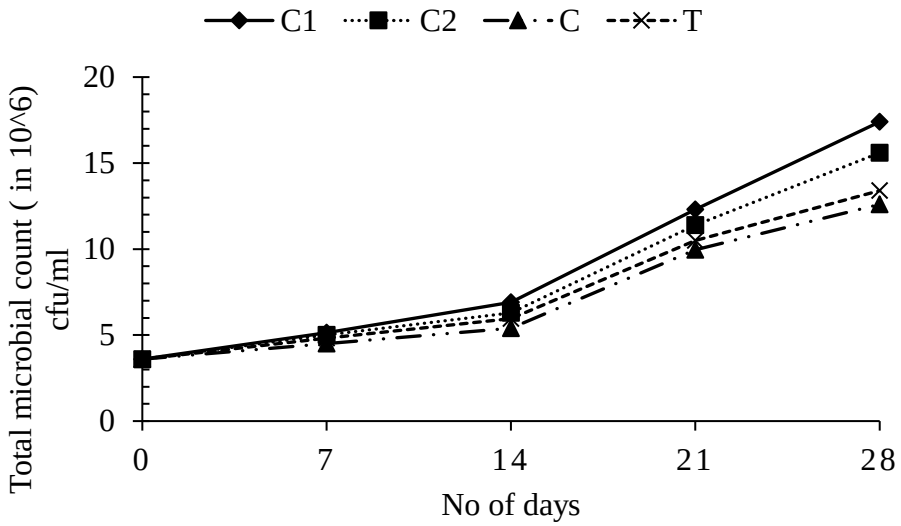

Fig 4.7: Effect of various spices essential oils on Total microbial count of Sugarcane juice during storage

C1- Control I C2- Control II C- Cardamom oil sample T- Tejpat oil sample

For the total microbial count analysis, total plate count method using plate count agar was employed. In the above Fig.4.7, it illustrates the change in the Total Microbial count of the sugarcane juice under storage conditions. It is evident from above graph that microbial count of all the samples of the sugarcane juices had rose during the time of storage. The Analysis of Variance (ANOVA) showed significant results ($p< 0.05$) among different sample and storage intervals. Least significance difference (LSD) at 5% level of significance showed that there was insignificant difference among the samples C2, C, T and the samples C1, C2, T. However, there were significant differences between the sample C1 and C.

In 7^{th} day, statistical analysis showed significant difference ($p<0.05$) between the sample C1 and C for total plate count. But, the sample C1, C2 and T and sample C2, C and T were no significantly different ($p>0.05$). On day 14^{th}, there was a significant difference ($p<0.05$) between the sample C1, C2 and C. The sample C1 and T also had a significant difference ($p<0.05$). However, the sample C2 and T and sample C and T had no significant difference ($p>0.05$). In 21^{st} and 28^{th} day, statistically there was a significant difference ($p<0.05$) between the sample C1, C2, C and T.

At the end of 28 days storage time, control sample C1 had the highest increase in the total plate count number of 383.33% and sample containing cardamom essential oil C had the least increment of 250% from the initial level. Similarly, the sample C2 and T had the percentage increment of 333.33% and 272.22%, respectively. This result depicts the fact that sample containing essential oils were effective in controlling the growth of microorganism than the control sample.

The microbial load in every sample rose as the storage duration increased. Nonetheless, it was shown that adding essential oils from different spices had positive impacts on the proliferation of microorganisms. Juice microbe development was able to be postponed by these additions. The action of essential oils is attributed to their increased percentage of monoterpene hydrocarbons, which operate synergistically to limit microbial development, as indicated by Ahmed and Eapen (1986). Kapoor *et al.* (2008) has also discussed the way in which flavonides, piperine, and numerous other elements work in tandem to suppress the development of these microorganisms. Microbial growth increased towards the end of the

storage period, which might have been caused by changes in the juice's pH and storage temperature. The increase may be partly due to high moisture content in fruit juices which has been found to promote the growth of yeast and bacteria. From the above graph, we can observe that the two samples containing essential oils i.e., C and T had lesser microbial load at the end of storage period with microbial load of 12.6×10^6 and 13.4×10^6, respectively. Among them, it was discovered that the sample containing essential oil of cardamom was the most successful in inhibiting microbial development. Comparing the graph, it is seen that the order of the samples for resisting the growth of the microorganisms are as follows:

C > T > C2 > C1

4.8 Effect on Yeast and mold count during storage

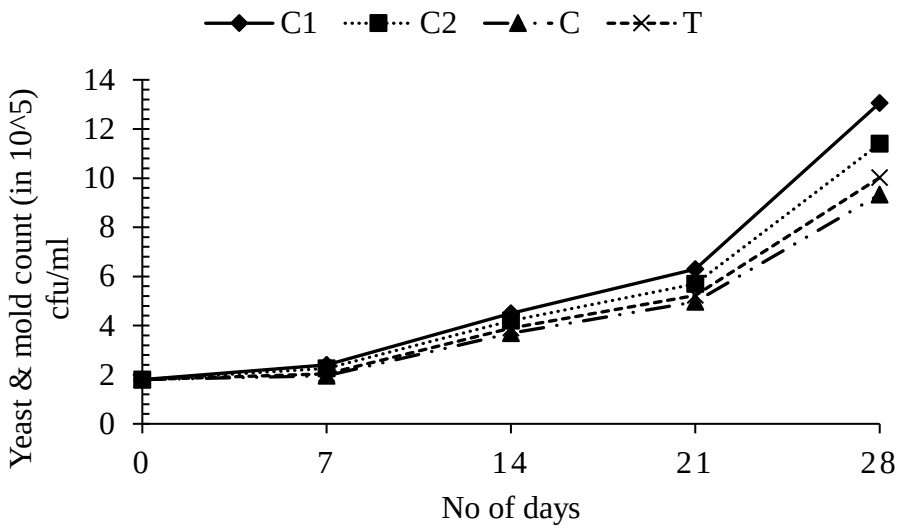

Fig 4.8: Effect of various spices essential oils on Yeast and mold count of Sugarcane juice during storage

C1- Control I C2- Control II C- Cardamom oil sample T- Tejpat oil sample

The yeast and mold count of the sugarcane juice samples were determined using malt extract medium. The variation in the amount of yeast and mold in sugarcane juice under storage conditions is depicted in the above Fig 4.8. The graph above makes clear that during the storage period, the yeast and mold counts of every sample of sugarcane juice increased. The Analysis of Variance (ANOVA) showed no significant differences ($p>0.05$) among the

samples. Least significance difference (LSD) at 5% level of significance also showed that there was insignificant difference among the samples C2, C, T and the samples C1, C2, T. However, there were significant differences between the sample C1 and C.

In 7^{th} day, statistical analysis showed that there was no significant difference ($p>0.05$) between the sample C1 and C2 and sample C2, C and T. But, the sample C1 was significantly different ($p<0.05$) from the sample C and T. On day 14^{th}, there was no significant difference ($p>0.05$) between the sample C1 and C2 and sample C and T. But, there was a significant difference ($p<0.05$) of sample C with C1 and C2 and of sample C1 with T. In 21^{st} day, there was a significant difference ($p<0.05$) between the sample C1, C2 and C. Similarly, there was a significant difference ($p<0.05$) between the sample C1 and T. However, no significant difference ($p>0.05$) was seen between the sample C2 and T and sample C and T. On day 28^{th}, all the samples were significantly different ($p<0.05$) from each other.

After the 28 days of storage period, the percentage increment of the yeast and mold counts from the initial counts in the sample C1, C2, C and T were 625.56%, 533.33%, 418.33% and 456.67%, respectively. This results showed that the control sample C1 had the highest increase in yeast and mold count while sample containing cardamom essential oil had the lowest. It indicates the antimicrobial effect of essential oil on the juice samples.

A longer storage duration results in higher levels of mold and yeast. This might be the result of mold and yeast development in addition to external contamination of the juice. The continuous rise in the growth of yeast and mold count is may be due to different factors like exposure to air, improper sealing conditions, temperature fluctuations and naturally present yeast and mold in environment. The proliferation of bacteria that produce acid may also be the cause of the increase in yeast and mold as well as the microbial contamination they have caused (Kapoor *et al.*, 2008). From our result, we had already seen the rise in acidity during the storage period which could be the reason for providing the favorable condition for yeast and molds to grow exponentially. Since yeast and mold grow best in the presence of sugar, the greater availability of reducing sugar content during storage may be the other cause of the increased proliferation of these two types of microorganisms. All of the samples had almost identical counts at first, but when the samples were stored longer, the control sample's growth of yeasts and molds was noticeably greater than that of the other treated samples.

