EFFECT OF PHYTOCONSTITUENTS OF HERBS AND SPICES EXTRACT ON STORAGE STABILITY OF MINCED CHICKEN MEAT UNDER REFRIGERATION STORAGE

by

Bikram Shrestha

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

2022

Effect of Phytoconstituents of Herbs and Spices Extract on Storage Stability of Minced Chicken Meat under Refrigeration Storage

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

by

Bikram Shrestha

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

April, 2022

Tribhuvan University

Institution of Science and Technology

Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This *dissertation* entitled *"Effect of Phytoconstituents of Herbs and Spices Extract on Storage Stability of Minced Chicken Meat under Refrigeration Storage*" presented by Bikram Shrestha has been accepted as the partial fulfillment of the requirements for Bachelor degree in Food Technology.

Dissertation committee

1. **Chairperson** _____

   (Mr. Nabin Gautam, Asst. Prof.)

2. **External Examiner** _____

   (Mr. Anup Halwai)

3. **Supervisor** _____

   (Mr. Devraj Acharya, Asst. Prof.)

4. **Internal Examiner** _____

   (Mr. Arjun Ghimire, Asst. Prof.)

5. **RMC Member** _____

   (Mr. Basanta Kumar Rai, Prof.)

April 18, 2022

Acknowledgements

I would like to express my special gratitude to my dissertation supervisor Mr. Devraj Acharya, Asst. Prof. CDFT, Hattisar, Dharan for his excellent guidance, encouragement and inspirations throughout my thesis work.

I owe my gratefulness to Assoc. Prof. Dil Kumar Limbu (Campus Chief, Central Campus of Technology), Asst. Prof. Nabin Gautam (HOD, Department of Food Technology) and Prof. Basanta Kumar Rai (Chairperson, Central Department of Food Technology) for providing facilities to perform this work successfully.

Furthermore, I would like to thank my friends Roshan Bokhim Limbu, Aadarsha Poudel, Mamata GC, Pragya Poudel, Asmita Bhandari and my senior Satish Mandal for their support and co-operation. I am indebted to all the teachers, technical and non-technical staffs of Central Campus of Technology and those who have directly and indirectly supported in my endeavor

I would also like to thank all my classmates and finally, I am indebted to my parents without whose constant encouragements, inspirations and affection, I would never have reached this far, and this work would have never seen the light of the day.

Date of submission: April, 2022

Bikram Shrestha

Abstract

Effect of phytochemical constituents of commonly found herbs and spices on shelf life of ground chicken meat was studied. Five different herbs and spices viz. onion leaves, garlic leaves, hattibar leaves, radish leaves and chiraito were collected and their methanolic extracts prepared. The extracts were then analyzed for total phenols, total flavonoids, total tannin and total radical scavenging capacity. The extract of each sample was incorporated in the ground meat at the concentration of 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm of total ground meat mass. Then the subsequent changes in thiobarbituric acid reactive substances (TBARS) value and total plate count (TPC) of the ground meat was studied at 0, 4, 7, 10 and 12 days in the refrigerated system at 4°C.

The highest amounts of phenols, flavonoids, tannins and antioxidant activity were found in the methanolic extract of onion leaves (99.34±2.92 mg GAE/g), radish leaves (53.04±0.41 mg QE/g), onion leaves (26.58±0.16 mg tannic acid/g) and chiraito (IC_{50}= 24.96±0.40 µg/ml) respectively. Similarly, the lowest amounts of phenols, flavonoids, tannins and antioxidant activity were found for methanolic extract of hattibar leaves (13.27±0.69 mg GAE/g), garlic leaves (16.62±2.97 mg QE/g), radish leaves (10.72±0.059 mg tannic acid/g) and hattibar leaves extract (IC_{50}=952.28± 1.43 µg/ml) respectively. The untreated meat sample exceeded the threshold TBARS (1 mg MDA/kg) value on 7^{th} days of storage and only 100 ppm of garlic leaves extract, chiraito extract and radish leaves extract incorporated samples exceeded threshold value on 12^{th} days of storage. Highest and lowest TBARS values were obtained from untreated meat sample (1.71±0.82 mg MDA/kg sample) and 500 ppm of chiraito extract incorporated meat (0.24±0.01 mg MDA/kg sample) on 12^{th} days of observation. In term of antimicrobial activity, only the control and 100 ppm of onion leaves incorporated samples exceeded legal threshold value (10^7cfu/g) on 4^{th} days and remaining samples exceeded on 10^{th} days of storage except 500 ppm chiraito extract incorporated which exceeded on 12^{th} days. And effectiveness increased with increased in concentration of each extract. Thus, garlic leaves extract, onion leaves extract, radish leave extract, chiraito extract and hattibar leaves extract significantly increased the shelf life of meat as compare to untreated meat sample.

Contents

List of Table

List of Figure

List of Plates

List of Abbreviations

Abbreviation	Full form
BHA	Butylated hydroxy anisole
BHT	Butylated hydroxyl toluene
CE	Chiraito extract
FAO	Food and agricultural organization
GLETBHQ	Garlic leaves extract
HLE	Hattibar leaves extract
Lox	Lipid oxidation
OLE	Onion leaves extract
RLE	Radish leaves extract
TAC	Total antioxidant capacity
TBARS	Thiobarbituric acid reactive substances
TBHQ	Tert-butyl hydroquinone
TPC	Total plate count

Part I

Introduction

1.1 General Introduction

The term "meat" refers to flesh, skeletal muscle, and any attached connective tissue or fat other than bone and bone marrow (Williams, 2007). Meat is a good source of protein, essential fatty acids, minerals and vitamins, but it is easy to spoil because it provides a suitable growth environment for various microorganisms (Bantawa *et al.*, 2018). Chicken meat is most favored by consumer around world because it has numerous advantageous dietary attributes like a low lipid content and somewhat high grouping of polyunsaturated fats (Bourre, 2005; Patsias *et al.*, 2008). Meat items are normally marketed at refrigerated temperatures (2–5 °C) (Petrou *et al.*, 2012). Spoilage of raw meat during refrigeration can occur in two ways: microbial growth and oxidative rancidity. The deterioration of the quality of fresh poultry meat has brought an economic burden to producers, and has led to the development of methods to extend the shelf life and general safety/quality as a major issue faced by poultry processing industry (Babuskin *et al.*, 2014).

Lipid oxidation, which is started in the unsaturated fats part in subcellular layers, is a significant reason for the disintegration and diminished time span of usability of meat items (Devatkal *et al.*, 2010). Lipid oxidation may produce changes in meat quality boundaries like tone, flavor, smell, surface, and surprisingly dietary benefit (Kolakowska, 2002). Likewise, meat and poultry items have as often as possible been discovered to be defiled with microorganisms during the butchering and assembling measure. These microorganisms produce unfortunate quality changes in meats, particularly corresponding to lactic corrosive microbes, a significant bacterial gathering related with meat decay (Doulgeraki *et al.*, 2012). By applying antioxidants and antibacterial agents to meat products, oxidation of lipids and proliferation of microorganisms during storage can be inhibited, which leads to delay in spoilage, longer life, maintaining quality and safety (Babuskin *et al.*, 2014). Food antioxidants are added to protect the lipid component from degradation. Synthetic antioxidants commonly used include butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butyl hydroquinone (TBHQ) (Peter and Shylaja, 2012).

Plant phenolic and polyphenolic constituents can be found in a wide range of foods, including vegetables, fruits, soybeans, grains, tea, coffee, red wine, and herbal extracts. Extracts from medicinal plant and culinary herb sources have very substantial antioxidant activity in vitro, according to (El-Alim *et al.*, 1999) due to presence of compounds such as polyphenolics, flavonoids, lignans, and terpenoids (Craig, 1999). There is a growing demand for natural antioxidants for the questionable action of these compounds as carcinogens. Antioxidants play a role in the body's defense mechanisms against cardiovascular disease, cancer, arthritis, asthma, and diabetes. Because many herbs and spices are known to be excellent sources of natural antioxidants, consumption of fresh herbs in your diet can contribute to your daily intake of antioxidants. Herbs and spices are an important source of antibacterial agents, and the use of spices, spice essential oils or active ingredients to control the growth of microorganisms in foodstuffs constitutes an alternative approach to chemical additives. The essential oils of some spices (used individually or in combination) are highly suppressed against selected pathogenic and putrefactive microorganisms (Peter and Shylaja, 2012).

Unlike synthetic compounds, natural preservatives obtained from spices are rich in phenolic compounds, reducing lipid oxidation and microbial growth and improving overall quality (Babuskin *et al.*, 2014).

1.2 Statement of the problem

Meat is one of the most important, nutritious and popular foods to help meet most physical requirements (Ahmad *et al.*, 2018). In recent years, rapid economic growth and the globalization of the food industry have increased interest in chicken production and consumption (Kamboh and Zhu, 2013). According to FAO (2010), the average per capita meat consumption in Nepal is 11.15 kg per year. Chicken accounts for 58% of total meat production in Nepal. Chicken is susceptible to perishing rapidly for high levels of protein and moisture. After that, the food industry may find a way to extend its lifespan recently. Various chemical preservatives are generally used to control the spoilage but they are undesirable to consumers for their adverse effects (Bazargani-Gilani *et al.*, 2015). Meat items are normally marketed at refrigerated temperatures (2–5°C). Lipid oxidation and microbial growth may occur during refrigeration storage (Zhang *et al.*, 2016) which causes the economic loss. Lipid oxidation is a highly complex set of free radical reactions between fatty acids and oxygen, which results in oxidative degradation of lipids,

also known as rancidity (Mozuraityte *et al.*, 2016). Lipid oxidation does not only produce offensive and flavour but also can decrease nutritional quality and safety by producing secondary lipid oxidation metabolite in food (Frankel, 1980).

Meat is also very much susceptible to spoilage due to chemical and enzymatic activities (Dave and Ghaly, 2011). Lipid oxidation is major cause of loss of quality of meat and meat product (Love and Pearson, 1971). Various synthetic antioxidants like BHT, PG, BHA, TBHQ etc. are used to control rancidity but they are undesirable to consumers for their adverse effects (Babuskin *et al.*, 2014). Thus, use of antioxidants obtained from different herbs and spices extract at specific amount can delay the lipid oxidation of chicken meat and increase shelf life of chicken meat.

1.3 Objectives

1.3.1 General objective

The general objective of study was to study effect of phytoconstituents of different herbs and spices extract on storage stability of minced chicken meat.

1.3.2 Specific objectives

The specific objectives were as follows:

1. To prepare the methanolic extract of onion leaves, garlic leaves, hattibar leaves, radish leaves and chiraito.
2. To find out the total phenol content, total flavonoids content, total tannin content and total antioxidant capacity of methanolic extracts of chiraito, garlic leaves, hattibar leaves, onion leaves and radish leaves.
3. To study the effect of phytoconstituents of herbs and spices on storage stability of minced chicken meat.

1.4 Significance of the study

This study will facilitate the promotion of use of natural ingredients (antioxidant) for the extension of shelf life of ground chicken meat. Because of the suspected action of synthetic antioxidant promoters of carcinogenesis, there is growing demand for natural antioxidants. Many herbs and spices are known as excellent sources of natural antioxidants. Various herbs and spices are rich in polyphenols and flavonoids which acts as natural antioxidant as they

have been used for human diet from thousands of year ago in traditional medicine and flavor, color, aroma of food (Babuskin *et al.*, 2014). Apart from this herbs and spices also have antimicrobial properties. By using the Phytochemicals extract of herbs and spice can increase the shelf life of meat by preventing lipid oxidation and controlling microbial spoilage. In the present scenario, the anti-diabetic, anti-hypercholesterolemic, anti-carcinogenic, anti-inflammatory effects of herbs and spices have paramount importance, as the key health issues of mankind nowadays are diabetes, cardio-vascular diseases, arthritis and cancer. The main significance of this study is to promote the use of natural antioxidant for preservation of ground chicken meat over synthetic antioxidant thus assuring food safety.

1.5 Limitations and delimitation of the study

- Phytochemical diffusion and retention kinetics were not studied.
- Phytochemical was extracted from few herbs and spices only.
- Variation in storage temperature of meat could not be done.
- Only methanolic extraction was used for the determination of phenols.

Part II

Literature review

2.1 Chicken meat

Meat is made up of fatty acids, amino acids and vitamins, and there are major food groups in the diet. Lean meat is the best source of bioavailable iron, which contains many key nutrients (McAfee *et al.*, 2010). In 2017, the production and consumption of poultry meat increased by 117 million tons as compared to beef (68 million tons) and pork (115 million tons) (Massingue *et al.*, 2018). This sign is likely related to nutritional properties, and the competitive price of chicken compared to lean meat. Increased prevalence of red meat consumption-related diseases, including meat and cardiovascular disease (CVD), diabetes and several types of cancer, and increased incidence of colorectal and pancreatic cancers (Akramzadeh *et al.*, 2020).

Chicken meat is a rich source of high-value protein, omega-3 polyunsaturated fatty acids, zinc, iron, selenium, potassium, magnesium, sodium, vitamin A, B-complex vitamins, and a variety of fats including folic acid. Its composition depends on the breed, age, the type of feed consumed, the climatic conditions and even the pieces of meat, resulting in significant differences in nutritional and sensory properties (Ahmad *et al.*, 2018). Composition of meat also depends on age, sex, feed, slaughter practices etc. (Mir *et al.*, 2017). Chen *et al.* (2016) reported that the moisture, ash, intramuscular fat (IMF) and protein content of different chicken breeds was in the range of 74.42 to 74.93, 1.11 to 1.40, 1.12 to 2.03, and 20.46 to 22.37 g/100 g, respectively for breast meat. The values so obtained for moisture content, crude protein, crude fat and ash content were slightly different then the result obtained by Ali *et al.* (2007) which were 75.47±1.44%, 22.04±0.48%, 1.05±0.30% and 1.07±0.04% respectively. According to Ahmad *et al.* (2018) the proximate of different parts of chicken meat are shown in Table 2.1

Table 2.1 Proximate of chicken meat, chicken breast with skin or without skin.

Meat cut	Protein (g)	Sat. fat (g)	Fat (g)	Energy (Kcal)	Vit.B_{12} (μg)	Na (mg)	Zc (mg)	P (mg)	Fe (mg)
Chicken Meat	22.8	0.6	1.9	113	0.7	78	1.4	202	0.7
Chicken breast, Raw	24.2	0.2	8.5	178	0.39	71	0.9	199	1.2
Chicken breast skinless Raw	23.8	0.4	1.28	109	0.40	59	0.7	218	0.4

Source: Ahmad *et al.* (2018)

2.2 Oxidation of lipid

Lipids are an important component of all types of meat, and are responsible for many desirable properties of meat (Amaral *et al.*, 2018). They are important for the taste and aroma profile of meat and contribute to tenderness and juiciness (Min and Ahn, 2005). Lipid Oxidation affects color, texture, nutritional value, flavor and aroma and induces rancidity, which causes offensive odors and unauthorized tastes, which are important reasons for consumer rejection (Amaral *et al.*, 2018). "Quality" and "health" are known as part of the, which are the most important factors influencing food choices, and the appearance, color, texture, taste and aroma of the are meat-accepting, controlling, or at least. Minimization of minimization of lipid oxidation process, has attracted great interest in the food industry (Brøndum *et al.*, 2000).

The development of oxidative rancidity in meat begins at the time of slaughter, when blood flow is interrupted, and the metabolic processes are blocked (Amaral *et al.*, 2018) Lipid oxidation is initiated in the unsaturated fatty acid fraction by the dissociation of hydrogen atoms and propagates as a radical mediated chain reaction. In fact, they form peroxide, which is also susceptible to oxidation, decomposes to form secondary reaction products of aldehydes, ketones, acids, alcohols, etc. In many cases, these compounds are involved in changes in taste, aroma, taste, and nutritional value that affect overall quality (Babuskin *et al.*, 2014). Oxidation begins with phospholipids and is catalyzed by heme

proteins, such as hemoglobin and myoglobin, cytochromes, free iron, enzymes, and sodium chloride. Phospholipids are found in cell membranes and are rich in polyunsaturated fatty acids; therefore, these are very susceptible to oxidation (Brøndum *et al.*, 2000). The nature and relative proportions of compounds formed by lipid oxidation depend on the characteristic lipid composition of the slaughtered animal, and they also depend on many other factors such as processing methods, storage conditions, types of ingredients, and presence and concentrations of pro- or antioxidants. It is important to mention that the animal lipid profile also varies according to a number of factors, including animal diet and lifestyle (Min *et al.*, 2005).

2.2.1 Mechanism of lipid oxidation

Lipid oxidation is the main cause of deterioration of meat adipose tissue. The direct impact on the commercial value and product of meat is a voluntary and unavoidable process. Lipids are one of the most chemically unstable food ingredients involved in oxidative reactions triggered by multiple factors through highly complex mechanisms. The main known factors associated with these reactions include the type of geological structure and the environment. Fatty acid saturation, light and heat exposure, molecular oxygen, the presence of pro-oxidants and antioxidants are factors that affect the oxidative stability of lipids (Min *et al.*, 2005). The nature and relative proportions of compounds formed by lipid oxidation depend upon the characteristic lipid composition of the slaughtered animal, and that they also rely upon many other factors like processing methods, storage conditions, sorts of ingredients, and presence and concentrations of pro- or antioxidants. It's important to say that the animal lipid profile also varies in line with variety of things, including animal diet and lifestyle (Min and Ahn, 2005).

Natural components found in muscle tissue such as iron, myoglobin (Mb), hydrogen peroxide (H_2O_2) and ascorbic acid induce lipid oxidation, act as catalysts and reactive oxygen species (ROS). Promotes the formation of. Oxidation reactions can be initiated by physical factors such as radiation and light. Therefore, in biological systems, lipids are oxidized through three major reactions: photooxidation, enzymatic oxidation and autoxidation (Amaral *et al.*, 2018).

Lipid Oxidation (LOx) is defined as a chain reaction of free radicals and consists of three stages: initiation, propagation, and termination. In the course of the reaction, there is a free radical that reacts with the hydrocarbon chain of the fatty acid forming peroxides, which, in turn, react with other hydrocarbon chains abstracting hydrogens originating hydroperoxides. The carbon chain, from which the hydrogens have been abstracted, will act as new peroxide, perpetuating the cycle (Estévez, 2015). Free radicals are highly reactive species of multiple free electrons that can exist independently over a short period of time. Some examples of these reactive oxygen species are hydroxyl radical (HO •), oxygen radical of organic compounds, furoxy (ROO •) and alkoxyl (RO •) radical, superoxide radical (O^2) and and its radical conjugate hydroperoxide acid ($HO•_2$), and singlet oxygen ($O^1{}_2$). These reactive oxygen molecules can occur intentionally or accidentally. In biological systems, it is produced during normal aerobic metabolism. Mitochondria consume molecular oxygen and reduce it in successive steps to produce ATP and H_2O. In this process, $O^1{}_2$, H_2O_2, HO is formed as unnecessary by-products. Meanwhile, cells protecting the body (phagocytes) deliberately produce $O^1{}_2$ and H_2O_2 inactivate bacteria and viruses (de Lima Júnior *et al.*, 2013).

In the starting step of LOx, a hydrogen atom (H•) leaves the double bond of a carbon unsaturated fatty acid at the adjacent (RH)alkyl R•(E1) to form a radical (Van Hecke *et al.*, 2017).

R: H + O: O + initial → R• (alkyl radical) + HOO • (1)

This alkyl radical can react with molecular oxygen to generate species of various radicals, such as the peroxyl (ROO•) radical (E2). If so, these radicals can find stability in subsequent propagation steps by pulling hydrogen atoms from other sensitive molecules such as adjacent RH and new R (E3) forming lipid hydroperoxides(ROOH) (Min *et al.*, 2005).

R • + O2 ≠ ROO • (2)

ROO • + RH → ROOH + R • (3)

This propagation mechanism can occur up to 100 times before the two R • combine and terminate the process. The radical species formed in the process have the potential to stabilize non-radical compounds. Peroxides normally formed as LOX secondary products are then subjected to cleavage, forming low molecular weight volatile and non-volatile

compounds (secondary LOX products) such as carbonyls, alcohols, hydrocarbons, francs, etc. Of these, aldehydes are among the most abundant products contained in meat, including hexanal malondialdehyde (MDA) and 4-hydroxy-2-trans-nonenol (Estévez, 2015).

Oxidative degradation occurs according to the above mechanism as immediately upon exceeding the antioxidant capacity of proteins and other redox components in the environment (Estévez, 2015). After slaughter, the antioxidant mechanism collapses and biochemical changes occur in vivo. This promotes the oxidation that occurs when converting muscle to meat flavour oxidation (Amaral *et al.*, 2018). Since H^+ can promote redox circulation of myoglobin, and can promote oxidation-promoting action, the decrease in pH promotes oxidation of muscle components. In addition to the lowering of pH, other post-mortem biochemical changes, such as changes in cell partitioning and release of free catalytic iron and oxidase, also contribute to the promotion of Lox (Zhang *et al.*, 2011). The degree of postmortem meat Lox is highly dependent on meat origin, muscle type and storage conditions (Estévez, 2015).

2.2.2 Peroxide value

Peroxide can be used to determine the degree of oxidation of lipids, fats, and oils. The peroxide value indicates the degree of peroxidation and quantifies the total amount of peroxides in the substance (Kouba and Mourot, 2011). Autoxidation is aided by the double bonds found in fats and oils. Oils with a high degree of unsaturation are the most prone to autoxidation. The peroxide value is the best test for autoxidation (oxidative rancidity). Peroxides are products of the autoxidation reaction. Autoxidation is a free radical reaction involving oxygen that causes fats and oils to deteriorate, resulting in off-flavors and off-odors. Peroxide value, or the concentration of peroxide in an oil or fat, is useful for determining the extent of spoilage (Chakrabarty, 2003).

2.2.3 Acid Value

The amount of free fatty acids in a fat is determined by the acid value. When freshly extracted from the source, natural fats and oils are mostly in the triglyceride form. However, when triglycerides are stored for an extended period of time, they begin to degrade, releasing free fatty acids (FFA). This hydrolysis is caused by a number of factors, including the presence of moisture in the oil, elevated temperatures, and, most importantly, lipases (enzymes) from the source or contaminating microorganisms. As a result, the neutral oil is transformed into

a complex mixture of triglycerides, diglycerides, monoglycerides, free fatty acids, and glycerol. Some fats and oils are fairly stable, but others, such as crude rice-bran oil, are notoriously susceptible to hydrolysis. Whichever the oil, presence of excess free fatty acids is a sure indicator to unnatural state of oil (Poudel, 2020).

2.2.4 Thiobarbituric acid reactive substances (TBARS number)

The primary oxidation products were discovered to be further oxidized to form secondary and tertiary oxidation products. These products contain a high concentration of aldehydes with small to large chain structures (Frankel and Edwin, 1987). One of the primary causes of rancidity in foods during preparation and storage is the presence of aldehydes and other reactive substances (Warner *et al.*, 2001). Malondialdehyde (MDA) is an important oxidation product that is thought to be the main marker in lipid peroxidation (Xiong *et al.*, 2015). Malondialdehyde (MDA) is one of the most abundant aldehydes produced during secondary lipid oxidation, and it is also one of the most commonly used oxidation markers (Barriuso *et al.*, 2013). The presence of oxidized lipids in human and animal diets increased the levels of thiobarbituric acid reactive substances (TBARS) in plasma and tissue (Ruban, 2009). MDA is frequently the most abundant individual aldehyde produced by lipid peroxidation in foods. Its concentration in meat and fish products could be as high as 300 µM or higher (Kanner, 2007).

Thiobarbituric acid (TBA) reacts with MDA to produce a color compound that can be measured spectrophotometrically, chromatographically, or using image processing techniques (Xiong *et al.*, 2015). Because of TBA's reactivity with several reactive substances in biological samples, a more widely accepted terminology known as thiobarbituric acid reactive substances (TBARS) is now widely used (Sun *et al.*, 2001). TBARS is now recognized as a reliable marker of lipid peroxidation-induced oxidative stress (Tsai and Huang, 2015). When frying meat or meat products, several oxidation products are produced, which can be measured using the TBA-MDA adduct and HPLC. There are several HPLC methods available for the TBARS assay (Al-Rimawi, 2015). The threshold TBARS value is 1 mg MDA/ kg of meat. Several study shows that MDA concentration over 0.5 mg/ kg of meat sample indicated some oxidation but there is not any legislative limit and values above 1.0 mg/kg as possibly unacceptable levels (Reitznerová *et al.*, 2017).

2.3 Herbs and spices

Herbs can be defined as plant materials (leaf, root, stem, flowering tips etc.) of aromatic plants used to impart flavour, aroma, taste to the food sometime with addition of colour. Herbs are lived plant that die down at the end of growing season. It is woody plant that is annual, biennial, perennial (Ravindran, 2017).

According to FAO(1994), spice can be defined as vegetable products such as leaves, roots, flowers, seeds etc. that are rich in essential oil and aromatic principle (Ravindran, 2017). The Food and Drug Administration Compliance Policy Guideline (FDA-CPG Sec. 525.750) recognizes the term spice to refer to aromatic vegetable substances in whole, broken, or ground form, whose significant function in food is seasoning rather than nutrition and from which no portion of any volatile oil or other flavoring principle has been removed (Atungulu and Pan, 2012).

2.4 The role of herbs and spices in health

The antioxidant properties of herbs and spices are of particular interest in view of the impact of oxidative modification of low-density lipoprotein cholesterol in the development of atherosclerosis. There is level III-3 evidence (National Health and Medical Research Council [NHMRC] levels of evidence1) that consuming a half to one clove of garlic (or equivalent) daily may have a cholesterol-lowering effect of up to 9%. There is level III-1 evidence that 7.2 g of aged garlic extract has been associated with anticlotting (in-vivo studies), as well as modest reductions in blood pressure (an approximate 5.5% decrease in systolic blood pressure). A range of bioactive compounds in herbs and spices have been studied for anticarcinogenic properties in animals, but the challenge lies in integrating this knowledge to ascertain whether any effects can be observed in humans, and within defined cuisines. Research on the effects of herbs and spices on mental health should distinguish between cognitive decline associated with ageing and the acute effects of psychological and cognitive function (Tapsell *et al.*, 2006).

There is level I and II evidence for the effect of some herbal supplements on psychological and cognitive function. There is very limited scientific evidence for the effects of herbs and spices on type 2 diabetes mellitus, with the best evidence being available for the effect of ginseng on glycaemia, albeit based on four studies. More research is required, particularly examining the effects of chronic consumption patterns. With increasing interest

in alternatives to non-steroidal anti-inflammatory agents in the management of chronic inflammation, research is emerging on the use of food extracts. There is level II evidence for the use of ginger in ameliorating arthritic knee pain; however, the improvement is modest and the efficacy of ginger treatment is ranked below that of ibuprofen. More definitive research is required. Public health and dietary implications Recommendations for intakes of food in the Australian guide to healthy eating do not yet include suggested intakes of herbs and spices (Tapsell *et al.*, 2006).

Future consideration should be given to including more explicit recommendations about their place in a healthy diet. In addition to delivering antioxidant and other properties, herbs and spices can be used in recipes to partially or wholly replace fewer desirable ingredients such as salt, sugar and added saturated fat in, for example, marinades and dressings, stir-fry dishes, casseroles, soups, curries and Mediterranean-style cooking. Vegetable dishes and vegetarian options may be more appetizing when prepared with herbs and spices (Tapsell *et al.*, 2006).

2.5 Phytochemicals

Phytochemicals consist of a large class of natural non-nutritive, biologically active compounds found in plants. As the prefix "Phyto" in the name implies, phytochemicals are basically produced only by plants. Phytochemicals serve as the natural defense system to host plants and provide color, aroma and taste. Plants use phytochemicals as natural protection against bacteria, fungi and viruses (Ramanthan *et al.*, 1989) More than 4,000 of these compounds have been discovered, and scientists are expected to find more phytochemicals in plant foods, such as fruits, kinds of vegetables, legumes, grains, herbs, and spices (Rowland, 1999).

Any phytochemicals are known, including: alkaloids, saponins, flavonoids, tannins, glycosides, anthraquinones, steroids and terpenoids. They not only protect plants, but also have tremendous physiological activities for humans and animals. These include cancer prevention, antibacterial, antifungal, antioxidant, hormone action, enzyme stimulation , etc. (Doss and Anand, 2012) Phytochemicals give hot pepper the burning sensation, onions and garlic the pungent flavour and tomatoes their red colour (Lesschaeve and Noble, 2005) Phytochemicals have far-reaching physiological effects, which can act as antioxidants,

simulate hormones in the body and inhibit the development of diseases in the body (Hayes, 2005).

2.5.1 Classes of major phytochemicals, food sources and nutritional benefits

There are many types of phytochemicals that can be found in all plant products, including fruits, kinds of vegetables, beans, grains, herbs and spices. No plant material naturally has all the important phytochemicals needed by humans. Therefore, it is recommended that you consume a variety of plant materials, including fruits, vegetables, grains, herbs, and spices to maximize the benefits of the rich combination of phytochemicals. Most of the plant materials in the human diet contain some important phytochemicals. Some good food sources phytochemicals are cabbage, lettuce, tomatoes, carrots, watermelon, mangoes, papaya, grapes, oranges, apples, cashew apples and nuts, mustard, pears, oats (Obeta, 2015).

According to Birt (2006), phytochemicals work in synergy and their effects when served together are stronger than the sum of the effects of parts served separately. The thousands of phytochemicals so far discovered are group-based. Table 2.2 shows some of these phytochemicals, their sources and biological function.

Table 2.2 Phytochemicals, their uses and functions

Phytochemicals Class	Phytochemicals	Sources	Potential nutritional Benefits
Carotenoids	β-carotene, & carotene, lutein, lycopene	Tomato, pumpkin, Carrot, watermelon. Guava, dark yellow pink and red coloured vegetative fruits	Act as antioxidant Reduce level of cancer Producing enzymes Inhibit spread of cancer
Polyphenols	Tannins	Fruits, Legumes, Green vegetable, black tea	Exhibit antimicrobial and antioxidant activities Increase antioxidant activity Prevent proliferation of cancer Help speed excretion of carcinogen from the body
Flavonoids	Anthocyanine, Anthoxanthin	Beans, citrus fruits	Block access of carcinogen, prevents Malignant change in cells Prevents cancer
Saponins	Panaxadiol, panaxatriol	Potato, tomato, soybean, beans	Reduce glucose and glycerol uptake in the gut.
Terpenes	Mono-terpenes	Garlic, maize, ginger	Help detoxify carcinogens, inhibit spread of cancer

Isothiocyanates	Allylisothiocyanate, indoles, sulforaphane	Cruciferous vegetables including cabbage, curliflower, broccoli	Suppress tumor growth, boost proliferation of cancer-fighting enzymes

Source: Birt (2006)

2.5.2 Polyphenols

Polyphenols, which include more than 8,000 compounds, are a family of natural compounds widely distributed in the outer layers of plant as suspected from their protective function in the plants (Manach *et al.*, 2004). Phenolic acids are secondary metabolites extensively spread throughout the plant kingdom. Phenolic compounds confer unique taste, flavour, and health promoting properties found in vegetables and fruits (Ghasemzadeh and Ghasemzadeh, 2011). They range from simple molecules such as phenolic acids to highly polymeric compounds such as tannins. Phenolic acid represents about one-third of the total amount of polyphenols in the human diet. These compounds have the ability to reduce free radicals, catalyze metal chelating; activating antioxidant enzymes, reducing α-tocopherol radicals and inhibiting oxidation (Oboh, 2006). As a result, they neutralize free radicals formed during normal physiological functioning of human body (Burns *et al.*, 2001).

