ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF GARLIC EXTRACTS AFTER COOKING WITH DIFFERENT SALTS AT DIFFERENT CONDITIONS

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BEds.

A Thesis Submitted in Partial Fulfilment of the Requirements for the Award of the Degree of Master of Science in Analytical Chemistry of the Department of Chemistry and Biochemistry, Moi University

October 2020

DECLARATION

Declaration by the Candidate

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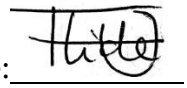
Signature:  Date: 15/04/2020

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DEDICATION

This work is dedicated to my parents, my Uncle Jabbar Alide, my Aunt Ms. Rehema Alide, my husband Mr. Idris Kassim, my daughter Rania Kassim and my siblings.

ABSTRACT

The use of natural food additives such as garlic is increasing over synthetic food additives due to fewer side effects if any and is also eco-friendly. Food is usually cooked in the presence of other additives such as salt. Cooking and additive-additive interactions may cause decrease in phytochemicals and antioxidant activity. This may result in consumption of decreased or lack of available antioxidants leading to health complications as a result of deviance in food preparation. The effect of cooking temperature and time on garlic in the presence of salt as a food additive is not documented. The study aimed at investigating the effect of cooking conditions (temperature and time) on phytochemicals and antioxidant activity (AOA) of garlic in the presence of refined salt (Kensalt) and unrefined salt (indigenous reed salt). The specific objectives were to; determine the total phenolic content (TPC) and total flavonoid content (TFC) of garlic, characterize phytochemical constituents of garlic, evaluate the effect of cooking temperature and time on phytochemicals and the AOA of garlic and to establish the effect of concentration of Kensalt and indigenous reed salt on phytochemicals and AOA of garlic. The phytochemicals of raw and cooked garlic were extracted using maceration with ethanol and distilled water. The water used for cooking at different conditions was analyzed separately. The TPC and TFC were determined using Folin-Ciocalteu's and Aluminium Chloride colorimetric methods respectively. Characterization was done using Fourier Transform Infrared (FTIR) spectroscopy and Liquid Chromatography tandem Mass spectroscopy (LC-MS/MS). The data was analyzed using SPSS software with ANOVA used to test the effect of cooking conditions. The TPC of fresh garlic was 637.91 ± 15.30 mg gallic acid equivalent (GAE)/100 g and 301.29 ± 6.58 mg GAE/100 g for the ethanolic extract and aqueous extract respectively. TFC was 258.21 ± 12.37 mg quercetin equivalent (QE)/100 g and 109.68 ± 6.78 QE/100 g for ethanolic and aqueous extracts respectively. FTIR spectral data showed absorptions in the range 3400-2400 cm^{-1} for carboxylic acids, 3650-3600 cm^{-1} for free O-H, bonded O-H at 3400-3200 cm^{-1}, C-O around 1300-1000 cm^{-1} for esters, carboxylic acids and alcohols. LC-MS/MS analysis revealed the presence of flavonoids; Quercetin, Hesperidin, Rutin and Catechin and Phenolic compounds; Ferulic acid, Gentisic acid, Caffeic acid, Gallic and Chlorogenic acid. Cooking temperature under the tested conditions had a significant effect (p-value < 0.01, alpha = 5%) on TPC and TFC while cooking time did not have a significant effect on phytochemicals and AOA (p-value = 0.511, alpha =5%). Salt had a significant effect on phytochemicals (p-value = 0.03, alpha = 5%) and AOA, however, the effect was higher on indigenous reed salt than iodized Kensalt. In conclusion, cooking at higher temperature in the presence of salt enhances extraction of phytochemicals and increases the AOA of garlic. It is recommended that garlic should be cooked at temperatures above 100°C in the presence of indigenous reed salt for optimum phytochemicals and AOA for more health benefits.

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LIST OF ACRONYMS

ACEII-PTRE	Africa Center of Excellence in Phytochemicals, Textile and Renewable Energy
ANOVA	Analysis of Variance
AOA	Antioxidant Activity
DPPH	2,2-DiPhenyl-1-PicrylHydrazyl
FTIR	Fourier Transform Infrared Spectroscopy
GAE	Gallic Acid Equivalent
HPLC	High Performance Liquid Chromatography
IUCEA	Interuniversity Council for East Africa
Kg	Kilograms
LOD	Limit of Detection
LOQ	Limit of Quantification
LSD	Least Square Difference
RSD	Relative Standard Deviation
SPSS	Statistical Package for Social Scientists
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
QE	Quercetin Equivalent

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It has been a long journey for me and my close relations. Starting my academic journey, I never thought I would make it this far. I would like to acknowledge those that immensely contributed to this work.Firstly, I am forever grateful to my maker Allah, whose endless mercy and blessings have seen me through.

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To my husband Idriss Kassim, there were times I felt like I was never going to make it, and it was in times like these that you encouraged me and stood by me. Your belief in my abilities has been a joy throughout this journey. A big thank you also goes to my parents; they are the reason I am here and made me a strong character. To Jabbar Alide, a man who dedicated his resources to make sure I reach this far without fail, a man who always supports me when called upon to do so; he has been nothing short of a father to me. Thanks to Elias Mwakilama of PAUSTI for his guidance in statistical analysis. I am forever indebted to my Aunt Rehema Alide, who with love, volunteered to take care of my seven weeks old daughter so that I can achieve my goals. To my siblings it has always been a pleasure growing up with you.

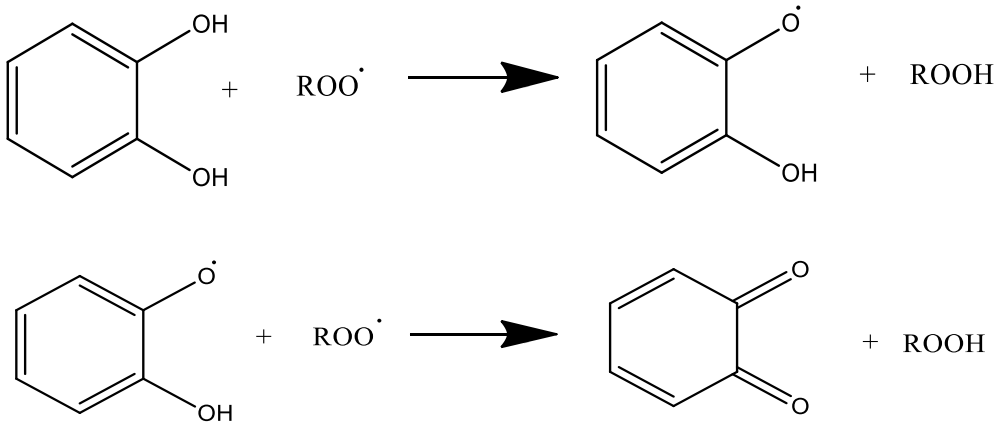
CHAPTER 1: INTRODUCTION

1.1 Background of the Study

Food additives are various chemical substances added to food to produce specific desirable effects. Additives are added to food to improve their quality and safety, to lengthen the shelf life, to change the taste, smell and appearance of food. These food additives can be natural or synthetic. Synthetic food additives are not preferred due to toxic and carcinogenic side effects for example butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiarybutylhydroquinone (TBHQ) (Tanvir *et al*., 2017). Natural food additives are more common and preferred due to fewer side effects if any and their friendliness to the environment.

Garlic is among the food additives that are very popular worldwide due to its aroma, and therapeutic properties (Prati *et al*., 2014). Garlic is well known for protection against degenerative diseases and antimicrobial properties which are attributed to the presence of antioxidants (Prati *et al*., 2014). These compounds that work as antioxidants in garlic are polyphenolic compounds (phenols and flavonoids) and are abundant in plant based foods hence common in our diet (Kim, Kang, & Gweon, 2012). According to (X. Lu, Ross, Powers, Aston, & Rasco, 2011) the antioxidant activity of most *Allium* species e. g garlic is due to organosulphur compounds and polyphenolic compounds. These compounds have interesting medicinal properties and are present in high amounts in garlic (Beato, Orgaz, Mansilla, & Montaño, 2011, Chen et al., 2013). When quantified, the polyphenolic compounds are referred to as total phenolic content and total flavonoid content for phenolic and flavonoid compounds respectively.

Antioxidants play an important role in inhibiting and scavenging free radicals in our bodies thereby providing protection against infection and degenerative diseases. Free radicals are defined as any molecular species that contains an unpaired electron (Lobo, Patil, Phatak, & Chandra, 2010). Because of the unpaired electron, they are highly unstable and reactive. They react by donating or accepting an electron from other molecules thereby behaving as oxidants (Lobo *et al*., 2010). Scheme 1.1 shows an oxidant scavenging radical with two active sites.



Scheme 1.1: Reaction mechanisms of Catechol as an oxidant.

Free radicals in our bodies are as a result of metabolic processes, exposure to sun rays, use of packaged food, smoking and exposure to environmental pollution (Ra, Daniela, Álová, Bezáková, & Fialová, 2009). There are antioxidant systems within our bodies most of which are enzymes. Vitamin A, Vitamin B and beta-carotene are the principle antioxidant micronutrient but our bodies cannot manufacture these nutrients hence the need to incorporate these antioxidant containing foods in our diet.

Direct consumption of raw food additives is limited due to the taste, aromatic and pungent properties, as such they are added to food and cooked as whole spices, chopped, powder or

extracts. The temperature and time at which the food is cooked as well as other food additives present for example salt can have either a positive or negative effect on the antioxidant activity of the food additive. Information on the effect caused by these conditions is lacking yet it is crucial in the nutritive value of these food additives. It is for this reason that this research was undertaken to investigate the effect of cooking conditions on garlic in the presence of indigenous reed salt and iodized commercial salt (Kensalt).

1.2 Problem Statement

Of recent, there has been a rampant increase of degenerative diseases such as cancer despite people's efforts of using food additives that have anticancer properties or antioxidant properties. In 2018 alone, it was estimated that the burden of cancer increased to 18.1 million new cases and 9.6 million deaths globally (Release, 2018). The ineffectiveness of the antioxidant capacity of the food additives could be due to preparation as well as presence of other food additives which may cause food additives interaction. This may cause consumption decrease or lack of available antioxidants thereby causing the oxidant-antioxidant imbalance. The heat and cooking time as well as the interaction with other food additives during preparation can cause the antioxidant activity to increase or decrease depending on the nature of the antioxidant. In order to obtain the maximum antioxidant activity of food additives, it is imperative that the right cooking temperature and time among other conditions be used to alleviate the emerging food preparation health issues.

1.3 Justification of the Study

The free radicals could be attributed to rise in cancer cases, which are becoming a concern in Kenya. Phytochemicals of garlic are promising candidates for cancAAer therapy (Y. Zhang & Liu, 2020). Garlic is highly nutritious and contains many compounds with potential

medicinal properties. The condition under which food is prepared will affect its medicinal and nutritional value. Research on the heat treatment of bioactive compounds has been done though little is known about the effects of heating and cooking time on the antioxidant activity of garlic in the presence of refined and unrefined salt. This research will mimic the cooking process and as such it will avail information/data on how best these food additives should be prepared for optimum antioxidant activity.

1.4 Significance of the Study

This study will avail data on the effect of heat and cooking time on phytochemicals and antioxidant activity of garlic in the presence of refined and unrefined salts. It will also provide the optimum cooking temperature that results in the highest retention of the antioxidant capacity of the bioactive components present in garlic and in turn alleviate the emerging food preparation health issues.

1.5 Objectives of the Study

1.5.1 Main Objective

The main objective of the study was to investigate the effect of cooking temperature and time on the phytochemicals and antioxidant activity of garlic in the presence of refined and unrefined salts.

1.5.2 Specific Objectives

The specific objectives were to:

i. Determine the total phenolic content, total flavonoid content and antioxidant activity of the aqueous and ethanolic extracts of fresh garlic (before cooking).

ii. Characterize the phytochemical constituents in garlic extract using High Performance Liquid Chromatography and Fourier transform infrared spectroscopy,

iii. Evaluate the effect of cooking temperature and time on total phenolic content, total flavonoid content and antioxidant activity of garlic.

iv. Establish the effect of the presence and concentration of refined salt and unrefined salt on phytochemicals and antioxidant activity of garlic.

1.5.3 Hypothesis

i. There are no differences in the TPC, TFC and AOA of aqueous and ethanolic extracts of fresh garlic.

ii. Garlic contains polyphenols and flavonoids.

iii. Cooking temperature and time have no effect on the TPC, TFC and AOA of garlic.

iv. Commercial kensalt and indigenous reed salt have no effect on TPC, TFC, and AOA of garlic.

CHAPTER 2: LITERATURE REVIEW

2.1 Food Additives

Food additives may be defined as natural or manufactured substances added to food for a variety of reasons (Street, 2010). There are different functional classes of food additives namely; sweeteners, colorants, preservatives, antioxidants, emulsifiers and stabilizers, flavorings and flavor enhancers (Street, 2010). All these functional classes of food additives are used when processing, storage and packaging to retain the original form or further improve the value of the food (Nam & Chan, 2014). Assigning a food additive to a particular functional class does not in any way prevent it from being used for other functions. For many years, people have used food additives for medicinal purposes because they were aware of the existence of their biologically active constituents from their own experience. However, of recent there has been an increase in the use of plant derived food additives due to the presence of bioactive compounds (Ilbäck, Busk, Ilback, & Busk, 2016). These bioactive compounds accord the various food additives with therapeutic properties. Plant derived food additives are mostly used unlike synthetic food additives due to their availability and less side effects if any (Ilbäck *et al*., 2016).

2.2 Chemistry of Garlic

Garlic is one of the mostly used food additive in the world due to its pungent smell as a seasoning or a condiment. It has been suggested and highly ranked as one of the highest sources of total phenolic compounds to human diet (Martins, Petropoulos, & Ferreira, 2016). Apart from its flavor, the main reason why garlic is widely consumed is its therapeutic and medicinal properties (Martins *et al*., 2016). These properties are attributed to the high content of biologically active organosulfur compounds present in garlic (Prati et al., 2014). These

organosulphur and polyphenolic compounds are bioactive hence its antioxidant activity and potential pharmacological properties (X. Lu *et al*., 2011). Unprocessed garlic or uncrushed garlic contains a limited number of organosulfur compounds, such as, alliin, methiin and γ-glutymyl-s-alkenyl cysteines (Martins *et al*., 2016). However, once garlic is processed or crushed, a cascade of chemical reactions are initiated and results into the formation of new organosulfur compounds such as alkenyl-polysulfides, ajoenes and vinyldithiins (Kodera, Matsuura, & Sumiyoshi, 2003).

Allicin is also one of the transformation compounds which bring about the garlic smell. However, due to its instability and reactiveness, it quickly decomposes into other organosulfur compounds and thus is considered as transient compound (Kodera *et al*., 2003). It is these organosulfur compounds that give garlic its therapeutic and medicinal properties such as anti-cancer, anti-diabetic, cardio-protective, anti-inflammatory, antimicrobial and immuno-modulatory (Kodera *et al*., 2003). The S-allyl group is the most active site in sulfur containing compounds derived from garlic and it plays an important role in the biological activity of garlic (Kodera *et al*., 2003).

2.3 Effect of Cooking Conditions on the Nutritive Value of Food

2.3.1 Temperature

Food processing in homes mostly involves heating the food to different temperatures. Many studies have investigated the effect of heat and various cooking methods or processes on the levels of antioxidant compounds and antioxidant activities. Heating affected the total phenolic content and antioxidant activity of curry paste in such a way that the total phenolic content of the extract increased after heating at 80°C, 90°C and 100°C for 10minutes,

20minutes and 30minutes and that the 2,2-diphenyl-1-picrylhydrazyl activity (DPPH) also increased (Faculty & Yai, 2011). The change was attributed to formation of new compounds as a result of the degradation and also release of bound antioxidants during the heating process.

Curcumin , which is the main component in turmeric, when heated, undergoes degradation due to roasting and its degradation products (i.e. 4-vynlguaiacol) exhibit significant biological activity such as antioxidant and anti-inflammatory properties (Esatbeyoglu, Ulbrich, Rehberg, Rohn, & Rimbach, 2015). In addition, turmeric oil showed significantly higher antioxidant activity after heating at 120 °C for one hour, indicating that the spice constituents were resistant to thermal degradation (Tiwari, Shanker, Srivastava, & Vankar, 2006).