The exponential proliferation of microorganisms during the log phase might be the cause of this.

In fact, the least amounts of spoiling microbes had been seen in cardamom oil. The main constituents of cardamom essential oil, such as 1,8-cineol (43.7%), α-terpineol (9.5%), terpinene-4-ol (3.2%), spathulenol (2.7%), and apinene (1.6%), may be responsible for the reduction of the growth of spoiling microorganisms in sugarcane juice (G. Singh *et al.*, 2004). At the end of 28 days, the highest count for yeast and mold were found on the control samle C1 with the count of 13.06×10^5 whereas the lowest count for yeast and mold count were found on the cardamom oil sample with the count of 9.33×10^5. Comparing the data from the graph above, it is seen that the order of the samples for resisting the growth of the yeasts and molds are as follows:

$$C > T > C2 > C1$$

4.9 Correlation plot between Total plate count and TSS

In **Fig 4.9** below, the correlation between TSS and Total Plate count for Control-I (Juice only) is illustrated. The graph shows a negative correlation i.e., with increase in microbial population, TSS of the juice reduced during 28 days of storage life. This result supports the observation of increasing acidity and decreasing total sugars in the samples.

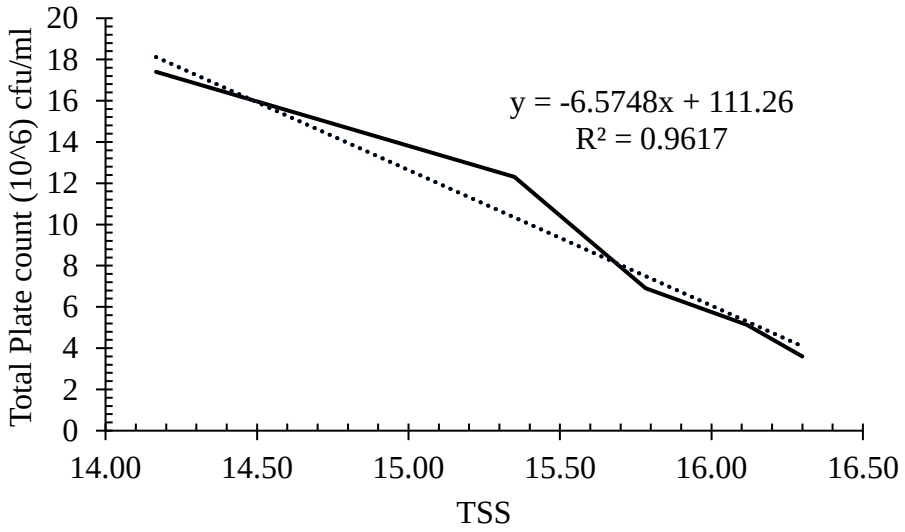

Fig 4.9: Correlation plot between Total plate count and TSS for control I

Below **Fig 4.10** is the correlation between TSS and Total Plate count for Control-II (Juice+ethanol). The graph shows a negative correlation i.e., with increase in microbial population, TSS of the juice reduced during 28 days of storage life.