Phenolic compounds regarded to be antioxidants play the very vital position of defensive organisms in opposition to dangerous outcomes of oxygen radicals and different surprisingly reactive oxygen species (Stratil *et al.*, 2006). The antioxidant activity of phenols stems from their redox properties, through which they act as hydrogen donors, singlet oxygen quenchers, reducing and metal chelating agents. There is a very positive correlation between total phenols and the antioxidant activity of more than plant materials. Polyphenols are the most abundant antioxidants in the diet and are widespread constituents of fruits, vegetables, cereals, dry legumes, chocolate, and beverages, such as tea, coffee, or wine (Scalbert *et al.*, 2005). The scavenging capacity of phenols is in particular because of the phenolic structure of hydroxyl substituent at the aromatic ring (Robbins, 2003). Furthermore, a definitely and surprisingly significant relationship among general phenolics and antioxidant activity changed into documented by Velioglu *et al.* (1998) which implied a compound with better content material of phenol possessed better antioxidant activity.

Phenolic phytochemicals are the largest category of phytochemicals and the most widely distributed in the plant kingdom (Shahidi, 2000). Structurally, they contain aromatic ring containing one or more hydroxyl groups (O'connell and Fox, 2001). Based on the number of carbon atoms present in its structure, phenolics are categorized into five major groups:

1 C_6 group: This group of phenol includes simple phenols and benzoquinones with six carbon atoms.

2 C_6C_n group: Phenolic acid and hydroxycinnamic acid derivatives are included in this group.

3 C_6-C_n-C_6 group: The largest group of phenolic compound includes flavonoids which have low molecular weight and are further of five types (flavones, flavonols, flavanols, flavanones and anthocyanins) on the basis of substitution pattern of carbon ring

4 $(C_6$-$C_3)_n$ group: This group consists of lignins and lignans.

5 Tannins: Tannins are high molecular weight phenols and classified into two main categories (hydrolysable and condensed tannins) (O'connell and Fox, 2001).

The antioxidant capacity of polyphenols in any diet is much higher than the combined antioxidant effect of beta-carotene, vitamins A and E in the same diet (Gülçin *et al.*, 2004). The total intake of polyphenols in a person's diet is 1 g per day, and the most common combined dietary intake of β-carotene, vitamin C and vitamin E is about 100 mg per day (King and Young, 1999). The important food sources of polyphenols are onions (flavonols), cocoa (proanthocyanidins), tea, apples and red wine (flavonols and catechins), citrus fruits (flavonoids), berries and cherries (anthocyanins) and soybean (isoflavones). Polyphenols such as gallic acid and catechins in natural substances are used by as a standard for determining the total phenol content of plants and plant materials (Zahin *et al.*, 2009). Several studies reported that the differences in polyphenol content could be attributable to biological factors (genotype, cultivars), as well as environmental (temperature, salinity, water stress and light intensity) conditions. Moreover, the extraction of phenolic compounds depends on the type of solvent used, the degree of polymerization of phenolics, and their interaction (Al Mamun *et al.*, 2016).

2.5.3 Significance of phenolic compound

Phenolic compounds play various role in plants, few of which are described in the following paragraph:

1. As antioxidant compounds: The primary and most important function of phenol is as an antioxidant. They act as free radical scavengers, which are formed as a result of excessive UV radiation.

2. As structural polymers: Lignin is the most important and widely distributed phenolic compound that serves as a plant structural unit.

3. As defensive compounds: Plants develop an astringent taste due to the presence of tannins. Tannins interact with and precipitate proteins, resulting in the bitter taste of plants. As a result, in the majority of cases, they act as a feed deterrent.

4. As signal molecules: Phenolic compounds serve as signal molecules in many biochemical metabolic pathways. In the salicylic acid pathway, for example, methyl salicylate (a phenolic compound) acts as a signaling compound. De-hydrodiconiferyl alcohol glucosidase has also been identified as a phenolic signaling compound (DCG).

5. As pollinator attractants: Simple phenolic acids with low molecular weight are responsible for flower aroma and attractive coloration, which attract pollinators.

6. As a UV screen: The phenolics present in the plant cuticle play an important role in reducing the amount of UV radiation that reaches the earth through the ozone layer (Manandhar, 2018).

2.5.4 Flavonoids

Flavonoids are a large family of hydroxylated polyphenolic compounds having a benzo-γ-pyrone structure and are ubiquitously present in plants (Kumar and Pandey, 2013; Pande, 2019). Flavonoids are a large class of complex polyphenol plant metabolites found in human food. They share a tricyclic structure consisting of two aromatic centers (rings A and B) and a central oxygen-containing heterocyclic ring. Part (Ring C) is composed some of the most widely studied of these compounds include flavanols, quercetin, myricetin, and kaempferol. The basic structure of flavonoids is shown in Fig 2.1. It is estimated that even in an industrial society, the intake of flavonoid may be as high as 1 gram per day(Gee and Johnson, 2001).

Flavonoid polymer is also called pro-anthocyanidin. Flavonoids exist as secondary plant metabolites and participate in pigmentation, antioxidants, antibacterial agents, anti-stress agents and UV protection(Vaya and Aviram, 2001). So far, more than 4000 flavonoids have been described, of which are found in plant parts commonly eaten by humans, and about 650 flavonoids and 1030 flavanols are known (Ghasemzadeh *et al.*, 2010).

Antioxidant activity of flavonoids is believed to be due to their ability to act as free radical acceptor and to complex metal ions (Hertog *et al.*, 1992). They are biologically active against liver toxins, tumors, viruses and other microbes, allergies and inflammation (De *et al.*, 1999). Table 2.3 shows some flavonoid subclasses, specific examples of such subclass and their food sources while Table 2.4 shows specific flavonoid phytochemicals and their functions.

Table 2.3 Flavonoids and their food sources

Flavonoids	Subclass	Food sources
Flavonones	Hesperetin	Orange
	Eriodicatol	Lemon
	Neringenin	
Flavones	Lutelin	Parsley, some cereals
	Epigenin	
Flavonols	Quercetin	Onion, tea, Red wine,
	Kaempferol	Apple
	Catechin (monoma)	
Flavanols	Proanthocyanin (also called tannin)	Green tea
Anthocyanidine	Cyanidine	Berries, red wine, cherries
Isoflavone	Genistein	Legumes, Soybean nuts, soy sauce
	Daidzein Equol	

Source: Rowland (1999)

Table 2.4 Some important flavonoids and their functions

S.N.	Flavonoids	Functions
1	Hesperitin	Raises blood level of the "good cholesterol and lowers blood level of the "bad" cholesterol Prevents inflammation and relieves pains Can prevent incidence of head and neck cancers
2	Quercetin	Protects the lungs from harmful effects of pollutants and cigarette smoke
3	Tangeritin	Induces cell death in cancer cells (Leukemia) but promotes the life of normal healthy cells
4	Resveratol	May reduce the risks of heart diseases, stroke and blood clots.
5	Flavanols (Anthocyanins)	Act as potent antioxidant Helps to improve balanced coordination and short- term memory in the elderly
6	Anthocyanins	Helps to prevent urinary tract infection.

Source: Hertog *et al.* (1992)

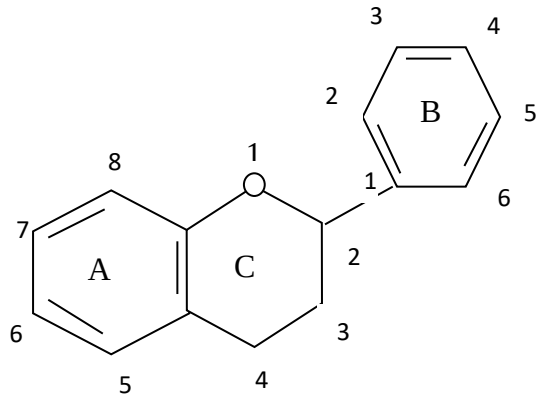


Fig 2.1 Basic structure of flavonoids

Source: Hertog *et al.* (1992)

2.5.5 Biological activity of flavonoids

Flavonoids have recently attracted people's attention due to their extensive biological and pharmacological activities. According to reports, they have a variety of biological properties, including antibacterial, cytotoxic, anti-inflammatory and anti-tumor activities, but the best descriptive property of almost every group of flavonoids is their ability to act as powerful antioxidants, protecting the human body from freedom The invasion of radicals and reactive oxygen species can protect species (Ramos, 2008). The ability of flavonoids as antioxidants depends on their molecular structure. The position and other characteristics of the hydroxyl group in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities (Heim *et al.*, 2002).

The β ring hydroxyl configuration is the most significant determinant of scavenging of ROS (Reactive Oxygen Species) and RNS (Reactive Nitrogen Species) because it donates hydrogen and an electron to hydroxyl, peroxyl, and peroxynitrite radicals, stabilizing them and giving rise to a relatively stable flavonoids radical (Kumari and Jain, 2015).

Mechanisms of antioxidant action may include

1. Suppression of ROS formation either by inhibition of enzymes or by chelating trace elements involved in free radical generation

2. Scavenging ROS

3. Regulation or protection of antioxidant defenses

The flavonoid action involves most of the mechanisms listed above. Some of the effects mediated by them may be the combined result of free radical scavenging activity and interaction with enzyme functions (Lewandowska *et al.*, 2016). Flavonoids inhibit the enzyme involved in the production of ROS, namely H. Microsomal monooxygenase, glutathione transferase, mitochondrial succinate oxidase, NADH oxidase, etc (Kumar and Pandey, 2013).

2.5.6 Tannins

Tannins are polyphenols, sometimes called plant polyphenols, although the name tannins originally referred to plant extracts that have an astringent taste without knowing its chemical structure (Haslam, 1989). Tannins are distinguished from other plant polyphenols,

which are essentially the properties of the former: binding to proteins, alkaline compounds, pigments, macromolecular compounds and metal ions, and antioxidant activity(Okuda and Ito, 2011). They are widely found in the flora. They are high molecular weight phenolic compounds. Tannin is soluble in water and alcohol and exists in the roots, bark, stems and outer layers of plant tissues. They form complexes with proteins, carbohydrates, gelatin and alkaloids. Due to their structural properties, tannins can be divided into four categories: gallic tannins, ellagitannins, compound tannins and condensed tannins (Saxena *et al.*, 2013).

1. Gallo-tannins: -These are polymers of galloyl units combined with various polyol units. The ubiquitous polyol group is derived from d glucose, and the hydroxyl functional group of the polyol group can be partially or completely replaced by galloyl units. In the case of meta-depsides, galloyl residues and polyol residues and one or more -linked galloyl units are esterified at the meta-position to the carboxyl group of the galloyl unit. Gallic tannins with residues of polyols coupled with cinnamoyl or coumarin are rare.

1 Ellagitannins: The characteristic unit of all ellagitannins, the HHDP group, is the product of the first biological oxidation of the galloyl group. The connection of one or two additional galloyl groups to the HHDP unit through the C-O or C-C bond leads to several changes in the HHDP group (Sieniawska and Baj, 2017).

2 Condensed tannins: Those are all oligomeric and polymeric pro-anthocyanidins formed by linkage of C-4 of one catechin with C-8 or C-6 of the next monomeric catechin(Saxena *et al.*, 2013)

3 Complex tannins: Among these, all oligomeric and polymerized pro anthocyanidins are formed by connecting the C-4 of a catechol (flavan3ol) with the C-8 or C-6 of the next monomer catechol (Sieniawska and Baj, 2017).

2.5.6.1 Activity of tannins

Tannins have multiple effects on biological systems because they are potential metal ion sequestrants, protein precipitants and biological antioxidants. Because tannin can play a variety of biological functions, and because of the huge structural changes, it is difficult to develop a model that can accurately predict its impact in any system (Skowyra, 2014).

Plant extracts containing tannins are used as astringents, antidiarrheals, diuretics, gastric and duodenal tumors, as well as anti-inflammatory, antiseptic, antioxidant and hemostatic

agents (Dolara *et al.*, 2005). Tannin has a variety of in vitro biological activities, of which anti-oxidation and antibacterial properties have been the most in-depth research on. Tannins are known to inhibit lipid peroxidation and have the ability to scavenge free radicals, which are important under cell pro-oxidation conditions. Most of the activities of tannins, including their free radical scavenging ability, depend to a large extent on their structure and degree of polymerization (Sieniawska and Baj, 2017).

2.5.7 Phytochemical metabolism in human

Most phytochemicals found in food come in many forms that affect their digestion and absorption. The most common are polyphenols, which exist in the form of glycoside conjugates. Some glycosides must be digested into aglycones (unbound form) before being reabsorbed. It is believed that some other forms of phytochemicals are absorbed in the intestines without the need for large amounts of digestion. It is believed that the absorption of most phytochemicals involves carriers. In addition, many glycosides are neither digested nor absorbed in the small intestine. It has been shown that those phytochemicals that are not absorbed by the small intestine are broken down by the microbiota of the large intestine (Ross and Kasum, 2002). Bacteria hydrolyze glycosides and produce aglycones, which can be further metabolized to form various aromatic compounds (Bradlow *et al.*, 1999).

2.6 Antioxidants

An antioxidant can be defined as "any substance that significantly delays or inhibits the oxidation of an oxidizable substrate when present at a low concentration compared to the concentration of the substrate." For simplicity, antioxidants are traditionally divided into two categories, primary or broken chain antioxidants and secondary or preventive antioxidants (Madhavi *et al.*, 1995). Secondary or preventive antioxidants are compounds that slow down the rate of oxidation. This can be achieved in a variety of ways, including substrate removal or singlet oxygen quenching (Frankel *et al.*, 2000). Primary antioxidants, AH, when present in trace amounts, may either delay or inhibit the initiation step by reacting with a lipid radical or inhibit the propagation step by reacting with peroxyl or alkoxyl radicals (Madhavi *et al.*, 1995).

$$LOO^{\cdot} + AH \longrightarrow LOOH + A^{\cdot}$$

$$LOO^{\cdot} + AH \longrightarrow LOOH + A^{\cdot}$$

$LO^{\bullet} + AH \longrightarrow LOH + A$

The antioxidant free radical may further interfere with chain-propagation reactions by forming peroxy antioxidant compounds:

$A^{\bullet} + LOO^{\bullet} \rightarrow LOOA$

$A^{\bullet} + LO^{\bullet} \rightarrow LOA$

The activation energy of the above reaction increases with the increase of the dissociation energy of AH and L-H bonds. Therefore, the effectiveness of antioxidants increases as the binding strength of AH decreases. Chain scission antioxidants can be produced naturally or synthetically, such as BHT, BHA, TBHQ and gallic acid esters. Synthetic antioxidants are widely used in the food industry and are present in human nutrition. Due to concerns about the safety of synthetic antioxidants, the use of natural antioxidants has been promoted. natural alternatives (such as plant bio phenols) have similar or even higher antioxidant activity than synthetic antioxidants (Antolovich *et al.*, 2002). For antioxidants, it is known to be its functional importance, the interest of antioxidants is high to protect edible oil to protect its derivatives, even if they are used for food to provide baking and cooking characteristics and nutrition services (Chu and Chen, 2006). Antioxidants are substances that generally prevent, delay or delay the onset of rancidity in food products due to the oxidation of unsaturated fatty acids present in food products. The use of antioxidants extends the shelf life of foods, minimizes waste and nutrient loss, and expands the range of uses of different oils/fats (Bhattacharya, 2003).

A quick, simple and inexpensive method for measuring the antioxidant capacity of foods involving the free radical scavenging 2,2Diphenyl1 picrylhydrazyl (DPPH) is widely used to test the ability of substances that act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity. The DPPH assay method is based on the reduction of DPPH, a stable free radical. The unpaired DPPH radical gives an absorbance (violet color) at 517 nm. When the antioxidants in the plant extract react with DPPH, it is reduced to DPPHH and causes a discoloration to yellow relative to the number of electrons captured. The absorbance of the color is the inverse of the radical scavenging activity of the sample extract. The trapping of DPPH by radical scavengers can be summarized as follows

DPPH • + FE DPPH ⟶ H + A• (1)

DPPH + A• DPPH ⟶ A (2)

A• + A• A ⟶ A (3)

Where FE is the determining substance of the extract and A • is the parent substance. The newly formed radical (A •) can mainly follow a radical interaction to make the molecule stable, through a disproportionate radical, the collision of radicals with the abstraction of an atom by a root of another equation (Chandra Shekhar and Anju, 2014).

2.6.1 Types of antioxidants

Antioxidants are available in both natural and synthetic forms which are discussed separately below in the following section.

2.6.1.1 Synthetic (artificial) antioxidant

Most of the synthetic antioxidants used are phenolic compounds, including butylated hydroxytoluene (BHT) and butylated hydroxy anisole (BHA), tertiary butylated hydroquinone (TBHQ) and propyl gallate (PG) are commonly used. A quantitative tolerance limit for this synthetic antioxidant is limited in federal regulations to a total content of 0.02% fat or oils alone or in combination. TBHQ, PG, BHA and BHT are the most widely used antioxidants in the food industry. BHA and BHT are not only oxidizable, but also fat soluble. Both molecules are incompatible with iron salts. In addition to preserving food, BHA and BHT are also used to preserve fats and oils in cosmetics and pharmaceuticals (Manandhar, 2018).

In recent years, several researchers have studied the potential toxicity of synthetic antioxidants. Butylated hydroxy anisole (BHA) has been banned. Most antioxidants have a phenolic structure and form relatively stable radicals and non-radical products by donating a hydrogen atom to the acyl group of the peroxy radical. Research carried out by Japan showed that the antioxidant promotes carcinogenesis in rats. Butylated hydroxytoluene has also been implicated as a tumor promoter (Manandhar, 2018).

2.6.1.1.1 Tert-butylhydroquinone

In food, TBHQ is used as a preservative for unsaturated vegetable oils and many animal fats. It does not cause discoloration, even in the presence of iron, and it does not modify the taste or smell of the material to which it is added and its chemical structure is shown in Fig 2.2. It can be combined with other preservatives such as butylated hydroxy anisole (BHA). As a food additive, its E number is E319. It is added to a wide variety of foods. Its main advantage is the extension of the useful life (O'brien, 2008).

TBHQ is certified safe for human consumption. In many large development organizations such as the FDA (Food and Drug Administration), FSIS (Food Safety and Inspection Service) and others, the use of TBHQ or combinations with concentrations of BHA or BHT of up to 0.02% by weight of fat or oil contained in food (Manandhar, 2018).

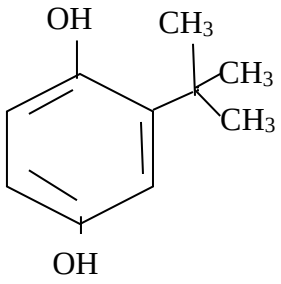


Fig 2.2 Chemical structure of TBHQ

Source: Burr and Burr (1929)

2.6.1.1.2 Butylated hydroxy anisole (BHA)

Butylated hydroxy anisole (BHA) is a mixture of two isomeric organic compounds, 2. tert-butyl4-hydroxyanisole and 3-tert-butyl-4-hydroxyanisole. It is made from 4. methoxyphenol and isobutylene whose chemical structure is shown in Fig 2.3. It is a white or pale yellow solid (crystal or flake) with a faint aromatic odor (Burr and Burr, 1929).

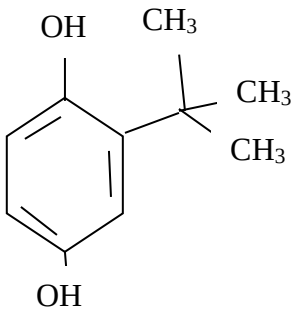


Fig 2.3 Chemical Structure Of BHA

Source: Burr and Burr (1929)

2.6.1.1.3 Butylated hydroxytoluene (BHT)

Butylated hydroxy anisole is a synthetic antioxidant that has been a widely used fat-soluble food preservative since 1947, with extensive biological activities. Prevents deterioration by reacting with oxygen delays development of off-flavors, odors, and color changes caused by oxidation. Protects against animal aganistic radiation and the acute toxicity of various xenobiotics and mutagens. Butylated hydroxy anisole (BHA) is a mixture of two isomeric organic compounds, 2-tert-butyl-4-hydroxyanisole and 3-tert-butyl-4-hydroxyanisole. is made from 4-methoxyphenol and isobutylene. It is generally insoluble in water, but can be converted to a soluble form for commercial applications. BHT was first used as a antioxidant food additive in 1954. An antioxidant is a substance that prevents -containing materials from being rust. Therefore, BHT prevents spoilage of the foods to which it is added. BHT has become very popular. Among food processors and is now used in a wide variety of products including breakfast cereals, gum, dried potato flakes, fortified rice, French fries, candy, sausage, freeze-dried meat and other foods that contain fat and oil. BHT is sometimes used in conjunction with a related compound (Manandhar, 2018).

2.6.1.2 Natural antioxidant

Natural antioxidants are widely used in foods and medicinal plants. These natural antioxidants, particularly polyphenols and carotenoids, have a wide range of biological effects, including anti-inflammatory, anti-aging, anti-atherosclerosis, and anti-cancer. Effective extraction and proper evaluation of antioxidants from foods and medicinal plants

are critical to exploring potential sources of antioxidants and promoting their use in functional foods, pharmaceuticals, and food additives (Xu *et al.*, 2017).

Natural antioxidants can be used in a number of applications even when no choice is left due to company guidelines or food laws and public interest groups. There is some scientific evidence alone that supports the use of natural antioxidants. The antioxidant activity from natural sources has been demonstrated in spices (Chang *et al.*, 1977); Plant extracts and vegetable proteins and their hydrolysates (Manandhar, 2018). The most commonly used natural antioxidants are not exactly natural, but are identical to nature. This means that their structure is identical to that of natural products, but that they were produced synthetically. Like other synthetic antioxidants, they are supplied in a relatively pure state. Tocopherol, Ascorbic Acid, and Citric Acid belong to this group. From the point of view of preparation for use, they can be viewed as pure synthetic substances that do not require any pre-treatment (Pokorný and Korczak, 2001). Mielnik *et al.* (2006) also reported that lipid oxidation can be prevented by increasing the concentration of grape seed extract on turkey meat. Burri *et al.* (2020) also reported that onion and beet root leaves were most efficient in inhibiting lipid oxidation with increasing concentration above 200ppm for two weeks.

2.6.1.2.1 Desirable properties of natural antioxidants

According to Embuscado (2015),Natural antioxidants that are ideal should have the following characteristics:

1. be safe for consumption, i.e., have no harmful physiological effects;
2. have no objectionable flavor, odor, or color, i.e., have little or no effect on the color, aroma, and flavor of the food;
3. be effective at low concentrations;
4. be stable during food preparation/processing and storage;
5. be economical;
6. be readily available;
7. be versatile for use in a variety of food applications**;**

2.6.1.3 Synergistic antioxidant

The preventive antioxidants which act with the aid of using lowering the rate of chain initiation is known as synergistic antioxidants despite the fact that they don't have any impact

as protectants whilst used alongside fats (Lee, 1975). These compounds enable to increase (improve) the capacity of the phenolic antioxidants to retard rancidity. Many additives showcase sequestrates (steel deactivating houses) in suitable for eating triglyceride oils as evidenced with the aid of using development in oxidative and/or flavor stability. Among those maximum essential is citric acid (Manandhar, 2018).

All metal inactivating compounds have free hydroxyl groups for carboxyl groups that are easily coordinated with salts of metal forms (Cowan, 1999). (Scalbert *et al.*, 2005) suggested metal inactivators, in fact they complex with the per-oxidizing metal and keep them in a ring or chelate structure with winter coordination complexes where the metal can no longer act as a per-oxidant (Manandhar, 2018).

2.7 Oxidative stress

Oxidative stress refers to an imbalance between the reactive oxygen species and the antioxidant system to detoxify the reactive intermediates or repair the resulting damage. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent Oxidation reactions are vital, they can also be harmful (Rafieian-Kopaei *et al.*, 2013). Insufficient amounts of antioxidants or inhibition of antioxidant enzymes cause oxidative stress, which can damage all components of the cell, including proteins, lipids, and deoxyribonucleic acid (Halliwell, 2012). Short-term oxidative stress can occur in tissues injured by trauma, infection, heat damage, hypertoxia, toxins, and excessive physical exertion. These injured tissues cause an increase in free radical-generating enzymes (eg, xanthine oxidase, lipogenase, cyclooxygenase), phagocyte activation, release of free iron, copper ions, or a disruption of phosphorylation electron transport chains. Oxidative, so that an excess of ROS is produced. The development, promotion, and progression of cancer, as well as the side effects of radiation and chemotherapy, have been linked to the imbalance between ROS and the antioxidant defense system. ROS have been associated with the induction and complications of diabetes mellitus, age-related eye diseases, and neurodegenerative diseases such as Parkinson's disease (Rao *et al.*, 2006).

2.8 Some herbs and spices

2.8.1 Chiraito (*Swertia chirayita*)

2.8.1.1 Background

This important medicinal herb is native to and native to the northern temperate regions (Singh, 2008). All-inclusive, this herb is found primarily in the high altitudes (1200 to 3600 m) of Asia, Europe, America, and Africa (Negi *et al.*, 2011). *Swertia chirayita*. (Gentianaceae) is widely disbursed in India withinside the temperate Himalayas among 4000 and 10,000 ft., from Kashmir to Bhutan, and in Khasia Hills among 4000 and 5000 ft. It additionally grows abundantly in Nepal. The plant is widely known for its makes use of withinside the Indian device of drugs for a selection of purposes (Ghosal *et al.*, 1973).

Swertia chirayita (chiraito) comprises 100 species, 27 of which are herbs valued in Nepal. It occupies an important place in the use of medicinal and aromatic plants. Approximately 9 types of them are for sale in the shopping malls of Nepal. It has a pungent bitter taste and is used as an astringent tonic. The main active ingredient is "chiratin" from the group of secoirodoid glycosides and it is said to be an anticancer (Ayurvedic drug) against cancer. It is a rich source of biologically active phytochemicals (xanthones, flavonoids, iridoids, secoiridoids, glycosides and terpenoids) (Ishwar and Karki, 2014).

2.8.1.2 Botanical description

Swertia L (*Gentianaceae - Gentianeae - Swertiinae)* is a morphologically diverse genus, but taxonomically different from. The taxon in its current description includes approximately 150 species and is an annual, biennial, or perennial herb with a size of 24 cm. up to over 1.5 m tall with 45 flowers, 1 or 2 nectaries at the base of characteristic rotating corolla lobes. The genus is found primarily in alpine or temperate habitats in Asia, Africa, and North America. The genus circumscription was often debated, leading to disagreement among taxonomists due to morphological similarities (nectar and rotating corolla lobes) between the *Swertia* species and related genera (Joshi and Joshi, 2008). Taxonomical position of chiraito is shown in Table 2.5.

Table 2.5 Taxonomical position of chiraito

Classification of *Swertia chirayita*	
Kingdom:	Plantae
Clade:	Tracheophytes
Division:	Angiosperms
Order:	Gentianales
Family:	Gentianaceae
Subtribe:	Swertiinae
Genus:	*Swertia* L.

Source: Anon. (2021b)

2.8.1.3 Phytochemicals of chiraito

Phenols, resins, flavonoids, terpenes, phytosterols, tannins and glycosides were screened with their respective tests from the methanol crude extracts of different species of chiraito (Khanal *et al.*, 2015).Table 2.6 show the phytochemicals composition of different spices of chiraito.

Table 2.6 Total phenolic content, total flavonoid content and IC50 values of methanol extracts of different Swertia species:

Swertia species	TFC (mg QE/g)	TPC (mg GAE/g)	IC50 (μg/ml)
S. chirayita	26.16 ± 0.25^{a}	67.49 ± 0.50^{a}	23.35 ± 0.59^{a}
S. angustifolia	18.41 ± 0.19^{b}	22.68 ± 0.78^{b}	45.81 ± 1.54^{b}
S. paniculate	25.18 ± 0.85^{a}	34.01 ± 0.67^{c}	29.53 ± 1.17^{c}
S. racemose	24.88 ± 1.26^{a}	66.91 ± 1.02^{a}	30.34 ± 1.14^{d}
S. nervosa	24.57 ± 0.19^{a}	54.36 ± 0.76^{d}	32.19 ± 0.63^{c}
S. ciliate	22.95 ± 0.48^{a}	42.53 ± 0.91^{e}	29.99 ± 0.96^{d}
S. dilatate	24.04 ± 1.26^{a}	67.00 ± 3.63^{a}	67.00 ± 3.63^{a}

In each column values with different letters are significantly different ($P < 0.05$) within different species.

Source: Khanal *et al.* (2015)

2.8.2 Onion (*Allium cepa* L.)

2.8.2.1 Background

The onion (*Allium cepa* L.) has been valued as a food and medicinal plant since ancient times. It is widely grown, second only to tomatoes, and is a vegetable onion plant known to most crops and consumed throughout the world. Onions have been used by humans since the Neolithic Age and are still used throughout the world. During this long period there have always been people who have appreciated the use of onions and have used them in considerable quantities, but there have also been those who detested and hated them (Pareek *et al.*, 2017). Onions have been cultivated in at least 175 countries around the world for about 5,000 years. The ancient Egyptians viewed the spherical light bulb as a symbol of the universe. The name probably comes from the Latin unus, which means "one", and the

Romans introduced the onion to Great Britain, from where it may have been brought to America (Burnie *et al.*, 1999). The earliest known written account of the onion comes from the Sumerians and dates from 2600 to 2100 BC (Pareek *et al.*, 2017).

2.8.2.2 Botanical description

Onion belongs to the family Amaryllidaceae; the plant is both biannual or perennial (depending at the cultivar), and scents while crushed (Organization, 1999). The plant has shallow adventitious fibrous roots bulb, and tubular leaves. The stem grows 100–200 cm tall at some point of the second year of the plant's life. The green leaves of the plant are an extension of the outer meal's storage leaves. The inflorescence is umbel-like and develops from a ring-like apical meristem. The umbel is the aggregation of flowers at various tiers of development, and it carries 200–600 small individual vegetation, even though this variety can variety from 50 to 1000. It is composed of white or greenish-white small flowers which develop on the tip of the stem in the second year of the plant. The onion bulb ranges in form from flat to globular to oblong, and the onions are commonly of 3 colors: red, white, and yellow (Fritsch, 2005). The culmination is capsule and contain black seeds. The bulb consists of fleshy and enlarged leaf bases. The safe to eat onion bulb can develop up to 10 cm in diameter, and its miles composed of several overlapping layers on a relevant core. The outer leaf bases of the bulb lose moisture and come to be scaly by the point of harvesting, and the internal leaves thicken because the bulb develops. The majority of the species of onion develop in open, sunny, and dry land, in particular in humid climates. However, the Allium species had been followed in other ecological niches of the world (Pareek *et al.*, 2017)..