Sengul *et al.* (2013), in their research investigated the effect of different cooking methods (boiling, steaming, stir-frying and microwaving) on the total phenolic content and antioxidant activity of selected vegetables (beet, red cabbage, broccoli, white cabbage, black radish, Kale, turnip and red radish) from Turkey. The total phenolic content and the antioxidant activities in cooked vegetables were lower than those of raw samples and that, antioxidants such as carotenoids and vitamin C were sensitive to heat and storage. It has also been concluded that boiling and microwaving results in the highest loss of antioxidants and antioxidant activity than other methods of cooking (Ra *et al*., 2009). Comparably, Agamy (2016), investigated the effect of boiling and microwave cooking on some antioxidant compounds in highly consumed vegetables (artichoke, green haricot, okra, pea, squash and tomato) in Egypt, it was reported that there was a statistically significant reduction in beta

carotene and Vitamin C when vegetables were boiled rather than when they were microwaved.

Total phenolic content significantly reduced in the vegetables. However, there was a small increase in the total phenolic content of boiled green haricot by 23.28% and microwaved green haricot by 16.72%. Boiling vegetable samples resulted in a decrease in total antioxidant capacity of green haricot, squash and tomato but led to the increase in total antioxidant activity of okra, pea and artichoke. From the above statements, it may be concluded that the increase in total phenolic content does not guarantee high antioxidant activity and vice-versa. Wangcharoen (2014) investigated the effect of heat on the antioxidant capacity of dry and wet heated garlic at 70ºC, 100ºC and 121ºC. The results showed that the antioxidant capacity was decreased by decomposition of some phenolic and sulfur-containing compounds. However, the antioxidant capacity increased when browning pigments developed but it was dependent on the degree of browning. On the same, increase in temperature has shown to increase the quality, amount of phenolic compounds and antioxidant activity of black garlic (X. Zhang, Li, Lu, Liu, & Qiao, 2018).

The above studies show that heating has an impact on the antioxidant activity of vegetables and other food additives. In conclusion, thermal stability of bioactive compounds depends on the type of spice, vegetable or food as well as the cooking conditions or methods as it has been evidenced in the studies above.

2.3.2 Time

As it has been stipulated in some research findings that heat processing has an effect on antioxidant activity of various vegetables and food additives, the length of processing time

also has an impact on the antioxidant activities of various vegetables and food additives. In a study conducted by G. Hwang *et al*. (2012), which investigated the various cooking methods and three cooking times (5, 10, 15 minutes) on the antioxidant properties of green paper, it was observed that boiling and steaming led to a significant loss on the antioxidant activity of red paper and also that the contents of red paper responsible for the antioxidant activity further decreased with prolonged cooking times. In another study, the effect of temperature and time to the antioxidant activity of *Plecranthus amboinicus* Lour were studied. It was determined that the antioxidant activity increased with the rise of temperature from 45°C to 100°C but dropped when the temperature was raised to 120°C. In addition, two hour boiling time gave the highest antioxidant activity but with no significant difference compared to 1 hour boiling time. However, 3 hours boiling significantly gave less antioxidant activity of the extract. Even if the time of processing has an effect on the antioxidant capacity of various vegetables and food additives, it all depends on the type of antioxidants present in the food.

2.3.3 Additives

Not only does heat affect the antioxidant capacity of foods or food additives but also the presence of other food additives. Normally, when cooking, salt is added to almost all dishes due to its ability to enhance flavor. Salt is also used in preservation of food due to its antimicrobial effect which is a result of its capacity to reduce water activity. It has been established that food additives and their degradation products can interact with other food additives and food constituents (additive-additive, additive-micronutrient) to form an array of products and these can also have an effect on the *antioxidant* activity (Taylor, Scotter, & Castle, 2007). In a study conducted by Wei *et al*., (2014), in which the effect of different

methods of processing (blanching, microwave, freezing, brining and prickling) on the antioxidant properties of selected herbs and vegetables (garlic, ginger, chili fruit, onion, mushroom cap and baby corn) were studied, it was found that brining with salt (salt is used as a preservative and softening agent) led to decline of antioxidant properties of vegetables and it was concluded that brining reduces the nutritional value of preserved vegetables because of the elimination of water soluble vitamins and minerals.

Burg & Oshrat (2015) also studied the effect of different salts on the antioxidant activity of red microalgal sulphated polysaccharide derived from porphyridium sp and porphyridium aerugineum in soybean based infant milk formula. It was noted that salt composition and concentration had an effect on the polysaccharide's antioxidant activity. The effect was because salt ions interfered with polysaccharide chains' interactions and alter their structure leading to a three new dimensional structure that better exposes antioxidant sites in comparison to the polysaccharide without salt supplement (Burg & Oshrat, 2015).

Similarly, some salts can have a positive effect on the antioxidant activity of other vegetables and other food additives due to the fact that they also possess some antioxidant properties as such there can be a combined effect. For example, bamboo salts which have been used for a longtime for the prevention and treatment of various diseases in Korea, are known to have antioxidant properties (A. Om & Jeong, 2007). In their study bamboo salts were found to have antioxidant properties and as such inhibited the formation of reactive oxygen species in human astrocyte U373G cells. Bamboo salts inhibited reactive oxygen species formation by 2.2 to 2.9 times compared with NaCl and even by 23 to 45% higher than vitamin E (A. Om & Jeong, 2007). Generally, different types of salts have different effects on the

antioxidant activity of foods due to presence of different minerals and different phytochemicals in different amounts.

2.4 Phytochemicals present in Food Additives

"Phyto" means plant and phytochemicals is a term that is used to refer to chemicals produced by plants. Plants have a lot of naturally produced chemicals that help to protect themselves from pest, injury and diseases. Phytochemicals are synthesized in different ways. Some phytochemicals are products of photosynthesis, glycolysis, citric acid cycle, acetyl CoA and some are derived from activated sugars (Jenzer & Sadeghi, 2016). These natural occurring compounds have become interesting candidates, not only for plant protection but also human health protection because of their lower toxicity or absence of toxicity (Oz & Kafkas, 2017). There are about 200,000 phytochemicals that are known and 20,000 of them are found in fruits, vegetables and grains. Vegetables are known to be among the most important sources of phytochemicals and food additives being one of them (Oz & Kafkas, 2017).

There are different types of phytochemicals present some of which include but not limited to vitamins, carotenoids, alkaloids, phenols, flavonoids and terpenoids (Oz & Kafkas, 2017). Phenolic compounds and their metabolites are common constituents of vegetables that play a vital role as to provide astringency and aroma properties. Alkaloids, phenolic compounds and terpenoids have so far gained importance due to their antioxidant, antiviral antibacterial and anticancer effects (Oz & Kafkas, 2017).

2.4.1 Alkaloids

Alkaloids are phytochemicals found in a huge variety of plants and are used for their pharmacological and psychotropic effects (Jenzer & Sadeghi, 2016). They are a class of

naturally occurring nitrogen containing organic compounds. Due to their medicinal properties, they are known to possess potential therapeutic effects against neurodegenerative diseases such as Alzheimers's disease, huntington disease, parkinson's disease, epilepsy, schizophrenia and stroke (Hussain et al., 2018). Some well-known examples of alkaloids include isoquinoline, indole, pyrroloindole, oxindole, piperidine, pyridine, aporphine, vinca, beta-carboline, methylxanthene, lycopodium and erythrine by products (Hussain *et al*., 2018).

Alkaloids are also known to exhibit a wide range of bioactivities such as anti-inflammatory, antibacterial, antidiabetes, antiulcer and anticancer agents such as berberine which is an isoquinoline alkaloid (J. Lu, Bao, Chen, Huang, & Wang, 2012). The ability of these compounds to mitigate pathophysiology of these illnesses is achieved by functioning as muscarinic and adenosine receptors agonists, antioxidants and antiamyloids (Hussain *et al*., 2018). Figure 2.1 shows caffeine which is a purine alkaloid found in coffee, tea, cola nuts, mate, guarana and cocoa.

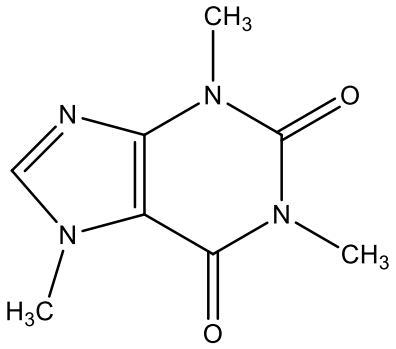


Figure 2.1: Structure of caffeine

2.4.2 Anthraquinones

Anthraquinones come from anthracene, a coal tar by product characterized by a chemical structure consisting of a polycyclic aromatic hydrocarbon and three fused rings of benzene. They naturally occur in plants, fungi and insects and contribute to the coloring pigments of such organisms (Patel & Patel, 2016). Due to the color property, anthraquinones are widely used commercially to manufacture dyes. Anthraquinones are derived from anthracene which is a chemical structure consisting of a polycyclic aromatic hydrocarbon and a three fused ring of benzene (Ebhardt & Rior, 2006). Figure 2.2 and Figure 2.3 shows anthracene and anthraquinones respectively.

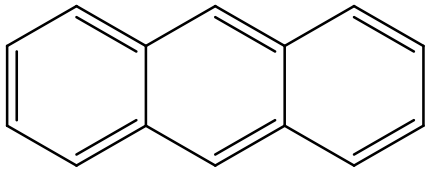


Figure 2.2: Structure of anthracene

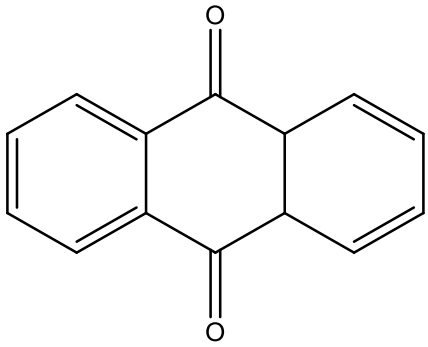


Figure 2.3: Structure of anthraquinone

2.4.3 Flavonoids

Flavonoids are phenolic substances found in plants and are characterized by a 15 carbon skeleton (Corradini *et al.*, 2011). The main structure in flavonoids is a 2-

phenylbenzopyranone. There are eight classes of flavonoids namely flavones, isoflavones, flavonols, anthocyanidins, flavanones, flavanols, chalcones and arones (Corradini *et al*., 2011). The major difference between these flavonoids classes lies in their degree of unsaturation and degree of oxidation (Corradini *et al*., 2011). Flavonoids are known to play different roles in plants of which include but not limited to defense system against insects harmful to the plant (Pietta, 2000). This role is achieved because of their astringency.

Examples of astringent flavonoids include catechins and flavanols. In animals, flavonoids are well known for their antioxidant activity. Flavonoids have lower redox potentials and as such, they are able to reduce highly oxidizing free radicals by forming less reactive flavonoid radicals and in turn prevent diseases which are as a result of oxidation reactions (Pietta, 2000, Corradini *et al*., 2011). Lastly, this class of compounds is also associated with anti-inflammatory (Serafini, Peluso, & Raguzzini, 2019), antiallergic, hepatoprotective, antithrombic, antiviral and antitumor activities (Pietta, 2000., Corradini *et al*., 2011., DW, W, ENM, & MP, 2016). Figure 2.4 shows the general structure of flavonoids.

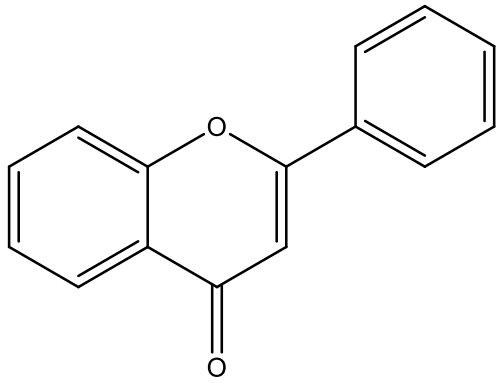


Figure 2.4: General structure of flavonoids

2.4.4 Glycosides

Glycosides are a large family of naturally occurring compounds and their core structure is comprised of a steroid nucleus with a five membered lactone ring (cardenolides) or a six membered lactone ring (bufadienolides) and sugar moieties. The main difference between the two core structures is the aglycone structure. The well-known examples of cardiac glycosides include digoxin, digitoxin, ouabain and oleandrin (Pongrakhananon, 2013). These compounds occur in minute amounts in seeds, leaves, stems, roots and bark of plants. Glycosides represent an interesting group of compounds that comprises the most drug-like molecules and as such they have been subjected to several investigations which have proved to be fruitful in developing potential drugs (Morsy, 2017). Furthermore, glycosides have been shown to have anticancer activities during various stages of carcinogenesis which include anti-proliferative, pro-apoptotic and chemotherapy sensitization (Pongrakhananon, 2013).

2.4.5 Phenols

Phenols are secondary metabolites whose core structure is a benzene ring with one or more hydroxyl groups. Phenolics range from simple phenolics to highly polymerized compounds. Medicinal herbs and dietary plants contain a large number of phenolics which include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans and quinones just to mention a few (Huang, Cai, Zhang, Huang, & Cai, 2009). Phenolics have occupied an important position in medicine due to a number of therapeutic effects they possess. Various bioactivities of phenols are responsible for their chemo-preventive properties which encompass antioxidant, anti-carcinogenic, anti-mutagenic and anti-inflammatory effects (Lin, Xiao, Zhao, Li, & Xing, n.d., Upadhyay & Dixit, 2015).

2.4.6 Saponins

Saponins are natural and high molecular weight glycosides of triterpene or steroids which are widely found in the plant kingdom, lower marine animals and some bacteria (Patel & Patel, 2016). Saponins are of two types namely; steroidal and triterpenoids saponins (Patel & Patel, 2016). One major characteristic of this class of compounds is their ability to foam. Saponins consists of a polycyclic aglycones attached to one or more sugar side chains (Marrelli, Conforti, Araniti, & Statti, 2016). The combination and/or interaction of the aglycone part which is hydrophobic and a hydrophilic part of the saponins is what brings about foaming (Patel & Patel, 2016).

There are some saponins which are toxins and as such they are referred to as sapotoxins. However, a wide number of saponins have been found to possess medicinal benefits such as anti-inflammation, anti-parasite, antivirus, antifungal, anticancer and anti-cardiovascular activity (Milugo *et al*., 2013., Moghimipour & Handali, 2015). In addition, saponins are also used in the cosmetic as well as food industries. In the cosmetic industry, they are used as stabilizers of cosmetic emulsion and as foam intensification in shampoos and conditioners. In food industries it is used as foaming agents in beverages and confectionary.

2.4.7 Steroids

Steroids are secondary metabolites that exert a wide range of biological activities and are produced by plants, animals and microorganisms (Gunaherath & Gunatilaka, 2014). They are made up of the tetracyclic 1,2-cyclopentanoperhydrophenanthrene carbon skeleton usually consisting of substituents at C-10 and C-13 and a side chain at C-17 (Gunaherath & Gunatilaka, 2014). Regardless of their source, all steroids are derived from s-squalene-2,3-epoxide, an intermediate of the acetate mevalonate pathway. Steroids play an important role

in plants for example, phytosterols, are an integral component of the plant cell membrane lipid by-layer that controls the membrane fluidity and permeability (Gunaherath & Gunatilaka, 2014). In addition to that, these compounds are known to exhibit biological activities. Phytosterols are reported to interfere with the intestinal absorption of cholesterol and in turn lower the blood cholesterol levels. Furthermore, they have also shown in-vitro anticancer properties and are alleged to inhibit the development of different types of cancers in human beings (Gunaherath & Gunatilaka, 2014).

There are also a number of medicinal plants known to exhibit anti-inflammatory properties due to the fact that they contain steroid like compounds. *Wathania Somnifera* is one type of plant species that contains chemical constituents with the steroidal structure and proved to be anti-inflammatory agents by modern clinical and pre-clinical studies (April, Patel, & Savjani, 2015). Withanolides steroids have also been found to show antitumor, immunomodulatory, anti-inflammatory, anti-leishmanial and antimicrobial activities (Gunaherath & Gunatilaka, 2014., April *et al*., 2015).

2.4.8 Tannins

Tannins are a group of phenolic compounds of molecular weight ranging from 500Da to more than 3000Da (Patel & Patel, 2016). They are commonly found in plant leaves, bark, fruits, wood and roots and are located in the tissues in the vacuoles. Tannins are mainly used by the plants for defense mechanisms against mammalian herbivores, birds and insects. In as much as other phenolics such as simple phenolics, flavonoids and neolignans are characterized and grouped according to their chemical structure, tannins are classified due to their ability to form complexes with proteins, polysaccharides, alkaloids, nucleic acids and minerals (S, Msa, & Selvakumar, 2018).