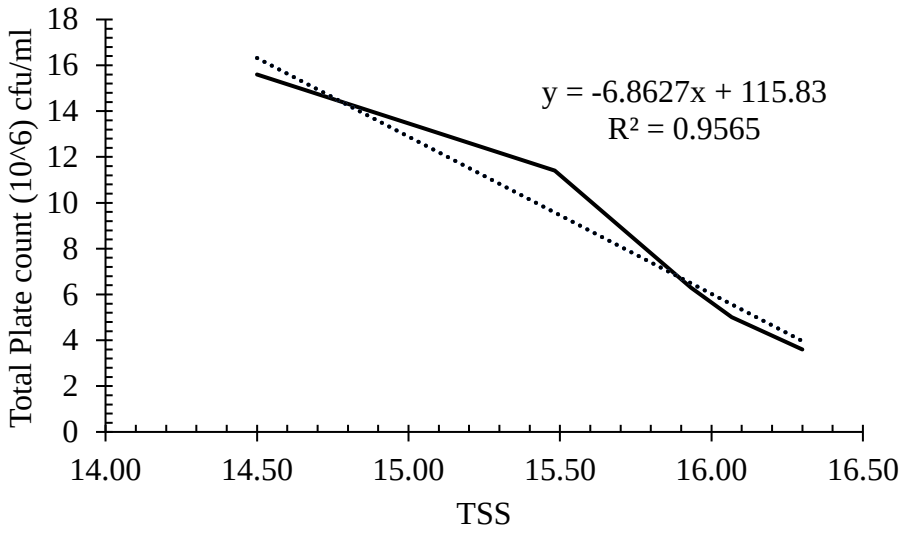

Fig 4.10: Correlation plot between Total plate count and TSS for control II

In **Fig 4.11** below, the correlation between TSS and Total Plate count for cardamom treated juice is shown. The graph shows a negative correlation i.e., with increase in microbial population, TSS of the juice reduced during 28 days of storage life. This sample C was the best sample for resisting the fall in the total sugar and increase in microbiological load during the storage period.

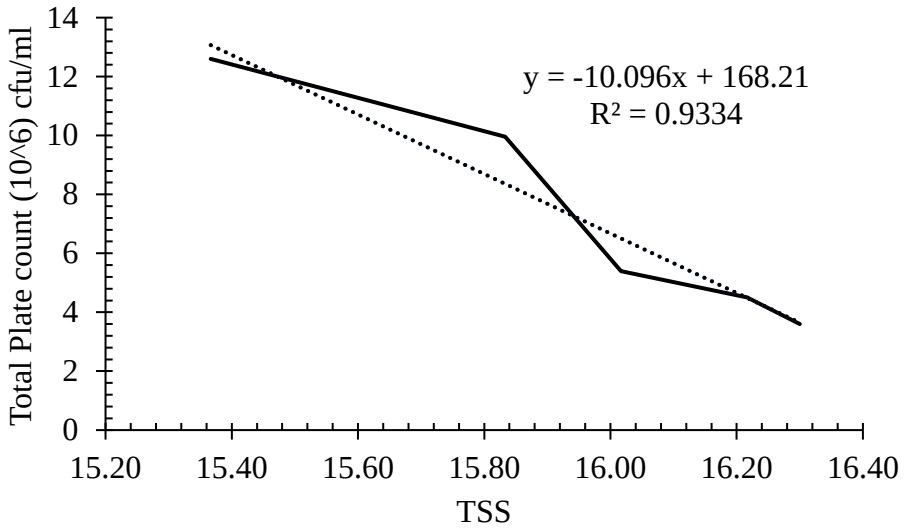

Fig 4.11: Correlation plot between Total plate count and TSS for cardamom oil treated juice

The correlation between TSS and Total Plate count for tejpat treated juice is illustrated in **Fig 4.12** below. The graph shows a negative correlation i.e., with increase in microbial population, TSS of the juice reduced during 28 days of storage life.

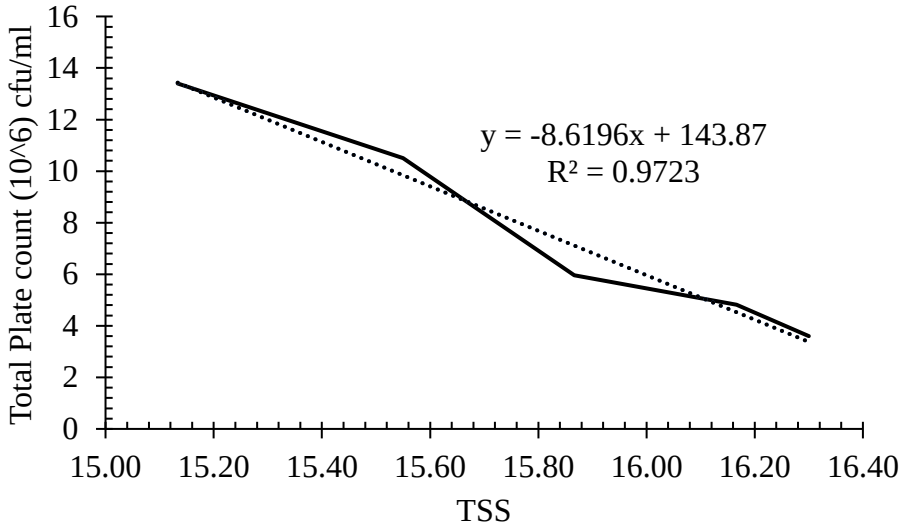

Fig 4.12: Correlation plot between Total plate count and TSS for tejpat oil treated juice