Allium contains more than 780 species (Burnie *et al.*, 1999) with large diversities in morphological characters. The chromosome number in onion is 16 (2n). It has been classified in hierarchical level as follows in Table 2.7

Table 2.7 Taxonomical position of onion

Classification of *Allum cepa L*	
Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Liliopodia
Subclass	Liliales
Order	Liliaceae
Genus	*Allium*
Species:	*cepa L*

Source: Burnie *et al.* (1999)

2.8.2.3 Phytochemicals of onion

Evidence from multiple investigations suggests that these biological and medicinal functions are primarily due to the high levels of organic sulfur compounds in onion. In addition to organic sulfur compounds, organoselenium compounds, flavanols (quercetin and its glycosides), and fiber (fructans and fructo-oligosaccharides (FOS)) have also been linked to the biological properties of onions. Additionally, additional onion ingredients, such as saponins and peptides, have recently been shown to have potentially beneficial health effects, including antifungal, antitumor, antispasmodic, and cholesterol-lowering effects, and the ability to inhibit the development and activity of osteoclasts in vitro (Corzo-Martínez and Villamiel, 2012).

According to Duan *et al.* (2015) the total phenolic content, total flavonoid content and IC_{50} (DPPH) of onion peel extract on 70% methanol were found as 94.24±0.59 mg GAE/g, 43.33±0.41 mg QE/g and 0.08±0.00 mg/ml respectively. Thampi *et al.* (2015) was found RSA IC_{50} for green onion leaves with a value of 69.965 µg/ml for methanol and 86.548 µg/ml for water. The flavonoid contents may be differ according to parts of plants because

quercetins are accumulate to varying degrees in plant tissues, and the levels found in different plant parts are affected by environmental factors (Hichri *et al.*, 2011)

2.8.3 Garlic (*Allium sativum)*

2.8.3.1 Background

The native land of garlic is Central Asia. There are a number of beliefs about the exact origin of garlic, such as that it comes from western China, around the Tien Shan mountains, to Kazakhstan and Kyrgyzstan (Petrovska and Cekovska, 2010).

The Sumerians (2600-2100 BC) actively used the healing properties of garlic and are believed to have brought garlic to China, from where it later spread to Japan and Korea. The spread of garlic probably took place first in the Old World and then in the New World. Even so, some historians still claim that garlic originated in China (Petrovska and Cekovska, 2010)**.**

2.8.3.2 Botanical description of garlic

Allium sativum var. *sativum*, also known as soft neck garlic, and *Allium sativum* var. *ophioscorodon*, generally known as hard neck garlic, are the two subspecies of *Allium sativum*. Both varieties are made up of cloves, which are prophylls enclosed by dry membrane skins and held together by a basal plate in an underground bulb. The bulb of hard neck garlic is made up of six to eleven cloves that are wrapped around a centered woody stem. The stem of this garlic type curls near the top, although it is usually plucked after it has curled one to three times. This is because if it continues to grow, there will be less energy available for the bulb. The scape would eventually produce bulbils, which contained microscopic cloves. White or light purple flowers are occasionally seen with the bulbils, but these are infertile. There is no flowering top on soft neck garlic, and each bulb can hold up to twenty-four cloves. The cloves are piled with larger ones on the outer, and the stem is central and delicate (Alam *et al.*, 2016).

As *Allium sativum* is sterile, it can be cultivated asexually from cloves and does not require pollination. Hard neck cultivars are better adaptable to cold conditions and grow best in moderate temperatures. *Allium sativum*, like the majority of the genus, is a perennial plant. Garlic is comprised of highly potent organosulfur compounds that act as secondary

metabolites. These compounds are liable for the very pungent odor and flavor of uncooked garlic and act as defenses against predators (Alam *et al.*, 2016).

Allium sativum, commonly known as garlic, is a species of the onion family Alliaceae (Saravanan *et al.*, 2010). Allium sativum is classified as shown in Table 2.8

Table 2.8 Taxonomical position of Garlic

Classification of *Allium sativum*	
Kingdom	Plantae
Division	Magnoliophyta
Class	Lilopsida
Order	Liliales
Family	Liliaceae
Genus	*Allium*
Species	*sativum*

Source : Saravanan *et al.* (2010)

2.8.3.3 Phytochemicals of Garlic

Medicinal plants are the richest biological source of medicines from traditional medical systems, modern medicines, nutraceuticals, dietary supplements, folk medicine, pharmaceutical intermediates, and chemicals for synthetic medicines (Ncube *et al.*, 2008). Plants are rich in various active principles with specific therapeutic effects. They are a source of chemical compounds such as tannins, flavonoids, saponin resins, alkaloids, etc. with healing properties (Doss, 2009). Fresh onion contains alliin, allicin and essential oils, when the garlic clove is crushed, the odorless compound alliin is converted to allicin through the enzyme alliinase. Allicin gives garlic its characteristic pungent odor. It contains vitamins, minerals and trace elements. Garlic, on the other hand, is a herbal medicine that is used to prevent and treat many illnesses such as cold and flu symptoms by strengthening the immune system and it has anticancer, antioxidant, anti-inflammatory, antimicrobial, antithrombotic,

hypocholesterolemia properties, hypoglycemic agents and hypotensive effects. And it is used to treat diabetes, atherosclerosis, hyperlipidemia, thrombosis, and high blood pressure. It is also effective against strokes, gastrointestinal neoplasms, blood clots (platelet aggregation inhibitors), etc. (Divya *et al.*, 2017).

Thampi *et al.* (2015) interpreted that the methanolic and aqueous extracts of garlic confirmed the minimal IC50 values of 64.033 µg/ml and 85.124 µg/ml respectively. Kim *et al.* (2016) also reported that total phenolic content, total flavonoid content and antioxidant capacity (IC_{50}) were 55.48±0.40 mg GAE/g, 8.06±0.32 mg QE/g and 0.24 mg/ml respectively for 70% methanolic extract of garlic peels. Al Mamun *et al.* (2016) also reported that the total flavonoid content of garlic leaves to be 11.92±1.6 mg QE/g. Gorinstein *et al.* (2008) reported that total phenolic content of methanolic extract of onion was found higher than garlic leaves. Nuutila *et al.* (2003) reported that methanolic extract of onion had higher antioxidant activity than methanolic extract of garlic but aqueous extract of garlic had higher antioxidant activity than aqueous extract of onion.

2.8.4 Radish

2.8.4.1 Background

Radishes are a fast-ripening root crop, possibly native to central and western China and India. It has been cultivated for thousands of years and was cultivated by the ancient Egyptians and Greeks. It was first cultivated in Western Europe in the mid-16th century. The main edible part of the plant is a super-bud and main root (Caballero *et al.*, 2003).

2.8.4.2 Botanical description

Raphanus sativus is an annual or biennial herb which exists in numerous exceptional forms. Four botanical varieties are regarded in the species, *R. sativus* L., particularly *radicula*, *Niger*, *mougri* and *oleifera*, the primary of which might be grown for their tuberous roots, at the same time as *oleifera* is grown normally for the oil in its seeds. Stems can be easy or branched, the basal leaves are lengthy, often pinnately lobed and coarsely toothed, however on occasion aren't serrated, at the same time as the cauline leaves are easy and linear. The plants are in lengthy terminal racemes, commonly white or lilac with red veins. The fruit are narrow, indehiscent, 2.5-7.5 cm lengthy and approximately 1.25 cm in diameter, with an extended tapering beak. There are commonly 6-12 globose seeds, yellow to chocolate-brown

in colour. Within the oriental forms, the faucet root is swollen and ranges in shape from nearly globular, cylindrical, or conical in shape, weighing up to 15 kg (except in var. *mougri*). The flesh is typically white, though in a few can be crimson to red. In the salad radish the pores and skin is commonly red (every so often white); in the Oriental radish it is typically white (Aruna *et al.*, 2012). Taxonomical position of radish is shown in Table 2.9

Table 2.9 Taxonomical position of radish

Classification of Radish	
Kingdom	Plantae
Class	Mangolioposida
Order	Brassicales
Family	Brassicaceae
Genus	*Raphanus*
Species	*Sativus*

Source Anon. (2008), Aruna *et al.* (2012)

2.8.4.3 Phytochemicals of Radish

Phytochemical examination of *Raphanus sativus* L. shows the presence of numerous phytochemicals viz. alkaloids, reducing sugar, flavonoids, glycosides, cardiac glycosides, tannins, saponin, protein, amino corrosive, terpenoids and steroids and so on. The radish leaves show a large portion of the phytoconstituents are available in aqueous and methanolic extract (Yadav *et al.*, 2011).

Total phenolic content of radish leaves was obtained to be 91.8 ± 2.9 mg GAE/g by Eugenio *et al.* (2017). Luo *et al.* (2018) reported that total phenolic content and IC_{50} ethyl acetate extract fraction of radish leaves was found to be 114.12 ± 1.31 mg GAE/g and 84.19 ± 2.12 µg/ml but chloroform extract showed 27.62 ± 0.63 mg GAE/g and 186.3 ± 3.78 µg/ml.

2.8.5 Hattibar

2.8.5.1 Background

Before the 1930s, plantation agaves were an important crop in the economies of some third world countries such as Mexico and Tanzania. However, the arrival of synthetic fibers has greatly reduced their economic value. Although their importance today cannot be compared to that of basic food crops, agaves are potentially useful plants for the future of semi-arid areas of the world because they are extremely robust and well adapted to the scarcity of water and also the source of multiple products (Robert *et al.*, 1992).

2.8.5.2 Botanical description

The genus *Agave L*. is a member of the Agavaceae family, order Asparagine's. It is classified into two subgenera, Littaea and Agave, and has 136 species that have been grouped together based on their close resemblance. However, this system is controversial and may not accurately reflect natural relationships (Robert *et al.*, 1992).

The center of origin and diversity of the genus *Agave* is limited to Mexico. However, *Agaves* have been introduced in virtually all subtropical areas of the world since the 17th century, mainly for ornamental purposes. The stems of these plants are thick shortened shoots, with the exception of a few arborescent varieties like *Agave karwinski* Zucc. The green leaves differ in size and shape from small, compact, and globose (*A. parryi Engelm*.) to massive plants (*A. mapisaga Tres*.) that can reach 2-2.5 m in height. The leaves are spade-shaped with a sharp thorn at the tip, though some plants (*A. cantala Roxb*) have long, narrow, and flexible leaves; they are thick and succulent with a spongy parenchyma for storing water and a waxy cover that prevents water loss and gives them a glaucous appearance. The edges can be smooth, as in some *A. cerulata Trel.* varieties, but they are more commonly covered with lateral prickles or "teeth" of various shapes and numbers. The meristematic area near the apex of the stem gives rise to the leaves. It takes several years for them to form in the bud, and once completely developed, they unfold in a spiral arrangement to form a rosette (Robert *et al.*, 1992). Taxonomical position of hattibar *(Agave americana)* is shown in Table 2.10.

Table 2.10 Taxonomical position of hattibar *(Agave americana)*

Classification of *Agave americana*	
Kingdom:	Plantae
Order:	Asparagales
Family:	Asparagaceae
Subfamily:	Agavoideae
Genus:	*Agave*
Species	*Americana*

Sources: Anon. (2021a)

2.8.5.3 Phytochemicals of hattibar

Agave has been used in the past as a raw ingredient for food and folkloric medicine. This plant is a rich source of saponins, which are anti-inflammatory agents as well as anticancer, antifungal, and anti-inflammatory agents. Polyphenols found in *Agave* have anticancer, cell strengthening, antidiabetic, alleviating, anti-parasitic, antibacterial, prebiotic, and co-adjuvant properties in mineral consumption. The comprehensive phytochemical representation of the sap, leaves, and side-effects produced from conventional food uses is expected to confirm the beneficial effects of *Agave* consumption and its potential use as a source of useful nutrients (Santos-Zea *et al.*, 2012).

Rizwan *et al.* (2012) reported that total phenol content of methanolic extract of *Agave attenuata* leaves was found to be 39.35 ± 0.69 mg GAE/100 g DW, flavonoid content as 304.8 ± 5.02 mg CE/100 g DW and DPPH (% inhibition, 0.1 mg/mL) 73.97 ± 1.49%. Dif (2016) obtained that the antioxidant capacity of ethyl acetate extract of *Agave* leaves was found to be 1.30 mg/ml but (Nasri and Salem, 2012) found total phenol of agave to be 4.49-12.30 mg GAE/g sample (Hamissa *et al.*, 2012; López-Romero *et al.*, 2018) reported total flavonoid content *of Agave americana* leaves ranged from 0.96 to 4.90 mg QE/g DW. Plant

antioxidant activity may be varied by species, and differences within the same species have been discovered depending on the solvent extraction, the physical condition of the plant material (fresh or dried), or environmental factors (Kratchanova *et al.*, 2010)

2.9 Total antioxidant capacity

Because measuring individual antioxidant molecules is impractical and their antioxidant effects are additive, total antioxidant capacity (TAC), total antioxidant activity (TAA), total antioxidant power (TAOP), total antioxidant status (TAS), total antioxidant status (TAS), or other synonyms are used to describe a sample's total antioxidant capacity (Erel, 2004).

TAC tests have the advantage of being able to assess the antioxidant components of a sample in a global manner. Measuring each antioxidant component individually is time-consuming and labor-intensive, and it necessitates the use of specialized and expensive procedures (Erel, 2004). TAC assays also have the advantages of being simple to use, cheap cost per sample, fast reactions, and the ability to be conducted utilizing automated, semi-automated, or manual procedures (Marques *et al.*, 2014).

2.9.1 DPPH radical scavenging assay

The DPPH assay assesses a substance's ability to scavenge the DPPH radical and convert it to hydrazine. When a substance that acts as a hydrogen atom donor is added to a DPPH solution, hydrazine is formed, with the color changing from violet to pale yellow (Formagio *et al.*, 2014). The DPPH• test is based on the ability of the stable free radical 2, 2-diphenyl1-picrylhydrazyl to react with hydrogen donors. • The DPPH radical demonstrates an UV-VIS absorption spectrum is intense. In this test, a radical solution is decolorized after it has been decolorized, reduction through the use of an antioxidant (AH) or a radical (R•) in accordance with the rules outlined scheme.

DPPH• + AH → DPPH• - H + A•,

DPPH• + R• → DPPH• - R

The DPPH• radical, which has a deep purple color, is one of the few stable organic nitrogen radicals. It is commercially available and does not need to be produced prior to assay. The reducing ability of antioxidants toward DPPH• is measured in this assay. The DPPH assay is thought to be primarily based on an electron transfer (ET) reaction, with hydrogen-atom

abstraction serving as a secondary reaction pathway. The test is simple and quick to perform, requiring only a UV-vis spectrophotometer, which explains its widespread use in antioxidant screening (Prior *et al.*, 2005; Sochor *et al.*, 2010).

2.9.2 Reducing power assay

The reducing power assay is a direct method of measuring the combined ("total") antioxidant activity of reductive (electron donating) antioxidants in a test sample that is relatively simple, quick, and inexpensive. The reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) is used as the signal, or indicator, reaction in the assay, and this is linked to a color change. In this assay, a key oxidant (in the form of a ferric salt in aqueous solution) is reduced by electron donating (reductive) antioxidants in the reaction mixture that have a redox potential lower than that of the half reaction under the reaction conditions used (Benzie and Devaki, 2018).

The flavonoids and phenolic acids found in medicinal plants have high antioxidant activity due to their ability to form complexes with metal atoms, particularly iron and copper. This method is based on the principle that as the absorbance of the reaction mixtures increases, so does the antioxidant activity. The antioxidant compound in the samples forms a colored complex with potassium ferricyanide, trichloroacetic acid, and ferric chloride, which is measured by a UV-Spectrophotometer at 700 nm (Vijayalakshmi and Ruckmani, 2016).

2.10 Microbial spoilage of meat and meat products

The shelf-life of meat and meat products is the amount of time that food can be stored and retains its quality characteristics until spoilage occurs. The shelf-life of products is strongly related to their deterioration, creating a line between an acceptable and unacceptable bacterial concentration, which determines off-odors, off-flavors, and an undesirable appearance. These sensorial changes are related to the number and type of microorganisms that are initially present, as well as their subsequent growth. The starting total microbial count for meat products is approximately 10^2-10^3 cfu/g, consisting of a wide range of species (Ray *et al.*, 2013).

Lower refrigeration temperatures inhibit bacterial growth and alter the composition of the microbiota on meat: at chill temperatures, psychotropic bacteria, such as Lactic acid bacteria, or Gram-negative bacteria, such as *Pseudomonas* spp., can grow (Doulgeraki *et al.*, 2012).

Under the refrigeration, raw chicken meat is safe for 48 h after the purchases (FSIS and USDA, 1997) and maximum permissible limit of microbial count for meat is 10^7 cfu/g. (Hussein *et al.*, 2019) Bacteria from the genera *Acinetobacter, Pseudomonas, Brochothrix, Flavobacterium, Psychrobacter, Moraxella, Staphylococcus, and Micrococcus*, as well as lactic acid bacteria and various *Enterobacteriaceae* genera, are the most common bacteria found on fresh meat. The composition of the atmosphere surrounding the meat has a significant impact on the survival and growth of these microbes (Pennacchia *et al.*, 2011).

2.11 Antimicrobial activity of herbs and spices

Globally, food spoilage as a result of microorganisms nevertheless broadly influences all sorts of food and reasons food waste and loss, even in devolved countries. It has been expected that every year losses of worldwide meals attain as much as 40% because of different factors which include spoilage through microorganisms (Gustavsson *et al.*, 2011). Food poisoning is considered as one of the most common cause of illness and death in developing countries (Sapkota *et al.*, 2012). Most of food poisoning reports are related to bacterial infection especially members of Gram negative bacteria like *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa* (Pandey and Singh, 2011) other Gram positive bacteria *Staphylococcus aureus* and *Bacillus cereus* have been also identified as the causal agents of food borne diseases or food spoilage (Braga *et al*., 2005) Prevention of food spoilage and their etiological agent is historically done via way of means of using chemical preservative (Shan *et al*., 2007; Yamamura *et al*., 2000) but plant extract can be used as natural antimicrobial agent which is healthy, safer and potentially effective (Hara-Kudo *et al*., 2004; Nasar-Abbas and Halkman, 2004). Many spices, including clove, oregano, thyme, cinnamon, and cumin, have been used to treat infectious diseases or protect food because they have antimicrobial properties against pathogenic and spoilage fungi and bacteria (Arora and Kaur, 1999; De *et al.*, 1999; Lai and Roy, 2004). Furthermore, the secondary metabolites of these spices are known as antimicrobial agents, the majority of which are generally recognized as safe food materials with minor side effects (Nabavi *et al.*, 2015). Antimicrobial effect of herbs and spices are given by the identified compounds in the extracts, including saponins, glycosides, terpenoids, steroids, flavonoids and tannins (López-Romero *et al.*, 2018).

Agave extracts have antimicrobial activity against Gram-positive and Gram-negative bacteria, which is one of their most notable properties. Several studies have shown that *A.*

sisalana extracts inhibited the growth of microorganisms such as *E. coli, Salmonella typhi,* and *Staphylococcus aureus*. and *Pseudomonas aeruginosa* (López-Romero et al., 2018). Rizwan *et al.* (2012) reported that the methanol extract showed strong inhibitory activity against *A. flavus* and *A. alternata,* with the highest inhibition zones minimum inhibitory concentration (27.5 and 20.75 mm) and the lowest minimum inhibitory concentration (MIC) values (18.4 and 69.4 mg/ml). Methanol extract showed no activity against *P. multocida* and *A. niger*. Essential oil extracts inhibited bacteria effectively, with garlic. Essential oil (EO) extracts inhibiting bacteria more effectively than onion EO extracts. *S. aureus* was less sensitive to the inhibitory activity of onions and garlic extracts than *S. enteritidis,* which was more inhibited at the same EO extract concentrations (Benkeblia, 2004). Radish contains raphanin, which is antibacterial and antifungal. Its root, leaves, seed has antimicrobial properties (Aruna *et al.*, 2012). Irkin and Arslan (2010) reported that 50% onion aqueous extract inhibited significantly the mesophilic aerobic bacteria of beef meat for 9 days at 4°C within legal limit. (Mahros *et al.*, 2021) also reported that microbial count of beef could be inhibited within the legal limit by using fresh garlic and garlic extract for 15 days at 4°C Methanol, ethyl acetate, and chloroform extracts of the root, stem, and leaf of white radish exhibit considerable antibacterial activity towards numerous foodborne and drug-resistant pathogenic microorganism together with *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. aureus*, *S. epidermidis*, *E. faecalis*, *S. typhimurium*, *K. pneumoniae*, *E. aerogenes*, and clinical isolates of E. cloaca (Jadoun *et al.*, 2016). *Swertia chirayita* (*S. chirayita*) has also shown to have antimicrobial activity against Gram-positive and Gram-negative bacteria. It is also used as a stringent tonic to the heart, liver, eyes, cough, scanty-urine, melancholia, dropsy, and skin diseases (Roy *et al.*, 2015). Methanol extract of Chiraito also showed significant antifungal activity with the zone of inhibition comparable to amphotericin (Shrestha *et al.*, 2015).

Part III

Materials and methods

3.1 Materials

3.1.1 Raw materials

The raw materials (chicken meat) were obtained from local market of Dharan. Onion (*Allium cepa*), garlic (*Allium sativum),* chiraito (*Swertia chirayita*), leaves of radius (*Raphanus sativus*) hattibar (Agave spps.) required for analysis were obtained from the locality of Itahari (Sunsari), Rampur (Udaypur), Ilam, Dharan (Sunsari), Bhojpur respectively.

3.1.2 Equipment and chemicals

All the apparatus and the chemicals required for the work were taken from the Central Campus of Technology, Dharan. List of the major apparatus, chemicals and equipment required are given in Appendix A.

3.2 Methods

3.2.1 Preparation of plant sample

Leaves of all four sample and chiraito (whole plant) were washed and are dried on mechanical dryer at 50ºC until moisture content was less than 10%. After drying leaves were crushed into power for extraction.

3.2.2 Preparation of plant extract

The crude plant extract was obtained by Soxhlet extraction. About 20 g of powdered plant material was evenly packed into a thimble and extracted with 250 ml of methanol solvent. Extraction continues for 24 h or until the solvent in the extractor siphon tube becomes colorless. Extract was then placed in a beaker and stored on a hot plate and heated at 30-40ºC until all the solvent had evaporated. The dried extract was stored in a refrigerator at 4ºC for further use in phytochemical analysis (Yadav *et al.*, 2011).

3.2.3 Preparation of meat sample

Chicken breast meat fillet was brought to laboratory from local market of Dharan immediately after slaughtering. Bones of chicken breast meat was removed and minced in mincer. Methanolic extract of each herb and spices were incorporated into boneless minced chicken meat at concentration of 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm. Then all samples were mixed properly and rested for 15 min. After that, all samples were packed in LDPE and stored at 4ºC for observation. TBARS and TPC values of each sample were determined in 0, 4, 7, 10 and 12 days of storage at 4°C.

3.2.4 Herbal extract analysis

3.2.4.1 Antioxidant Activity of herbs and spices

Duan *et al.* (2015) developed a method for measuring DPPH radical scavenging activity. Samples (2.0 ml) were vigorously shaken after being mixed with 0.4 mm DPPH (2.0 ml). At 37°C water bath, the mixture solution was left in the dark for 30 min. Ascorbic acid was used as a positive control. The reaction's absorbance is then calculated. A spectrophotometer was used to read the mixture at 517 nm. Following formula was used to calculate radical scavenging activity based on the control reading:

Radical scavenging activity of DPPH (%) = $(1-A_s/A_c)\times100$

where As represents the absorbance of the sample, and Ac is the absorbance of reaction control. Methanol was placed as blank. The IC_{50} value is a parameter widely used to measure the antioxidant activity of test samples. It is calculated as the concentration of antioxidants needed to decrease the initial DPPH concentration by 50%. Thus, the lower IC_{50} value the higher antioxidant activity (Rivero-Cruz *et al.*, 2020).

3.2.4.2 Determination of Total Phenolic Content

1 ml sample (1 mg/ml) was mixed with 1 ml Folin-Ciocalteu reagent. After 3 min, 1ml of saturated Na_2CO_3 solution was added to the mixture, and then 7 ml of distilled water was added. The mixture was kept in the dark for 90 min and the absorbance was read at 725 nm. The total phenol content (TPC) was determined using a standard curve generated by gallic acid. The evaluation of phenolic compounds was performed in triplicate. The result is the

mean ± standard deviation and expressed as mg gallic acid equivalent/g extract (Miladi and Damak, 2008).

3.2.4.3 Determination of Total Flavonoid Content

The total flavonoid contents of the spice extracts were determined by a modified colorimetric method described by Sakanaka *et al.* (2005) using quercetin as a standard. The extract or standard solution (250 µL) was mixed with distilled water (1.25 ml) and 75 µL 5% sodium nitrite ($NaNO_2$) solution. After 5 minutes, 150 µL 10% aluminum chloride ($AlCl_3$) solution was added. After 6 min, add 0.5 ml 1 M sodium hydroxide (NaOH) and 0.6 ml distilled water. The solution was then mixed and the absorbance measured at 510 nm. The results are expressed as mg quercetin/g sample. All determinations were performed in triplicates.

3.2.4.4 Determination of Tannin Content

Tannins were determined by the Folin-Ciocalteu method Approximately 0.1 ml of the sample extract was transferred to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of sodium carbonate solution at 35% and diluted to 10 ml. with distilled water. Mixture was stirred well and kept at room temperature for 30 minutes. a set of tannic acid reference standard solutions (20, 40, 60, 80, 100 µg/ml) were prepared in the same manner as described above. The absorbance of the test solution and the standard solution was measured against the blank at 700 nm using a UV/Visible spectrophotometer. The estimation of tannin content was performed in triplicate. The tannin content is expressed in mg tannic acid equivalents/g dry sample(CI and Indira, 2016).

3.2.5 Shelf-life Analysis

3.2.5.1 Determination of TBARS

Thiobarbituric acid reactive substances (TBARS) were used to assess lipid oxidation, with some modification to the method reported by Ahn *et al.* (1998). 5 g of sample was homogenized in 15 ml distilled water and centrifuged at 2000 rpm for 15 min. 1 mL of the resulting slurry was transferred to a test tube containing 2 ml of trichloroacetic acid/thiobarbituric acid (TCA/TBA) solution consisting of 15% TCA (w/v) and 0.375% TBA (w/v) in 0.25 M HCl and 50 µl of 7.2% butylated hydroxytoluene (w/v) prepared in

absolute ethanol then mixture was vortexed and incubated at 100°C for 15 min at water bath to develop color. Then test-tube content was cooled rapidly down to room temperature and centrifuged at 2000rpm for 10 min. Then, absorbance was measured at 531 nm with a spectrophotometer against blank (1 ml distilled water and 2 ml TCA-TBA-HCl solution). TBARS were calculated using 1,1,3,3-tetraethoxypropane standard curve and expressed as mg MDA/kg meat.

3.2.5.2 Determination of Total Plate Count

For determination of microbial counts, 10 g of meat sample was homogenized with 90 ml, 0.1% distilled water. Serial 10-fold dilutions were prepared by diluting 1 ml of homogenate in 9 ml of distilled water. Appropriate serial dilutions were duplicate plated (Pour plate method) with plate count agar (PCA). Plates were incubated at 37°C for 48 h and colony was counted (Naveena *et al.*, 2006).

3.3 Statistical analysis

Experiment was conducted in three replications. One Way ANOVA at 5% level of significance was carried out on the obtained data using IBM SPSS statistics 20 for phytochemicals analysis. Again, One Way ANOVA at 5% level of significance was carried out on the obtained data using R- Programming version 4.02 and JMP version 14 software for TBARS and TPC values. Post hoc test was done by Tukey Honest Test to evaluate the significant difference between the samples and within the samples of TBARS and TPC values. Bar graphs and line graphs were constructed by using MS-Excel 365.

Part IV

Results and Discussions

Leaves of onion (*Allium cepa*), garlic (*Allium sativum)*, chiraito (*Swertia chirayita*), radius (*Raphanus sativus*), hattibar (*Agave* spps.) and chicken breast meat were collected and brought to laboratory. All sample were dried and grinded into powder and used in extraction of phytochemicals. Proximate of meat, DPPH radical scavenging capacity, total phenols, total flavonoids of all plant sample were determined. Then 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm of each sample were mixed to minced meat and packed in LDPE and stored at refrigeration. TBARS number and total plate count of ground meat were studied on every 3 days interval up to 12 days.

4.1 Proximate analysis of meat

The proximate analysis of chicken breast was carried out. The following Table 4.1 shows the proximate analysis of fresh chicken breast meat.

Table 4.1 Proximate analysis of chicken breast meat

Components	Value % (wb)	Value % (db)
Moisture (%)	73.13 ±0.73	73.13 ±0.73
Crude fat (%)	1.15 ±0.22	4.27±0.22
Crude protein (%)	23 ±0.20	85.59±0.20
Total ash (%)	1.05 ±0.21	3.90±0.21

*Values are the means of three determinations and the figures in the parentheses are their standard deviation. Means with different superscripts are different ($p < 0.05$).

From the proximate analysis of meat; percentage of moisture, crude fat, crude protein and total ash were found to be 73.13±0.73%, 1.15±0.22%, 23±0.20% and 1.05±0.21% respectively. The values so obtained for moisture content, crude protein, crude fat and ash content were slightly different then the result obtained by Ali *et al.* (2007). Ahmad *et al.* (2018) also reported similar result for fat and protein. Chen *et al.* (2016) also reported

slightly lower result for protein and slighter higher for moisture and ash content. This may be due to age, sex, feed, slaughter practices etc. (Mir *et al.*, 2017).

4.2 Total phenol content

Phenolic compounds regarded to be antioxidants play the very vital position of defensive organisms in opposition to dangerous outcomes of oxygen radicals and different surprisingly reactive oxygen species (Stratil *et al.*, 2006). The scavenging capacity of phenols is in particular because of the phenolic structure of hydroxyl substituent at the aromatic ring (Robbins, 2003). Furthermore, a definitely and surprisingly significant relationship among general phenolics and antioxidant activity changed into documented by Velioglu *et al.* (1998) which implied a compound with better content material of phenol possessed better antioxidant activity.

Total phenol contents of methanolic extracts of spices are shown in Fig 4.1. The total phenol contents of spices extracts were found to be significantly different ($p < 0.05$).