2.4.9 Terpenoids

Terpenoids are also a group of naturally occurring organic compounds in plants with the general formula $(C_5H_8)_n$ (Yadav, Yadav, & Goyal, 2014). They are colorless; they have fragrance, volatile and easily oxidized. Terpenoids also undergo addition reaction and the products of these reactions have antiseptic properties (Yadav *et al*., 2014). In plants and other photosynthetic organisms, these compounds are vital for the growth and survival due to the fact that they play an important role in the conversion of light into chemical energy for assembly and function of photosynthetic reaction centers (Pattanaik & Lindberg, 2015). Many terpenoid compounds have been found to possess anti-inflammatory properties and are of interest in the fields of pharmaceuticals as anti-inflammatory drugs and nutrition and even potentially as biofuels (Pattanaik & Lindberg, 2015). Figure 2.5 shows the structure of Geraniol which is a monoterpene.

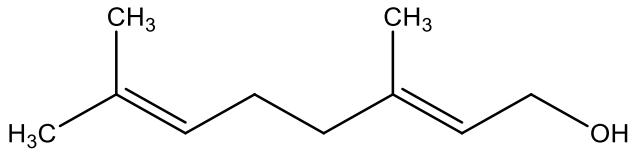


Figure 2.5: Structure of Geraniol which is a monoterpene

2.5 Antioxidants

Antioxidants are compounds that inhibit the process of oxidation (A. Om & Jeong, 2007). Even in small amounts, antioxidants are capable of reacting with reactive oxygen species (ROS) in our bodies hence they are of a great physiological importance to the body. Oxidative processes occurring in human organism lead to the formation of oxygen reactive forms such as peroxides, superoxide, hydroxyl radical and singlet oxygen among others (El ż bieta Sikora, Ewa Cie ś lik, 2008). Some other factors that contribute to the formation of

free radicals include; exposure to sun rays, use of packaged food, smoking and exposure to environment (Ra *et al*., 2009).

The ability of our bodies to fight against free radicals depends on the amount of antioxidants present. If our bodies contain more antioxidants than free radicals then chronic infections are reduced unlike when the antioxidants are less. When there is oxidant-antioxidant imbalance which results in the presence of more radicals then the susceptibility to chronic diseases will increase because these radicals damage important proteins in the bodies. During times of stress the levels of these reactive species can increase drastically and lead to what is known as oxidative stress which is the culprit of inflammatory disease, Cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts, autism and ageing (Atta, Mohamed, & Abdelgawad, 2017). Antioxidants react with oxygen reactive species and convert them to less reactive species thereby protecting our bodies from radical related diseases (El ż bieta Sikora, Ewa Cie ś lik, 2008). They can either stop the process of oxidation by chain breaking or by preventing oxidation by reducing the rate of chain initiation(Atta *et al*., 2017). Fruits, vegetables, tea and food additives have been found to be a great source of natural radical scavenging antioxidants.

2.6 Methods used in Phytochemical Analysis

There are various methods that are employed to qualitatively and quantitatively analyze phytochemicals. The methods include; Spectrophotometry, Gas chromatography, Thin-layer chromatography, Liquid chromatography, Fourier Transform Infrared Spectroscopy and Capillary electrophoresis (Kafkas, Kosar, Öz, & Mitchell, 2018).

2.6.1 Fourier Transform Infrared spectroscopy (FTIR)

FTIR is the most powerful tool for the identification of chemical bonds and/ or functional groups in a compound (Ashokkumar & Ramaswamy, 2014). Different functional groups absorb at different wavelengths of light and this can be seen in a spectrum (Ashokkumar & Ramaswamy, 2014). The chemical bonds in a molecule can be determined by interpreting the infrared absorption spectrum.

2.6.2 Spectrophotometry

Spectrophotometry is a simple and rapid method for quantitative measurement of phytochemicals in plant materials (Cong-cong, Bing, Yi-qiong, Jian-sheng, & Tong, 2017). It is based on different principles for measuring the various structures present in the phytochemicals (Cong-cong *et al*., 2017). This method employs the use of assays to detect the phytochemicals in plants. For example, Folin-Ciocalteu's reagent is one of the assays that have been used for a long time in the detection of phenolic compounds in plant materials.

2.6.3 Chromatographic Techniques

Chromatographic techniques such as liquid chromatography, gas chromatography and capillary electrophoresis with different detectors such as Ultraviolet, Diode array, mass spectrometry and florescence are also used to identify, separate and quantify phytochemicals (Kafkas *et al*., 2018). Depending on the detector used various information about a particular compound can be found. Analyses by chromatographic techniques are achieved by the use of different stationary phases and mobile phases. The nature of the phytochemical to be analyzed also dictates which chromatographic technique to be used. For example, volatile compounds are analyzed using gas chromatography whereas non-volatile are analyzed using Liquid chromatography though derivatization techniques can also be employed at times.

Thin-layer chromatography (TLC) is a low cost chromatographic technique which is used for fast separation and detection of multiple substances on the same TLC plate within a short period of time (Cong-cong *et al*., 2017). TLC can be used both for qualitative and quantitative analysis. Maleš, T, & T (2013) used high performance thin layer chromatography (HPTLC) for the identification and quantification of individual polyphenols (quercitrin, isoquercitrin, hyperoside and chlorogenic acid) in *Arbutus unedo* L.

Capillary electrophoresis is an advanced and high resolution analytical technique that is used to separate charged compounds for example phenolic compounds in plants (Cong-cong *et al*., 2017). Separation happens in a narrow capillary column with solutions of ions which is suitable for separating and quantifying polar and charged compounds with low to medium molecular weight. Just like GC and HPLC, Capillary electrophoresis also uses various detectors. The inability to discriminate compounds with close charge to mass ratio remains a disadvantage of CE as such solvents can be modified to enhance separation (Cong-cong *et al*., 2017).

CHAPTER 3: METHODOLOGY

3.1 Materials and Methods

3.1.1 Study Area

The samples were all from Kenya except for garlic which was bought in Kenya but is of Chinese origin. The study was done at the Directorate of Government Analytical Laboratory (DGAL) in Uganda.

3.1.2 Reagents and Chemicals

Below are the chemicals, reagents, apparatus and analytical instruments that were used in the research.

These include; ammonium sulphate, Sodium carbonate, Folin-Ciocalteu reagent, Chloroform, Distilled water, Ferric chloride, Hydrochloric acid, Magnesium metal, Mercuric Chloride, Potassium Iodide, absolute ethanol (99.9%), Sulphuric acid, Sodium hydroxide, glacial acetic acid, Bromine water, Lead acetate, Olive oil, n-hexane, Ethyl acetate, Potassium bromide, Aluminum chloride, DPPH reagent, Quercetin hydrate (≥ 95%), Ascorbic acid, Gallic acid (≥ 99.9%). The standards were all of analytical grade and were purchased from Sigma Aldrich. Folin-Ciocalteu's reagent (phenol reagent) was from India Loba laboratory reagents.

3.2 Research Design

In this study, both qualitative and quantitative research was undertaken using the experimental research design.

3.3 Sampling

Random sampling technique was used for sample collection. Garlic bulbs and commercial iodized (Kensalt) were purchased from a supermarket in Eldoret town, Kenya. Indigenous reed salt from *Typha latifolia* was purchased from vendors in Busia county of Western Kenya. A total of three samples weighing 7 kg, 1 kg and 0.5 kg of garlic, indigenous reed salt and Kensalt respectively, were collected.

3.4 Preparation of the Extract

A mass of 100.00g of chopped garlic was used. A stainless garlic chopper was used to cut garlic bulbs into pieces measuring 2.00 mm by 2.00 mm. Aqueous and ethanol garlic extracts were prepared in an effort to determine the effect of the following conditions: time, temperature, salt additive and its concentration on the phytochemical composition and antioxidant levels. Chopped garlic samples were used for extraction.

3.4.1 Effect of Temperature and Cooking Time

Fresh chopped garlic was macerated in ethanol, and water for 24 hours to determine the total phenolic content, total flavonoid content and antioxidant activity before cooking which acted as a control for the experiment. The cooking of chopped garlic samples was done at respective temperatures of 25ºC (room temperature) as a control, 50ºC, 75ºC, 100ºC, 125ºC and 150ºC. The garlic samples were heated for; 15, 30, 45 and 60 minutes for the 6 temperature conditions. The heated samples were filtered using Whatman No.1 filter paper. The filtrate (aqueous extract) was analyzed whilst the residues were macerated with absolute ethanol (ethanolic extracts) for 24 hours. The ethanolic extract from the residue was also analyzed. This means that there were 25 aqueous extracts and 25 ethanol extracts for analysis. A total of 50 samples were obtained from the above preparations for analysis to

ascertain the effect of cooking time and temperature on the garlic phytochemical levels. The ethanolic extracts were then concentrated under reduced pressure at 50°C using a rotary vacuum evaporator. The concentrated extracts were collected in a petri dish and air-dried at room temperature. The extracts were stored in a refrigerator at 4°C awaiting analysis. All analyses were done in triplicate and data was presented as mean ± standard deviation.

3.4.2 Salt additive and Concentration

For the effect of salt additive, optimum conditions from (3.4.1) above were used. Two sets of garlic extracts were used; chopped with and without addition of selected salts at different concentrations of 0.00 M, 0.05 M, 0.10 M, 0.20 M and 0.40 M. This gave a total of 9 samples (with no salt, with indigenous reed salt and with Kensalt at the mentioned concentrations).

3.4 Phytochemical Screening

A total of 3 samples of garlic were subjected to phytochemical screening to test for the presence of alkaloids, saponin, phenolics, tannins, anthraquinones, terpenes, flavonoids, steroids and glycosides using the standard method of Obwonge *et al*. (2012). The samples were garlic macerated in water, ethanol and cooked garlic.

3.4.1 Alkaloids

Measured 1.50 g of Mercuric chloride was dissolved in 60 ml of distilled water. Then 5.00 mg of potassium iodide was also dissolved in 10 ml of distilled water. The two solvents were then mixed and diluted with distilled water up to the 100 ml mark. Few drops of the prepared reagent were added to 1 ml of the extract. Appearance of a pale-yellow precipitate signified the presence of alkaloids.

3.4.2 Anthraquinones

Measured 1 ml of each sample was shaken with 10 ml of ferric chloride solution and 5 ml of hydrochloric acid. Each mixture was heated in a water bath for 10-15 minutes, filtered and allowed to cool. The filtrate was extracted with chloroform and manually shaken gently. The clear layer at the base was poured into a test tube and 2 ml of ammonium sulphate was added. A delicate pink rose color indicated the presence of anthraquinones.

3.4.3 Flavonoids

Measured 0.5 ml of the extract was added to a test tube followed by 5 drops of diluted hydrochloric acid to the extract together with a small amount of magnesium metal. The solution was then boiled for 3 minutes. The appearance of a reddish-pink or brown color indicated the presence of flavonoids.

3.4.4 Steroids

A mass of 100 mg of dried extract was dissolved in 2 ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish-brown color at the interface was indicative of the presence of steroidal ring.

3.4.5 Glycosides

Two (2) ml of concentrated sulphuric acid was be added to the extract. Formation of a reddish-brown color indicated the presence steroidal aglycone, which is a part of glycoside.

3.4.6 Tannins

A total of 10 ml of bromine water was added to 0.5 ml of extract. Discoloration of bromine water indicated the presence of tannins.

Lead Acetate test: 3 few drops of 1% lead acetate were added to 5 ml of the extract. Formation of a yellow or red precipitate indicated the presence of tannins.

3.4.7 Terpenoids

Two (2) ml of chloroform and 1 ml of concentrated sulphuric acid were added to 2 ml of the extract. Appearance of a reddish-brown color indicated the presence of terpenoids.

3.4.8 Saponins

Distilled water (2 ml) was added to 2 ml of the extract in a test tube. The test tube was shaken vigorously. The formation of persistent form indicated the presence of saponins.

3.4.9 Phenolics

To 2 ml of ethanolic extract, 1 ml of 1% ferric chloride solution was added. A blue or green color formation indicated the presence of phenols.

3.5 Determination of Total Phenolic Content

Total phenolic content of the extracts was determined by using the Folin-Ciocalteu (FC) assay (Taylor, Memnune, Yildiz, & Kavaz, 2014) with some modifications. FC reagent (1.50 ml, 10 times dilution) and sodium carbonate (1.20 ml, 7.5% w/v) was introduced into test tubes containing 0.20 ml of extracts. Absorbance was measured at 760 nm using UV-1900 UV-Vis Spectrophotometer (Shimadzu Corporation, Japan) after incubating for 30 minutes in the dark. Total phenolic content has been expressed as Gallic acid equivalent (GAE) in mg/100g (Wei *et al*., 2014). TPC was calculated from a calibration curve that was obtained using Gallic acid as the standard (Hwang, Shin, Lee, Lee, & Yoo, 2012). All extracts were analyzed in triplicate.

3.6 Determination of Total Flavonoid Content

Aluminum Chloride colorimetric method was used for the determination of Total Flavonoid Content (TFC) of the samples (Chandra *et al*., 2014). Quercetin was used to make standard calibration curve. Stock quercetin solution was made by dissolving 5.00 mg in 1 ml of ethanol, and then the standard solutions of quercetin were prepared by serial dilutions using ethanol (5-200 mg/ml). Measured 1 ml of extracts were separately mixed with 1.00 ml 0f 2 % aluminum chloride after mixing the solution was incubated for 60 minutes at room temperature. The absorbance of the reaction mixture was measured against a blank at 420 nm using UV-1900 UV-Vis Spectrophotometer (Shimadzu Corporation, Japan). The concentration of the total flavonoid content in the test samples was calculated from the calibration plot and expressed as mg quercetin equivalent (QE/mg) of dried plant material.

3.7 Determination of Total Antioxidant Activity

The determination of the antioxidant activity was done according to the method of (Shimada, Yahara, Nakamurat, Science, & Corporation, 1992) with some modifications. The extracts of garlic samples obtained in 3.4.1 above were used for determination of total antioxidant activity. The antioxidant activity of garlic was determined and evaluated using DPPH radical scavenging activity because DPPH method is simple, rapid, reproducible, sensitive, sample polarity independent and does not require expensive reagents or instrument (Zingiber, 2010). Measured 1 ml of the extract was mixed with 1.2 ml of 0.003% DPPH in ethanol. The reducing ability of the antioxidants towards DPPH was evaluated by monitoring the absorbance at 517 nm using UV-1900 UV-Vis Spectrophotometer (Shimadzu Corporation, Japan). The percentage of DPPH inhibition was calculated using the Equation 1.

$$\text{Inhibition (\%)} = \frac{(A_c - A_s)}{A_c} \times 100 \quad (1)$$

Where A_s is the absorbance of the sample and A_c is the absorbance of the control.

3.8 Fourier Transform Infrared (FTIR) Spectroscopy Analysis

Fourier transform infrared spectroscopy was used to determine the functional groups in the garlic extracts obtained in 3.4.1 above to confirm the identity/presence or absence of respective constituents (Banu & Cathrine, 2015). The method of Ashokkumar *et al.* (2014) was used with some modifications. Dried powders of ethanolic and aqueous extract of garlic were used. Exactly 10.00 mg of the dried extract powder was encapsulated in 100.00 mg of KBr pellet, in order to prepare translucent sample disk. The powdered garlic sample was then loaded on Attenuated Total Reflection (ATR) crystal and spectra read spectrophotometrically (4500-400 cm^{-1}) using a FT-IR spectrometer (Nicolet NEXUS 470, Thermo Scientific, USA). The frequencies of different components present in each sample were recorded. The resolution was 4 cm^{-1} for 20 scans on each sample. The analysis was repeated twice for confirmation of spectra. The peaks obtained were compared with published data of functional groups (Silva, Rosa, & Ferreira, 2014).

3.9 Liquid Chromatography-Mass Spectroscopy (LC-MS/MS) Analysis

A targeted qualitative analysis of the extracts was performed using the method of Sighn *et al.*, (2002). Reverse phase chromatographic analysis was carried out using C-18 reverse phase column. Sample clean-up was done using C-18 Solid Phase Extraction. The C-18 cartridge was conditioned with 60.00 ml of 10% methanol in acidified water (0.1% formic acid). Exactly 50.00 ml of the extract solution was loaded into a C-18 column and allowed to flow through under gravity at a flow rate of approximately 1ml/min. The co-extracted

substances were eluted from the sorbent with 100.00 ml of aqueous acetic acid (2% v/v). The column was dried by passing nitrogen gas for 5 minutes and total retained phenols were eluted with acidified methanol (0.1% formic acid) (Rajauria, Foley, & Abu-ghannam, 2016). The purified extracts were concentrated under a rotary evaporator at 38℃ and reconstituted in 1.20 ml of 10% acidified methanol and then filtered through 0.45µm filter prior to LC-MS/MS analysis.