Part V

Conclusions and recommendations

5.1 Conclusions

As stated in the objectives, essential oils of cardamom and tejpat were extracted. On the basis of total soluble solids, total sugar, reducing sugar, pH, titratable acidity, total microbiological count, and yeast mold counts, the effects of essential oils on sugarcane juice were studied. Comparing the effects of the essential oils on sugarcane juice, the best essential oil was determined for preservative purpose among the cardamom and tejpat oils. The objectives and results of my study led me to develop the following conclusions:

1. There was a significant differences in % total sugar, % reducing sugar, pH, titratable acidity, total microbial count and yeast and mold count among the samples. However, no significant difference was observed statistically in TSS among the samples.
2. The addition of essential oils was effective in controlling the growth of microorganisms which indicate the preservative action of the essential oils. It may be deduced that, in comparison to the control samples, the essential oil exhibited comparable or greater antioxidant and antibacterial activity.
3. During observations, % reducing sugar, acidity, total microbial count and yeast & molds count were increased. However, total soluble solids, % total sugar and pH decreased across all samples.
4. Sugarcane juice sample containing cardamom essential oils was found to be superior than the sample containing tejpat essential oils.

5.2 Recommendations

Based on the result obtained from study, following things are recommended for betterment and improvement of the study:

1. Preservative properties of oleoresins and essential oils of different spices for different fruit juices could be studied.
2. Sensory analysis could be done.
3. Enzymatic changes and study could be done.

Part VI

Summary

Nepal is predominantly an agricultural nation. Crops of different kinds are grown around the country. Among several crops grown in Nepal, Sugarcane is one of the most important one. It is one the largest industrial crop that plays a pivotal role in national economy. Sugarcane juice degrades quickly due to microbial activity that initiates the fermentation. This is because sugarcane juice has a high concentration of nutrients. Fruit juices have been kept using various chemical preservatives, some of which may cause allergies in certain individuals and have other negative health effects, in an effort to combat the problem of spoiling. However, nowadays Customers' desire for fewer synthetic preservative-free items, such as spice's essential oils with natural antioxidant and antimicrobial agent, has developed recently due to health concerns.

Spices viz. cardamom and tejpat were collected, grounded and essential oils were extracted using hydro-distillation process from them. Sugarcane stalk were also collected, sorted, washed and then juices was extracted using mechanical juice extractor. The extracted juice was run through many layers of muslin fabric to filter it. The extracted filtered sugarcane juice was divided into four equal batches of 50 ml each in the conical flasks. Essential oils were not used during the preparation of control sample (I). Sugarcane juice was mixed with 100 µL of ethanol to create the control sample (II). In order to prepare the essential oils included juice sample, two batches of sugarcane juice were homogeneously combined with 10 µL of each essential oil (Tejpat and Cardamom) that had been pre-dissolved in 90 µL of ethanol using a rotary shaker. The samples were thereafter refrigerated for a period of 28 days, with observations being made every 7 days.

At day 28, control sample I and II showed inferiority in every aspects of physico-chemical and microbiological test than the sample incorporated with essential oils. And among the two essential oils, cardamom essential oils incorporated sample showed the best results. These findings indicate that the use of essential oils could be effective in preventing the sugarcane juice from rapid deterioration at refrigerated conditions.

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Appendices

Appendix A

List of Chemicals:

1. 97% Ethanol
2. Sodium Hydroxide
3. Oxalic Acid
4. Phenolphthalein
5. Lead Acetate
6. Potassium Oxalate
7. Carrez I and Carrez II
8. Fehling A and Fehling B
9. Dextrose
10. Methylene Blue
11. Citric Acid
12. Plate Count Agar
13. Malt Extract

List of Equipment:

1. Abbe Refractometer
2. Refrigerator
3. Weighing Balance
4. pH meter
5. Glassware
6. Hot Air Oven
7. Incubator
8. Rotary Shaker
9. Autoclave
10. Micropipette

Appendix B

Statistical analysis of the physico-chemical and microbiological changes occurring during storage.