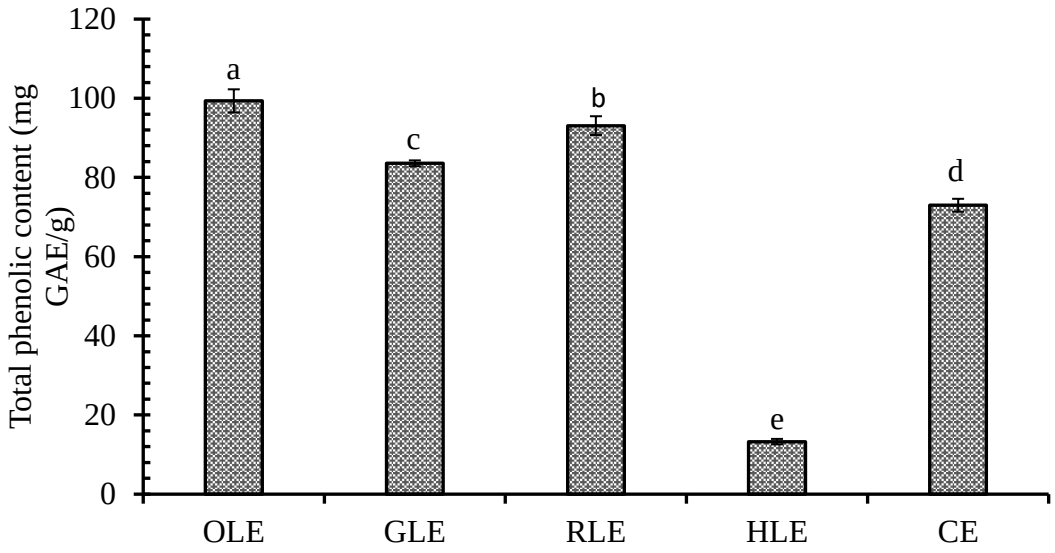


Fig. 4.1 Total phenolic content (mg GAE/g) of methanolic extract of herbs and spices

*OLE, GLE, RLE, HLE and CE denote the methanolic extract of onion leaves, garlic leaves, radish leaves, hattibar leaves and chiraito respectively. Vertical error bars represent ± standard deviation. Bars with similar alphabets at the top are not significantly different.

Statistical analysis at 5% level of significance shows that the total phenol content is significantly different with species. Highest value and lowest value of phenolic content were

obtained for onion leaves (99.34±2.92) and hattibar (agave) leaves 13.27±0.69 respectively. From the phytochemical analysis, the total phenolic content of onion leaves, garlic leaves, radish leaves, chiraito (*Swertia chirayita)* and hattibar leaves extract on 80% methanol were found to be 99.34±2.92, 83.58±0.73, 93.09±2.37, 72.98±1.63 and 13.27±0.69 respectively. Khanal *et al.* (2015) found total phenolic content of *swertia chirayita* (Chiraito) to be 67.49±0.50 mg GAE/g which was slightly lower than our finding. Duan *et al.* (2015) was found total phenol content (mg GAE/g) to be 94.24±0.59 of 70% methanolic extract of onion (Allium cepa L.) peels but we found 99.34±2.92^{e} mg GAE/g for onion leaves. Gorinstein *et al.* (2008) also found that total phenolic content of methanolic extract of onion was found higher than garlic leaves. Nasri and Salem (2012) reported similar result for *agave americana* (hattibar) and Eugenio *et al.* (2017) also found similar result for radish leaves as our result but our finding was lower than result obtained by Luo *et al.* (2018). Kim *et al.* (2016) was found lower value for GLE than ours finding. Several studies reported that the differences in polyphenol content could be attributable to biological factors (genotype, cultivars), as well as environmental (temperature, salinity, water stress and light intensity) conditions. Moreover, the extraction of phenolic compounds depends on the type of solvent used, the degree of polymerization of phenolics, and their interaction (Al Mamun *et al.*, 2016).

4.3 Total flavonoid content

Flavonoids are a large family of hydroxylated polyphenolic compounds having a benzo-γ-pyrone structure and are ubiquitously present in plants (Kumar and Pandey, 2013; Pande, 2019). Antioxidant activity of flavonoids is believed to be due to their ability to act as free radical acceptor and to complex metal ions (Hertog *et al.,* 1992). They are biologically active against liver toxins, tumors, viruses and other microbes, allergies and inflammation (De *et al.,* 1999). The flavonoid content of methanolic extract of herbs and spices are shown in Fig 4.2.

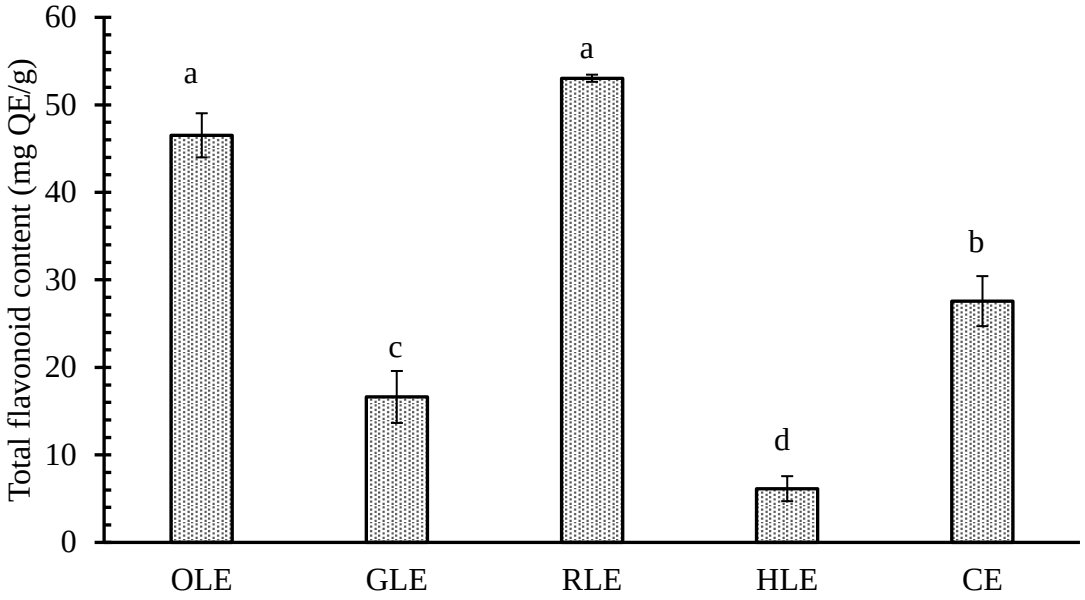


Fig. 4.2 Total flavonoid content (mg QE/g) of methanolic extract of herbs and spices

*OLE, GLE, RLE, HLE and CE denote the methanolic extract of onion leaves, garlic leaves, radish leaves, hattibar leaves and chiraito respectively. Vertical error bars represent ± standard deviation. Bars with similar alphabets at the top are not significantly different.

Statistical analysis at 5% level of significance shows that the total flavonoid content is significantly different with plant species. Highest value and lowest value of flavonoid content were obtained for radish leaves (53.04±0.41) mg QE/g and hattibar leaves (6.14±1.43) mg QE/g respectively. Duan *et al.* (2015) reported total flavonoid content in 70% methanolic extract of onion peels to be 43.33±0.41 mg QE/g and in 70% ethanolic extract of onion peels to be 49.63±0.55 mg QE/g and we found the total flavonoid content in methanolic extract of onion leaves to be 47.52±2.52 mg QE/g which is slightly higher than methanolic extract of onion peels because quercetins are accumulate to varying degrees in plant tissues, and the levels found in different plant parts are affected by environmental factors (Hichri *et al.*, 2011). Al Mamun *et al.* (2016) reported that the total flavonoid content of garlic leaves to be 11.92±1.6 mg QE/g which slightly lower than our result (16.62±2.97 mg QE/ g) and Kim *et al.* (2016) obtained even more lower result for 70% methanolic extract of garlic leaves. The total flavonoid of chiraito was found to be 27.57±2.86 mg QE/g and Khanal *et al.* (2015) also found the similar results. Hamissa *et al.* (2012) found slightly lower result than our finding for *Agave americana.*

At 5% level of significance, total flavonoids contents were found to be in following order: RLE > OLE > CE > GLE > HLE.

4.4 Total tannin content

Plant extracts containing tannins are used as astringents, antidiarrheals, diuretics, gastric and duodenal tumors, as well as anti-inflammatory, antiseptic, antioxidant and hemostatic agents (Dolara *et al.*, 2005). Tannins have been shown to prevent lipid peroxidation and scavenge free radicals, both of which are significant under pro-oxidation conditions in cells. The majority of tannin's actions, including their ability to scavenge free radicals, are largely determined by their structure and degree of polymerization (Sieniawska and Baj, 2017). The total tannin content of methanolic extract of herbs and spices were shown in Fig 4.3

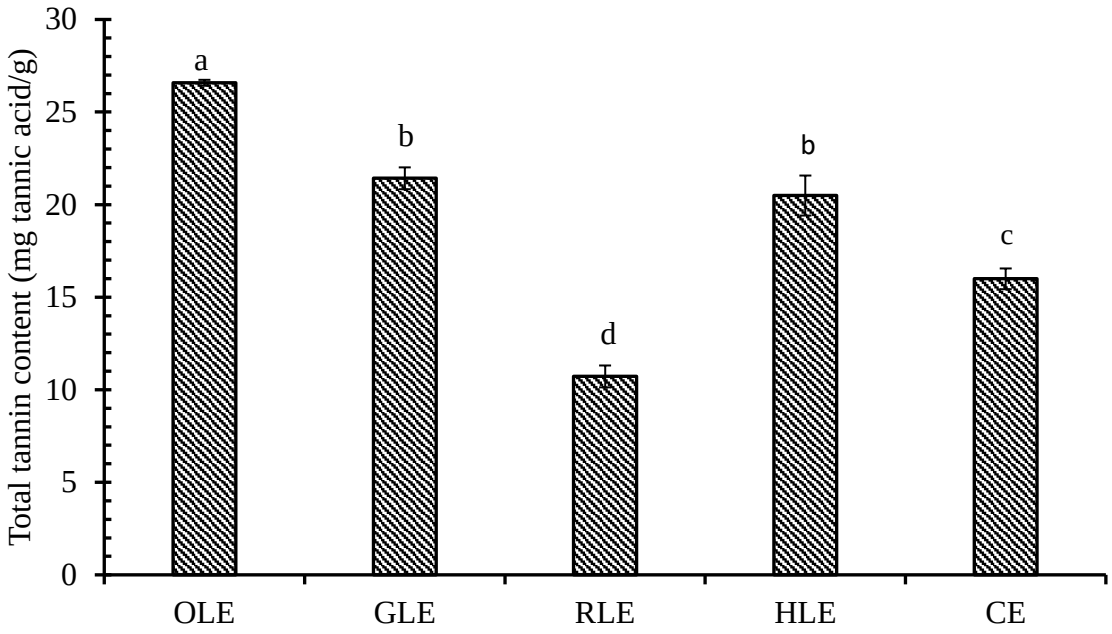


Fig. 4.3 Total tannin content of methanolic extract of herbs and spices.

*OLE, GLE, RLE, HLE and CE denote the methanolic extract of onion leaves, garlic leaves, radish leaves, hattibar leaves and chiraito respectively. Vertical error bars represent ± standard deviation. Bars with similar alphabets at the top are not significantly different.

Statistical analysis at 5% level of significance shows that the total tannin content is significantly different with species but garlic leaves extract and hattibar leaves extract showed similar result. The total tannin content of methanolic extract of onion leaves, garlic leaves, hattibar leaves, chiraito and radish leaves were found to be 26.58 ± 0.16^{a}, 21.42 ± 0.61^{b},

20.49 ± 1.08^{b}, 15.99 ± 0.56^{c} and $10.72\pm.059^{d}$ mg tannic acid/ g respectively. At 5% level of significance, total flavonoids contents were found to be in following order: OLE > GLE > HLE > CE > RLE.

4.5 Total antioxidant activity

Total antioxidant activity of methanolic extracts of herbs and spices are shown in Table 4.5. The activity of herbs and spices extracts were found to be significantly different ($p < 0.05$).

The IC_{50} value is a parameter widely used to measure the antioxidant activity of test samples. It is calculated as the concentration of antioxidants needed to decrease the initial DPPH concentration by 50%. Thus, the lower IC_{50} value the higher antioxidant activity (Rivero-Cruz *et al.*, 2020). Total antioxidant activity of methanolic extracts of herbs and spices was shown in Fig 4.4.

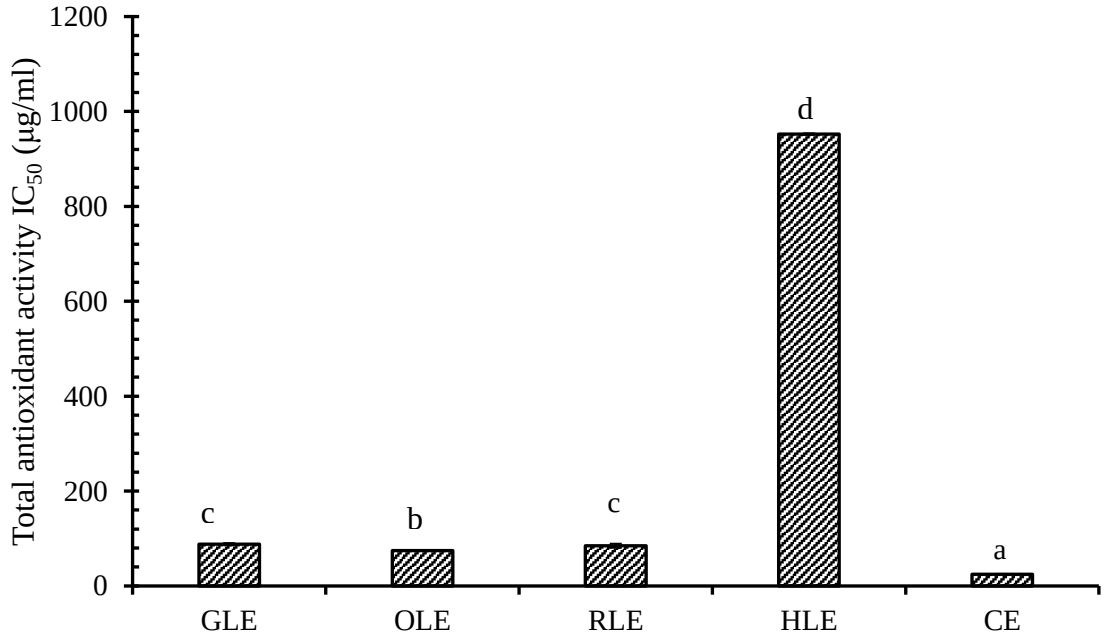


Fig. 4.4 Total antioxidant activity of methanolic extracts of herbs and spices

*OLE, GLE, RLE, HLE and CE denote the methanolic extract of onion leaves, garlic leaves, radish leaves, hattibar leaves and chiraito respectively. Vertical error bars represent ± standard deviation. Bars with similar alphabets at the top are not significantly different.

Statistical analysis at 5% level of significance shows that the total antioxidant activity is significantly different with species. Lowest amount of antioxidant activity (IC_{50}) was found

for hattibar leaves (952.28±1.43 µg/ml) and highest antioxidant activity (IC_{50}) for chiraito (24.96±0.40 µg/ml). Khanal *et al.* (2015) was found 23.35± 0.59 µg/ml for *Swertia chirayita* which was similar to our finding. IC_{50} of onion leaves was 74.79±0.45 µg/mg. Duan *et al.* (2015) was found that IC_{50} for methanolic extract of onion peels as 0.08±0.00 mg/ml which was slightly higher than our result. According to Thampi *et al.* (2015), IC_{50} of green onion leaves with a value of 69.965 µg/ml for methanol and 86.548 µg/ml for water extract. Our result for onion leaves was slightly lower than aqueous extract but slightly higher than methanolic extract of green onion leaves as compare to result found by Thampi *et al.* (2015). IC_{50} of garlic leaves was found to be 88.033±2.25 which was higher than result (64.033 µg/ml) obtained by Thampi *et al.* (2015). A study found that aqueous extract of garlic had higher antioxidant activity than aqueous extract of onion. Another study found that methanol extract of onion had higher antioxidant activity than methanol extract of garlic (Nuutila *et al.*, 2003). IC_{50} of hattibar (*Agave americana*) was found to be 952.28±1.43 µg/ml which was lower than result obtained by Dif (2016). This is due to plant antioxidant activity varies by species, and differences within the same species have been discovered depending on the solvent extraction, the physical condition of the plant material (fresh or dried), or environmental factors (Kratchanova *et al.*, 2010). IC_{50} of methanolic extract of radish leaves was found to be 84.79±4.27 µg/ml and similar result was obtained by Luo *et al.* (2018). Chiraito have strong antioxidant activity followed by onion, radish, garlic and hattibar leaves.

4.6 Estimation of change in TBARS value of the ground meat over time

4.6.1 Effect of various concentration of extracts on TBARS value over the time

4.6.1.1 Effect of chiraito extract on TBARS value over the time

In the five samples of ground meat incorporated with 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm of radish extract, TBARS values were recorded over time for 0,4,7,10,12 days. The trend of change in TBARS values of CE treated meat samples were presented in Fig. 4.5.

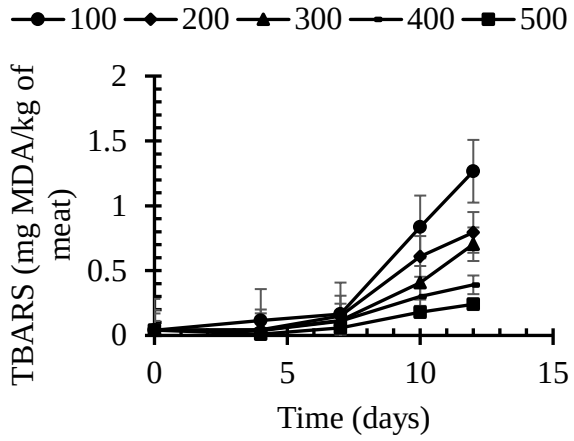


Fig. 4.5 Change in TBARS value over time for CE incorporated sample

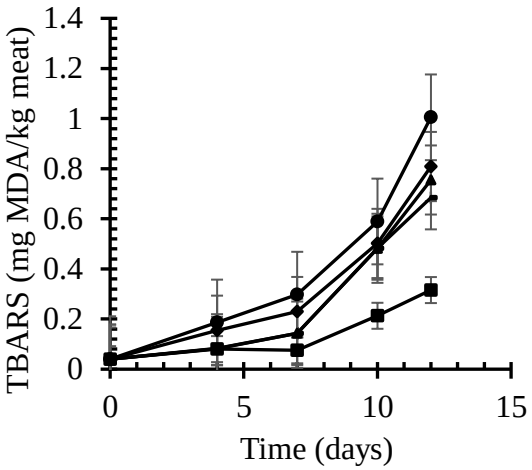


Fig. 4.6 Change in TBARS value over time for GLE incorporated sample

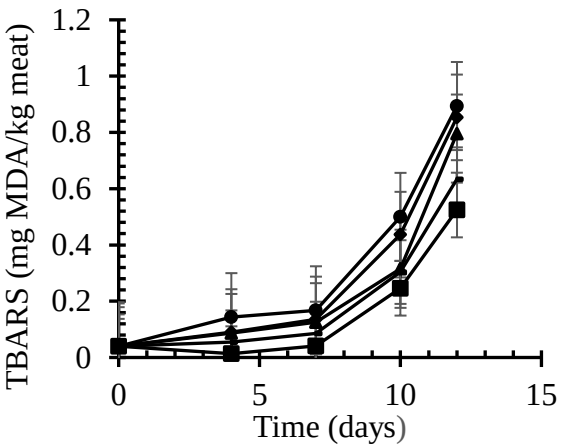


Fig. 4.7 Change in TBARS value over time for HLE incorporated sample

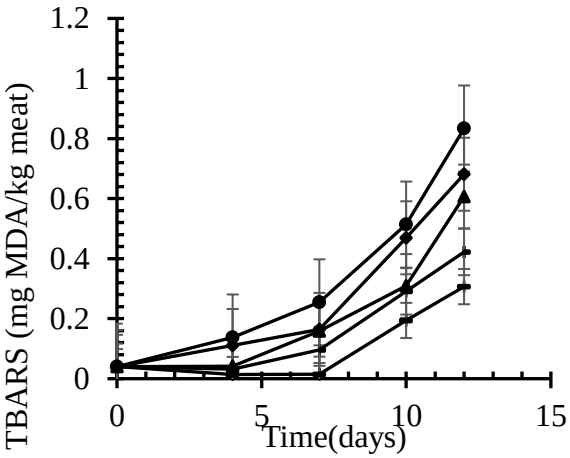


Fig. 4.8 Change in TBARS value over time for OLE incorporated sample

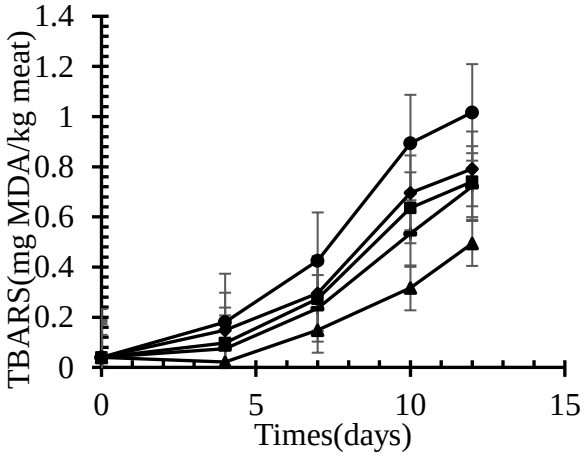


Fig. 4.9 Change in TBARS value over time for RLE incorporated sample

At 5% level of significance, both concentration of methanolic extract of chiraito extract and storage time significantly affected TBARS values of ground meat. Highest value and lowest value were obtained for the ground meat incorporated with 100 ppm and 500 ppm extract respectively. 100 ppm CE incorporated sample crossed the threshold value 1 mg MDA/kg sample on 12 days of storage at 4^0C whereas other samples did not cross threshold value on 12th days of storage. 100 ppm samples showed steeply rise in TBARS value after 7th days of storage similarly 200 ppm and 300ppm also showed rise in TBARS value after 7th days of storage but 400 ppm and 500 ppm were almost no increment for 7th days after that small increment in TBARS value. TBARS values were lower with higher concentration of antioxidant because antioxidant slow down the rate of oxidation (Frankel *et al.*, 2000).

4.6.1.2 Effect of garlic leaves extract on TBARS value over the time

In the five samples of ground meat incorporated with 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm of garlic leaves extract, TBARS values were recorded over time for 0,4,7,10,12 days. The trend of change in TBARS value of GLE treated meat was presented in Fig. 4.6.

At 5% level of significance, both concentration of garlic extract and the storage time significantly affected TBARS values of ground meat. Highest and lowest TBARS value were obtained from 100 ppm and 500 ppm incorporated ground meat sample respectively. 100ppm GLE incorporated sample (1.0051±0.0011 mg MDA/kg) crossed the threshold value on 12th days of storage under refrigeration respectively. On 4th days, there were no significantly different TBARS value found for 300 ppm, 400 ppm and 500 ppm incorporated samples. On 7th days, all sample showed significantly different result except 300ppm and 400ppm sample. On 10th and 12th days only 300 ppm and 400 ppm incorporated samples were not significantly different. All GLE incorporated samples showed increased TBARS value after 4th days but 500 ppm incorporated sample increased significantly after 7th days whereas the control showed steeply increased after 4th days of storage but with increased in concentration of extract increment was at lower rate.

4.6.1.3 Effect of hattibar leaves extract on TBARS value over the time

In the five samples of ground meat incorporated with 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm of hattibar leaves extract, TBARS values were recorded over time for 0,4,7,10,12 days. The trend of change in TBARS value of HLE treated meat was presented in Fig. 4.7.

At 5% level of significance, both concentration of hattibar leaves extract and the storage time significantly affected TBARS values of ground meat. Highest and lowest TBARS value were obtained from the 100 ppm and 500 ppm incorporated ground meat sample respectively. None of the HLE incorporated samples crossed the threshold value which mean they can be stored further if microbial load was within control. Highest and lowest TBARS values were observed for the control and 500 ppm incorporated ground meat respectively with storge time. There were not such a significantly increment in TBARS values of GLE treated samples up to 7^{th} days after that significantly increased whereas untreated meat sample (control) was steeply rise in TBARS value. At 5% level of significance, TBARS values were found to be in following order; control >100 ppm > 200 ppm > 300 ppm > 400 ppm > 500 ppm.

4.6.1.4 Effect of onion leaves extract on TBARS value over the time

In the five samples of ground meat incorporated with 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm of radish extract, TBARS values were recorded over time for 0,4,7,10,12 days. The trend of change in TBARS value of OLE treated meat was presented in Fig. 4.8.

At 5% level of significance, both concentration of onion leaves extract and the storage time significantly affected TBARS values of ground meat. Highest and lowest TBARS value were obtained from the 100 ppm and 500 ppm incorporated ground meat sample respectively. None of OLE incorporated sample exceeded the threshold TBARS value even after the end of 12^{th} days of storage. OLE treated samples showed slightly increment of TBARS value after 4^{th} days except 500 ppm OLE treated sample which showed almost no increment up to 7^{th} days after that slightly increment in TBARS value. The highest value of TBARS was obtained from the control and followed by 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm incorporated meat.

4.6.1.5 Effect of radish leaves extract on TBARS value over the time

In the five samples of ground meat incorporated with 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm of radish extract, TBARS values were recorded over time for 0,4,7,10,12 days. The trend of change in TBARS value of RLE treated meat is presented in Fig. 4.9.

At 5% level of significance, both concentration of radish leaves extract and the storage time significantly affected TBARS values of ground meat. Highest value was found for the

ground meat incorporated with 100 ppm OLE and lowest value was observed for ground meat incorporated with 500 ppm OLE. Only 100ppm extract incorporated meat (1.016667±0.082mg MDA/kg of sample) exceeded the threshold value on 12th days which means it cannot be stored further. TBARS value were significantly increased with the time of storage but less increased in higher concentration of extract. 100 ppm RLE treated sample showed slightly increased of TBARS value after 4thdays. 200 ppm, 300 ppm and 400 ppm treated samples showed similar result 7th days which showed slightly increment after 7th days of storage. 500 ppm incorporated sample showed slightly increment in TBARS value after 7th days of storage.

Most of samples incorporated with plant extract did not cross threshold value (1 mg MDA/kg meat) after 12th days of storage except 100 ppm of OLE, RLE and CE incorporated meat. It was found that TBARS value was generally increased with time and decreased in increased of concentration of plant extract. The control rapidly increased in TBARS values throughout the refrigerated storage. Similar result was obtained by Ahn *et al.* (2004) for beef meat. These natural extracts were effective antioxidants in raw ground meat as compare to the control. Similar result was reported by Ahn *et al.* (2004). Mielnik *et al.* (2006) also reported that lipid oxidation can be prevented by increasing the concentration of grape seed extract on turkey meat. Burri *et al.* (2020) also reported that onion and beet root leaves were most efficient in inhibiting lipid oxidation with increasing concentration above 200 ppm for two weeks.

4.6.2 Effect of various sample on TBARS value over the time

4.6.2.1 Effect of 100 ppm on TBARS value over the time

In the six samples of ground meat incorporated with 100 ppm of extract of chiraito, garlic leaves, hattibar leaves, onion leaves, radish leaves and control, TBARS values were recorded over time for 0,4,7,10,12 days. The trend of change in TBARS value of garlic treated meat is presented in Fig. 4.10.

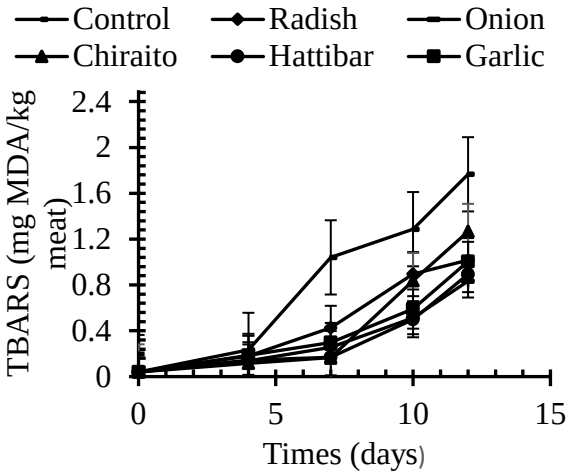


Fig. 4.10 Effect of 100 ppm of various sample on TBARS value over time.

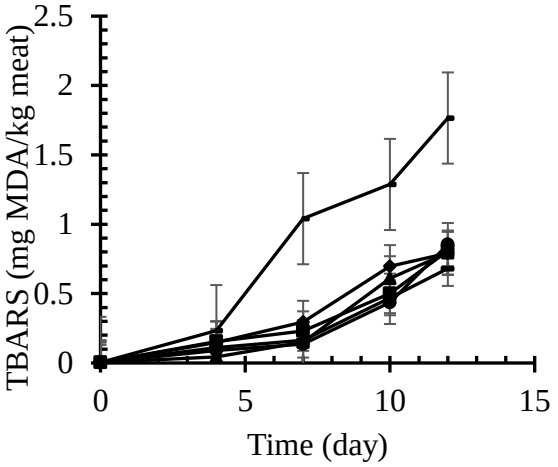


Fig. 4.11 Effect of 200 ppm of various sample on TBARS value over time.

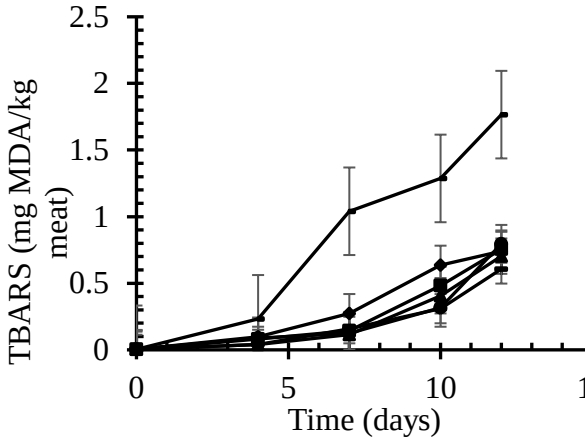


Fig. 4.12 Effect of 300 ppm of various sample on TBARS value over time.

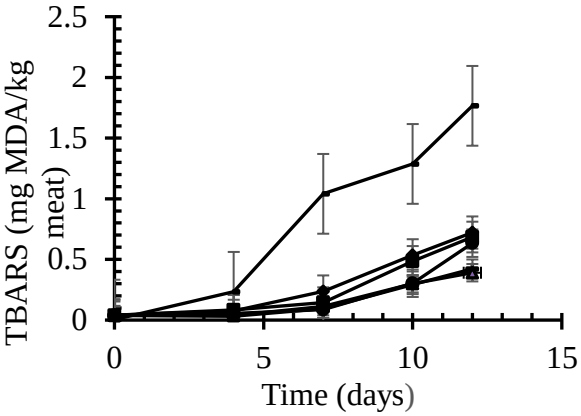


Fig. 4.13 Effect of 400 ppm of various sample on TBARS value over time.

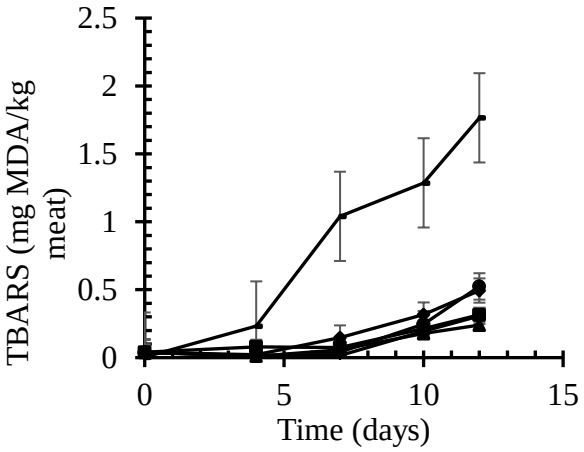


Fig. 4.14 Effect of 500 ppm of various sample on TBARS value over time.