LC-MS/MS was used for characterization of the phenolic compounds. Sample solutions of 20.00 µl were injected into a C-18 reverse phase column (rapid resolution HD; 2.1 x 50 mm 1.8 micron) at 25℃. Solvent A was made of 0.1% formic acid in water; solvent B was acetonitrile at a flow rate of 0.80 ml/min. Identification of phenolic compounds was based on retention time and mass to charge ratio in reversed phase LC and MS spectra features (Zhong & Hodgson, 2019).

3.10 HPLC-UV Analysis

3.10.1 Sample preparation

The samples were first centrifuged to obtain a supernatant. Extraction of phenolics was then done by passing 15 ml of the supernatant through Supelco C-18 SPE cartridges. The SPE cartridges were first conditioned using methanol and DI water and the samples loaded at a drop-wise rate. Elution was done using 1ml of 100% methanol. The extracts were filtered through 0.45-micron PTFE filters prior to HPLC analysis.

3.10.2 Standard preparation

Coumarin, Cinnamic acid and Quercetin standards were prepared in 20% methanol. Standard concentration prepared was 20 ppm for each of the compounds.

3.10.3 HPLC conditions

HPLC instrument used was Shimadzu Prominence LC, LC mode: Isocratic (80% Mobile phase A), Mobile phase A: 1% Formic acid in DI water, Mobile phase B: 100 % Methanol, Column: Shimpack C-18, 150 mm x 4.6 mm, 5 µm, Column oven temperature: 30 ^{0}C and Run time: 12 minutes.

3.11 Quality Control and Quality Assurance

3.11.1 Calibration Control and Verification

Quality control procedures that affirm the accuracy of calibrations were followed. The recommended procedures included use of analytical grade reagents and also checking that the slope, intercept and correlation coefficient of the calibration curves were within the acceptable ranges.

3.11.2 Precision

Three sets of calibration standards were prepared and analyzed to provide a measure of precision and reproducibility (Tahir, Kalkotwar, Naikwade, & Shaikh, 2013). Precision was expressed as the relative standard deviation (RSD) which is given by Equation 2.

$$\text{RSD} = \frac{s}{\mu} \times 100 \qquad (2)$$

Where s is the standard deviation and µ is the mean.

3.11.3 Accuracy

Accuracy was determined by means of recovery experiments which involved spiking the sample with a known concentration of the standard solution (Rathod, Rani, Kartheek, &

Kumar, 2014). The concentrations before and after spiking were measured. There after the percent recovery was calculated using Equation 3.

$$\%R = \frac{(S-U)}{C_{sa}} \times 100 \qquad (3)$$

Where S = measured concentration in spiked aliquot, U = measured concentration in unspiked aliquot and Csa = actual concentration of spike added.

3.11.4 Linearity and Range

Standard solution of gallic acid and quercetin at different concentrations were prepared. A calibration curve was constructed by plotting concentration versus absorbance. It was expected that the results will show an excellent correlation between absorbance and concentration of garlic acid within a concentration range. The resulting plot slope, intercept and correlation coefficient provided the desired information on linearity (Rathod *et al*., 2014).

3.11.5 Sensitivity, Limit of Detection and Limit of Quantification

The sensitivity of measurement was estimated in terms of the limit of detection (LOD) and limit of quantification (LOQ) (Rathod *et al*., 2014). The limit of detection and limit of quantification were expressed as in Equations 4 and 5.

$$LOD = \frac{3.3\delta}{S} \qquad (4)$$

$$LOQ = \frac{10\delta}{S} \qquad (5)$$

Where δ = standard deviation of the response,

S = slope of the calibration curve

3.11.6 Repeatability

Repeatability was achieved by preparing and analyzing three sets of standard solutions (Value, Sharma, Agrawal, & Gupta, 2012).

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Phytochemical screening, total phenolic and total flavonoid contents of garlic

The qualitative phytochemical screening of garlic revealed that the aqueous extracts of garlic contained glycosides, steroids, flavonoids, alkaloids, phenolic compounds, tannins and terpenes. Saponins were not present. The ethanolic extracts of the garlic also contained all the phytochemicals mentioned above except for steroids. These results agree with those of Nazir & Chauhan (2019), whose research focused on qualitative phytochemical analysis of *Allium sativum* (garlic) and *Curcuma longa* (turmeric) which revealed that the aqueous and ethanolic extracts of garlic contain saponins, alkaloids, tannin, steroids and flavonoids. The only difference was the absence of saponins in the aqueous extract and steroids in the ethanolic extracts which could be attributed to the type of garlic, origin and maturity at the time of analysis (Mahmood, Anwar, Abbas, & Saari, 2012). Table 4.1 summarizes the results on qualitative phytochemical analyses of garlic.

Table 4.1: Phytochemical analysis of fresh garlic

Phytochemical	Aqueous Extract	Ethanol Extract
Glycosides	+	+
Steroids	++	_
Flavonoids	+	+++
Alkaloids	+	+++
Saponins	-	+
Phenolics	+	+++
Tannins	+	+
Terpenes	++	+

Key: +++ = abundant ++ = moderate + = least - = not detected

The total phenolic content and total flavonoid content of the fresh garlic ethanolic and aqueous extracts were calculated using the calibration curves (Figures 4.1 and 4.2).

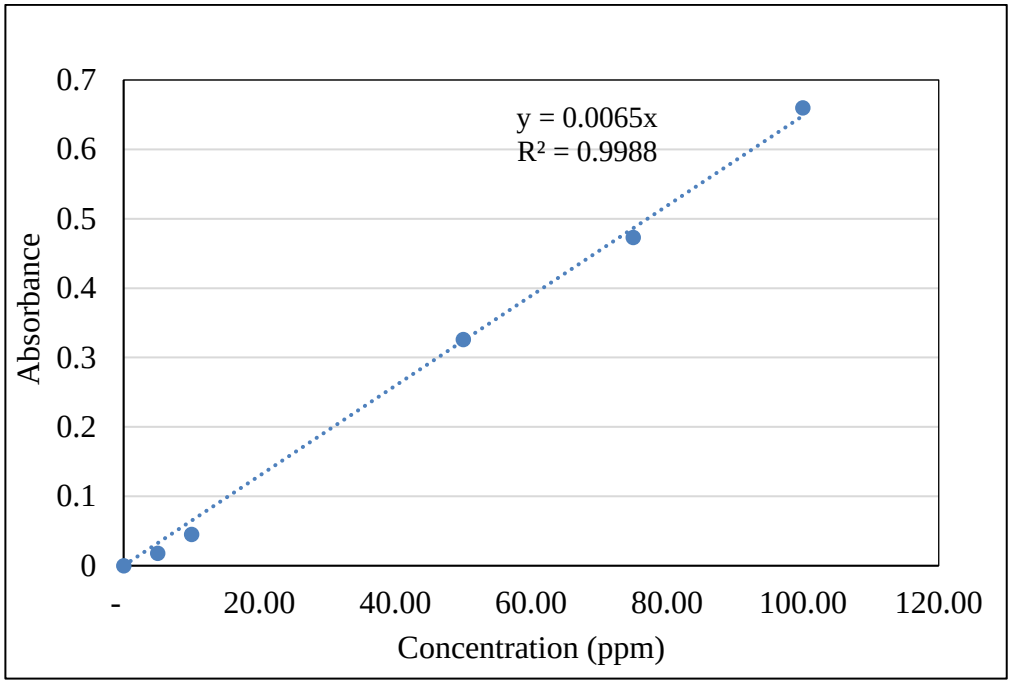


Figure 4.1**:** Gallic acid calibration curve

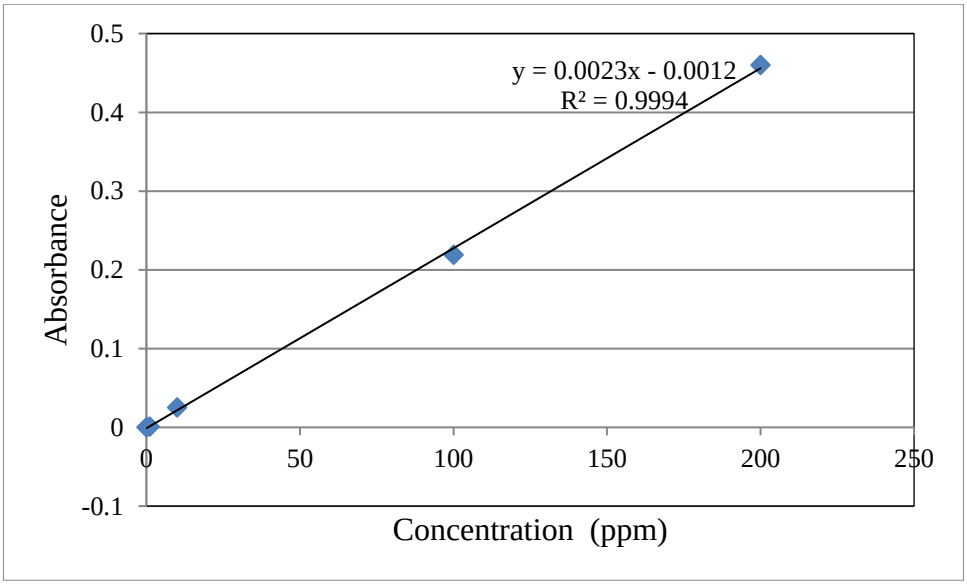


Figure 4.2: Quercetin calibration curve

The total phenolic content of the ethanolic extract of fresh garlic was found to be 637.91 ± 15.30 mg GAE/100g and that of the aqueous extract was 301.29 ± 6.58 mg GAE/100g which is less than that of ethanol. This indicates that ethanol is a better solvent or extracts more phytochemicals than water. Similarly, the total flavonoid content of ethanol extract of fresh garlic was 258.21 ± 12.37 mg QE/100g and that of aqueous extract was 109.68 ± 6.78 mg QE/100g which is 57.52 % lower than for ethanol extract.

A different result from the above was reported by Mishra, Tripathi, & Khan (2017). The TPC of fresh garlic was 78.45 mg GAE/100g which is eight times lower than the TPC reported in this study. Similar to that, are the findings of Wei *et al.* (2014) which study focused on the antioxidant activity of selected fresh and processed herbs and vegetables. They found that the TPC of fresh garlic was 154 ± 10 mg GAE/100g and TFC was 8.3 ± 0.6 mg CE/100g. On the other hand, a higher TPC (32.17 mg GAE/g) in Chinese garlic has been reported by Akan (2019). In addition, distilled water registered a higher total phenolic content and flavonoid content in both non-aged and aged garlic than ethanol with TPC and TFC greater in aged garlic than non-aged garlic. The variations in the findings could be attributed to difference in the cultivars (Akan, 2019), location, climate and maturation at the time of analysis.

4.2 Characterization of the phytochemicals in garlic

4.2.1 FTIR Analysis

The FTIR analysis in the 4000 cm^{-1} to 400 cm^{-1} region for both the aqueous extract and ethanol extract showed an absorption in the range 3400-2400 cm^{-1} which is characteristic of carboxylic acids. Peaks were also identified in the range 3650-3600 cm^{-1} for free O-H;

bonded O-H at 3400-3200 cm^{-1}; C-O around 1300-1000 cm^{-1} for esters, carboxylic acids and alcohols; S=O for sulphoxides at around 1050 cm^{-1} (Pavia, Lampman, Kriz, & Vyvyan, 2013). Figures 4.3 and 4.4 show the FTIR spectra of the aqueous and ethanolic extracts of garlic.

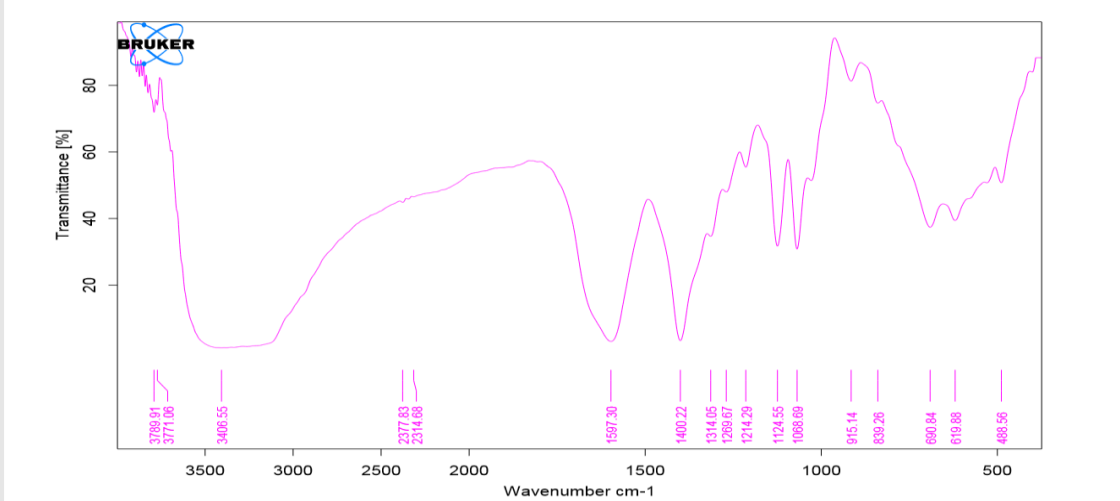


Figure 4.3: FTIR spectrum for aqueous extracts of fresh garlic

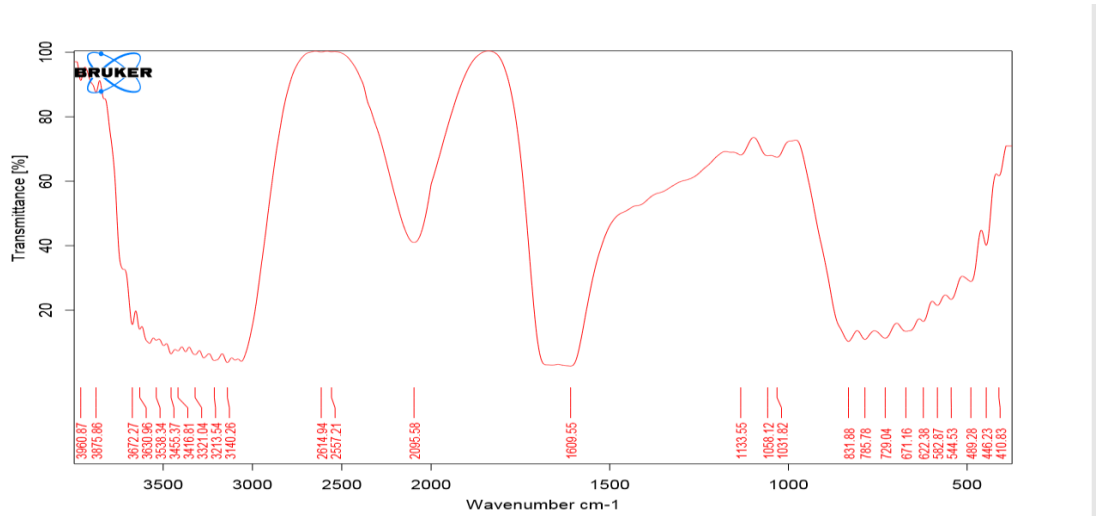


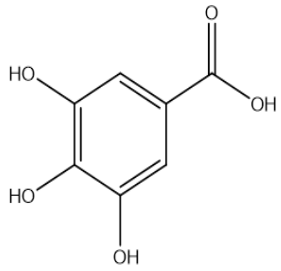
Figure 4.4: FTIR spectrum for ethanolic extracts of fresh garlic

The reported functional groups are associated with phenolic and flavonoid compounds. Divya, Bukke, & Venkataswamy (2017) in their study on phytochemicals, functional groups

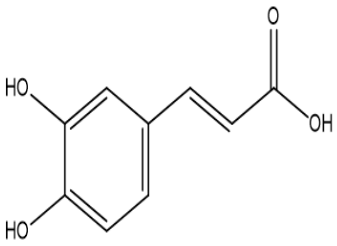
and mineral composition of garlic, showed a peak at 3265 cm^{-1} which is due to O-H stretching of a hydroxyl group. This indicated the presence of polyhydroxy compounds such as flavonoids. A peak at 2926 cm^{-1} was due to asymmetric stretching of C-H groups of aromatic compounds and at 1619 cm^{-1} was due to C=O stretching of peptide linkages or stretching of carbonyl and carboxylic groups. Another peak at 1395 cm^{-1} was as a result of O-H bend of carboxylic acids whereas a peak at 1036 cm^{-1} was due to S=O for the presence of organo sulphur compounds.