Table B.1 ANOVA output for Total soluble solids of the juice samples at 5% level of significance (two way no blocking).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Sample	3	0.46160	0.15387	2.94	0.076	0.3153
No. of days	4	5.73750	1.43438	27.41	<.001	0.3525
Residual	12	0.62806	0.05234			
Total	19	6.82715				

Table B.2 Tukey test for Total soluble solids of the juice samples (95% confidence intervals)

Sample	Mean
Control I (C1)	15.54 a
Control II (C2)	15.66 a
Cardamom oil sample (C)	15.95 a
Tejpat oil sample (T)	15.80 a

* Similar alphabets a, b, c and d indicate no significant difference between the samples

Table B.3 ANOVA output for % total sugar of the juice samples at 5% level of significance (two way no blocking).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Sample	3	6.2351	2.0784	14.14	<.001	0.5282
No. of days	4	59.6300	14.9075	101.46	<.001	0.5906
Residual	12	1.7632	0.1469			
Total	19	67.6283				

Table B.4 Tukey test for % total sugar of the juice samples (95% confidence intervals)

Sample	Mean
Control I (C1)	13.46 a
Control II (C2)	13.71 ab
Cardamom oil sample (C)	14.91 c
Tejpat oil sample (T)	14.29 bc

* Similar alphabets a, b, c and d indicate no significant difference between the samples

Table B.5 ANOVA output for % reducing sugar of the juice samples at 5% level of significance (two way no blocking).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Sample	3	0.107567	0.035856	7.69	0.004	0.0941
No. of days	4	0.545959	0.136490	29.28	<.001	0.1052
Residual	12	0.055939	0.004662			
Total	19	0.709464				

Table B.6 Tukey test for % reducing sugar of the juice samples (95% confidence intervals)

Sample	Mean
Control I (C1)	0.7233 b
Control II (C2)	0.6293 ab
Cardamom oil sample (C)	0.5247 a
Tejpat oil sample (T)	0.5773 a

* Similar alphabets a, b, c and d indicate no significant difference between the samples

Table B.7 ANOVA output for pH of the juice samples at 5% level of significance (two way no blocking).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Sample	3	0.020797	0.006932	4.57	0.023	0.05366
No. of days	4	0.836686	0.209171	137.96	<.001	0.05999
Residual	12	0.018194	0.001516			
Total	19	0.875677				

Table B.8 Tukey test for pH of the juice samples (95% confidence intervals)

Sample	Mean
Control I (C1)	4.936 a
Control II (C2)	4.957 ab
Cardamom oil sample (C)	5.022 b
Tejpat oil sample (T)	4.985 ab

* Similar alphabets a, b, c and d indicate no significant difference between the samples

Table B.9 ANOVA output for titratable acidity of the juice samples at 5% level of significance (two way no blocking).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Sample	3	0.0072886	0.0024295	6.55	0.007	0.02654
No. of days	4	0.0430514	0.0107628	29.02	<.001	0.02967
Residual	12	0.0044498	0.0003708			
Total	19	0.0547897				

Table B.10 Tukey test for titratable acidity of the juice samples (95% confidence intervals)

Sample	Mean
Control I (C1)	0.3387 b
Control II (C2)	0.3152 ab
Cardamom oil sample (C)	0.2899 a
Tejpat oil sample (T)	0.2959 a

* Similar alphabets a, b, c and d indicate no significant difference between the samples

Table B.11 ANOVA output for Total Microbial Count of the juice samples at 5% level of significance (two way no blocking).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Sample	3	9.9830	3.3277	4.54	0.024	1.180
No. of days	4	349.6829	87.4207	119.32	<.001	1.319
Residual	12	8.7919	0.7327			
Total	19	368.4578				

Table B.12 Tukey test for Total Microbial Count of the juice samples (95% confidence intervals)

Sample	Mean
Control I (C1)	9.066 b
Control II (C2)	8.382 ab
Cardamom oil sample (C)	7.212 a
Tejpat oil sample (T)	7.656 ab

* Similar alphabets a, b, c and d indicate no significant difference between the samples

Table B.13 ANOVA output for Yeast and mold count of the juice samples at 5% level of significance (two way no blocking).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Sample	3	4.6640	1.5547	3.72	0.042	0.891
No. of days	4	219.3489	54.8372	131.16	<.001	0.996
Residual	12	5.0170	0.4181			
Total	19	229.0299				

Table B.14 Tukey test for Yeast and mold count of the juice samples (95% confidence intervals)

Sample	Mean
Control I (C1)	5.612 b
Control II (C2)	5.070 ab
Cardamom oil sample (C)	4.348 a
Tejpat oil sample (T)	4.596 ab

* Similar alphabets a, b, c and d indicate no significant difference between the samples