At 5% level of significance, both sample and the storage time significantly affected TBARS values of ground meat. Highest value was observed in control and exceeded threshold value (1 mg MDA/kg meat) at 7th days of storage at 4°C. TBARS value of 100ppm incorporated samples with CE, GLE and RLE were exceeded at 12th days of storage. After 4th days, all 100 ppm incorporated samples and control were significantly different but 100 ppm of GLE & RLE incorporated samples showed similar result and 100 ppm of HLE and OLE incorporated samples also showed similar result. On 7th days, 100 ppm of GLE & OLE incorporated showed similar result. Similarly, 100 ppm of HLE & CE incorporated samples showed similar result. At 10th days, 100 ppm of OLE and HLE treated sample showed similar result. On 12th days, all samples were significant except RLE and GLE treated meat. Control showed highest value (1.71±0.082 mg MDA/kg of meat) and OLE incorporated sample showed lowest value (0.83±0.015 mg MDA/kg meat) at 12th days. So even at 12th days of storage, OLE and HLE incorporated were safe to consume but Control was rancid at 7th days of storage.

4.6.2.2 Effect of 200 ppm on TBARS value over the time

In the six samples of ground meat incorporated with 200 ppm of extract of chiraito, garlic leaves, hattibar leaves, onion leaves, radish leaves and control, TBARS values were recorded over time for 0,4,7,10,12 days. The trend of change in TBARS value of garlic treated meat is presented in Fig. 4.11.

At 5% level of significance, both sample and the storage time significantly affected TBARS values of ground meat. Control exceeded the threshold value at 7th days of storage whereas none of the 200ppm incorporated samples were exceeded the threshold value which mean all sample can be consumed after 12th days of storage. There was significantly difference ($p<0.005$) among the TBARS value of various samples. On 4th days, all 200ppm incorporated samples were significantly difference except GLE and RLE. On 7th days, 200ppm of OLE, CE and HLE incorporated samples showed similar result but other were significantly different whereas on 10th days, 200ppm of OLE incorporated samples showed the connected result with GLE and HLE. On 12th days, CE, RLE and GLE were showed similar result.

4.6.2.3 Effect of 300 ppm on TBARS value over the time

In the six samples of ground meat incorporated with 300 ppm of extract of chiraito, garlic leaves, hattibar leaves, onion leaves, radish leaves and control, TBARS values were recorded over time for 0,4,7,10,12 days. The trend of change in TBARS value of garlic treated meat is presented in Fig. 4.12.

At 5% level of significance, both sample and the storage time significantly affected TBARS values of ground meat. None of the sample crossed threshold value. On 4^{th} days, all samples were significantly different with control but HLE was connected to RLE and GLE and OLE and CE showed similar result. On 7^{th} days OLE, GLE, HLE and CE showed similar result. On 10^{th} days all samples were significantly different except HLE and OLE. On 12^{th} days, all sample were significantly different.

4.6.2.4 Effect of 400 ppm on TBARS value over the time

In the six samples of ground meat incorporated with 400 ppm of extract of chiraito, garlic leaves, hattibar leaves, onion leaves, radish leaves and control, TBARS values were recorded over time for 0,4,7,10,12 days. The trend of change in TBARS value of garlic treated meat is presented in Fig. 4.13

At 5% level of significance, both sample and the storage time significantly affected TBARS values of ground meat. Highest value was observed in control and exceeded threshold value (1 mg MDA/kg meat) at 7^{th} days of storage 4ºC. None of the sample crossed threshold value. On 4^{th} days, all samples were significantly different with control but GLE and RLE as well as CE and OLE showed similar result. On 7^{th} days, GLE, CE and OLE were connected to RLE and HLE. On 10^{th} days HLE, CE and OLE showed similar result. On 12^{th} days, all plant extract incorporated sample were interconnected.

4.6.2.5 Effect of 500 ppm on TBARS value over the time

In the six samples of ground meat incorporated with 500 ppm of extract of chiraito, garlic leaves, hattibar leaves, onion leaves, radish leaves and control, TBARS values were recorded over time for 0,4,7,10,12 days. The trend of change in TBARS value of garlic treated meat is presented in Fig. 4.14.

At 5% level of significance, both sample and the storage time significantly affected TBARS values of ground meat Highest value was observed in control and exceeded threshold value (1 mg MDA/kg meat) at 7th days of storage 4ºC. None of the sample crossed threshold value. After 4th days, all samples were significantly different with control. On 4th days, RLE, OLE, HLE and CE incorporated samples showed similar TBARS result. On 7th days, CE and OLE were inter-related with CE and HLE. Similarly, on 10th days, OLE and CE were showed similar result and GLE was connected to HLE and OLE. On 12th days, all sample were significantly different except GLE and OLE.

4.7 Estimation of total plate count of the ground meat over time

4.7.1 Effect of various sample extract on TPC of ground meat over the time

4.7.1.1 Effect of chiraito extract on TPC of ground meat over the time.

In the five samples of ground meat incorporated with 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm of methanolic extract of chiraito, TPC were recorded over time for 0,4,7,10,12 days. The trend of change in TPC of CE incorporated meat is presented in Fig. 4.15.

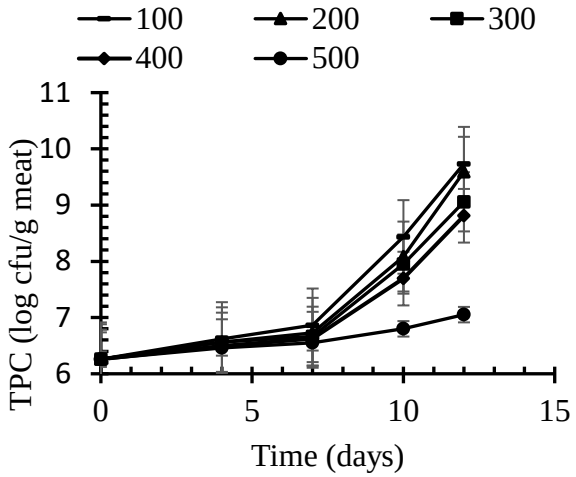


Fig. 4.15 Change in TPC of CE incorporated meat over the time

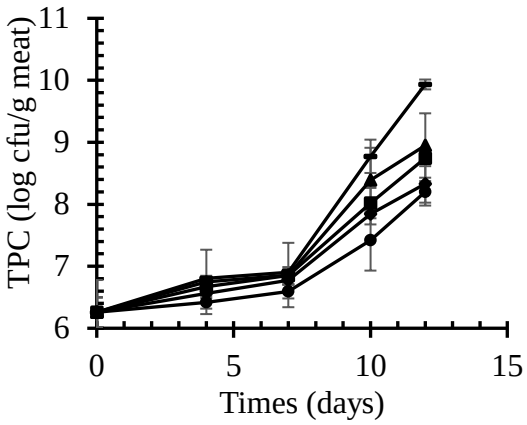


Fig. 4.16 Change in TPC of RLE incorporated meat over the time

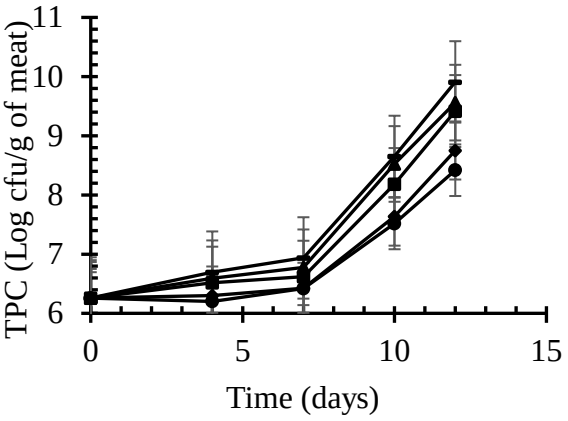


Fig. 4.17 Change in TPC of HLE incorporated meat over the time

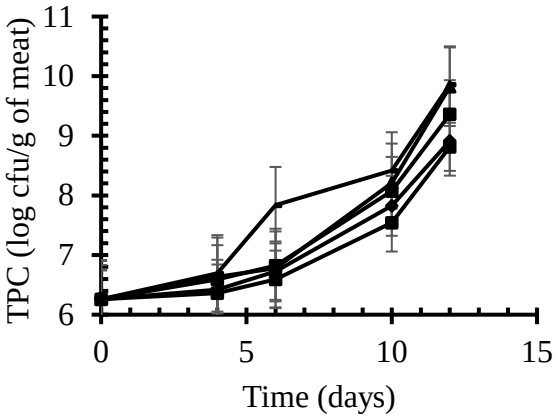


Fig. 4.18 Change in TPC of OLE incorporated meat over the time

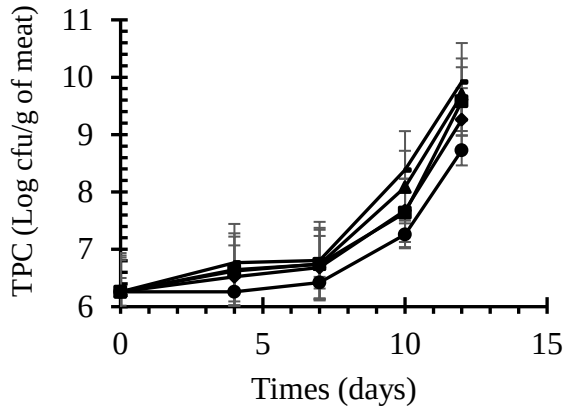


Fig. 4.19 Change in TPC of RLE incorporated meat over the time

At 5% level of significance, both concentration of extract and the storage time significantly affected TPC values of ground meat. Highest and lowest TPC values were obtained for the ground meat incorporated 100 ppm and 500 ppm respectively. All CE incorporated sample exceeded the threshold value (10^7 cfu/g) on 10^{th} days of storage except 500 ppm incorporated samples had just exceeded on 12^{th} days. There were slightly increased in TPC up to 7^{th} days of storage after that steeply raised in TPC values of CE incorporated sample except 500 ppm CE incorporated meat which was slightly increased stored at 4°C. The lowest TPC value were obtained for the 500 ppm incorporated sample (7.05±0.22 log cfu/g) respectively on 12^{th} days.

CE showed significant antimicrobial effect as compare to the control. Roy *et al.* (2015) reported that chiraito was effective against gram-positive and gram-negative bacteria.

4.7.1.2 Effect of garlic leaves extract on TPC of ground meat over the time

In the five samples of ground meat incorporated with 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm of methanolic extract of garlic leaves, TPC were recorded over time for 0,4,7,10,12 days. The trend of change in TPC of garlic leaves extract treated meat is presented in Fig. 4.16.

At 5% level of significance, both concentration of extract and the storage time significantly affected TPC values of ground meat. Highest and lowest TPC values were obtained for the 100 ppm and 500 ppm incorporated samples respectively. All GLE incorporated samples exceeded the threshold limit on 10^{th} days but 400 ppm and 500 ppm sample had just exceeded threshold value after 10^{th} days of storage. There were slightly increased in TPC up to 7^{th} days of storage after that steeply raised in TPC values of GLE incorporated samples at 4°C. On 12^{th} days, the lowest TPC were found for 500 ppm GLE incorporated sample (8.02±0.17 log cfu/ml).

GLE significantly inhibited microbial growth as compare to the control. Benkeblia (2004) reported that essential oil of GLE was more effective to gram-positive bacteria as compare to OLE essential oil. Mahros *et al.* (2021) also reported that microbial count of beef could be inhibited within the legal limit by using fresh garlic and garlic extract for 15 days at 4°C.

4.7.1.3 Effect of hattibar leaves extract on TPC of ground meat over the time

In the five samples of ground meat incorporated with 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm of methanolic extract of hattibar leaves, TPC were recorded over time for 0,4,7,10,12 days. The trend of change in TPC of hattibar leaves treated meat is presented in Fig. 4.17.

At 5% level of significance, both concentration of extract and the storage time significantly affected TPC values of ground meat. Highest and lowest TPC values were obtained for the ground meat incorporated with 100 ppm and 500 ppm incorporated sample respectively. All HLE incorporated samples exceeded on 10^{th} days of storage but 400 ppm and 500 ppm incorporated sample had just exceeded the threshold limit. On 4^{th} days, 100 ppm, 200 ppm and 300 ppm incorporated samples were not significantly different and slightly increase in TPC value but in case of 400 ppm and 500 ppm almost no increment up to 4^{th} days of storage after that there were significantly increased in TPC value of all samples and TPC value of the control increased from beginning. The lowest TPC values were obtained from the 500 ppm HLE incorporated sample (8.35±0.1 log cfu/ml) respectively on 12^{th} days.

HLE significantly inhibited the microbial growth as compare to the control. López-Romero *et al.* (2018) reported *Agave* spp showed strong antimicrobial properties against Gram-positive and Gram-negative bacteria.

4.7.1.4 Effect of onion leaves extract on TPC of ground meat over the time

In the five samples of ground meat incorporated with 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm of methanolic extract of onion leaves, TPC were recorded over time for 0,4,7,10,12 days. The trend of change in TPC of onion leaves treated meat is presented in Fig. 4.18.

At 5% level of significance, both concentration of extract and the storage time significantly affected TPC values of ground meat. Highest and lowest TPC values were obtained for the 100 ppm and 500 ppm incorporated samples respectively. 100 ppm GLE incorporated samples and other samples exceeded the threshold limit on 7^{th} and 10^{th} respectively but 400 ppm and 500 ppm sample had just exceeded threshold value after 10^{th} days of storage. 100 ppm and 200 ppm OLE extract increased slightly up to 4^{th} days and 300

ppm, 400 ppm and 500 ppm were almost no increment up to 4th days. 100 ppm OLE extract incorporated sample were increased steeply after 4th days and other OLE treated sample were increased after 7th days of storage at 4°C. On 12th days, the lowest TPC values were obtained from the 500 ppm (8.91± 0.13 log cfu/ml) OLE incorporated samples respectively.

OLE extract significantly inhibited the microbial growth as compare to the control. OLE significantly inhibited the *E-coil*, yeast and mold (Irkin and Arslan, 2010). Irkin and Arslan (2010) reported that 50% onion aqueous extract inhibited significantly the mesophilic aerobic bacteria of beef meat for 9 days at 4°C within legal limit.

4.7.1.5 Effect of radish leaves extract on TPC of ground meat over the time

In the five samples of ground meat incorporated with 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm of methanolic extract of radish leaves, TPC were recorded over time for 0,4,7,10,12 days. The trend of change in TPC of radish leaves extract treated meat is presented in Fig. 4.19.

At 5% level of significance, both concentration of extract and the storage time significantly affected TPC values of ground meat. Highest and lowest TPC values were obtained for the control and ground meat incorporated with 500 ppm respectively. All samples showed significantly different ($p<0.05$) result from the control on 4th.days of storage. All RLE incorporated samples exceeded the threshold limit on 10th but 300 ppm, 400 ppm and 500 ppm sample had just exceeded threshold value after 10th days of storage. All RLE incorporated sample except 500 ppm increased slightly up to 6th days and 500 ppm RLE incorporated sample was almost no increment up to 4th days and slightly increased up to 7th days of storage. All RLE extract incorporated samples were increased steeply after 7th days of storage at 4°C. The lowest TPC values were shown by the 500 ppm (8.72±0.26 log cfu/ml) incorporated samples.

4.7.2 Effect of various concentration of sample on TPC of ground meat over the time

4.7.2.1 Effect of 100 ppm on TPC of ground meat over the time

In the five samples of ground meat incorporated with 100 ppm of methanolic extract of chiraito, garlic leaves, hattibar leaves, onion leaves and radish leaves and control, TPC were recorded over time for 0,4,7,10,12 days. The trend of change in TPC of 100 ppm treated meat is presented in Fig. 4.20

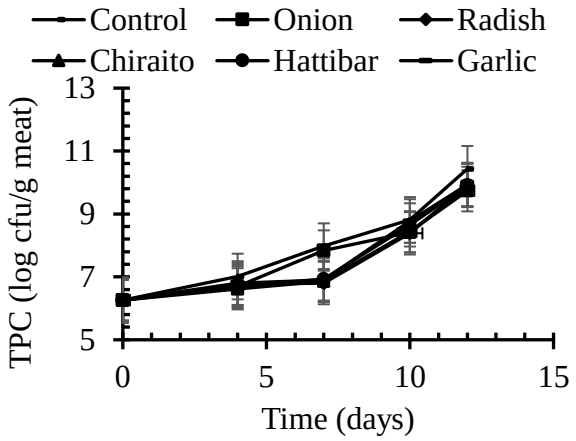


Fig. 4.20 Effect of 100 ppm of various sample on TPC value over time

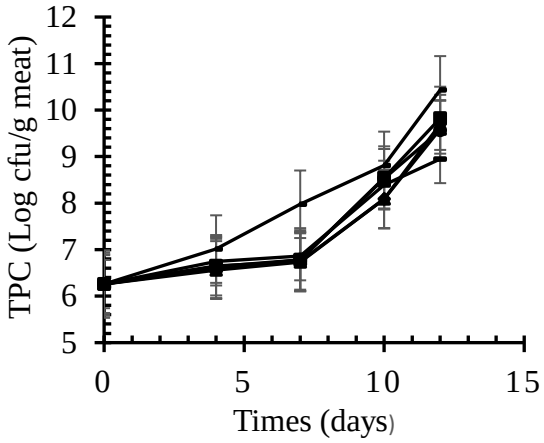


Fig. 4.21 Effect of 200 ppm of various sample on TPC value over time

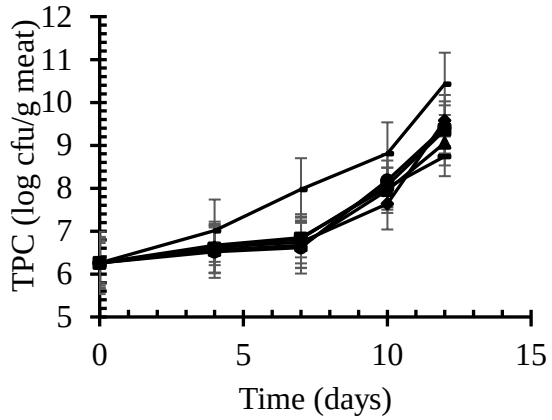


Fig. 4.22 Effect of 300 ppm of various sample on TPC value over time

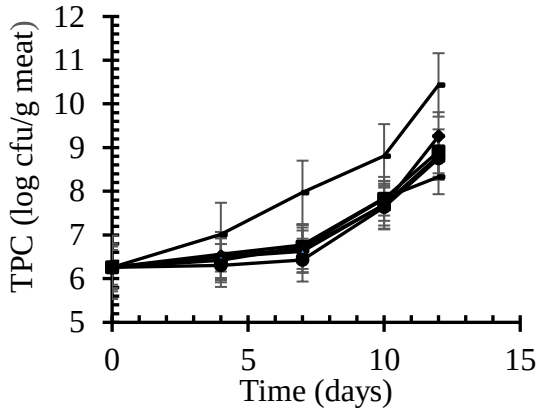


Fig. 4.23 Effect of 400 ppm of various sample on TPC value over time

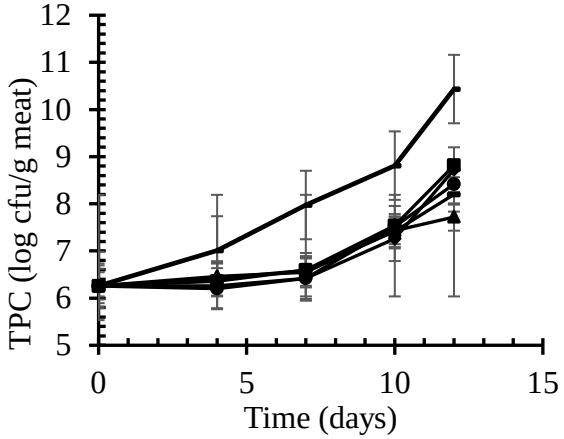


Fig. 4.24 Effect of 500 ppm of various sample on TPC value over time

At 5% level of significance, both sample and the storage time significantly affected TPC values of ground meat. Control exceeded the threshold at 4th days of observation whereas 100 ppm of CE, GLE, HLE, OLE and RLE incorporated meat exceeded threshold value (7 log_{10}) at 10th days of storage. Thus, using plant extract increase the shelf-life significantly. On 4th days, TPC value of CE incorporated sample significantly different to the control. But remaining sample showed relation between both control and chiraito extract incorporated meat. On 7th days control and all samples showed significantly different but all samples were significantly similar result. On 10th days, Chiraito, onion leaves, radish leaves extract treated meat showed significantly similar result whereas garlic and hattibar leaves extract treated sample showed interconnected result between control and other three sample. On 12th days, there were significant differences between samples and control but significantly similar within samples. The control showed rise in TPC value steeply from beginning but all samples showed steeply rise in TPC after 7th days of storage but OLE incorporated sample showed steeply rise in TPC value after 4th days of storage. On 12th days, highest and lowest TPC values were obtained from Control (11.1±0.17 log cfu/g) and CE (9.73±0.24 log cfu/g) respectively.

4.7.2.2 Effect of 200 ppm on TPC of ground meat over the time

In the five samples of ground meat incorporated with 200 ppm of methanolic extract of chiraito, garlic leaves, hattibar leaves, onion leaves and radish leaves and control, TPC were recorded over time for 0,4,7,10,12 days. The trend of change in TPC of 200 ppm treated meat is presented in Fig. 4.21.

At 5% level of significance, both sample and the storage time significantly affected TPC values of ground meat. Control exceeded the threshold at 4th days of observation whereas other all samples exceeded the threshold value after 7th days of storage. On 4th days, the control was significantly different than extract incorporated samples whereas CE and HLE were significantly similar and GLE, RLE and OLE were connected to both the control and CE & HLE. On 7th days, there were significantly different between samples and control but significantly similar within samples. On 10th days, there were significantly different between samples and control but significantly similar within samples. On 12th days, there were significant different between TPC of control and samples. Except garlic leaves extract incorporated sample, all herbs and spices incorporated sample gave similar result. The control showed rise in TPC value steeply from beginning but all samples showed steeply

rise in TPC after 7th days of storage. On 12th days, highest and lowest TPC values were obtained from Control (11.1±0.17 log cfu/g) and GLE (8.94±0.31 log cfu/g) respectively.

4.7.2.3 Effect of 300 ppm on TPC of ground meat over the time

In the five samples of ground meat incorporated with 300 ppm of methanolic extract of chiraito, garlic leaves, hattibar leaves, onion leaves and radish leaves and control, TPC were recorded over time for 0,4,7,10,12 days. The trend of change in TPC of 300 ppm treated meat is presented in Fig. 4.22.

At 5% level of significance, both sample and the storage time significantly affected TPC values of ground meat. Control exceeded the threshold at 4th days of observation and other samples exceeded after 10th days of storage. on 4th days, HLE and CE extract incorporated sample showed significantly similar result and remaining samples showed the connected result between this two sample and control. On 7th days, there was significance difference between samples and control but significant similar within samples. On 10th days, there was significance difference between samples and control but significantly similar within samples. On 12th days, all samples were significantly different but HLE, OLE and CE extract incorporated samples were showed connected result between RLE and GLE incorporated samples. The control showed rise in TPC value steeply from beginning but all samples showed steeply rise in TPC after 7th days of storage. On 12th days, highest and lowest TPC values were obtained from Control (11.1±0.17 log cfu/ml) and (8.74±0.12 log cfu/ml) respectively.

4.7.2.4 Effect of 400 ppm on TPC of ground meat over the time

In the six samples of ground meat incorporated with 400 ppm of methanolic extract of chiraito, garlic leaves, hattibar leaves, onion leaves and radish leaves and control, TPC were recorded over time for 0,4,7,10,12 days. The trend of change in TPC of 400 ppm treated meat is presented in Fig. 4.23.

At 5% level of significance, both sample and the storage time significantly affected TPC values of ground meat. Control exceeded the threshold at 4th days of observation whereas others samples exceeded at 10th days of observation. On 4th days, TPC of OLE, HLE incorporated samples were significantly difference than control whereas RLE, GLE and CE were connected to both control and OLE and HLE. On 7th days and 10th days, all samples

were significantly difference ($p<0.0001$) than control but significantly similar within the sample. On 12th days, samples and control were significant but OLE, HLE and GLE incorporated sample were inter-related with CE and RLE. The control showed rise in TPC value steeply from beginning but all samples showed steeply rise in TPC after 7th days of storage. On 12th days, highest and lowest TPC values were obtained from Control (11.1±0.17 log cfu/g) and CE (8.085±0.39 log cfu/g) respectively.

4.7.2.5 Effect of 500 ppm on TPC of ground meat over the time

In the six samples of ground meat incorporated with 500 ppm of methanolic extract of chiraito, garlic leaves, hattibar leaves, onion leaves and radish leaves and control, TPC were recorded over time for 0,4,7,10,12 days. The trend of change in TPC of 500 ppm treated meat is presented in Fig. 4.24

At 5% level of significance, both sample and the storage time significantly affected TPC values of ground meat. Control exceeded the threshold at 4th days of observation whereas others samples exceeded at 10 days of observation. From 4th to 12th days all samples were significantly different as compare to control. From 4th to 10th days TPC of all sample were significantly different from the control but significantly similar within samples. On 12th days, OLE and RLE showed significantly similar result. GLE and HLE were connected to CE and RLE & OLE. CE showed lowest TPC at 12th days. The control showed rise in TPC value steeply from beginning but all sample showed almost no increment up to 7th days but slightly increased after 7th to 10th days after that steeply increased except CE incorporated meat. The lowest and highest TPC were obtained for CE (7.72± 0.26 log cfu/g) and control (11.1± 0.17 log cfu/g) respectively.

It had been found that plant extract could delay lipid oxidation at least 12 days but could not stop microbial spoilage for 12 days. 500 ppm of CE incorporated was only sample which was safe for 12th days in terms of TBARS and TPC value but other were spoiled. Most of the plant extract incorporated samples inhibited microbial growth as compare to the control and microbial growth was lower with increased in concentration of concentration. Jadoun *et al.* (2016) reported that methanol, ethyl acetate, and chloroform extracts of the root, stem, and leaf of white radish exhibit considerable antibacterial activity towards numerous foodborne and drug-resistant pathogenic microorganism together with *B. subtilis*, *E. coli*, *P.*

aeruginosa, S. aureus, S. epidermidis, E. faecalis, S. typhimurium, K. pneumoniae, E. aerogenes. All concentration of plant extract could delay lipid oxidation at least 12 days

Part V

Conclusions and recommendations

5.1 Conclusions

As per the objectives, methodologies stated in the methods were carried out for the results. Based on the obtained results, the following conclusions have been drawn:

- The highest amount of phenol was found in onion leaves followed by radish leaves, garlic leaves, chiraito, and hattibar leaves extract.
- The highest amount of flavonoids content was found in radish leaves followed by onion leaves, hattibar leaves, chiraito leaves and garlic leaves extract.
- The highest amount of tannin content was found in onion leaves followed by garlic leaves, hattibar leaves, chiraito and radish leaves extract.
- The highest amount of antioxidant activity was found in chiraito followed by onion leaves, radish leaves, garlic leaves and hattibar leaves extract.
- The extract of herbs and spices inhibited the lipid oxidation significantly which was studied in terms of TBARS as compare to the control (untreated meat).
- The herbs and spices extract exhibited significant antimicrobial activity except 100 ppm of OLE in terms of TPC as compare to the control (untreated meat).

5.2 Recommendations

- 500 ppm of CE incorporated meat is most effective up to 12^{th} days of storage at $4^{\circ}C$ on basis of TBARS (0.24±0.01 mg MDA/kg sample) and TPC (7.05±0.22 log cfu/g) result.
- Inclusion of herbs and spices in meat product increases storage life of meat and meat products.
- Other plants (herbs and Spices) can be used as natural preservatives in meat.

Part VI

Summary

Chicken meat is most favoured by consumer around world because it has numerous advantageous dietary attributes like a low lipid content and somewhat high grouping of polyunsaturated unsaturated fats. So that chicken meat has promising market in Nepal. But there is problem of lipid oxidation and microbial spoilage of meat and meat product which reduces its shelf life. To address these issues, synthetic antioxidants such as BHA and BHT, as well as other preservatives, have been widely used in recent decades, as they are less expensive and more effective at low concentrations. However, consumers are now concerned about its use due to various health risks, and they are more drawn to preservatives derived from natural sources. Among these natural sources, chiraito, onion leaves, garlic leaves, hattibar leaves, radish leaves carry the significance amount of antioxidative and antimicrobial properties.

Some herbs and spices viz. garlic leaves, onion leaves, hattibar leaves, radish leaves and chiraito were collected and methanolic extraction of these samples were carried out. The extract was then analyzed for total phenols, total flavonoids and tot-al radical scavenging capacity. 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm of each sample was incorporated in chicken breast meat and compared with control over the time of storage.

The highest amount of total phenol was found for onion leaves (99.34±2.92 mg GAE/g) and lowest for hattibar leaves (13.27±0.69 mg GAE/g). The highest amount of total flavonoid content was found for radish leaves (53.04±0.41 mg GAE/g) and the lowest garlic leaves (16.62±2.97 mg GAE/g). The highest amount of tannin was found for onion leaves (26.58±0.16 mg tannin acid/g) and the lowest for radish leaves (10.72±0.059 mg tannic acid/g). Chiraito (IC_{50}=24.96±0.40 µg/ml) was found to have the highest amount of antioxidant activity and lowest was found in hattibar leaves (IC_{50}=952.28±1.43 µg/ml).

The control sample reached the threshold TBARS value (1 mg MDA/kg) at 7 days of observation but 100 ppm of garlic, chiraito and radish extract incorporated meat reached the threshold TBARS value at 12 days of storage and remaining sample could not cross the threshold value after 12 days of storage. Control (1.71±0.82 mg MDA/kg sample) showed highest and 500 ppm of CE incorporated sample (0.24±0.01 mg MDA/kg sample) showed

lowest TBARS values as compare to extract incorporated sample at 12^{th} days. Within each sample, 500 ppm and 100 ppm incorporated samples showed lowest and highest TBARS value with change time. In terms of antimicrobial activity, the control sample exceeded the legal threshold (10^7 cfu/g) 4^{th} days of observation. 100 ppm of onion extract incorporated sample exceeded threshold value before 7^{th} days but other sample exceeded on 10^{th} days of storage except 500 ppm CE incorporated meat which exceeded on 12^{th} days but cfu/g value was lower in higher concentration of extract of each sample and vice versa. These results show that the use of natural antioxidant sources could be effective in preventing ground meat against lipid oxidation at refrigerated storage as well as the inhibitory activity of extract of garlic leaves, onion leaves, hattibar leaves, radish leaves and chiraito towards microorganisms was significant. The effectiveness increased with the increase in concentration of each type of extract.