4.2.2 LC-MS/MS Analysis

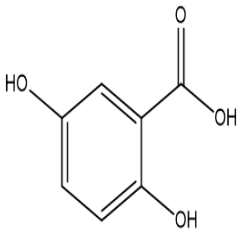
A targeted LC-MS/MS analysis of phenolic compounds and flavonoids revealed the presence of four flavonoids which are quercetin, hesperidin, rutin and catechin.



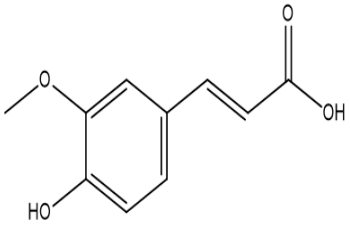
Gallic



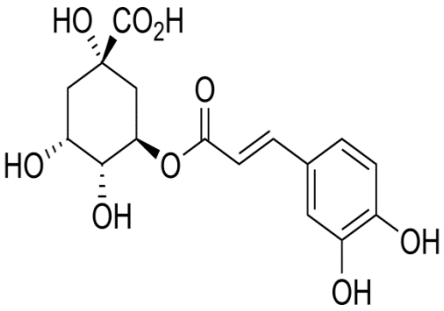
Caffeic acid



Gentisic



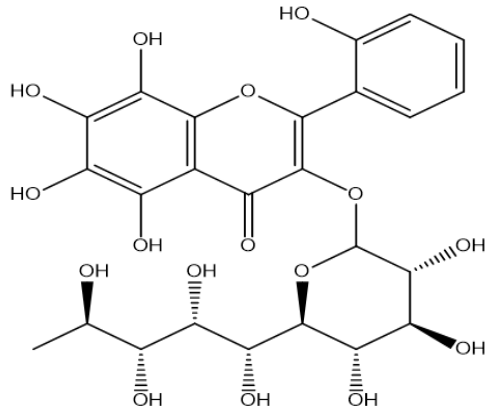
Ferulic



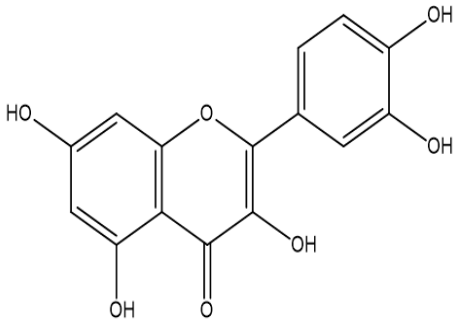
Chlorogenic

Figure 4.5: Structure of phenolic compounds identified in garlic.

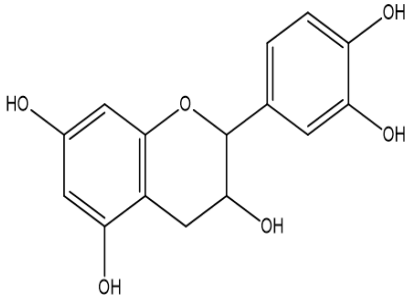
Phenolic compounds namely; isoferulic acid, gentisic acid, caffeic acid, gallic acid and chlorogenic acid were also identified. Refer to appendix 1(a) and (b) for the retention times, m/z values and fragmentation patterns of the above-mentioned compounds. Figures 4.5 and 4.6 show the structures of the phenolic and flavonoid compounds identified with functional groups as earlier depicted by FTIR.



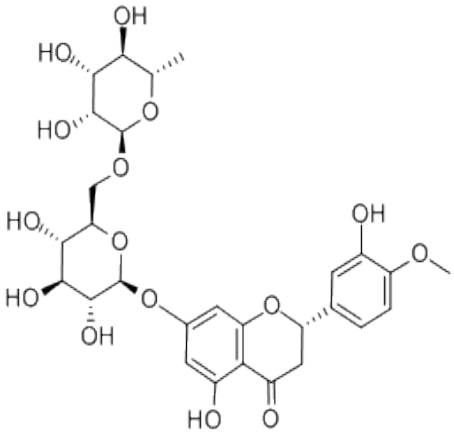
Rutin



Querceti



Catechin



Hesperidin

Figure 4.6: Structure of flavonoids identified in garlic

Some of the compounds identified have also been reported previously. Feng, Chun-Hua, Fu-Rong, Pei-Pei & Rong-Hai (2018) found that HPLC analysis of Black Garlic Residue (BGR) indicated the presence of gallic acid and coumaric acid by comparison with standard phenolic compounds. They also reported that coumaric acid was the main ingredient in BGR

(Feng, Chun-Hua, Fu-Rong, Pei-Pei, Rong-Hai, 2018). In addition, The & Asia (2018) reported that HPLC analysis of garlic extracts revealed the presence of highest amounts of syringic acid and p-hydroxybenzoic acid derivatives. Further, it was reported that Chinese cultivar contained highest amounts of gallic acid and p-coumaric acid and that the analyzed extracts were reported to contain significant amounts of epicatechin and catechin.

In another study aimed at the determination of phenolic compounds in artichoke, garlic and spinach by Ultra High Performance Liquid Chromatography (UHPLC) coupled to tandem mass spectroscopy, quercetin was the only flavanol detected and that caffeic acid was the predominant phenolic acid followed by ferulic acid (Alarcón-flores, Romero-González, Luis, Vidal, & Frenich, 2014). Cultivars *et al*., (2019) in their study on phytochemical characteristics and antimicrobial activity of Australian grown garlic cultivars identified vanillic acid, caffeic acid, p-coumaric acid, ferulic acid and sinapic acid in the garlic samples. As it can be noted from literature, there are differences in the findings which can be attributed to the reasons that have been mentioned earlier.

4.2.3 HPLC-UV Analysis

With the available standards, HPLC with a UV detector was used to qualitatively analyze coumarin, cinnamic acid and quercetin in the samples. Refer to appendix 3 for the chromatograms for the mentioned standards and chromatograms for aqueous and ethanol extracts of fresh garlic.

For cinnamic acid determination in the ethanol extract, the main peak’s retention time was at 2.079 minutes and the next peak was at 2.523 minutes. There was band broadening and tailing between the two peaks. The same applies to chromatograms for quercetin and

coumarin determination in the ethanol extracts of fresh garlic. Band broadening and tailing were observed between the main peak and the next. This resulted in poor separation and unresolved peaks.

The chromatograms for the aqueous extracts of fresh garlic were more different as compared to those of ethanolic extracts. Band broadening was more apparent in the aqueous extracts ethanolic extracts hence resulting in poor separation and unresolved peaks. Though this is the case, it showed that the aqueous samples contained coumarin, cinnamic acid and quercetin. For example, the chromatogram for quercetin determination in the sample showed a main peak at 2.125 minutes with intense tailing up to 2.550 minutes. The retention time for quercetin standard is 2.152 minutes which means that its peak was not well resolved due to the broadening and tailing. Generally, co-elution was a major challenge in identifying these compounds due to close retention times.

4.3 Effect of temperature and cooking time on phytochemicals and antioxidant activity of Garlic

4.3.1 Effect of Cooking Temperature on Total Phenolic and Total Flavonoid Content

Total phenolic and total flavonoid content for the aqueous and ethanol extracts varied irregularly with increase in cooking temperature and time. Table 4.2 and 4.3 shows results that were obtained at different cooking temperatures.

Table 4.2: Total Phenolic Content at different cooking temperatures and time for ethanolic extracts

Time (minutes)	Cooking Temperature					
	25 ºC	50 ºC	75 ºC	100 ºC	125 ºC	150 ºC
15	394.42 ±10.86[a]	381.00 ± 4.58[a]	385.90 ± 5.01[a]	379.02 ± 6.53[a]	293.83 ± 10.65[c]	266.34 ± 1.23[c]
30	377.95±14.47[a]	382.51 ± 10.17[a]	387.66 ± 7.72[a]	370.20 ± 4.96[a]	285.30 ± 17.52[c]	266.42 ± 9.43[c]
45	383.80 ± 1.24[a]	381.20 ± 5.38[a]	375.46 ± 9.51[a]	290.00 ± 5.06[a]	255.18 ± 17.94[c]	234.66 ± 12.86[c]
60	355.31 ± 9.88[a]	367.40 ± 8.22[a]	352.72 ± 13.22[a]	272.77 ± 10.74[a]	230.48 ± 22.40[c]	141.64 ± 3.72[c]

Different letters in the same row mean significant difference ($p \leq 0.05$) as per post hoc table

Table 4.3: Total Phenolic Content at different cooking temperatures and time for aqueous extracts

Time (minutes)	Cooking Temperature					
	25 ºC	50 ºC	75 ºC	100 ºC	125 ºC	150 ºC
15	365.49 ± 13.61[a]	517.28 ± 9.15[b]	701.39 ± 20.71[b]	753.68 ± 13.07[b]	878.65 ± 9.83[c]	933.60 ± 23.41[c]
30	367.08 ± 14.71[a]	632.86 ± 18.34[b]	753.91 ± 7.42[b]	797.67 ± 17.36[b]	973.775 ± 18.12[c]	1016.86 ± 4.18[c]
45	369.48 ± 21.48[a]	793.75 ± 33.21[b]	880.43 ± 6.55[b]	768.47 ± 17.71[b]	1091.46 ± 15.75[c]	1199.95 ± 20.78[c]
50	376.68±20.04[a]	798.52 ± 20.87[b]	897.36 ± 10.51[b]	932.66 ± 13.29[b]	1200.87 ± 39.92[c]	1273.30 ± 17.87[c]

Means followed by a different letter in a row are significantly different at $p \leq 0.05$

Subjected to different cooking temperatures, phenolic content from aqueous extracts was observed to be increasing with increase in temperature. These results differ with the total phenolic content from garlic residues subjected to or macerated in ethanol. The contents were in decreasing order.

For the hypothesis that temperature has no significant effect on TPC for aqueous extracts, ANOVA test was applied to test for this hypothesis. Based on appendix 3(a), it was found that at alpha = 5% significance level, there is a significance difference of total phenolic content between groups of temperature levels where p-value < 0.01.

While ANOVA test showed significant differences, a post hoc test was done to identify those notable differences. According to appendix 4(a), these differences were notable between high and low temperature levels. For instance, concentrations at 25°C were more significantly different from those at other remaining temperatures (p-value < 0.01). However, concentrations at 50°C were not significantly different from those at 75°C (p-value=0.148 > 0.05).

This therefore means that increase in cooking temperature increases the total phenolic content of garlic by allowing bound phenolic compounds to be released in the water. This statement is supported by results from Table 4.2 above which shows that the total phenolic content of the cooked/heated garlic residues (ethanolic extracts) were decreasing with increasing temperature. Likewise, under ethanolic extracts, significant differences were noted in total phenolic content due to different boiling temperatures that were applied earlier on to the garlic samples under aqueous extracts (p-value < 0.01) according to appendix 3.

Much differences were as well noted between samples that were residues from high temperature boiling in water and those from low temperature boiling in water. For instance,

significant differences were observed between residues from 25°C aqueous extract and those of 125°C (p-value < 0.01) and 150°C (p-value < 0.01) unlike those that were subjected to 75°C (p-value=0.925) and 100°C (p-value = 0.067) (refer to appendix 4(b)). In addition, an LC-MS/MS analysis for the garlic sample (aqueous extract) boiled at 150°C indicated the presence of more phenolic compounds namely; gallic acid, chlorogenic acid, caffeic acid, ferulic acid, ellargic acid, gentisic acid and tannic acid as compared to the fresh garlic samples (not cooked).

A phytochemical screening of the garlic cooked at 150 °C showed that most of the phytochemicals were abundant at that temperature as compared to phytochemical screening of samples at temperatures below 100 °C. This is a clear indication that most of the phytochemicals leached in the cooking water and that more compounds were extracted at higher temperatures. Table 4.4 shows the phytochemical screening at different temperatures.

Table 4.4: Phytochemical screening of garlic extracts cooked at different temperatures

Phytochemical	Ethanol extract (25 °C)	Aqueous extract (25 °C)	Ethanol extract (150 °C)	Aqueous extract (150 °C)
Glycosides	+	+	+	+
Steroids	-	++	+	+
Flavonoids	+++	+	+	+++
Alkaloids	+++	+	+	+++
Saponins	+	_	+	+++
Phenolics	+++	+	+	++
Tannins	+	+	_	+
Terpenes	+	++	+	+

Key: +++ = abundant ++ = moderate + = least - = not detected

For different temperature levels, there was an increase in total flavonoid content under aqueous extracts. For ethanolic extracts, the amount of total flavonoid content decreased as the temperature increased. Table 4.5 and 4.6 shows the results for the effect of cooking temperature on total flavonoid content for aqueous and ethanolic extracts respectively. Table 4.5: Total Flavonoid Content (TFC) at different cooking temperatures and time for aqueous extracts

Time (minutes)	Cooking temperature					
	25 ºC	50 ºC	75 ºC	100 ºC	125 ºC	150 ºC
15	133.82 ± 13.09^{a}	148.85 ± 15.74^{ab}	229.64 ± 8.50^{bc}	246.14 ± 6.47^{cd}	379.54 ± 9.08^{de}	374.13 ± 15.43^{e}
30	136.62 ± 7.95^{a}	211.47 ± 12.50^{ab}	246.87 ± 7.63^{bc}	309.25 ± 0.00^{cd}	395.66 ± 12.09^{de}	367.11 ± 9.63^{e}
45	165.02 ± 8.37^{a}	253.72 ± 6.30^{ab}	290.39 ± 21.17^{bc}	374.39 ± 4.48^{cd}	392.10 ± 15.46^{de}	490.36 ± 10.99^{e}
60	179.61 ± 3.57^{a}	268.79 ± 8.79^{ab}	296.62 ± 18.37^{bc}	374.92 ± 14.72^{cd}	392.28 ± 1.41^{de}	522.57 ± 15.59^{e}

Different letters in the same row mean significant difference (alpha < 0.05) as per post hoc table

Table 4.6: Total Flavonoid Content (TFC) at different cooking temperatures and time for ethanolic extracts

Time (minutes)	Cooking temperature					
	25 ºC	50 ºC	75 ºC	100 ºC	125 ºC	150 ºC
15	437.92 ± 4.99^{a}	392.78 ± 1.56^{b}	324.57 ± 7.06^{c}	252.56 ± 13.29^{d}	125.54 ± 7.53^{e}	125.07 ± 3.71^{f}
30	444.27 ± 3.41^{a}	347.34 ± 7.41^{b}	291.27 ± 5.79^{c}	246.02 ± 8.13^{d}	183.89 ± 20.96^{e}	86.37 ± 17.08^{f}
45	428.96 ± 1.58^{a}	349.19 ± 6.99^{b}	274.27 ± 4.17^{c}	192.05 ± 9.35^{d}	182.55 ± 20.33^{e}	71.95 ± 4.02^{f}
60	414.98 ± 20.16^{a}	324.53 ± 11.08^{b}	247.75 ± 9.54^{c}	180.61 ± 4.39^{d}	129.26 ± 2.94^{e}	69.10 ± 6.03^{f}

Different letters in the same row mean significant difference (alpha < 0.05) as per post hoc table

The variations in TFC at different temperatures for aqueous extracts were significant (p-value < 0.01) according to appendix 3 and the differences are noted through the LSD tests (refer to appendix 4(c)). For example, total flavonoid contents under aqueous extracts with 25 ºC significantly differ from those at 75 ºC (p-value = 0.005) or even 150 ºC (p-value <

0.01). Similarly, amounts of total flavonoid contents significantly differed with varying temperatures under ethanol extracts (p-value < 0.01) according to appendix 3.

Such differences were notable between the levels of temperatures, evidenced by the LSD test (see appendix 4(d)). For instance, significant differences were noted between total flavonoid contents under varying temperatures between groups of temperature levels. For example, at 25 ºC, total flavonoid contents significantly differed with those obtained at other temperatures (p-value < 0.01). Figure 4.7 provides a summary on the effect of cooking temperature on total phenolic and flavonoid content of garlic.