Color Plates

Plate 1 Extraction of essential oils using cleavenger apparatus

Plate 2 Mixing of the juice sample with essential oils using rotary shaker

Plate 3 Four different prepared samples

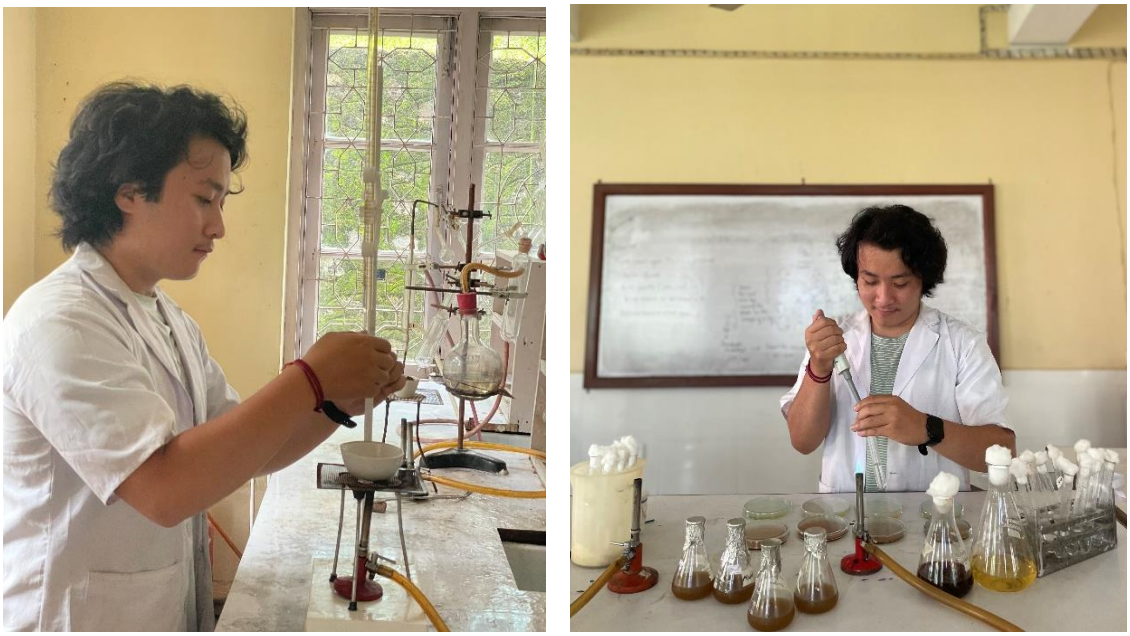

Plate 4 Carrying out titrimetric and microbial analysis

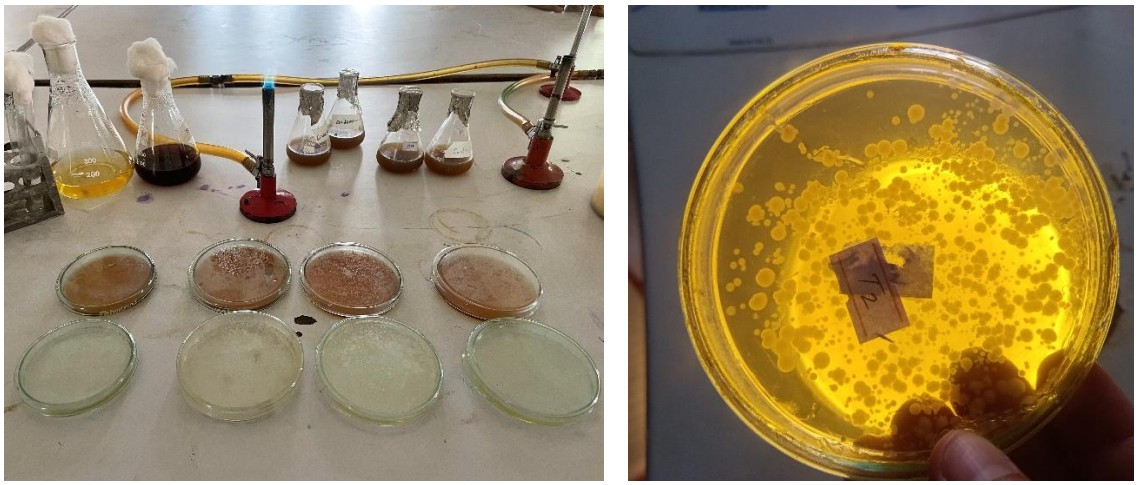

Plate 5 Microbiological analysis