References

Ahmad, R. S., Imran, A. and Hussain, M. B. (2018). Nutritional composition of meat. *Meat Sci. Nutr.* **61**. [doi:10.5772/intechopen.77045]

Ahn, Juhee, Grün, I. U. and Mustapha, A. (2004). Antimicrobial and antioxidant activities of natural extracts in vitro and in ground beef. *J. Food Protec.* **67** (1), 148-155.

Ahn, U, D., Olson, D., Jo, C., Chen, X., Wu, C. and Lee, J. (1998). Effect of muscle type, packaging, and irradiation on lipid oxidation, volatile production, and color in raw pork patties. *Meat Sci*. **49** (1), 27-39.

Akramzadeh, N., Ramezani, Z., Ferdousi, R., Akbari-Adergani, B., Mohammadi, A., Karimian-Khosroshahi, N., Famenin, B. K., Pilevar, Z. and Hosseini, H. (2020). Effect of chicken raw materials on physicochemical and microbiological properties of mechanically deboned chicken meat. *Vet. Res. Forum*. **11**, 153.

Al-Rimawi, F. (2015). Development and validation of a simple reversed-phase HPLC-UV method for determination of malondialdehyde in olive oil. *J. Am. Oil Chem. Soc.* **92** (7), 933-937.

Al Mamun, Nazmul Hasan, Belal Hazrat, Rokon Ul Karim, Dobirul Islam, Sayela Afroz, Ariful Islam, Tabassum Ara, Sirajam Munira, Masudul Hasan Khan and Islam, A. (2016). Investigation on phytochemical content and antioxidant activity of locally grown garlic (Allium sativum L.) in Bangladesh. *Int. J. Biol. Res.* **1** (5), 37-42.

Alam, K., Hoq, O. and Uddin, S. (2016). Medicinal plant Allium sativum. A review. *J. Med. Plants Stud.* **4** (6), 72-79.

Ali, M., Kang, G.-H., Yang, H.-S., Jeong, J.-Y., Hwang, Y.-H., Park, G.-B. and Joo, S.-T. (2007). A comparison of meat characteristics between duck and chicken breast. *Asian-Australasian J. Anim. Sci*. **20** (6), 1002-1006.

Amaral, A. B., Silva, M. V. d. and Lannes, S. C. d. S. (2018). Lipid oxidation in meat: mechanisms and protective factors–a review. *Food Sci. Technol.* **38**, 1-15.

Anon. (2008). New World Encyclopedia contributors. New World Encyclopedia,. Retrieved from

https://www.newworldencyclopedia.org/p/index.php?title=Radish&oldid=768274. (Last update 23 July 2008). [Accessed 19 November 2021].

Anon. (2021a). Agave. Wikipedia, The Free Encyclopedia. Retrieved from https://en.wikipedia.org/w/index.php?title=Agave&oldid=1056039737. (Last update 19 November 2021). [Accessed 24 November 2021].

Anon. (2021b). Swertia. Wikipedia, The Free Encyclopedia. Retrieved from https://en.wikipedia.org/w/index.php?title=Swertia&oldid=1026407365. (Last update 2 June 2021). [Accessed 24 November].

Antolovich, M., Prenzler, P. D., Patsalides, E., McDonald, S. and Robards, K. (2002). Methods for testing antioxidant activity. *Analyst*. **127** (1), 183-198.

Arora, D. S. and Kaur, J. (1999). Antimicrobial activity of spices. *Int. J. Antimicrobial Agents*. **12** (3), 257-262.

Aruna, G., Yerragunt, V. G. and Raju, A. B. (2012). Photochemistry and pharmacology of *Raphanus sativus*. *Int. J. Drug Formulation Res.* **3** (1), 43-52.

Atungulu, G. and Pan, Z. (2012). Microbial decontamination of nuts and spices. *In:* "Microbial decontamination in the food industry". (G. Atungulu, Ed.). pp. 125-162. University of California Davis, USA. Elsevier.

Babuskin, S., Babu, P., Saravana, A., Sasikala, M., Sabina, K., Archana, G., Sivarajan, M. and Sukumar, M. (2014). Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. *Int. J.Food Microbiol.* **171**, 32-40.

Bantawa, K., Rai, K., Limbu, D. S. and Khanal, H. (2018). Food-borne bacterial pathogens in marketed raw meat of Dharan, eastern Nepal. *BMC Res. Notes*. **11** (1), 1-5.

Barriuso, B., Astiasarán, I. and Ansorena, D. (2013). A review of analytical methods measuring lipid oxidation status in foods: a challenging task. *Eur. Food Res. Technol*. **236** (1), 1-15.

Bazargani-Gilani, B., Aliakbarlu, J. and Tajik, H. (2015). Effect of pomegranate juice dipping and chitosan coating enriched with Zataria multiflora Boiss essential oil on

the shelf-life of chicken meat during refrigerated storage. *Innovative Food Sci. Emerging Technol.* **29**, 280-287. [doi.10.1016/j.ifset.2015.04.007].

Benkeblia, N. (2004). Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *Food Sci.Tech.* **37** (2), 263-268.

Benzie, I. F. and Devaki, M. (2018). "The Ferric Reducing Antioxidant Power (FRAP) Assay for Non-enzymatic Antioxidant Capacity: Concepts, Procedures, Limitations and Applications". Vol. 11. Wiley. New York.

Bhattacharya, D. (2003). Antioxidants in oils and fats: Some technical aspects. *Ic-antioxidant-03. Kolkata and Jadavpur Univ.. July* 15-16.

Birt, D. F. (2006). Phytochemicals and cancer prevention: from epidemiology to mechanism of action. *J. Am. Diet. Assoc.* **106** (1), 20-21. [doi:10.1016/j.jada.2005.11.005].

Bourre, J. (2005). Where to find omega-3 fatty acids and how feeding animals with diet enriched in omega-3 fatty acids to increase nutritional value of derived products for human: what is actually useful. *J. Nutr. Health Aging*. **9** (4), 232-242.

Bradlow, H. L., Telang, N. T., Sepkovic, D. W. and Osborne, M. P. (1999). Phytochemicals as modulators of cancer risk. *Adv. Nutr. Cancer 2*. 207-221.

Braga, L., Shupp, J., Cummings, C., Jett, M., Takahashi, J., Carmo, L., Chartone-Souza, E. and Nascimento, A. (2005). Pomegranate extract inhibits *Staphylococcus aureus* growth and subsequent enterotoxin production. *J. Ethnoparmacol*. **96** (1-2), 335-339.

Brøndum, J., Byrne, D., Bak, L., Bertelsen, G. and Engelsen, S. (2000). Warmed-over flavour in porcine meat—A combined spectroscopic, sensory and chemometric study. *Meat Sci.* **54** (1), 83-95.

Burnie, G., Forrester, S. and Greig, D. (1999). "Botanica: The Illustrated A-Z of Over 10,000 Garden Plants:[and how to Cultivate Them]". Könemann. [ISBN 3829030681].

Burns, J., Gardner, P. T., Matthews, D., Duthie, G. G., Lean, J. and Crozier, A. (2001). Extraction of phenolics and changes in antioxidant activity of red wines during vinification. *J. Agric. Food Chem.* **49** (12), 5797-5808.

Burr, G. and Burr, M. (1929). Progress in the chemistry of fats and other lipids. *J. Biol. Chem*. **9** (4), 556-565.

Burri, S. C., Ekholm, A., Bleive, U., Püssa, T., Jensen, M., Hellström, J., Mäkinen, S., Korpinen, R., Mattila, P. H. and Radenkovs, V. (2020). Lipid oxidation inhibition capacity of plant extracts and powders in a processed meat model system. *Meat Sci.* **162**, 108033. [doi.10.1016/j.meatsci.2019.108033].

Caballero, B., Trugo, L. C. and Finglas, P. M. (2003). "Encyclopedia of Food Sciences and Nutrition" (2 ed.). Academic. [ISBN 978-0-122-27055-0].

Chakrabarty, M. (2003). "Chemistry and Technology of Oils & Fats". Vol. 1. Allied Publishers. [ISBN 978-8-177-64495-5].

Chandra Shekhar, T. and Anju, G. (2014). Antioxidant Activity by DPPH Radical Scavenging Method of Ageratum conyzoides Linn. *Am. J. Ethnomed.* **1** (4), 244-249.

Chang, S. S., Ostric-Matijasevic, B., Hsieh, O. A. and Huang, C. L. (1977). Natural antioxidants from rosemary and sage. *J. Food Sci.* **42** (4), 1102-1106.

Chen, Y., Qiao, Y., Xiao, Y., Chen, H., Zhao, L., Huang, M. and Zhou, G. (2016). Differences in physicochemical and nutritional properties of breast and thigh meat from crossbred chickens, commercial broilers, and spent hens. *Asian-Australasian J. Anim. Sci.* **29** (6), 855.

Chu, S.-C. and Chen, C. (2006). Effects of origins and fermentation time on the antioxidant activities of kombucha. *Food Chem*. **98** (3), 502-507.

CI, K. C. and Indira, G. (2016). Quantitative estimation of total phenolic, flavonoids, tannin and chlorophyll content of leaves of *Strobilanthes kunthiana* (Neelakurinji). *J. Med. Plants*. **4**, 282-286.

Corzo-Martínez, M. and Villamiel, M. (2012). "An Overview on Bioactivity of Onion". Onion Consumption and Health. 1ª Ed. Nueva York: Nova Science Publishers, Inc. [ISBN 978-1-62100-836-1].

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* **12** (4), 564-582.

Craig, W. J. (1999). Health-promoting properties of common herbs. *Am. J. Clin. Nutr.* **70** (3), 491s-499s.

Dave, D. and Ghaly, A. E. (2011). Meat spoilage mechanisms and preservation techniques: a critical review. *Am. J. Agric. Biol. Sci.* **6** (4), 486-510.

de Lima Júnior, D. M., do Nascimento Rangel, A. H., Urbano, S. A. and Moreno, G. M. B. (2013). Oxidação lipídica e qualidade da carne ovina. *Acta Veterinaria Brasilica*. **7** (1), 14-28.

De, M., Krishna De, A. and Banerjee, A. (1999). Antimicrobial screening of some Indian spices. *Phytother. Res.* **13** (7), 616-618.

Devatkal, Suresh K and Naveena, B. (2010). Effect of salt, kinnow and pomegranate fruit by-product powders on color and oxidative stability of raw ground goat meat during refrigerated storage. *Meat Sci.* **85** (2), 306-311.

Dif, M. (2016). Phenolic quantification and *Agave americana* leaves de geoclimatic area. *Adv.in Environ. Biol.* **10** (9), 194-200.

Divya, B., Suman, B., Venkataswamy, M. and Thyagaraju, K. (2017). A study on phytochemicals, functional groups and mineral composition of *Allium sativum* (garlic) cloves. *Int. J. Curr. Pham. Res.* **9** (3), 42-45.

Dolara, P., Luceri, C., De Filippo, C., Femia, A. P., Giovannelli, L., Caderni, G., Cecchini, C., Silvi, S., Orpianesi, C. and Cresci, A. (2005). Red wine polyphenols influence carcinogenesis, intestinal microflora, oxidative damage and gene expression profiles of colonic mucosa in F344 rats. *Mutation Res. Fund. Mol. Mech. of Motagenesis*. **591** (1-2), 237-246.

Doss, A. (2009). Preliminary phytochemical screening of some Indian medicinal plants. *Ancient Sci. Life* **29** (2), 12.

Doss, A. and Anand, S. (2012). Preliminary phytochemical screening of *Asteracantha longifolia* and *Pergularia daemia*. *World Appl. Sci.* . **18** (2), 233-235.

Doulgeraki, A. I., Ercolini, D., Villani, F. and Nychas, G.-J. E. (2012). Spoilage microbiota associated to the storage of raw meat in different conditions. *Int. J. Food Microbiol.* **157** (2), 130-141.

Duan, Y., Jin, D.-H., Kim, H.-S., Seong, J.-H., Lee, Y.-G., Kim, D.-S., Chung, H.-S. and Jang, S.-H. (2015). Analysis of total phenol, flavonoid content and antioxidant activity of various extraction solvents extracts from onion (*Allium cepa* L.) peels. *J. Kor. Appl. Sci. Technol.* **32** (3), 418-426.

El-Alim, S. S. L. A., Lugasi, A., Hóvári, J. and Dworschák, E. (1999). Culinary herbs inhibit lipid oxidation in raw and cooked minced meat patties during storage. *J. Sci. Food Agric.* **79** (2), 277-285.

Embuscado, M. (2015). Herbs and spices as antioxidants for food preservation. *In:* "Handbook of Antioxidants for Food Preservation" (1 ed.). (F. Shahidi, Ed.). pp. 251-283. Elsevier. [ISBN 978-1-782-42089-7].

Erel, O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin. Biochem.* **37** (4), 277-285.

Estévez, M. (2015). Oxidative damage to poultry: from farm to fork. *Poultry Sci.* **94** (6), 1368-1378. [doi: 10.3382/ps/pev094].

Eugenio, M. H. A., Pereira, R. G. F. A., Abreu, W. C. d. and Pereira, M. C. d. A. (2017). Phenolic compounds and antioxidant activity of tuberous root leaves. *Int. J. Food Prop.* **20** (12), 2966-2973.

Formagio, A. S. N., Volobuff, C. R. F., Santiago, M., Cardoso, C. A. L., Vieira, M. D. C. and Valdevina Pereira, Z. (2014). Evaluation of antioxidant activity, total flavonoids, tannins and phenolic compounds in Psychotria leaf extracts. *Antioxidants.* **3**(4), 745-757.

Frankel. (1980). Lipid oxidation. *Progress Lipid Res.* **19** (1-2), 1-22. [doi.10.1016/0163-7827(80)90006-5].

Frankel and Edwin, N. (1987). Secondary products of lipid oxidation. *Chem. Phy. Lipids*. **44** (2-4), 73-85.

Frankel, Edwin, N. and Meyer, A. S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *J. Sci. Food Agric.* **80** (13), 1925-1941.

FSIS and USDA. (1997). safe storage of meat and poultry. Retrieved from https://rvs.umn.edu/Uploads/EducationalMaterials/6e870938-2072-47f1-a589-e4a3f346d31b.pdf. [Accessed May, 1998].

Gee, J. and Johnson, I. (2001). Polyphenolic compounds: interactions with the gut and implications for human health. *Curr. Med. Chem.* **8** (11), 1245-1255.

Ghasemzadeh, A. and Ghasemzadeh, N. (2011). Flavonoids and phenolic acids: Role and biochemical activity in plants and human. *J. Med. Plants Res.* **5** (31), 6697-6703.

Ghasemzadeh, A., Jaafar, H. Z. and Rahmat, A. (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). *Mol.* **15** (6), 4324-4333.

Ghosal, S., Sharma, P., Chaudhuri, R. and Bhattacharya, S. (1973). Chemical constituents of the gentianaceae V: Tetraoxygenated xanthones of *Swertia chirata* Buch.-Ham. *J. Pharmacol. Sci.* **62** (6), 926-930.

Gorinstein, S., Leontowicz, H., Leontowicz, M., Namiesnik, J., Najman, K., Drzewiecki, J., Cvikrová, M., Martincová, O., Katrich, E. and Trakhtenberg, S. (2008). Comparison of the main bioactive compounds and antioxidant activities in garlic and white and red onions after treatment protocols. *J. Agric. Food Chem.* **56** (12), 4418-4426.

Gülçin, I., Küfrevioğlu, Ö. İ., Oktay, M. and Büyükokuroğlu, M. E. (2004). Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle *(Urtica dioic*a L.). *J. Ethnopharmacol.* **90** (2-3), 205-215.

Gustavsson, J., Cederberg, C., Sonesson, U., Van Otterdijk, R. and Meybeck, A. (2011). "Global Food Losses and Food Waste". FAO Rome. Rome, Italy. [ISBN 978-92-5-107205-9].

Halliwell, B. (2012). Free radicals and antioxidants: updating a personal view. *Nutr. Rev.* **70** (5), 257-265.

Hamissa, A. M. B., Seffen, M., Aliakbarian, B., Casazza, A. A., Perego, P. and Converti, A. (2012). Phenolics extraction from *Agave americana* (L.) leaves using high-temperature, high-pressure reactor. *Food Bioprod. Process.* **90** (1), 17-21.

Hara-Kudo, Y., Kobayashi, l., Sugita-Konishi, Y. and Kondo, K. (2004). Antibacterial activity of plants used in cooking for aroma and taste. *J. Food Prot.* **67** (12), 2820-2824. [doi: 10.4315/0362-028X-67.12.2820].

Haslam, E. (1989). "Plant Polyphenols". Cambridge University Press, Cambridge

Hayes, D. P. (2005). The protective role of fruits and vegetables against radiation-induced cancer. *Nutr. Rev.* **63** (9), 303-311. [doi: 10.1111/j.1753-4887.2005.tb00145.x].

Heim, K. E., Tagliaferro, A. R. and Bobilya, D. J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J. Nutr. Biochem.* **13** (10), 572-584.

Hertog, M. G., Hollman, P. C. and Katan, M. B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J. Agric. Food Chem.* **40** (12), 2379-2383.

Hichri, I., Barrieu, F., Bogs, J., Kappel, C., Delrot, S. and Lauvergeat, V. (2011). Recent advances in the transcriptional regulation of the flavonoid biosynthetic pathway. *J. Exp. Bot.* **62** (8), 2465-2483.

Hussein, K., Friedrich, L., Pinter, R., Németh, C., Kiskó, G. and Dalmadi, I. (2019). Effect of linalool and piperine on chicken meat quality during refrigerated conditions. *Acta Alimentaria* **48** (4), 431-440.

Irkin, R. and Arslan, M. (2010). Effect of onion (*Allium cepa* L.) extract on microbiological quality of refrigerated beef meat. *J. Muscle Foods*. **21** (2), 308-316.

Ishwar, S. and Karki, T. (2014). Medicinal importance of *Swertia chirayita* (Chiraito). *Food Science and Technology for Agro-entrepreneurship Development. 7th National Conference of Food Science & Technology (Food Conference-2014), 13-14 June 2014, Kathmandu, Nepal*. 189-194.

Jadoun, J., Yazbak, A., Rushrush, S., Rudy, A. and Azaizeh, H. (2016). Identification of a new antibacterial sulfur compound from Raphanus sativus seeds. *Evidence-Based Complementary and Alternative Med.* **2016**.

Joshi, K. and Joshi, A. (2008). *Swertia* L.(*Gentianaceae*) in Nepal Himalaya: Checklist, phytogeography, ethnobotany and conservation status. *Ethnobotanical Leaflets*. **2008** (1), 43.

Kamboh, A. and Zhu, W.-Y. (2013). Effect of increasing levels of bioflavonoids in broiler feed on plasma anti-oxidative potential, lipid metabolites, and fatty acid composition of meat. *Poultry Sci.* **92** (2), 454-461.

Kanner, J. (2007). Dietary advanced lipid oxidation endproducts are risk factors to human health. *Mol. Nutr. Food Res.* **51** (9), 1094-1101.

Khanal, S., Shakya, N., Thapa, K. and Pant, D. R. (2015). Phytochemical investigation of crude methanol extracts of different species of Swertia from Nepal. *BMC Res. Notes*. **8** (1), 1-9.

Kim, G.-H., Duan, Y., Lee, S.-C. and Kim, H.-S. (2016). Assessment of antioxidant activity of garlic (*Allium sativum* L.) peels by various extraction solvents. *J. Kor. Appl. Sci. Technol.* **33** (1), 204-212.

King, A. and Young, G. (1999). Characteristics and occurrence of phenolic phytochemicals. *J. Am. Diet. Assoc.* **99** (2), 213-218.

Kolakowska. (2002). "Chemical Functional Properties of Food Lipids" (1st ed.). CRC Press. Boca Raton. [ISBN 978-1-003-04039-2].

Kouba, M. and Mourot, J. (2011). A review of nutritional effects on fat composition of animal products with special emphasis on n-3 polyunsaturated fatty acids. *Biochimie*. **93** (1), 13-17. [doi: 10.1016/j.biochi.2010.02.027].

Kratchanova, M., Denev, P., Ciz, M., Lojek, A. and Mihailov, A. (2010). Evaluation of antioxidant activity of medicinal plants containing polyphenol compounds. Comparison of two extraction systems. *Acta Biochimica Polonica* **57** (2), 229-234. [doi: 10.18388/abp.2010_2399].

Kumar, S. and Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *Sci. World J.* **2013**. 1-11. [doi: 10.1155/2013/162750]

Kumari, M. and Jain, S. (2015). Screening of potential sources of tannin and its therapeutic application. *Int J Nutr Food Sci*. **4** (2), 26-29. [doi: 10.11648/j.ijnfs.s.2015040201.15].

Lai, P. and Roy, J. (2004). Antimicrobial and chemopreventive properties of herbs and spices. *Curr. Med. Chem.* **11** (11), 1451-1460.

Lee, F. (1975). "Basic Food Chemistry, The AVI Pub. Co". Inc., Westport, Conn

Lesschaeve, I. and Noble, A. C. (2005). Polyphenols: factors influencing their sensory properties and their effects on food and beverage preferences. *Am. J. Clin. Nutr.* **81** (1), 330S-335S.

Lewandowska, H., Kalinowska, M., Lewandowski, W., Stępkowski, T. M. and Brzoska, K. (2016). The role of natural polyphenols in cell signaling and cytoprotection against cancer development. *J. Nutr. Biochem.* **32**, 1-19.

López-Romero, J. C., Ayala-Zavala, J. F., González-Aguilar, G. A., Peña-Ramos, E. A. and González-Ríos, H. (2018). Biological activities of *Agave* by-products and their possible applications in food and pharmaceuticals. *J. Sci. Food Agric.* **98** (7), 2461-2474.

Love, J. D. and Pearson, A. (1971). Lipid oxidation in meat and meat products—A review. *J. Am. Oil Chem. Soc.* **48** (10), 547-549.

Luo, X., Zhang, H., Duan, Y. and Chen, G. (2018). Protective effects of radish (*Raphanus sativus* L.) leaves extract against hydrogen peroxide-induced oxidative damage in human fetal lung fibroblast (MRC-5) cells. *Biomed. Pharmacotherapy*. **103**, 406-414.

Madhavi, D. L., Deshpande, S. and Salunkhe, D. K. (1995). "Food antioxidants: Technological: Toxicological and Health Perspectives". CRC Press. [ISBN 082479351X].

Mahros, M., Eltanahy, A., Abd-Elghany, S. and Sallam, K. (2021). The antimicrobial effect of fresh garlic and garlic oil supplemented with ground beef. *Mansoura Vet. Med. J.* **22** (2), 48-51. [doi: 10.35943/mvmj.2021.67329.1045].

Manach, C., Scalbert, A., Morand, C., Rémésy, C. and Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* **79** (5), 727-747.

Manandhar, N. (2018). Phytochemical and anioxidanr activity of common spices and their mix. B.Tech (Food) Dissertation. Tribhuvan Univ., Nepal.

Marques, S. S., Magalhães, L. M., Tóth, I. V. and Segundo, M. A. (2014). Insights on antioxidant assays for biological samples based on the reduction of copper complexes—The importance of analytical conditions. *Int. J. Mol. Sci.* **15** (7), 11387-11402.

Massingue, A. A., de Almeida Torres Filho, R., Fontes, P. R., Ramos, A. d. L. S., Fontes, E. A. F., Perez, J. R. O. and Ramos, E. M. (2018). Effect of mechanically deboned poultry meat content on technological properties and sensory characteristics of lamb and mutton sausages. *Asian-Australasian J. Anim. Sci.* **31** (4), 576.

McAfee, A. J., McSorley, E. M., Cuskelly, G. J., Moss, B. W., Wallace, J. M., Bonham, M. P. and Fearon, A. M. (2010). Red meat consumption: An overview of the risks and benefits. *Meat Sci.* **84** (1), 1-13.

Mielnik, M., Olsen, E., Vogt, G., Adeline, D. and Skrede, G. (2006). Grape seed extract as antioxidant in cooked, cold stored turkey meat. *LWT-Food Sci. Technol.* **39** (3), 191-198.

Miladi, S. and Damak, M. (2008). In vitro antioxidant activities of *Aloe vera* leaf skin extracts. *J. Soc. Chim. Tunisie* **10** (10), 101-109.

Min, B. and Ahn, D. (2005). Mechanism of lipid peroxidation in meat and meat products-A review. *Food Sci. Biotechnol.* **14** (1), 152-163.

Min, B., Ahn, D. J. F. S. and Biotechnology. (2005). Mechanism of lipid peroxidation in meat and meat products-A review. *Food Sci. Biotechnol.* **14** (1), 152-163.

Mir, N. A., Rafiq, A., Kumar, F., Singh, V. and Shukla, V. (2017). Determinants of broiler chicken meat quality and factors affecting them: a review. *J. Food Sci. Technol.* **54** (10), 2997-3009.

Mozuraityte, R., Kristinova, V. and Rustad, T. (2016). Oxidation of Food Components. *In:* "Encyclopedia of Food and Health". (B. Caballero, P. M. Finglas and F. Toldrá, Eds.). pp. 186-190. Oxford. Academic Press. [ISBN 978-0-12-384953-3].

Nabavi, S. F., Di Lorenzo, A., Izadi, M., Sobarzo-Sánchez, E., Daglia, M. and Nabavi, S. M. (2015). Antibacterial effects of cinnamon: From farm to food, cosmetic and pharmaceutical industries. *Nutr*. **7** (9), 7729-7748.

Nasar-Abbas, S. and Halkman, A. K. (2004). Antimicrobial effect of water extract of sumac (*Rhus coriaria* L.) on the growth of some food borne bacteria including pathogens. *Int. J. of Food Microbiol.* **97** (1), 63-69.

Nasri, S. and Salem, H. B. (2012). Effect of oral administration of *Agave americana* or *Quillaja saponaria* extracts on digestion and growth of Barbarine female lamb. *Livestock Sci*. **147** (1-3), 59-65.

Naveena, B., Muthukumar, M., Sen, A., Babji, Y. and Murthy, T. (2006). Improvement of shelf-life of buffalo meat using lactic acid, clove oil and vitamin C during retail display. *Meat Sci.* **74** (2), 409-415.

Ncube, N., Afolayan, A. and Okoh, A. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *Afr. J. Biotechnol.* **7** (12), 1797-1806.

Negi, J. S., Singh, P. and Rawat, B. (2011). Chemical constituents and biological importance of *Swertia*: a review. *Curr. Res. Chem.* **3** (1), 1-15.

Nuutila, A. M., Puupponen-Pimiä, R., Aarni, M. and Oksman-Caldentey, K.-M. (2003). Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. *Food Chem.* **81** (4), 485-493.

O'brien, R. D. (2008). "Fats and Oils: Formulating and Processing for Applications". CRC Press. [ISBN 1420061674].

O'connell, J. and Fox, P. (2001). Significance and applications of phenolic compounds in the production and quality of milk and dairy products: a review. *Int. Dairy J.* **11** (3), 103-120.

Obeta, N. A. (2015). Effect of moist heating and drying processing on the proximate and phytochemical composition of *Vernonia amygdalina* and *Gongronema latifolium* leaves. *Agric. Sci. Res. J.* **5** (11), 153-165.

Oboh, G. (2006). Antioxidant properties of some commonly consumed and underutilized tropical legumes. *Eur. Food Res. Technol.* **224** (1), 61-65.

Okuda, T. and Ito, H. (2011). Tannins of constant structure in medicinal and food plants—hydrolyzable tannins and polyphenols related to tannins. *Mol.* **16** (3), 2191-2217.

Organization, W. H. (1999). "WHO Monographs on Selected Medicinal Plants". Vol. 2. World Health Organization. [ISBN 9241545372].

Pande, S. S. (2019). Effect of alcoholic fermentation on phytochemical(polyohenol/ flavonoid, vitamin C and FOS) levels and radiacal scarvenging activity of yacon (*Smallanthus sonchifolius*) root slices. B. Tech (Food) Dissertation. Tribhuvan Univ., Nepal.

Pandey, A. and Singh, P. (2011). Antibacterial activity of *Syzygium aromaticum* (clove) with metal ion effect against food borne pathogens. *Asian J. Plant Sci. Res.* **1** (2), 69-80.

Pareek, S., Sagar, N. A., Sharma, S. and Kumar, V. (2018). Onion (*Allium cep*a L.). *In:* "Fruit and Vegetable Phytochemicals: Chemistry and Human Health" (2nd ed., Vol. 2). (E. M. Yahia, Ed.). pp. 1145-1162. Hariyana, India. John Wiley & Sons Ltd.

Patsias, A., Badeka, A., Savvaidis, I. and Kontominas, M. (2008). Combined effect of freeze chilling and MAP on quality parameters of raw chicken fillets. *Food Microbiol.* **25** (4), 575-581.

Pennacchia, C., Ercolini, D. and Villani, F. (2011). Spoilage-related microbiota associated with chilled beef stored in air or vacuum pack. *Food Microbiol.* **28** (1), 84-93.

Peter, K. and Shylaja, M. (2012). Introduction to herbs and spices: definitions, trade and applications. *In:* "Handbook of Herbs and Spices" (Vol. 1). (K. Peter, Ed.). pp. 1-24. Elsevier. [doi: 10.1533/9780857095671.1].

Petrou, S., Tsiraki, M., Giatrakou, V. and Savvaidis, I. (2012). Chitosan dipping or oregano oil treatments, singly or combined on modified atmosphere packaged chicken breast meat. *Int. J. Food Microbiol.* **156** (3), 264-271.

Petrovska, B. B. and Cekovska, S. (2010). Extracts from the history and medical properties of garlic. *Pharmacognosy Rev.* **4** (7), 106.

Pokorný, J. and Korczak, J. (2001). Preparation of natural antioxidants. *Antioxidants Food*. 311-330.

Poudel, N. (2020). Effect of phytochemical constituents of pomegranate peel extract on the shelf life of ground buffalo meat. M. Tech (Food) Dissertation. Tribhuvan Univ., Nepal.

Prior, R. L., Wu, X. and Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **53** (10), 4290-4302.

Rafieian-Kopaei, M., Baradaran, A. and Rafieian, M. (2013). Oxidative stress and the paradoxical effects of antioxidants. *J. Res. Med. Sci.* **18** (7), 628.

Ramanthan, R., Lau, K. and Da, N. (1989). Antiperoxidative action of flavonoids and related products in ground pork (abstract) proceedings of III Int. Symp. *III Int. Symp*. 56.

Ramos, S. (2008). Cancer chemoprevention and chemotherapy: dietary polyphenols and signalling pathways. *Mol. Nutr. Food Res.* **52** (5), 507-526.

Rao, A., Bharani, M. and Pallavi, V. (2006). Role of antioxidants and free radicals in health and disease. *Adv. Pharmacol. Toxicol.* **7** (1), 29-38.

Ravindran, P. (2017). The Encyclopedia of Herbs and Spices. Vol. 2. CABI. India.

Ray, B.B and A. (2013). "Fundamental Food Micrology" (5 ed.). Boca Roton. FL,USA.

Reitznerová, A., Šuleková, M., Nagy, J., Marcinčák, S., Semjon, B., Čertík, M. and Klempová, T. (2017). Lipid peroxidation process in meat and meat products: a comparison study of malondialdehyde determination between modified 2-thiobarbituric acid spectrophotometric method and reverse-phase high-performance liquid chromatography. *Mol.* **22** (11), 1988.