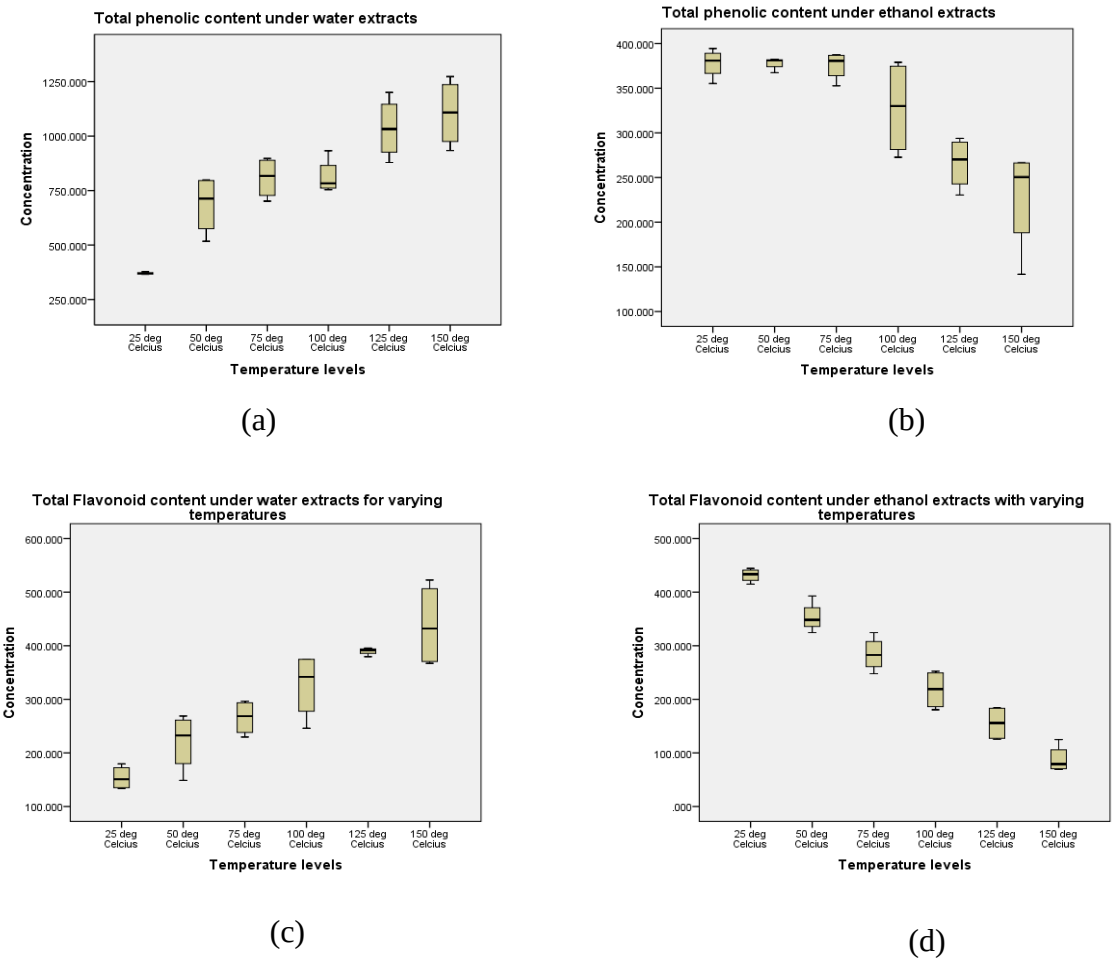


Figure 4.7: Effect of cooking temperature on total phenolic content and total flavonoid content

Comparably, these results agree with those of (Shaima GA, Mahmoud MS, Mohamed MR, 2016) where they investigated the effect of heat treatment on phenolic and flavonoid compounds and antioxidant activities of some Egyptian sweet and chilli pepper. The data revealed that total phenolic and total flavonoid contents increased after boiling as well as antioxidant activity which has a positive relationship with total phenolic content and total flavonoid content. In addition, black garlic produced by heating garlic bulbs at 70 ^{o}C for 30 days exhibited a significant increase in total polyphenol and total flavonoids content than raw garlic (Choi, Cha, & Lee, 2014) . On the contrary, (Mishra et al., 2017), found out that the total phenolic content of garlic after boiling reduced by 34.18-52.87 mg/100g. This could be the case because as per the methodology, it showed that after boiling, the analysis was done on the garlic residues rather than on the water that was used for boiling so that the total phenolic content of the water could also be considered. In addition to that, boiling and steaming significantly reduced the total phenolic content of red pepper as compared with raw pepper (Hwang et al., 2012). The highest loss in TPC was observed after boiling than steaming. On the other hand, stir frying and roasting did not significantly affect TPC.

4.2.2 Effect of Cooking Time on Total Phenolic and Total Flavonoid Content

Under varying cooking times, total phenolic contents differed for both aqueous and ethanolic extracts. However, there was no significant difference on total phenolic contents for both aqueous and ethanolic extracts (refer to appendix 3).

Under varying times, total flavonoid contents were also different under aqueous extract. Total flavonoid contents under ethanolic extracts decreased with increase in time of cooking. However, these differences were not significant under aqueous extracts (p-value= 0.485) and (p-value=0.919) ethanolic extracts.

Figure 4.8 summarizes the effect of time on total phenolic and total flavonoid content of garlic.

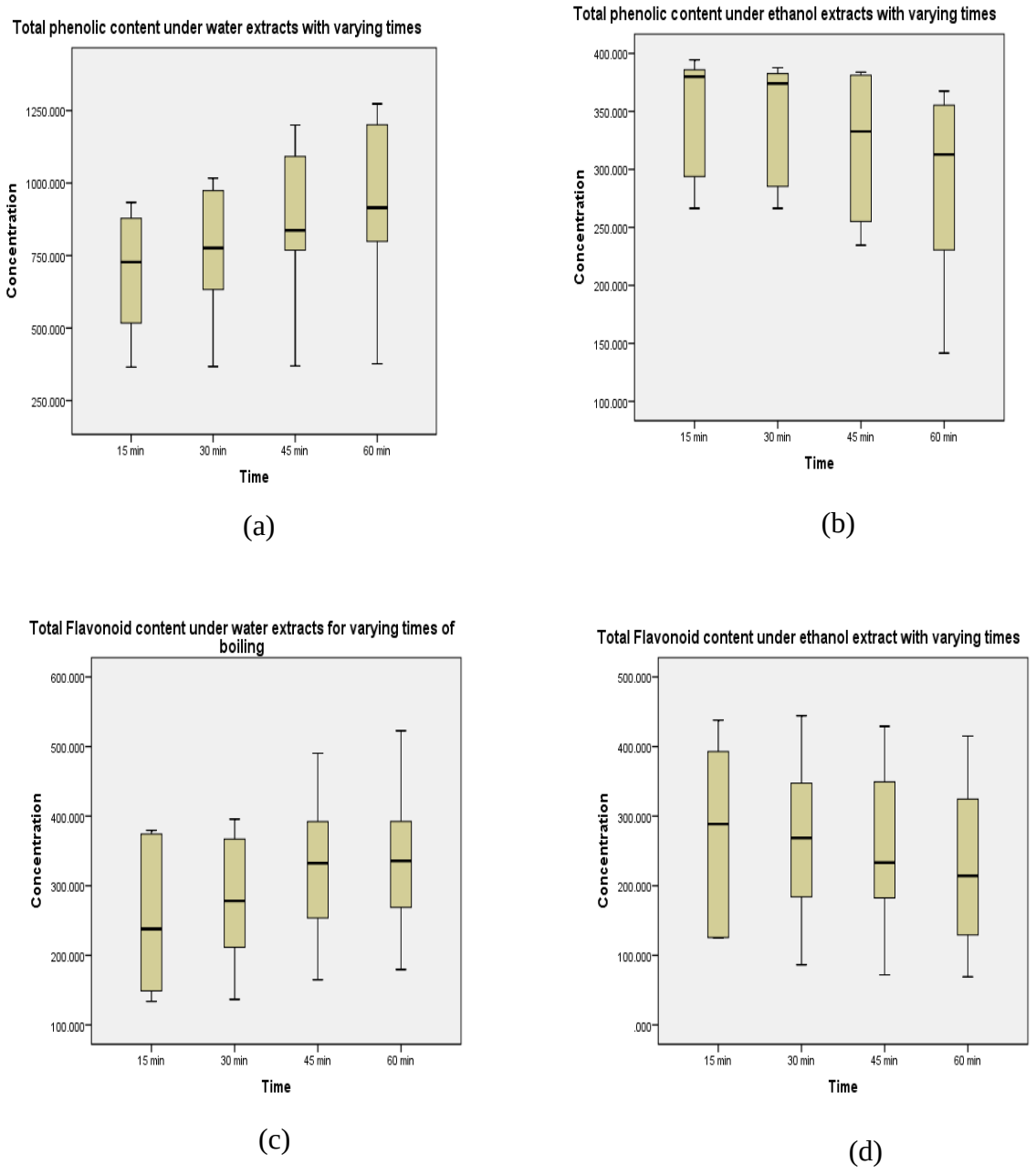


Figure 4.8: Effect of cooking time on total phenolic content and total flavonoid content of garlic

Generally, time did not have a significant effect on the total phenolic content and total flavonoid content. This result is in agreement with a study by Food (2017) which showed

that the cooking time did not significantly affect the TPC and antioxidant activity of selected edible mushrooms. This was the case because the cooking time considered (≤5 minutes) might be too short to affect significantly the levels of phenolic compounds and antioxidant activity. Conversely, Hwang *et al*., (2012), in a study which aimed at determining the effect of different cooking methods and time (5, 10, 15 minutes) on the contents and antioxidant activity of red pepper found that boiling reduced the total phenolic content (13.9-54.9%) and that prolonged cooking times decreased the contents of red paper.

4.3.3: Effect of Cooking Temperature and Time on Phytochemicals and Total Antioxidant Activity of Garlic

To assess the effect of cooking temperature and time on antioxidant activity of garlic, ANOVA test was carried out. As the cooking temperature increased, the antioxidant activity was also increasing in the aqueous extracts. However, the antioxidant activity of garlic in the ethanolic extracts was decreasing with increase in cooking temperature. Tables 4.7 and 4.8 shows the antioxidant activity of aqueous and ethanolic extracts at different temperatures.

Table 4.7: Antioxidant activity of garlic at different cooking temperatures and time for ethanolic extracts

Time (minutes)	Cooking temperature					
	25 ºC	50 ºC	75 ºC	100 ºC	125 ºC	150 ºC
15	48.95 ± 1.22^{a}	47.77 ± 0.49^{a}	46.04 ± 1.34^{ab}	46.64 ± 0.57^{b}	31.05 ± 0.45^{c}	3.33 ± 1.70^{d}
30	47.68 ± 0.31^{a}	49.14 ± 0.95^{a}	46.49 ± 0.28^{ab}	46.79 ± 0.46^{b}	31.39 ± 0.91^{c}	2.13 ± 1.26^{d}
45	47.53 ± 0.74^{a}	47.27 ± 0.22^{a}	47.20 ± 0.79^{ab}	27.73 ± 0.37^{b}	16.74 ± 2.82^{c}	2.24 ± 1.22^{d}
60	37.97 ± 1.84^{a}	46.97 ± 4.57^{a}	32.03 ± 6.46^{ab}	27.58 ± 0.37^{b}	15.25 ± 3.16^{c}	2.24 ± 0.80^{d}

Different letters in the same row mean significant difference (alpha < 0.05) as per post hoc table

Table 4.8: Antioxidant activity of garlic at different cooking temperatures and time for aqueous extracts

Time (minutes)	Cooking temperature					
	25 ºC	50 ºC	75 ºC	100 ºC	125 ºC	150 ºC
15	42.90 ± 0.72^{a}	50.26 ± 0.47^{b}	60.50 ± 4.12^{bc}	67.97 ± 0.30^{cd}	72.31 ± 0.27^{d}	73.95 ± 3.90^{d}
30	48.99 ± 4.50^{a}	52.13 ± 0.61^{b}	59.42 ± 1.22^{bc}	71.38 ± 0.18^{cd}	75.00 ± 2.06^{d}	75.75 ± 2.12^{d}
45	48.06 ± 0.79^{a}	65.88 ± 0.44^{b}	66.22 ± 0.32^{bc}	71.52 ± 0.10^{cd}	75.90 ± 1.77^{d}	76.98 ± 0.40^{d}
60	48.02 ± 0.55^{a}	66.07 ± 3.74^{b}	72.80 ± 0.19^{bc}	71.82 ± 0.57^{cd}	76.31 ± 0.69^{d}	81.95 ± 0.68^{d}

Different letters in the same row mean significant difference (alpha < 0.05) as per post hoc test.

ANOVA test was done to test the effect. According to appendix 3, it shows the effect of temperature on both the aqueous (p-value < 0.01, alpha = 5%) and ethanol (p-value < 0.01, alpha = 5%) extracts was significant.

Much as the antioxidant activity was seen to be increasing with increase in cooking time, the differences were insignificant for both ethanolic and aqueous extracts. The results above indicate that the antioxidant activity of garlic was increasing significantly with the increase in temperature. Similarly, the antioxidant activity of black garlic increased significantly as it was being heated until the 21st day(Choi *et al*., 2014). The increase in antioxidant activity of the black garlic was due to the fact that there was also an increase in total polyphenol and flavonoid content. Different results have also been reported in literature. Onreal & Arc (2009) in their study on the influence of cooking methods on antioxidant activity of vegetables, concluded that boiling led to the greatest losses (-14%) in the antioxidant activity of the vegetables. However, it was shown that garlic, which was one of the vegetables studied, maintained its antioxidant activity even after boiling. This was the case because according to the methodology, garlic was cooked/boiled whole without crushing or chopping

as a result the bioactive compounds responsible for the antioxidant activity were not activated or released hence the effect could not be properly accounted for.

Further to that, Sutana *et al.* (2007) argued that such differences could be as a result of the vegetables themselves (bioactive structures), the cooking method and the bioavailability of the phenolic compounds (Onreal & Arc, 2009). Similarly, Food (2017), in their study concluded that the %DPPH scavenging activity of mushrooms decreased significantly ($p \leq 0.05$) when temperature was increased. This result corresponded with the decrease in the total phenolic content of the mushrooms as the temperature was being increased. The difference in the results could be due to the fact that, the phenolic compounds that leached in the cooking water were not taken into consideration as it has been discussed earlier hence the decrease in total phenolic content and antioxidant activity. Likewise, the DPPH scavenging activity of red paper reduced by 42.0-60.5%, 23.5-30.3%, 11.6-15.4% and 4.6-15.8% for boiling, steaming, stir frying and roasting respectively (Hwang *et al*., 2012). Boiling registered the highest loss in antioxidant activity due to the fact that it also led to a higher loss in TPC as these are compounds responsible for the antioxidant activity. Generally, increase in temperature and cooking time increased the antioxidant activity of garlic. This is because temperature enhances the extraction of bound phenolic compounds thereby increasing the phytochemicals and antioxidant activity of garlic. This is true if the leached phytochemicals are considered and provided decanting is not involved as a method of cooking. Figure 4.9 and 4.10 below provides a summary on the effect of temperature and time on the antioxidant activity of garlic.