Rivero-Cruz, J. F., Granados-Pineda, J., Pedraza-Chaverri, J., Pérez-Rojas, J. M., Kumar-Passari, A., Diaz-Ruiz, G. and Rivero-Cruz, B. E. (2020). Phytochemical constituents, antioxidant, cytotoxic, and antimicrobial activities of the ethanolic extract of Mexican brown propolis. *Antioxidants*. **9** (1), 70.

Rizwan, K., Zubair, M., Rasool, N., Riaz, M., Zia-Ul-Haq, M. and De Feo, V. (2012). Phytochemical and biological studies of *Agave attenuata*. *Int. J. Mol. Sci.* **13** (5), 6440-6451.

Robbins, R. J. (2003). Phenolic acids in foods: an overview of analytical methodology. *J. Agric. Food Chem.* **51** (10), 2866-2887.

Robert, M., Herrera, J., Chan, J. and Contreras, F. (1992). Micropropagation of *Agave* spp. *In:* "High-Tech and Micropropagation III" (1st ed.). (Y. P. S. Bajaj, Ed.). pp. 306-329. A-137, New Friends Colony, New Delhi India. Springer. [ISBN 978-3-540-53660-4].

Ross, J. A. and Kasum, C. M. (2002). Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annu. Rev. Nutr.* **22** (1), 19-34.

Rowland, I. (1999). Optimal nutrition: fibre and phytochemicals. *Proceed. Nutr. Soc.* **58** (2), 415-419. [doi: 10.1017/s0029665199000543].

Roy, P., Abdulsalam, F. I., Pandey, D. K., Bhattacharjee, A., Eruvaram, N. R. and Malik, T. (2015). Evaluation of antioxidant, antibacterial, and antidiabetic potential of two traditional medicinal plants of India: *Swertia cordata* and *Swertia chirayita*. *Pharmacognosy Res.* **7** (Suppl 1), S57-62. [doi: 10.4103/0974-8490.157997].

Ruban, S. (2009). Lipid peroxidation in muscle foods-an overview. *Global Veterinaria* **3**(6), 509-513.

Sakanaka, S., Tachibana, Y. and Okada, Y. (2005). Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). *Food Chem.* **89** (4), 569-575.

Santos-Zea, L., Maria Leal-Diaz, A., Cortes-Ceballos, E. and Alejandra Gutierrez-Uribe, J. (2012). *Agave* (*Agave spp*.) and its traditional products as a source of bioactive compounds. *Curr. Bioactive Compounds*. **8** (3), 218-231.

Sapkota, R., Dasgupta, R. and Rawat, D. (2012). Antibacterial effects of plants extracts on human microbial pathogens & microbial limit tests. *Int. J. Res. Pharm. Chem.* **2** (4), 926-936.

Saravanan, P., Ramya, V., Sridhar, H., Balamurugan, V. and Umamaheswari, S. (2010). Antibacterial activity of *Allium sativum* L. on pathogenic bacterial strains. *Global veterinaria*. **4** (5), 519-522.

Saxena, M., Saxena, J., Nema, R., Singh, D. and Gupta, A. (2013). Phytochemistry of medicinal plants. *J. Pharmacognosy Phytochem.* **1** (6).

Scalbert, A., Manach, C., Morand, C., Rémésy, C. and Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. **45** (4), 287-306.

Shahidi, F. (2000). Antioxidants in food and food antioxidants. *Food/nahrung*. **44** (3), 158-163.

Shan, B., Cai, Y.-Z., Brooks, J. D. and Corke, H. (2007). The in vitro antibacterial activity of dietary spice and medicinal herb extracts. *Int. J. Food Microbiol.* **117** (1), 112-119.

Shrestha, P., Bista, M., Sharma, P., Shrestha, S., Lamichhane, B., Adhikari, S., Pandey, B. R. and Shrestha, B. G. (2015). Phytochemical screening, antimicrobial activity and cytotoxicity of Nepalese medicinal plants *Swertia chirayita* and *Dendrobium amoenum*. *Nepal J. Biotechnol.* **3** (1), 48-57.

Sieniawska, E. and Baj, T. (2017). Tannins. *In:* "Pharmacognosy". (S. Badal and R. Delgoda, Eds.). pp. 199-232. Elsevier. [ISBN 978-0-12-802104-0].

Singh, A. (2008). Phytochemicals of *Gentianaceae:* a review of pharmacological properties. *Int. J. Pharm. Sci. Nanotechnol.* **1** (1), 33-36.

Skowyra, M. (2014). Antioxidant properties of extracts from selected plant materials (*Caesalpinia spinosa, Perilla frutescens, Artemisia annua and Viola wittrockiana*) in vitro and in model food systems. Ph.D Doctoral thesis. Universitat Politècnica de Catalunya, Spain.

Sochor, J., Ryvolova, M., Krystofova, O., Salas, P., Hubalek, J., Adam, V., Trnkova, L., Havel, L., Beklova, M. and Zehnalek, J. (2010). Fully automated spectrometric protocols for determination of antioxidant activity: Advantages and disadvantages. *Mol.* **15** (12), 8618-8640.

Stratil, P., Klejdus, B. and Kubáň, V. (2006). Determination of total content of phenolic compounds and their antioxidant activity in vegetables evaluation of spectrophotometric methods. *J. Agric. Food Chem.* **54** (3), 607-616.

Sun, Q., Faustman, C., Senecal, A., Wilkinson, A. and Furr, H. (2001). Aldehyde reactivity with 2-thiobarbituric acid and TBARS in freeze-dried beef during accelerated storage. *Meat Sci.* **57** (1), 55-60.

Tapsell, L. C., Hemphill, I., Cobiac, L., Sullivan, D. R., Fenech, M., Patch, C. S., Roodenrys, S., Keogh, J. B., Clifton, P. M. and Williams, P. G. (2006). Health benefits of herbs and spices: the past, the present, the future. *J. Australian Med. Assoc.* **185** (4), 1-21.

Thampi, Nivetha, Jeyadoss and S, V. (2015). Comparative investigation of total antioxidant and free radical scavenging activities of two *Allium* species. *Asian J. Pharm. Clin. Res.* **8** (4), 148-151.

Tsai, M.-C. and Huang, T.-L. (2015). Thiobarbituric acid reactive substances (TBARS) is a state biomarker of oxidative stress in bipolar patients in a manic phase. *J. Affective Disorders*. **173**, 22-26.

Van Hecke, T., Van Camp, J. and De Smet, S. (2017). Oxidation during digestion of meat: interactions with the diet and helicobacter pylori gastritis, and implications on human health. *Comprehensive Rev. Food Sci. Food Safety*. **16** (2), 214-233.

Vaya, J. and Aviram, M. (2001). Nutritional antioxidants mechanisms of action, analyses of activities and medical applications. *Curr. Med. Chem.-Immunology, Endocrine & Metab. Agents* **1**(1), 99-117.

Velioglu, Y., Mazza, G., Gao, L. and Oomah, B. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* **46** (10), 4113-4117.

Vijayalakshmi, M. and Ruckmani, K. (2016). Ferric reducing anti-oxidant power assay in plant extract. *Bangladesh J. Pharmacol*. **11** (3), 570-572.

Warner, K., Neff, W. E., Byrdwell, W. C. and Gardner, H. W. (2001). Effect of oleic and linoleic acids on the production of deep-fried odor in heated triolein and trilinolein. *J. Agric. Food Chem*. **49** (2), 899-905.

Williams, P. (2007). Nutritional composition of red meat. *Nutr. Diet*. **64**, S113-S119.

Xiong, Z., Sun, D.-W., Pu, H., Xie, A., Han, Z. and Luo, M. (2015). Non-destructive prediction of thiobarbituric acid reactive substances (TBARS) value for freshness evaluation of chicken meat using hyperspectral imaging. *Food Chem.* **179**, 175-181.

Xu, D.-P., Li, Y., Meng, X., Zhou, T., Zhou, Y., Zheng, J., Zhang, J.-J. and Li, H.-B. (2017). Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. *Int. J. Mol. Sci.* **18** (1), 96.

Yadav, RNS Agarwala and Munin. (2011). Phytochemical analysis of some medicinal plants. *J. Phytology*. **3** (12).

Yamamura, A., Murai, A., Takamatsu, H. and Watabe, K. (2000). Antimicrobial effect of chemical preservatives on enterohemorrhagic *Escherichia coli* O157: H7. *J. Health Sci.* **46** (3), 204-208.

Zahin, M., Aqil, F. and Ahmad, I. (2009). The in vitro antioxidant activity and total phenolic content of four Indian medicinal plants. *Int. J of Pham. and Pharmacol. Sci.* **1** (1), 88-95.

Zhang, Huiyun, Wu, J. and Guo, X. (2016). Effects of antimicrobial and antioxidant activities of spice extracts on raw chicken meat quality. *Food Sci. Human Wellness*. **5** (1), 39-48.

Zhang, Wangang, Xiao, S., Lee, E. J. and Ahn, D. U. (2011). Consumption of oxidized oil increases oxidative stress in broilers and affects the quality of breast meat. *J. Agric. Food Chem.* **59** (3), 969-974.

Appendices

Appendix A

List of chemicals used

Chemicals/reagents	Specification/assay	Manufacturer/Supplier
DPPH	85%	HI media, India
Ethanol	99.9%	Changshu Hongsheng Fine Chemical Col Ltd, China
Folin-Ciocalteu	A.R. grade	Fisher Scientific, India
Gallic acid	99.5%	Loba Chemie, India
Hydrochloric acid	> 35%	Emplura®
Methanol	> 99%	Emplura® Merck Life Science Pvt. Ltd.
Metaphosphoric acid	~ 33.6%	Loba Chemie, India
Quercetin	> 98%	Himedia, India
Sodium hydroxide	99.5%	Qualigens, India
Sodium carbonate	98%	Fizmerk India Chemicals
Sodium nitrite	>98%	Merck Specialties Pvet. Ltd
Sulfuric acid	>98%	Qualigens, India
Tannic acid		
Trichloroacetic acid		
TBA		

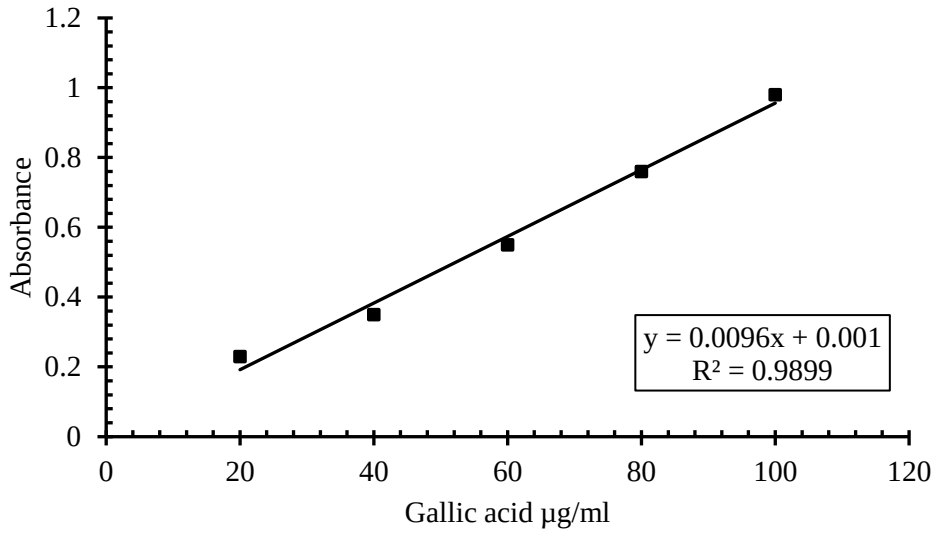
Potassium iodide		
Petroleum ether		

List of equipments used

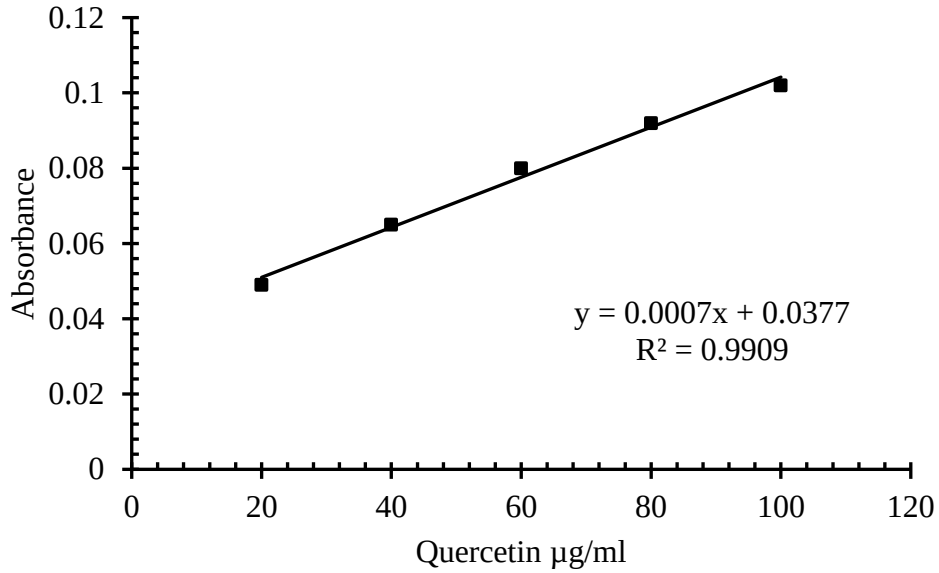
Equipment/glassware/apparatus	Specification	Manufacturer/Supplier
Burette/pipette/other glassware, etc	Certified	Borosil, India
Centrifuge	5000rpm	
Digital balance		
Electric cabinet dryer		
Micro-kjeldahl set	Manual	H. L. Scientific, India
Micropipette	50-1000 μL	Proline®, Finland
Mortar-pestle	Marble	Zeal International
Muffle furnace	~ 1200^0C	Suntech Enterprise, India
Spectrophotometer		
Thermometer	± 0.1^0C	MexTech®
Vacuum packaging machine	Table top	
Vortex mixer/ Homoginizer		

Appendix B

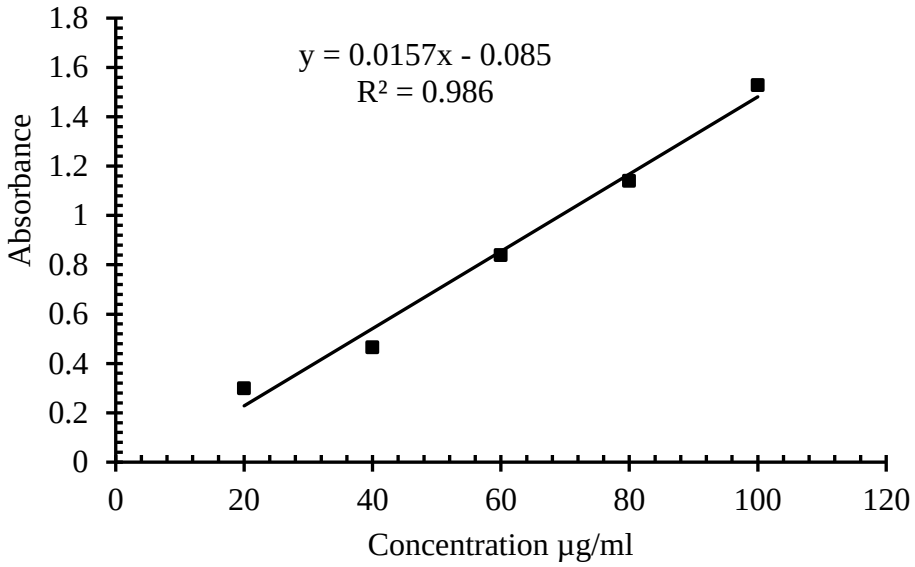
B.1 Calibration curve of gallic acid



B.2 Calibration curve of quercetin



B.3 Calibration curve of tannic acid



Appendix C

C.1 Significance test for phenol

Dependent Variable: total phenolic content

Table C.1.1 Test of between-subject effects

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	14328.561[a]	4	3582.140	1006.704	.000
Intercept	78738.051	1	78738.051	22128.087	.000
Sample	14328.561	4	3582.140	1006.704	.000
Error	35.583	10	3.558		
Total	93102.195	15			
Corrected Total	14364.144	14			

a. R Squared = .998 (Adjusted R Squared = .997)

Table C.1.2 Post hoc test

Tukey HSD

sample	N	Subset				
		1	2	3	4	5
hattibar	3	13.2639				
chiraito	3		72.9861			
Garlic	3			83.5764		
Radish	3				93.0903	
Onion	3					99.3403
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 3.558.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

C 2 Significance test for flavonoid

Dependent Variable: flavonoid

Table C 2.1 Test of between subject effects

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	4776.079[a]	4	1194.020	234.052	.000
Intercept	13662.305	1	13662.305	2678.084	.000
sample	4776.079	4	1194.020	234.052	.000
Error	51.015	10	5.102		
Total	18489.399	15			
Corrected Total	4827.095	14			

a. R Squared = .989 (Adjusted R Squared = .985)

Table C 2.2 Post hoc test

Tukey HSD

sample	N	Subset			
		1	2	3	4
hattibar	3	6.1433			
Garlic	3		16.6200		
chiraito	3			27.5680	
Onion	3				47.5233
Radish	3				53.0443
Sig.		1.000	1.000	1.000	.079

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 5.102.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

C 3 Significance test for tannin

Dependent Variable: tannin

Table C 3.1 Test of between subject effects

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	429.557[a]	4	107.389	240.295	.000
Intercept	5438.509	1	5438.509	12169.265	.000
sample	429.557	4	107.389	240.295	.000
Error	4.469	10	.447		
Total	5872.535	15			
Corrected Total	434.026	14			

a. R Squared = .990 (Adjusted R Squared = .986)

Table C.3.2 Post hoc test

Tukey HSD

sample	N	Subset			
		1	2	3	4
Radish	3	10.7220			
Chiraito	3		15.9870		
Hattibar	3			20.4883	
Garlic	3			21.4267	
Onion	3				26.5820
Sig.		1.000	1.000	.465	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .447.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

C4 Significance test for antioxidant activity

Dependent Variable: IC_{50} (µg/ml)

Table C 4.1.1 Tests f between subject effects

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	1883843.793[a]	4	470960.948	91465.852	.000
Intercept	900163.822	1	900163.822	174821.822	.000
Sample	1883843.793	4	470960.948	91465.852	.000
Error	51.490	10	5.149		
Total	2784059.106	15			
Corrected Total	1883895.284	14			

a. R Squared = 1.000 (Adjusted R Squared = 1.000)

Table C.4.2 Post hoc test

Tukey HSD

Sample	N	Subset			
		1	2	3	4
Chiraito	3	24.9567			
Onion	3		74.7900		
Radish	3			84.7913	

Garlic	3			88.0333	
Hattibar	3				952.2850
Sig.		1.000	1.000	.449	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 5.149.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Appendix D

D.1 Significance tests for effect of various sample to TBARS value with time

D.1.1 Within 100ppm

Table no D.1.1.1 Analysis of variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	0	0	0.000	1.000
Error	12	0.00000004	3.3333e-9		
C. Total	17	0.00000004			

Table no D.1.1.2 Post hoc test at 0 day

Level		Mean
Chiraito	A	0.00396667
Control	A	0.00396667
Garlic	A	0.00396667
Hattibar	A	0.00396667
Onion	A	0.00396667
Radish	A	0.00396667

Levels not connected by same letter are significantly different.

Table no D.1.1.3 Analysis of variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	0.02706351	0.005413	146.8537	<.0001*
Error	12	0.00044229	0.000037		
C. Total	17	0.02750580			

Table no D.1.1.4 Post hoc test at 4 days

Level				Mean
Control	A			0.23293333
Garlic		B		0.18633333
Radish		B		0.18100000
Hattibar			C	0.14333333
Onion			C	0.13733333
Chiraito			D	0.11533333

Table No D.1.1.5 Analysis of variance at 7 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	1.6527580	0.330552	867.7654	<.0001*
Error	12	0.0045711	0.000381		
C. Total	17	1.6573291			

Table No D.1.1.6 Post hoc test at 7 days

Level				Mean
Control	A			1.0403333
Radish		B		0.4253333
Garlic			C	0.2973000
Onion			C	0.2543333
Hattibar			D	0.1676667
Chiraito			D	0.1656667

Levels not connected by same letter are significantly different.

Table No D.1.1.7 Analysis of variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	1.3753380	0.275068	807.8344	<.0001*
Error	12	0.0040860	0.000340		

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
C. Total	17	1.3794240			

Table No D.1.1.8 Post hoc test at 10 days

Level						Mean
Control	A					1.2866667
Radish		B				0.8943333
Chiraito			C			0.8366667
Garlic				D		0.5893333
Onion					E	0.5133333
Hattibar					E	0.4996667

Levels not connected by same letter are significantly different.

Table No D.1.1.9 Analysis of variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	1.5869106	0.317382	3002.543	<.0001*
Error	12	0.0012685	0.000106		
C. Total	17	1.5881791			

Table No D.1.1.10 Post hoc test at 12 days

Level						Mean
Control	A					1.7119333
Chiraito		B				1.2660000
Radish			C			1.0166667
Garlic			C			1.0051333
Hattibar				D		0.8936667
Onion					E	0.8333333

Levels not connected by same letter are significantly different.

D.1.2 Within 200ppm

Table No D.1.2.1 Analysis of variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	0	0	0.000	1.000
Error	12	0.00000004	3.3333e-9		
C. Total	17	0.00000004			

Table No D.1.2.2 Post hoc at 0 day

Level		Mean
Chiraito	A	0.00396667
Control	A	0.00396667
Garlic	A	0.00396667
Hattibar	A	0.00396667
Onion	A	0.00396667
Radish	A	0.00396667

Levels not connected by same letter are significantly different.

Table No D.1.2.3 Analysis of variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	0.06323905	0.012648	329.1462	<.0001*
Error	12	0.00046111	0.000038		
C. Total	17	0.06370017			

Table No D.1.2.4 Post hoc test at 4 days

Level				Mean
Control	A			0.23293333
Garlic		B		0.15526667
Radish		B		0.14866667
Onion			C	0.11066667

Level			Mean
Hattibar	D		0.09056667
Chiraito		E	0.04300000

Levels not connected by same letter are significantly different.

Table No D.1.2.5 Analysis of variance at 7 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	1.8408729	0.368175	990.8353	<.0001*
Error	12	0.0044590	0.000372		
C. Total	17	1.8453319			

Table No D.1.2.6 Post hoc test at 7 days

Level					Mean
Control	A				1.0403333
Radish		B			0.2946667
Garlic			C		0.2299333
Onion				D	0.1643333
Chiraito				D	0.1493333
Hattibar				D	0.1353333

Levels not connected by same letter are significantly different.

Table No D.1.2.7 Analysis of variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	1.5228074	0.304561	1006.606	<.0001*
Error	12	0.0036308	0.000303		
C. Total	17	1.5264381			

Table No D.1.2.8 Post hoc test at 10 days

Level					Mean
Control	A				1.2866667
Radish		B			0.6960000
Chiraito			C		0.6092333
Garlic				D	0.5017333
Onion				D	E	0.4693333
Hattibar					E	0.4366667

Levels not connected by same letter are significantly different.

Table No D.1.2.9 Analysis of variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	2.1904711	0.438094	4085.768	<.0001*
Error	12	0.0012867	0.000107		
C. Total	17	2.1917578			

Table No D.1.2.10 Post hoc test at 12 days

Level					Mean
Control	A				1.7119333
Hattibar		B			0.8533333
Garlic			C		0.8086667
Chiraito			C		0.7943333
Radish			C		0.7943333
Onion				D	0.6810000

Levels not connected by same letter are significantly different.

D.1.3 Within 300ppm

Table No D.1.3.1 Analysis of variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	0	0	0.000	1.000
Error	12	0.00000004	3.3333e-9		
C. Total	17	0.00000004			

Table No D.1.3.2 Post hoc at 0 day

Level		Mean
Chiraito	A	0.00396667
Control	A	0.00396667
Garlic	A	0.00396667
Hattibar	A	0.00396667
Onion	A	0.00396667
Radish	A	0.00396667

Levels not connected by same letter are significantly different.

Table No D.1.3.3 Analysis of variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	0.07521303	0.015043	657.6252	<.0001*
Error	12	0.00027449	0.000023		
C. Total	17	0.07548752			

Table No D.1.3.4 Post hoc test at 4 days

Level					Mean
Control	A				0.23293333
Radish		B			0.09700000
Hattibar		B	C		0.08700000
Garlic			C		0.08162667
Onion				D	0.04113333
Chiraito				D	0.04100000

Levels not connected by same letter are significantly different.

Table No D.1.3.5 Analysis of variance at 7 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	1.9724848	0.394497	1042.875	<.0001*
Error	12	0.0045393	0.000378		
C. Total	17	1.9770241			

Table No D.1.3.6 Post hoc test at 7 days

Level					Mean
Control	A				1.0403333
Radish		B			0.2733333
Onion			C		0.1583333
Garlic			C		0.1433667
Hattibar			C		0.1253333
Chiraito			C		0.1153333

Levels not connected by same letter are significantly different.

Table No D.1.3.7 Analysis of variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	2.0566017	0.411320	1287.408	<.0001*
Error	12	0.0038339	0.000319		
C. Total	17	2.0604356			

Table No D.1.3.8 Post hoc test at 10 days

Level					Mean
Control	A				1.2866667
Radish		B			0.6363333
Garlic			C		0.4823000
Chiraito				D	0.4060000

Level		Mean
Hattibar	E	0.3153333
Onion	E	0.3090000

Levels not connected by same letter are significantly different.

Table No D.1.3.9 Analysis of variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	2.6077718	0.521554	15814.53	<.0001*
Error	12	0.0003958	0.000033		
C. Total	17	2.6081675			

Table No D.1.3.10 Post hoc test at 12 days

Level						Mean
Control	A					1.7119333
Hattibar		B				0.7956667
Radish			C			0.7206667
Chiraito				D		0.7033333
Garlic					E	0.6812333
Onion						F 0.6070000

Levels not connected by same letter are significantly different.

D.1.4 Within 400ppm

Table No D.1.4.1 Analysis of variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	0	0	0.000	1.000
Error	12	0.00000004	3.3333e-9		
C. Total	17	0.00000004			

Table No D.1.4.2 Post hoc at 0 day

Level		Mean
Chiraito	A	0.00396667
Control	A	0.00396667
Garlic	A	0.00396667
Hattibar	A	0.00396667
Onion	A	0.00396667
Radish	A	0.00396667

Levels not connected by same letter are significantly different.

Table No D.1.4.3 Analysis of variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	0.08410352	0.016821	654.7286	<.0001*
Error	12	0.00030829	0.000026		
C. Total	17	0.08441182			

Table No D.1.4.4 Post hoc test at 4 days

Level					Mean
Control	A				0.23293333
Garlic		B			0.08260000
Radish		B			0.07463333
Hattibar			C		0.05433333
Chiraito				D	0.03733333
Onion				D	0.03183333

Levels not connected by same letter are significantly different.

Table No D.1.4.5 Analysis of variance at 7 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	2.0975944	0.419519	1135.028	<.0001*
Error	12	0.0044353	0.000370		
C. Total	17	2.1020298			

Table No D.1.4.6 Post hoc test at 7 days

Level				Mean
Control	A			1.0403333
Radish		B		0.2353333
Garlic		C		0.1433333
Chiraito		C	D	0.1113333
Onion		C	D	0.0953333
Hattibar			D	0.0850000

Levels not connected by same letter are significantly different.

Table No D.1.4.7 Analysis of variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	2.2082487	0.441650	1431.862	<.0001*
Error	12	0.0037013	0.000308		
C. Total	17	2.2119500			

Table No D.1.4.8 Post hoc test at 10 days

Level				Mean
Control	A			1.2866667
Radish		B		0.5336667
Garlic		C		0.4833333
Hattibar			D	0.3036667
Chiraito			D	0.2996667

Level		Mean
Onion	D	0.2910000

Levels not connected by same letter are significantly different.

Table No D.1.4.9 Analysis of variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	3.5404205	0.708084	49.9176	<.0001*
Error	12	0.1702209	0.014185		
C. Total	17	3.7106414			

Table No D.1.4.10 Post hoc test at 12 days

Level				Mean
Control	A			1.7119333
Radish		B		0.7213333
Garlic		B	C	0.6843333
Hattibar		B	C	0.6336667
Onion		B	C	0.4221667
Chiraito			C	0.3903333

Levels not connected by same letter are significantly different.

D.1.5 Within 500ppm

Table No D.1.5.1 Analysis of variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	0	0	0.000	1.000
Error	12	0.00000004	3.3333e-9		

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
C. Total	17	0.00000004			

Table No D.1.5.2 Post hoc at 0 day

Level		Mean
Chiraito	A	0.00396667
Control	A	0.00396667
Garlic	A	0.00396667
Hattibar	A	0.00396667
Onion	A	0.00396667
Radish	A	0.00396667

Levels not connected by same letter are significantly different.

Table No D.1.5.3 Analysis of variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	0.11576303	0.023153	607.2195	<.0001*
Error	12	0.00045755	0.000038		
C. Total	17	0.11622058			

Table No D.1.5.4 Post hoc test at 4 days

Level				Mean
Control	A			0.23293333
Garlic		B		0.08000000
Radish			C	0.02133333
Onion			C	0.01393333
Hattibar			C	0.01360000
Chiraito			C	0.00966667

Levels not connected by same letter are significantly different.

Table No D.1.5.5 Analysis of variance at 7 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	2.3975882	0.479518	1333.389	<.0001*
Error	12	0.0043155	0.000360		
C. Total	17	2.4019037			

Table No D.1.5.6 Post hoc test at 7 days

Level				Mean
Control	A			1.0403333
Radish		B		0.1480000
Garlic			C	0.0753000
Chiraito			C	D	0.0576667
Hattibar			C	D	0.0407000
Onion				D	0.0146667

Levels not connected by same letter are significantly different.

Table No D.1.5.7 Analysis of variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	2.8287668	0.565753	1862.951	<.0001*
Error	12	0.0036442	0.000304		
C. Total	17	2.8324111			

Table No D.1.5.8 Post hoc test at 10 days

Level					Mean
Control	A				1.2866667
Radish		B			0.3170000
Hattibar			C		0.2463333
Garlic			C	D	0.2130000
Onion				D	0.1940000
Chiraito				D	0.1785333

Levels not connected by same letter are significantly different.

Table No D.1.5.9 Analysis of variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	4.6495723	0.929914	13390.23	<.0001*
Error	12	0.0008334	0.000069		
C. Total	17	4.6504056			

Table No D.1.5.10 Post hoc test at 12 days

Level						Mean
Control	A					1.7119333
Hattibar		B				0.5243333
Radish			C			0.4940000
Garlic				D		0.3156667
Onion				D		0.3066333
Chiraito					E	0.2400000

Levels not connected by same letter are significantly different.

D.2 Significance tests for effect of various concentration sample to TBARS value with time

D.2.1 Within Chiraito

Table No D.2.1.1 Analysis of variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	0.00001475	2.9502e-6	885.0625	<.0001*
Error	10	3.33333e-8	3.3333e-9		
C. Total	15	0.00001478			

Table No D.2.1.2 Post hoc at 0 day

Level		Mean
100	A	0.00396667
200	A	0.00396667
300	A	0.00396667
400	A	0.00396667
500	A	0.00396667

Table No D.2.1.3 Analysis of variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	14.651324	2.93026	523261.6	<.0001*
Error	10	0.000056	5.6e-6		
C. Total	15	14.651380			

Table No D.2.1.4 Post hoc test at 4 days

Level			Mean
100	A		0.1153333
200		B	0.0430000
300		B	0.0410000
400		B	0.0373333
500		C	0.0096667

Table No D.2.1.5 Analysis of variance at 7 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	44.398504	8.87970	686574.8	<.0001*
Error	10	0.000129	0.000013		
C. Total	15	44.398634			

Table No D.2.1.6 Post hoc test at 7 days

Level					Mean
100	A				0.1656667
200		B			0.1493333
300			C		0.1153333
400			C		0.1113333
500				D	0.0576667

Table No D.2.1.7 Analysis of variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	86.031167	17.2062	1231068	<.0001*
Error	10	0.000140	0.000014		
C. Total	15	86.031306			

Table No D.2.1.8 Post hoc test at 10 days

Level						Mean
100	A					0.836667
200		B				0.609233
300			C			0.406000
400				D		0.299667
500					E	0.178533

Table No D.2.1.9 Analysis of variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	122.06251	24.4125	1440.980	<.0001*
Error	10	0.16942	0.0169		
C. Total	15	122.23192			

Table No D.2.1.10 Post hoc test at 12 days

Level				Mean
100	A			1.266000
200		B		0.794333
300		B	C	0.703333
400			C	D	0.390333
500				D	0.240000

D.2.2 Within Garlic

Table No D.2.2.1 Analysis of variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	0.00001475	2.9502e-6	885.0625	<.0001*
Error	10	3.33333e-8	3.3333e-9		
C. Total	15	0.00001478			

Table No D.2.2.2 Post hoc at 0 day

Level		Mean
100	A	0.00396667
200	A	0.00396667
300	A	0.00396667
400	A	0.00396667
500	A	0.00396667

Table No D.2.2.3 Analysis of variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	14.164355	2.83287	92212.97	<.0001*
Error	10	0.000307	3.072e-5		
C. Total	15	14.164662			

Table No D.2.2.4 Post hoc test at 4 days

Level				Mean
100	A			0.1863333
200		B		0.1552667
400			C	0.0826000
300			C	0.0816267
500			C	0.0800000

Table No D.2.2.5 Analysis of variance at 7 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	43.722549	8.74451	403381.8	<.0001*
Error	10	0.000217	2.168e-5		
C. Total	15	43.722766			

Table No D.2.2.6 Post hoc test at 7 days

Level				Mean
100	A			0.2973000
200		B		0.2299333
300			C	0.1433667
400			C	0.1433333
500			D	0.0753000

Table No D.2.2.7 Analysis of variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	85.672815	17.1346	918743.3	<.0001*
Error	10	0.000187	1.865e-5		
C. Total	15	85.673002			

Table No D.2.2.8 Post hoc test at 10 days

Level					Mean
100	A				0.589333
200		B			0.501733
400			C		0.483333
300			C		0.482300
500				D	0.213000

Table No D.2.2.9 Analysis of variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	120.49008	24.0980	568144.0	<.0001*
Error	10	0.00042	4.242e-5		
C. Total	15	120.49051			

Table No D.2.2.10 Post hoc test at 12 days

Level					Mean
100	A				1.005133
200		B			0.808667
400			C		0.684333
300			C		0.681233
500				D	0.315667

D.2.3 Within Hattibar

Table No D.2.3.1 Analysis of variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	0.00001475	2.9502e-6	885.0625	<.0001*
Error	10	3.33333e-8	3.3333e-9		
C. Total	15	0.00001478			

Table No D.2.3.2 Post hoc at 0 day

Level		Mean
100	A	0.00396667
200	A	0.00396667
300	A	0.00396667
400	A	0.00396667
500	A	0.00396667

Table No D.2.3.3 Analysis of variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	14.450063	2.89001	236073.6	<.0001*
Error	10	0.000122	1.224e-5		
C. Total	15	14.450186			

Table No D.2.3.4 Post hoc test at 4 days

Level					Mean
100	A				0.1433333
200		B			0.0905667
300		B			0.0870000
400			C		0.0543333
500				D	0.0136000

Table No D.2.3.5 Analysis of variance at 7 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	44.523553	8.90471	651214.8	<.0001*
Error	10	0.000137	1.367e-5		
C. Total	15	44.523690			

Table No D.2.3.6 Post hoc test at 7 days

Level					Mean
100	A				0.1676667
200		B			0.1353333
300		B			0.1253333
400			C		0.0850000
500				D	0.0407000

Table No D.2.3.7 Analysis of variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	87.245893	17.4492	760865.3	<.0001*
Error	10	0.000229	0.000023		
C. Total	15	87.246122			

Table No D.2.3.8 Post hoc test at 10 days

Level					Mean
100	A				0.499667
200		B			0.436667
300			C		0.315333
400			C		0.303667
500				D	0.246333

Table No D.2.3.9 Analysis of variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	119.15269	23.8305	987453.2	<.0001*
Error	10	0.00024	2.413e-5		
C. Total	15	119.15293			

Table No D.2.3.10 Post hoc test at 12 days

Level						Mean
100	A					0.893667
200		B				0.853333
300			C			0.795667
400				D		0.633667
500					E	0.524333

D.2.4 Within Onion

Table No D.2.4.1 Analysis of variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	0.00001475	2.9502e-6	885.0625	<.0001*
Error	10	3.33333e-8	3.3333e-9		
C. Total	15	0.00001478			

Table No D.2.4.2 Post hoc at 0 day

Level		Mean
100	A	0.00396667
200	A	0.00396667
300	A	0.00396667
400	A	0.00396667
500	A	0.00396667

Table No D.2.4.3 Analysis of variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	14.536582	2.90732	154617.1	<.0001*
Error	10	0.000188	1.88e-5		
C. Total	15	14.536770			

Table No D.2.4.4 Post hoc test at 4 days

Level					Mean
100	A				0.1373333
200		B			0.1106667
300			C		0.0411333
400			C		0.0318333
500				D	0.0139333

Table No D.2.4.5 Analysis of variance at 7 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	44.246834	8.84937	767286.1	<.0001*
Error	10	0.000115	1.153e-5		
C. Total	15	44.246949			

Table No D.2.4.6 Post hoc test at 7 days

Level					Mean
100	A				0.2543333
200		B			0.1643333
300		B			0.1583333
400			C		0.0953333
500				D	0.0146667

Table No D.2.4.7 Analysis of variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	87.416692	17.4833	229039.4	<.0001*
Error	10	0.000763	7.633e-5		
C. Total	15	87.417456			

Table No D.2.4.8 Post hoc test at 10 days

Level						Mean
100	A					0.513333
200		B				0.469333
300			C			0.309000
400			C			0.291000
500				D		0.194000

Table No D.2.4.9 Analysis of variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	123.00178	24.6004	99886.13	<.0001*
Error	10	0.00246	0.000246		
C. Total	15	123.00424			

Table No D.2.4.10 Post hoc test at 12 days

Level						Mean
100	A					0.833333
200		B				0.681000
300			C			0.607000
400				D		0.422167
500					E	0.306633

D.2.5 Within Radish

Table No D.2.5.1 Analysis of variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	0.00001475	2.9502e-6	885.0625	<.0001*
Error	10	3.33333e-8	3.3333e-9		
C. Total	15	0.00001478			

Table No D.2.5.2 Post hoc at 0 day

Level		Mean
100	A	0.00396667
200	A	0.00396667
300	A	0.00396667
400	A	0.00396667
500	A	0.00396667

Table No D.2.5.3 Analysis of variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	14.273297	2.85466	2390837	<.0001*
Error	10	0.000012	1.194e-6		
C. Total	15	14.273309			

Table No D.2.5.4 Post hoc test at 4 days

Level						Mean
100	A					0.1810000
200		B				0.1486667
300			C			0.0970000
400				D		0.0746333
500					E	0.0213333

Table No D.2.5.5 Analysis of variance at 7 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	42.516895	8.50338	388874.0	<.0001*
Error	10	0.000219	2.187e-5		
C. Total	15	42.517114			

Table No D.2.5.6 Post hoc test at 7 days

Level						Mean
100	A					0.4253333
200		B				0.2946667
300			C			0.2733333
400				D		0.2353333
500					E	0.1480000

Table No D.2.5.7 Analysis of variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	83.106508	16.6213	681200.9	<.0001*
Error	10	0.000244	2.44e-5		
C. Total	15	83.106752			

Table No D.2.5.8 Post hoc test at 10 days

Level						Mean
100	A					0.894333
200		B				0.696000
300			C			0.636333
400				D		0.533667
500					E	0.317000

Table No D.2.5.9 Analysis of variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	119.08588	23.8172	1318294	<.0001*
Error	10	0.00018	0.000018		
C. Total	15	119.08606			

Table No D.2.5.10 Post hoc test at 12 days

Level					Mean
100	A				1.016667
200		B			0.794333
400			C		0.721333
300			C		0.720667
500				D	0.494000

Appendix E

E.1 Significance tests for effect of various sample to TBARS value with time

E.1.1 With in 100ppm

Table no E.1.1.1 Analysis of variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	1.4199e-29	2.84e-30	0.0000	1.0000
Error	12	0.69821200	0.058184		
C. Total	17	0.69821200			

Table no E.1.1.2 Post hoc test at 0 day

Level		Mean
Chiraito	A	6.2593333
Control	A	6.2593333
Garlic	A	6.2593333
Hattibar	A	6.2593333
Onion	A	6.2593333
Radish	A	6.2593333

Levels not connected by same letter are significantly different.

Table no E.1.1.3 Analysis of Variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	0.27961361	0.055923	2.7803	0.0681
Error	12	0.24136733	0.020114		
C. Total	17	0.52098094			

Table no E.1.1.4 Post hoc test at 4 days

Level			Mean
Control	A		7.0110000
Garlic	A	B	6.8033333
Radish	A	B	6.7670000

Level			Mean
Hattibar	A	B	6.6963333
Onion	A	B	6.6930000
Chiraito		B	6.6190000

Levels not connected by same letter are significantly different.

Table no E.1.1.5 Analysis of Variance at 7 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	5.2148856	1.04298	40.1253	<.0001*
Error	12	0.3119160	0.02599		
C. Total	17	5.5268016			

Table no E.1.1.6 Post hoc test at 7 days

Level			Mean
Control	A		8.3080000
Hattibar		B	6.9360000
Garlic		B	6.9036667
Chiraito		B	6.8620000
Onion		B	6.8366667
Radish		B	6.8033333

Levels not connected by same letter are significantly different.

Table no E.1.1.7 Analysis of Variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	2.5813638	0.516273	3.8608	0.0256*
Error	12	1.6046460	0.133721		
C. Total	17	4.1860098			

Table no E.1.1.8 Post hoc test at 10 days

Level			Mean
Control	A		9.4770000
Garlic	A	B	8.7740000
Hattibar	A	B	8.6513333
Chiraito		B	8.4336667
Onion		B	8.4183333
Radish		B	8.3850000

Levels not connected by same letter are significantly different.

Table no E.1.1.9 Analysis of Variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	3.8508933	0.770179	38.6134	<.0001*
Error	12	0.2393507	0.019946		
C. Total	17	4.0902440			

Table no E.1.1.10 Post hoc test at 12 days

Level			Mean
Control	A		11.100333
Garlic		B	9.933000
Radish		B	9.920667
Hattibar		B	9.910667
Onion		B	9.859000
Chiraito		B	9.736333

Levels not connected by same letter are significantly different.

E.1.2 Within 200ppm

Table no E.1.2.1 Analysis of Variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	1.4199e-29	2.84e-30	0.0000	1.0000
Error	12	0.69821200	0.058184		
C. Total	17	0.69821200			

Table no E.1.2.2 Post hoc test at 0 day

Level		Mean
Chiraito	A	6.2593333
Control	A	6.2593333
Garlic	A	6.2593333
Hattibar	A	6.2593333
Onion	A	6.2593333
Radish	A	6.2593333

Levels not connected by same letter are significantly different.

Table no E.1.2.3 Analysis of Variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	0.47678628	0.095357	3.7833	0.0274*
Error	12	0.30246133	0.025205		
C. Total	17	0.77924761			

Table no E.1.1.4 Post hoc test at 4 days

Level			Mean
Control	A		7.0110000
Garlic	A	B	6.6666667
Radish	A	B	6.6243333
Onion	A	B	6.5926667
Chiraito		B	6.5603333
Hattibar		B	6.5186667

Levels not connected by same letter are significantly different.

Table no E.1.2.5 Analysis of Variance at 7 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	6.2585100	1.25170	42.2539	<.0001*
Error	12	0.3554800	0.02962		
C. Total	17	6.6139900			

Table no E.1.2.6 Post hoc at 7 days

Level			Mean
Control	A		8.3080000
Garlic		B	6.8520000
Onion		B	6.8226667
Radish		B	6.7436667
Chiraito		B	6.6666667
Hattibar		B	6.6190000

Levels not connected by same letter are significantly different.

Table E.1.2.7 Analysis of Variance at 10 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	6.1688520	1.23377	8.1874	0.0014*
Error	12	1.8082940	0.15069		
C. Total	17	7.9771460			

Table No E.1.2.8 Post hoc test at 10 days

Level			Mean
Control	A		9.4770000
Hattibar		B	8.1853333
Onion		B	8.0730000
Garlic		B	8.0193333
Chiraito		B	7.9513333
Radish		B	7.6360000

Levels not connected by same letter are significantly different.

Table no E.1.2.9 Analysis of Variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	10.042600	2.00852	28.2747	<.0001*
Error	12	0.852432	0.07104		
C. Total	17	10.895032			

Table no E.1.2.10 Post hoc test at 12 days

Level				Mean
Control	A			11.100333
Radish		B		9.577333
Hattibar		B	C	9.418333
Onion		B	C	9.359667
Chiraito		B	C	9.058667
Garlic			C	8.744667

Levels not connected by same letter are significantly different.

E.1.3 Within 300ppm

Table no E.1.3.1 Analysis of Variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	1.4199e-29	2.84e-30	0.0000	1.0000
Error	12	0.69821200	0.058184		
C. Total	17	0.69821200			

Table no E.1.3.2 Post hoc at 0 day

Level		Mean
Chiraito	A	6.2593333
Control	A	6.2593333
Garlic	A	6.2593333
Hattibar	A	6.2593333
Onion	A	6.2593333
Radish	A	6.2593333

Levels not connected by same letter are significantly different.

Table no E.1.3.3 Analysis of Variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	0.47678628	0.095357	3.7833	0.0274*
Error	12	0.30246133	0.025205		

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
C. Total	17	0.77924761			

Table no E.1.3.4 Post hoc test at 4 days

Level			Mean
Control	A		7.0110000
Garlic	A	B	6.6666667
Radish	A	B	6.6243333
Onion	A	B	6.5926667
Chiraito		B	6.5603333
Hattibar		B	6.5186667

Levels not connected by same letter are significantly different.

Table no E.1.3.5 Analysis of Variance at 7 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	6.2585100	1.25170	42.2539	<.0001*
Error	12	0.3554800	0.02962		
C. Total	17	6.6139900			

Table no E.1.4.6 Post hoc test at 7 days

Level			Mean
Control	A		8.3080000
Garlic		B	6.8520000
Onion		B	6.8226667
Radish		B	6.7436667
Chiraito		B	6.6666667
Hattibar		B	6.6190000

Levels not connected by same letter are significantly different.

Table E.1.3.7 Analysis of Variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	6.1688520	1.23377	8.1874	0.0014*
Error	12	1.8082940	0.15069		
C. Total	17	7.9771460			

Table no E.1.3.8 Post hoc test at 10 days

Level			Mean
Control	A		9.4770000
Hattibar		B	8.1853333
Onion		B	8.0730000
Garlic		B	8.0193333
Chiraito		B	7.9513333
Radish		B	7.6360000

Levels not connected by same letter are significantly different.

Table no E.1.3.9 Analysis of Variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	10.042600	2.00852	28.2747	<.0001*
Error	12	0.852432	0.07104		
C. Total	17	10.895032			

Table no E.1.3.10 Post hoc test at 12 days

Level				Mean
Control	A			11.100333
Radish		B		9.577333
Hattibar		B	C	9.418333
Onion		B	C	9.359667
Chiraito		B	C	9.058667
Garlic			C	8.744667

Levels not connected by same letter are significantly different.

E.1.4 Within 400ppm

Table no E.1.4.1 Analysis of Variance at 0 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	1.4199e-29	2.84e-30	0.0000	1.0000
Error	12	0.69821200	0.058184		
C. Total	17	0.69821200			

Table no E.1.4.2 Post hoc test at 0 day

Level		Mean
Chiraito	A	6.2593333
Control	A	6.2593333
Garlic	A	6.2593333
Hattibar	A	6.2593333
Onion	A	6.2593333
Radish	A	6.2593333

Levels not connected by same letter are significantly different.

Table no E.1.4.3 Analysis of Variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	0.8888149	0.177763	4.8057	0.0121*
Error	12	0.4438787	0.036990		
C. Total	17	1.3326936			

Table no E.1.4.4 Post hoc test at 4 days

Level			Mean
Control	A		7.0110000
Garlic	A	B	6.5603333
Radish	A	B	6.5186667
Chiraito	A	B	6.4923333
Onion		B	6.4183333
Hattibar		B	6.3010000

Levels not connected by same letter are significantly different.

Table no E.1.4.5 Analysis of Variance at 7 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	7.1257072	1.42514	41.7676	<.0001*
Error	12	0.4094493	0.03412		
C. Total	17	7.5351565			

Table no E.1.4.6 Post hoc test at 7 days

Level			Mean
Control	A		8.3080000
Garlic		B	6.7740000
Onion		B	6.7253333
Radish		B	6.6830000
Chiraito		B	6.6250000
Hattibar		B	6.4236667

Levels not connected by same letter are significantly different.

Table no E.1.4.7 Analysis of Variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	7.6957372	1.53915	11.8669	0.0003*
Error	12	1.5564153	0.12970		
C. Total	17	9.2521525			

Table no E.1.4.8 Post hoc test at 10 days

Level			Mean
Control	A		9.4770000
Garlic		B	7.8410000
Onion		B	7.8260000
Chiraito		B	7.6930000
Radish		B	7.6776667
Hattibar		B	7.6343333

Levels not connected by same letter are significantly different.

Table no E.1.4.9 Analysis of Variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	17.389746	3.47795	49.6582	<.0001*
Error	12	0.840453	0.07004		
C. Total	17	18.230199			

Table no E.1.4.10 Post hoc test at 12 days

Level				Mean
Control	A			11.100333
Radish		B		9.259333
Onion		B	C	8.914000
Hattibar		B	C D	8.751667
Garlic			C D	8.333333
Chiraito			D	8.085000

Levels not connected by same letter are significantly different.

E.1.5 Within 500ppm

Table E.1.5.1 Analysis of Variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	1.4199e-29	2.84e-30	0.0000	1.0000
Error	12	0.69821200	0.058184		
C. Total	17	0.69821200			

Table E.1.5.2 Post hoc at 0 day

Level		Mean
Chiraito	A	6.2593333
Control	A	6.2593333
Garlic	A	6.2593333
Hattibar	A	6.2593333
Onion	A	6.2593333
Radish	A	6.2593333

Levels not connected by same letter are significantly different.

Table no E.1.5.3 Analysis of Variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	1.2674738	0.253495	7.5028	0.0021*
Error	12	0.4054427	0.033787		
C. Total	17	1.6729165			

Table no E.1.5.4 Post hoc at 4 days

Level		Mean
Control	A	7.0110000
Chiraito	B	6.4600000
Garlic	B	6.4183333
Onion	B	6.3596667
Radish	B	6.2593333
Hattibar	B	6.2006667

Levels not connected by same letter are significantly different.

Table no E.1.5.5 Analysis of Variance at 7 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	8.1368538	1.62737	51.4735	<.0001*
Error	12	0.3793887	0.03162		
C. Total	17	8.5162425			

Table no E.1.5.6 Post hoc test at 7 days

Level		Mean
Control	A	8.3080000
Garlic	B	6.5926667
Onion	B	6.5926667
Chiraito	B	6.5510000
Hattibar	B	6.4183333
Radish	B	6.4183333

Levels not connected by same letter are significantly different.

Table no E.1.5.7 Analysis of Variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	10.580693	2.11614	19.2775	<.0001*
Error	12	1.317267	0.10977		
C. Total	17	11.897960			

Table no E.1.5.8 Post hoc test at 10 days

Level				Mean
Control	A			9.4770000
Onion		B		7.5410000
Hattibar		B		7.5186667
Chiraito		B		7.4336667
Garlic		B		7.4183333
Radish		B		7.2593333

Levels not connected by same letter are significantly different.

Table no E.1.5.9 Analysis of Variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	21.732755	4.34655	98.7916	<.0001*
Error	12	0.527966	0.04400		
C. Total	17	22.260721			

Table no E.1.5.10 Post hoc test at 12 days

Level					Mean
Control	A				11.100333
Onion		B			8.813667
Radish		B			8.725333
Hattibar		B	C		8.359667
Garlic			C	D	8.026333
Chiraito				D	7.725333

Levels not connected by same letter are significantly different.

E.2 Significance tests for effect of various concentration of sample to TBARS value with time

E.2.1 With in Chiraito

Table no E.2.1.1 Analysis of variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	36.730550	7.34611	126.2558	<.0001*
Error	10	0.581843	0.05818		
C. Total	15	37.312394			

Table no E.2.1.2 Post hoc test

Level		Mean
100	A	6.2593333
200	A	6.2593333
300	A	6.2593333
400	A	6.2593333
500	A	6.2593333

Table no E.2.1.3 Analysis of Variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	6.0879390	1.21759	63.4636	<.0001*
Error	10	0.1918560	0.01919		
C. Total	15	6.2797950			

Table no E.2.1.4 Post hoc Test

Level		Mean
100	A	6.6190000
200	A	6.5603333
300	A	6.5603333
400	A	6.4923333

Level		Mean
500	A	6.4600000

Table no E.2.1.5 Analysis of variance at 7 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	0.25738367	0.051477	5.5441	0.0106*
Error	10	0.09284933	0.009285		
C. Total	15	0.35023300			

Table no E.2.1.6 Post hoc test

Level			Mean
100	A		6.8620000
200	A	B	6.7286667
300	A	B	6.6666667
400	A	B	6.6250000
500		B	6.5510000

Table no E.2.1.7 Analysis of Variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	5.7989217	1.15978	11.0704	0.0008*
Error	10	1.0476440	0.10476		
C. Total	15	6.8465657			

Table no E.2.1.8 Post hoc Test

Level			Mean
100	B		8.433667
200	B	C	8.085000
300	B	C	7.951333
400	B	C	7.693000
500		C	7.433667

Table no E.2.1.9 Analysis of variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	19.054595	3.81092	45.2663	<.0001*
Error	10	0.841889	0.08419		
C. Total	15	19.896484			

Table no E.2.1.10 Post hoc test

Level			Mean
100	B		9.736333
200	B		9.592667
300	B		9.058667
400		C	8.085000
500		C	7.725333

E.2.2 Within in Garlic

Table no E.2.2.1 Analysis of variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	36.730550	7.34611	126.2558	<.0001*
Error	10	0.581843	0.05818		
C. Total	15	37.312394			

Table no E.2.2.2 Post hoc test

Level		Mean
100	A	6.2593333
200	A	6.2593333
300	A	6.2593333
400	A	6.2593333
500	A	6.2593333

Table no E.2.2.3 Analysis of Variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	6.8153967	1.36308	294.0629	<.0001*
Error	10	0.0463533	0.00464		
C. Total	15	6.8617500			

Table no E.2.2.4 Post hoc Test

Level				Mean
100	A			6.8033333
200	A	B		6.7486667
300	A	B		6.6666667
400		B	C	6.5603333
500			C	6.4183333

Table no E.2.2.5 Analysis of variance at 7 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	0.22048867	0.044098	4.7595	0.0174*
Error	10	0.09265133	0.009265		
C. Total	15	0.31314000			

Table no E.2.2.6 Post hoc test

Level			Mean
100	A		6.9036667
200	A	B	6.8590000
300	A	B	6.8520000
400	A	B	6.7740000
500		B	6.5926667

Table no E.2.2.7 Analysis of Variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	6.6554704	1.33109	8.0555	0.0028*
Error	10	1.6524113	0.16524		
C. Total	15	8.3078818			

Table no E.2.2.8 Post hoc Test

Level				Mean
100	A	B		8.774000
200	A	B	C	8.392000
300		B	C	8.019333
400		B	C	7.841000
500			C	7.418333

Table no E.2.2.9 Analysis of variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	15.992359	3.19847	51.0136	<.0001*
Error	10	0.626985	0.06270		
C. Total	15	16.619343			

Table no E.2.2.10 Post hoc test

Level				Mean
100	B			9.933000
200		C		8.948333
300		C		8.744667
400		C	D	8.333333
500			D	8.026333

E.2.3 Within Hattibar

Table no E.2.3.1 Analysis of variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	36.730550	7.34611	126.2558	<.0001*
Error	10	0.581843	0.05818		
C. Total	15	37.312394			

Table no E.2.3.2 Post hoc test

Level		Mean
100	A	6.2593333
200	A	6.2593333
300	A	6.2593333
400	A	6.2593333
500	A	6.2593333

Table no E.2.3.3 Analysis of Variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	6.1902273	1.23805	30.9471	<.0001*
Error	10	0.4000527	0.04001		
C. Total	15	6.5902800			

Table no E.2.3.4 Post hoc Test

Level		Mean
100	A	6.6963333
200	A	6.5926667
300	A	6.5186667
400	A	6.3010000
500	A	6.2006667

Table no E.2.3.5 Analysis of variance at 7 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	0.73363510	0.146727	6.3860	0.0065*
Error	10	0.22976333	0.022976		
C. Total	15	0.96339844			

Table no E.2.3.6 Post hoc test

Level			Mean
100	A		6.9360000
200	A	B	6.7780000
300	A	B	6.6190000
400		B	6.4236667
500		B	6.4183333

Table no E.2.3.7 Analysis of Variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	6.5119928	1.30240	28.1841	<.0001*
Error	10	0.4621047	0.04621		
C. Total	15	6.9740974			

Table no E.2.3.8 Post hoc Test

Level				Mean
100	B			8.651333
200	B			8.524000
300	B	C		8.185333
400		C	D	7.634333
500			D	7.518667

Table no E.2.3.9 Analysis of variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	12.118458	2.42369	75.0524	<.0001*
Error	10	0.322933	0.03229		

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
C. Total	15	12.441392			

Table no E.2.3.10 Post hoc test

Level			Mean
100	B		9.910667
200	B		9.560333
300	B		9.418333
400		C	8.751667
500		C	8.359667

E.2.4. Within Onion

Table no E.2.4.1 Analysis of variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	36.730550	7.34611	126.2558	<.0001*
Error	10	0.581843	0.05818		
C. Total	15	37.312394			

Table no E.2.4.2 Post hoc test

Level		Mean
100	A	6.2593333
200	A	6.2593333
300	A	6.2593333
400	A	6.2593333
500	A	6.2593333

Table no E.2.4.3 Analysis of Variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	6.2936811	1.25874	143.2194	<.0001*

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Error	10	0.0878887	0.00879		
C. Total	15	6.3815698			

Table no E.2.4.4 Post hoc Test

Level				Mean
100	A			6.6930000
200	A	B		6.6343333
300	A	B	C	6.5926667
400		B	C	6.4183333
500			C	6.3596667

Table no E.2.4.5 Analysis of variance at 7 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	3.0284531	0.605691	0.8964	0.5191
Error	10	6.7566007	0.675660		
C. Total	15	9.7850538			

Table no E.2.4.6 Post hoc test

Level		Mean
100	A	7.8366667
300	A	6.8226667
200	A	6.7820000
400	A	6.7253333
500	A	6.5926667

Table no E.2.4.7 Analysis of Variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	5.0912924	1.01826	9.2286	0.0017*
Error	10	1.1033673	0.11034		

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
C. Total	15	6.1946598			

Table no E.2.4.8 Post hoc Test

Level		Mean
100	B	8.418333
200	B	8.207667
300	B	8.073000
400	B	7.826000
500	B	7.541000

Table no E.2.4.9 Analysis of variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	9.4455224	1.88910	67.7098	<.0001*
Error	10	0.2790000	0.02790		
C. Total	15	9.7245224			

Table no E.2.4.10 Post hoc test

Level					Mean
100	B				9.859000
200	B	C			9.822667
300		C	D		9.359667
400			D	E	8.914000
500				E	8.813667

E.2.5 Within Radish

Table no E.2.5.1 Analysis of variance at 0 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	36.730550	7.34611	126.2558	<.0001*
Error	10	0.581843	0.05818		

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
C. Total	15	37.312394			

Table no E.2.5.2 Post hoc test

Level		Mean
100	A	6.2593333
200	A	6.2593333
300	A	6.2593333
400	A	6.2593333
500	A	6.2593333

Table no E.2.5.3 Analysis of Variance at 4 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	6.5990393	1.31981	87.9493	<.0001*
Error	10	0.1500647	0.01501		
C. Total	15	6.7491040			

Table no E.2.5.4 Post hoc Test

Level			Mean
100	A		6.7670000
200	A		6.6466667
300	A		6.6243333
400	A	B	6.5186667
500		B	6.2593333

Table no E.2.5.5 Analysis of variance at 7 day

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	0.37178108	0.074356	13.1407	0.0004*
Error	10	0.05658467	0.005658		
C. Total	15	0.42836575			

Table no E.2.5.6 Post hoc test

Level				Mean
100	A	B		6.8033333
300	A	B		6.7436667
200	A	B		6.7416667
400		B		6.6830000
500			C	6.4183333

Table no E.2.5.7 Analysis of Variance at 10 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	6.7738806	1.35478	9.0943	0.0018*
Error	10	1.4897013	0.14897		
C. Total	15	8.2635819			

Table no E.2.5.8 Post hoc Test

Level				Mean
100		B		8.385000
200		B	C	8.085000
400		B	C	7.677667
300		B	C	7.636000
500			C	7.259333

Table no E.2.5.9 Analysis of variance at 12 days

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Column 1	5	8.7401038	1.74802	34.5643	<.0001*
Error	10	0.5057307	0.05057		
C. Total	15	9.2458344			

Table no E.2.5.10 Post hoc test

Level				Mean
100	B			9.920667
200	B	C		9.695000
300	B	C		9.577333
400		C	D	9.259333
500			D	8.725333

Appendix F

Plate no. H.1 Hattibar leaves

Plate no H.2 Garlic leaves

Plate no H.3 Onion leaves

Plate no H.4 Chiraito

Plate no H.5 Radish leaves

Plate no H.6 Sample prepared for TBARS analysis

Plate no H.7 Taking spectrophotometer reading