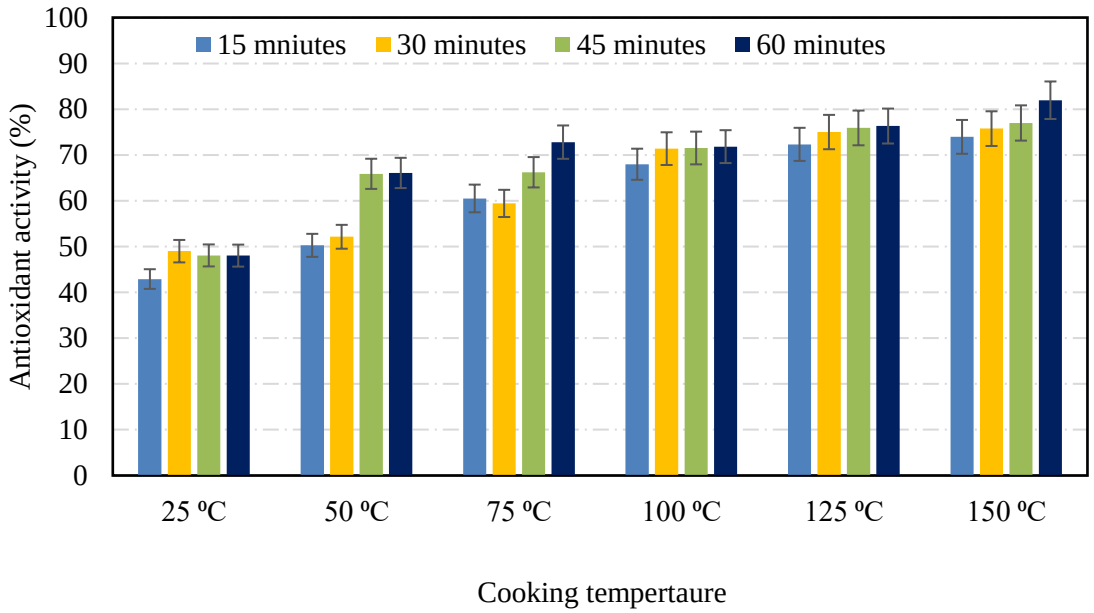


Figure 4.9: Effect of temperature and time on the antioxidant activity of garlic for aqueous extract

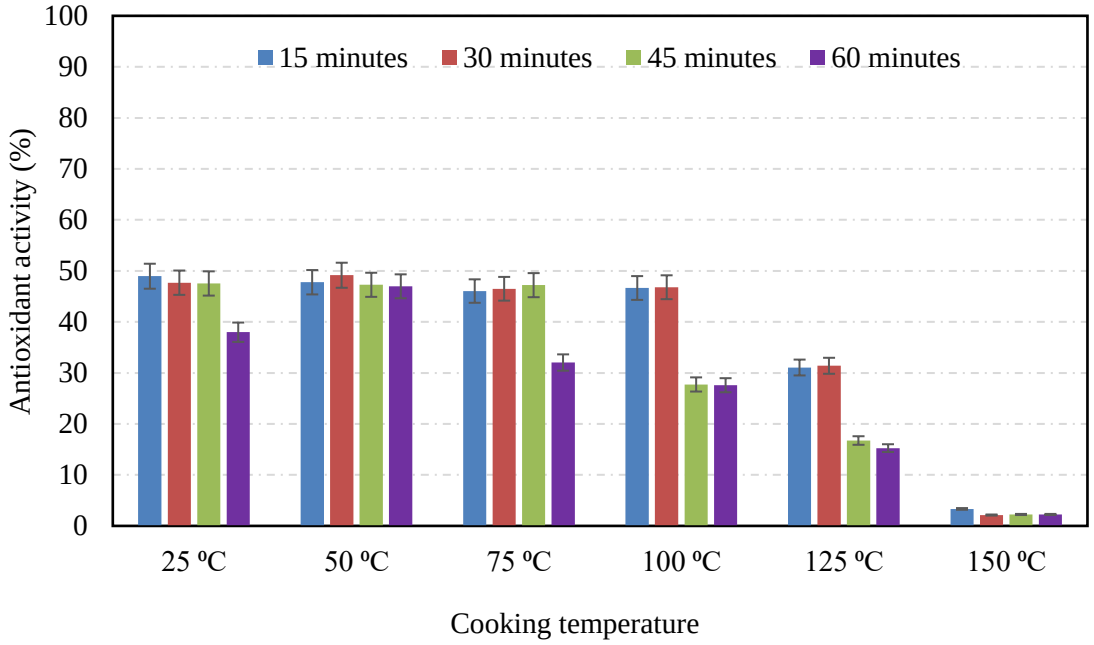


Figure 4.10: Effect of temperature and time on the antioxidant activity of garlic for ethanolic extracts

4.4: Effect of the Indigenous Reed Salt and Iodized Commercial salt and their concentrations on Phytochemicals and Antioxidant Activity

4.4.1: Effect of different types of salts on Phytochemicals and antioxidant activity of garlic

The effect of salt was studied at optimum cooking temperature and time (150 °C, 1 hour) as it gave the highest total phenolic content, total flavonoid content and antioxidant activity. The effect of the iodized kensalt and indigenous reed salt on phytochemical contents were tested.

Generally, TPC and TFC increased with the addition of indigenous reed salt more than the addition of iodized Kensalt with TPC > TFC in each case. However, without addition of any type of salt, TPC and TFC are on the lower side than where salt is added/present regardless of the type of salts.

Further, the effect of different types of salts on TPC and TFC was verified using the independent-sample t-test under no equal variance assumption as per Appendix 3.

Based on the t-test outputs, it was confirmed that these differences were significant ($p = 0.003$). However, the mean differences were insignificant for the effect of salt types on TFC ($p = 0.662$). This might be the case for TFC because flavonoids are usually in small quantities than phenolic compounds hence the difference that the presence/absence of the different types of salts brought about could be very small. The increase in TPC after the addition of salt regardless of the type could be explained by the fact that salt is a tenderizer as such it

softens tissues thereby enhancing the extraction of phytochemicals. By touching, garlic residues that were cooked in the presence of salt were much softer than those cooked without salt.

A similar study aimed at determining the effect of soaking in salt solutions on water absorption, pH, and cooking time on African yam bean seeds (*Sphenostylis stenocarpa* Hochst ex A Rich Harms) reported that salt had tenderizing effects. In addition, under the same salt concentration, the reduction in cooking time was much higher in akanwu (sodium sesquicarbonate) soaked beans unlike sodium chloride salt signifying that the former salt is a better tenderizer than the latter (Onyeike & Uzogara, 2016). Tables 4.9 and 4.10 summarize the effect of indigenous reed salt and Kensalt on TPC and TFC.

Table 4.9: Effect of salt and its concentration on Total Phenolic Content (TPC)

Concentration	Kensalt	Indigenous reed salt
0.00 m	1255.52 ± 5.87^{a}	1255.52 ± 5.87^{a}
0.05 m	1268.75 ± 17.27^{b}	2190.29 ± 17.50^{c}
0.10 m	1348.63 ± 12.08^{b}	2464.62 ± 8.83^{c}
0.20 m	1435.33 ± 6.48^{b}	2710.21 ± 6.50^{c}
0.40 m	1434.06 ± 8.64^{b}	2887.68 ± 15.03^{c}

Different letters in the same row and column mean significant difference (alpha < 0.05) as per independent sample t-test

4.10: Effects of salt and its concentration on Total Flavonoid Content

Concentration	Kensalt	Indigenous reed salt
0.00 m	397.68 ± 8.78[a]	397.68 ± 8.78[a]
0.05 m	413.17 ± 3.69[a]	433.91 ± 0.88[a]
0.10 m	487.75 ± 0.00[a]	497.25 ± 16.60[a]
0.20 m	538.74 ± 0.87[a]	555.08 ± 6.27[a]
0.40 m	558.87 ± 20.25[a]	601.87 ± 1.54[a]

Different letters in the same row and column mean significant difference (alpha < 0.05) as per independent sample t-test

Using independent sample t-test, the results showed that there was a significant difference between the amounts of phytochemicals in the presence of salts with antioxidant activity (p-value < 0.01, alpha = 5%) at higher salt concentrations and hence it can be concluded that the salts had an effect on antioxidant activity of garlic and that the indigenous reed salt has also antioxidant properties since it is a salt derived from plants. This is supported by a research done by Om & Jeong (2007) which reported that bamboo salt used for prevention and treatment of various diseases in Korea has anti-oxidative effect more than vitamin E. Table 4.11 shows results from the independent sample t-test on the effect of salt and salt concentration of antioxidant activity of garlic.

Table 4.11: Effect of salt and its concentration on antioxidant activity

Salt concentration	Kensalt	Indigenous reed salt
0.00 m	73.65 ± 0.43[a]	73.65 ± 0.43[a]
0.05 m	73.48 ± 0.75[b]	86.66 ± 0.42[c]
0.10 m	76.53 ± 0.33[b]	90.32 ± 0.38[c]
0.20 m	76.71 ± 0.82[b]	95.03 ± 0.20[c]
0.40 m	80.12 ± 0.08[b]	97.03 ± 0.20[c]

Different letters in the same row and column mean significant difference (alpha < 0.05) as per independent sample t-test

Different salt concentrations of the two types of salts were measured for different effects on phenolic and flavonoid content. The results, for total phenolic content increased with an increase in salt concentration levels in both Iodized Kensalt and Indigenous Reed salt but the differences were more with indigenous reed salt. However, the differences were not significant (p-value =0.204, at alpha = 5%). These results are in agreement with a study by Food (2017) which looked at the effect of cooking time, temperature and salt concentration on phenolic content and antioxidant activity of selected edible mushrooms which found that Salt concentration did not significantly affect the levels of phenols and antioxidant activity.

However a different result was reported by Burg & Oshrat (2015) which demonstrated that salt composition and concentration affected the antioxidant activity of red microalgal polysaccharides and thus it was postulated that the salt interfered with the polysaccharide chains hence exposing the antioxidant sites. These differences could be as a result of the different concentration ranges used and also the antioxidant compounds of interest. Figures 4.11, 4.12, and 4.13 below summarize the effect of salt and its concentration on TPC, TFC and AOA of garlic extracts.

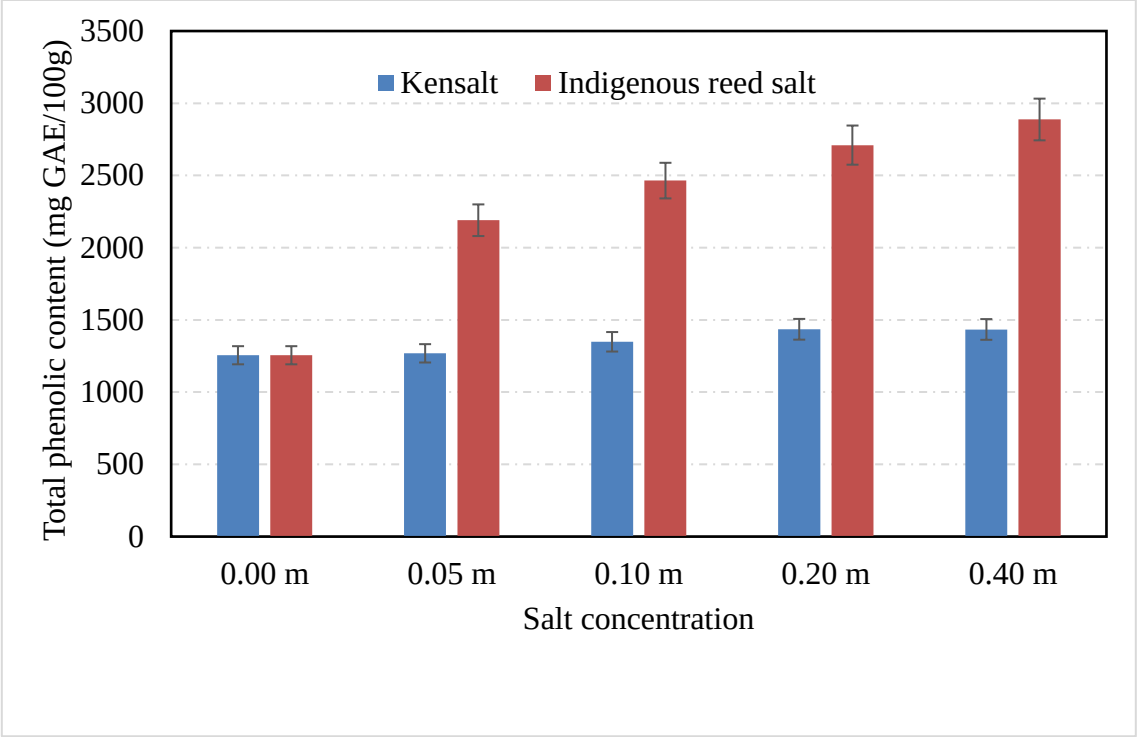


Figure 4.11: Effect of salt and its concentration on TPC of garlic extracts

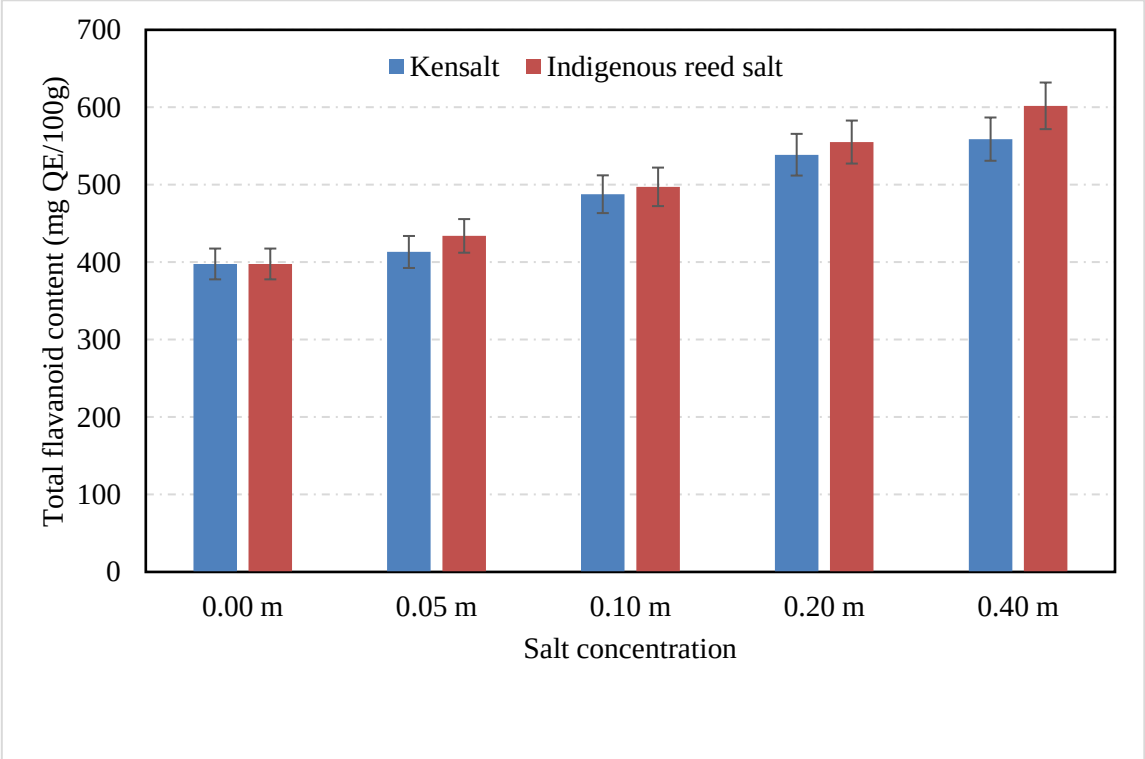


Figure 4.12: Effect of salt and its concentration on TFC of garlic extract

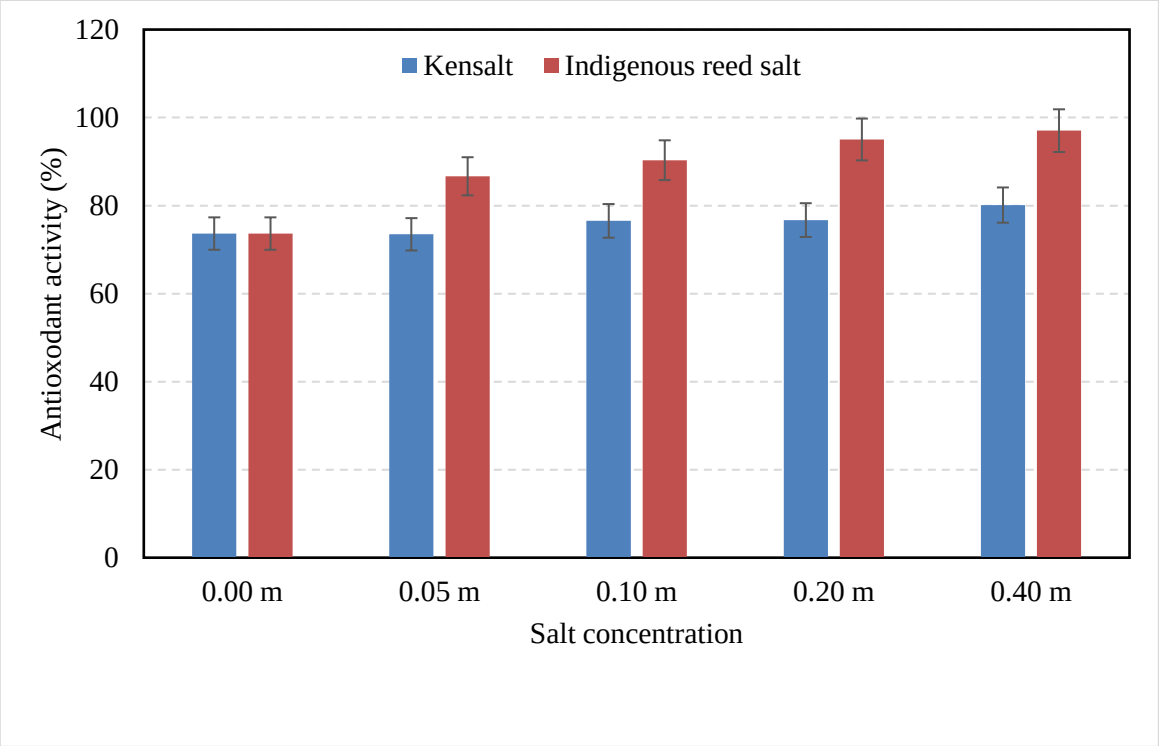


Figure 4.13: Effect of salt and its concentration on the antioxidant activity of garlic extract

4.5 Quality Control and Quality Assurance

Quality control procedures were followed which included the use of analytical grade reagents. The slope, intercept and correlation coefficient of the calibration curve are within the acceptable ranges. The R^2 values for gallic acid and quercetin standard calibration curves were 0.9976 and 0.9994, respectively.

4.5.1 Accuracy, Precision and Repeatability

Three sets of calibration standards were prepared and analyzed to provide a measure of precision and reproducibility. Precision was expressed as the relative standard deviation (RSD) where a %RSD of the range 0.794-8.33 and 0-7.80 was obtained for gallic acid and quercetin calibration curve respectively. The calculated RSD was within the acceptable ranges hence allowing for repeatability.

4.5.2 Linearity and Range

Standard solution of gallic acid (5-100 mg/ml) and quercetin (1-200 mg/ml) at different concentrations were prepared. A calibration curve was constructed by plotting concentration versus absorbance. The results showed an excellent correlation between absorbance and concentration of garlic acid within the concentration range. The correlation coefficients for gallic acid and quercetin calibration curves were 0.9976 and 0.994 respectively.

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In view of the results gathered, the following conclusions are drawn;

1. Ethanol is a very good solvent/extractant than water due to the fact that ethanol gave a higher total phenolic content (637.91 ± 50.30 mg GAE/100g) and total flavonoid content 258.21 ± 12.37 mg QE/100 g than water which gave 301.29 ± 6.58 mg GAE/100 g of Total phenolic content and 109.68 ± 6.78 mg QE/100 g of total flavonoid content.
2. Both ethanolic and aqueous extracts of garlic contain phytochemicals (phenolic compounds and flavonoids). This was evidenced by results from FTIR screening which showed characteristic peaks of the compounds with S=O, C=O, OH, COOH functional groups in the range of 4000 cm^{-1} to 400 cm^{-1}. In addition, LC-MS/MS analysis of phenolic compounds and flavonoids revealed the presence of four flavonoids which are Quercetin, Hesperidin, Rutin and Catechin, and Phenolic compounds namely ferulic acid, gentisic acid, caffeic acid, Gallic and chlorogenic acids were also identified with the functional groups depicted by FTIR.
3. Cooking temperature has a significant effect on total phenolic content, total flavonoid content and antioxidant activity of garlic whereas cooking time has no significant effect. Increase in cooking temperature significantly increased the phytochemical content (p value <0.01, alpha = 5%) and antioxidant activity of garlic. This was evidenced by the fact that, as the cooking temperature increased, total phenolic and total flavonoid content of the cooking water increased, so did the antioxidant activity whilst the total phenolic and total flavonoid content of the garlic residues were

significantly decreasing (p value<0.01, alpha =5%) with increasing cooking temperature. This has also shown that the phytochemicals are resistant to heat and that heat/temperature enhances the extraction of these phytochemicals because aqueous extracts for the fresh garlic had less total phenolic content and flavonoid content whereas the aqueous extracts cooked at higher temperatures had higher total phenolic and total flavonoid content.

4. It is recommended that garlic should be cooked at higher temperatures in the presence of indigenous reed salt for optimum phytochemicals and AOA for more health benefits. Much as the commercial iodized salt is common on our tables, the use of indigenous reed salt should be encouraged as it adds nutritional value to the food. Further studies should investigate the effects of salt on phytochemicals and antioxidant activity of food additives and/or vegetables.

5.2 Recommendations

The following recommendations are made from this research;

i. There is need to work with pure compounds from garlic so that the effect of the cooking conditions can be well established.

ii. Isolation and characterization of the phytochemicals at each temperature and cooking time should be done so as to determine the different kinds of phenolic compounds and flavonoids present at a particular temperature and time unlike working with crude extracts where the antioxidant activity is as a result of a synergistic effect of compounds other than phenolic compounds and flavonoids such as polysaccharide chains which have also been reported in literature.

iii. More studies have to be done on the additive–additive and/or additive-matrix effect on phytochemicals and antioxidant activity of food additives and/or vegetables since there is limited literature on this.

iv. There is need to determine the composition of the indigenous salt and establish the chemical(s) responsible for increasing the phenolic and flavonoid contents when salts are added.

REFERENCES

Agamy, N. F. (2016). Effect of boiling and microwave cooking on some antioxidant compounds in highly consumed vegetables in Egypt. *Central European Researchers Journal*, *2*(2), 76–84.

Akan, S. (2019). Evaluation and Comparison of Some Parameters in Four Garlic Varieties. *Journal of the Institute of Science and Technology*, *4*(November), 1866–1875. https://doi.org/10.21597/jist.541783

Alarcón-flores, M. I., Romero-González, R., Luis, J., Vidal, M., & Frenich, A. G. (2014). *Determination of Phenolic Compounds in Artichoke , Garlic and Spinach by Ultra-High-Performance Liquid Chromatography Coupled to Tandem Mass Spectrometry*. https://doi.org/10.1007/s12161-014-9852-4

April, M.-, Patel, S. S., & Savjani, J. K. (2015). *Systematic review of plant steroids as potential anti- inflammatory agents : Current status and future perspectives*. *4*(2), 121–125.

Ashokkumar, R., & Ramaswamy, M. (2014). *Original Research Article Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants*. *3*(1), 395–406.

Atta, E. M., Mohamed, N. H., & Abdelgawad, A. A. M. (2017). *Antioxidants : An Overview on the Natural and Synthetic Types. 6(8)*, 365–375. https://doi.org/10.17628/ecb.2017.6.365-375

Banu, K. S., & Cathrine, L. (2015). General Techniques Involved in Phytochemical Analysis. *International Journal of Advanced Research in Chemical Science*, *2*(4), 25–32.

Beato, V. M., Orgaz, F., Mansilla, F., & Montaño, A. (2011). *Changes in Phenolic Compounds in Garlic (Allium sativum L .) Owing to the Cultivar and Location of Growth*. 218–223. https://doi.org/10.1007/s11130-011-0236-2

Burg, A., & Oshrat, L. (2015). Salt Effect on the Antioxidant Activity of Red Microalgal Sulfated Polysaccharides in Soy-Bean Formula. *Marine Drugs*, *13*, 6425–6439. https://doi.org/10.3390/md13106425

Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M. H., Elsohly, M. A., & Khan, I. A. (2014). *Assessment of Total Phenolic and Flavonoid Content , Antioxidant Properties , and Yield of Aeroponically and Conventionally Grown Leafy Vegetables and Fruit Crops : A Comparative Study*. *2014*.

Chen, S., Shen, X., Cheng, S., Li, P., Du, J., & Chang, Y. (2013). *Evaluation of Garlic Cultivars for Polyphenolic Content and Antioxidant Properties*. *8*(11). https://doi.org/10.1371/journal.pone.0079730

Choi, I. S., Cha, H. S., & Lee, Y. S. (2014). *Physicochemical and Antioxidant Properties of*

Black Garlic. 16811–16823. https://doi.org/10.3390/molecules191016811

Cong-cong, X. U., Bing, W., Yi-qiong, P. U., Jian-sheng, T. A. O., & Tong, Z. (2017). Advances in extraction and analysis of phenolic compounds from plant materials. *Chinese Journal of Natural Medicines*, *15*(10), 721–731. https://doi.org/10.3724/SP.J.1009.2017.00721

Corradini, E., Foglia, P., Giansanti, P., Gubbiotti, R., Samperi, R., Laganà, A., … Gubbiotti, R. (2011). *Flavonoids : chemical properties and analytical methodologies of identification and quantitation in foods and plants*. *6419*(July 2017). https://doi.org/10.1080/14786419.2010.482054

Cultivars, A. S. L., Dao, A., Phan, T., Netzel, G., Chhim, P., Netzel, M. E., & Sultanbawa, Y. (2019). Phytochemical Characteristics and Antimicrobial Activity of Australian Grown Garlic. *Foods*.

Divya, B., Bukke, S., & Venkataswamy, M. (2017). A Study on Phytochemicals , Functional Groups and Mineral Composition of Allium sativum (Garlic) Cloves. *International Journal of Current Pharmaceutical Research*, *9*(3). https://doi.org/10.22159/ijcpr.2017v9i3.18888

DW, N., W, A., ENM, N., & MP, N. (2016). *Research and Reviews : Journal of Pharmacognosy and Phytochemistry Medicinally Important Phytochemicals : An Untapped Research Avenue*. *4*(1).

Ebhardt, S. U. E. G., & Rior, R. O. L. P. (2006). *Concentrations of Anthocyanins in Common Foods in the United States and Estimation of Normal Consumption*.

El ż bieta Sikora, Ewa Cie ś lik, K. T. (2008). The Sources of Natural Antioxidants. *ACTA Scientiarum Polonorum*, *7*(1), 5–17.

Esatbeyoglu, T., Ulbrich, K., Rehberg, C., Rohn, S., & Rimbach, G. (2015). Thermal stability, antioxidant, and anti-inflammatory activity of curcumin and its degradation product 4-vinyl guaiacol. *Food and Function*, *6*(3), 887–893. https://doi.org/10.1039/c4fo00790e

Faculty, A., & Yai, H. (2011). *Stability of antioxidant and antibacterial properties in heated turmeric-chili paste and its ingredients*. *404*, 397–404.

Feng, Chun-Hua, Fu-Rong, Pei-Pei, Rong-Hai, H.-L. (2018). Study on the Ageing Method and Antioxidant Activity of Black Garlic Residues. *Czech Journal of Food Science*, *2018*(1402001), 88–97.

Gunaherath, G. M. K. B., & Gunatilaka, A. A. L. (2014). *Plant Steroids : Occurrence , Biological Significance , and Their Analysis*. (Table 1). https://doi.org/10.1002/9780470027318.a9931

Huang, W., Cai, Y., Zhang, Y., Huang, W., & Cai, Y. (2009). *Natural Phenolic Compounds From Medicinal Herbs and Dietary Plants : Potential Use for Cancer Prevention Natural Phenolic Compounds From Medicinal Herbs and Dietary Plants : Potential*

Use for Cancer Prevention. (November 2013), 37–41. https://doi.org/10.1080/01635580903191585

Hussain, G., Rasul, A., Anwar, H., Aziz, N., Razzaq, A., & Wei, W. (2018). *Role of Plant Derived Alkaloids and Their Mechanism in Neurodegenerative Disorders*. *14*. https://doi.org/10.7150/ijbs.23247

Hwang, I. G., Shin, Y. J., Lee, S., Lee, J., & Yoo, S. M. (2012). Effects of different cooking methods on the antioxidant properties of red pepper (Capsicum annuum L.). *Preventive Nutrition and Food Science*, *17*(4), 286–292. https://doi.org/10.3746/pnf.2012.17.4.286

Ilbäck, N., Busk, L., Ilback, B. N., & Busk, L. (2016). *Food additatives . Use , intake and safety Food additives . Use , intake and safety*. *7878*(2000). https://doi.org/10.3402/fnr.v44i0.1778

Jenzer, H., & Sadeghi, L. (2016). Phytochemicals : Sources and biological functions. *Journal of Pharmacognosy and Phytochemistry*, *5*(5), 339–341.

Kafkas, N. E., Kosar, M., Öz, A. T., & Mitchell, A. E. (2018). Advanced Analytical Methods for Phenolics in Fruits. *Journal of Food Quality*, *2018*, 1–6. https://doi.org/10.1155/2018/3836064

Kim, J., Kang, O., & Gweon, O. (2012). Comparison of phenolic acids and flavonoids in black garlic at different thermal processing steps. *Journal of Functional Foods*, *5*(1), 80–86. https://doi.org/10.1016/j.jff.2012.08.006

Kodera, Y., Matsuura, H., & Sumiyoshi, H. (2003). Garlic Chemistry : Chemical and Biological Properties of Sulfur-Containing Compounds Derived from Garlic. *Food Factors in Health Promotion and Disease Prevention*, *851*, 346–357. https://doi.org/10.1021/bk-2003-0851.ch030

Lin, D., Xiao, M., Zhao, J., Li, Z., & Xing, B. (n.d.). *An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes*. (Figure 2). https://doi.org/10.3390/molecules21101374

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). *Free radicals , antioxidants and functional foods : Impact on human health*. https://doi.org/10.4103/0973-7847.70902

Lu, J., Bao, J., Chen, X., Huang, M., & Wang, Y. (2012). Alkaloids Isolated from Natural Herbs as the Anticancer Agents. *Evidence-Based Complementary and Alternative Medicine*, *2012*. https://doi.org/10.1155/2012/485042

Lu, X., Ross, C. F., Powers, J. R., Aston, D. E., & Rasco, B. A. (2011). *Determination of Total Phenolic Content and Antioxidant Activity of Garlic (Allium sativum) and Elephant Garlic (Allium ampeloprasum) by Attenuated Total Reflectance À Fourier Transformed Infrared Spectroscopy*. 5215–5221. https://doi.org/10.1021/jf201254f

Mahmood, T., Anwar, F., Abbas, M., & Saari, N. (2012). Effect of Maturity on Phenolics (Phenolic Acids and Flavonoids) Profile of Strawberry Cultivars and Mulberry Species

from Pakistan. *International Journal of Molecular Sciences*, *13*, 4591–4607. https://doi.org/10.3390/ijms13044591

Maleš, C., T, D. Š., & T, M. B. (2013). *Quantitative Determination of Flavonoids and Chlorogenic Acid in the Leaves of Arbutus unedo L . Using Thin Layer Chromatography*. *2013*, 1–5.

Marrelli, M., Conforti, F., Araniti, F., & Statti, G. A. (2016). Effects of Saponins on Lipid Metabolism : A Review of Potential Health Benefits in the Treatment of Obesity. *Molecules*, *21(10)*. https://doi.org/10.3390/molecules21101404

Martins, N., Petropoulos, S., & Ferreira, I. C. F. R. (2016). *Chemical composition and bioactive compounds of garlic (Allium sativum L .) as affected by pre- and post-harvest conditions : A review*. *211*, 41–50. https://doi.org/10.1016/j.foodchem.2016.05.029

Milugo, T. K., Omosa, L. K., Ochanda, J. O., Owuor, B. O., Wamunyokoli, F. A., Oyugi, J. O., & Ochieng, J. W. (2013). *Antagonistic effect of alkaloids and saponins on bioactivity in the quinine tree (Rauvolfia caffra sond .): further evidence to support biotechnology in traditional medicinal plants*.

Mishra, N., Tripathi, R., & Khan, Z. (2017). Physicochemical and Antioxidant Potential of Garlic : Heat Processing Effects. *Journal of Agricultural Engineering and Food Technology*., *4*(2), 127–133.

Moghimipour, E., & Handali, S. (2015). Saponin : Properties , Methods of Evaluation and Applications. *Annual Research and Review in Biology*, *5*(3), 207–220. https://doi.org/10.9734/ARRB/2015/11674

Morsy, N. (2017). Cardiac Glycosides in Medicinal Plants. *Chapter from Aromatic and Medicinal Plants-Back to Nature, Edited vol*. https://doi.org/10.5772/65963

Nam, P., & Chan, A. (2014). *Chemical Properties and Applications of Food Additives : Preservatives , Dietary Ingredients , and Processing Aids Introduction : Foods and Food Additives*. 1–20. https://doi.org/10.1007/978-3-642-41609-5

Nazir, I., & Chauhan, R. S. (2019). Qualitative phytochemical analysis of Allium sativum (Garlic) and Curcuma longa (Turmeric). *Journal of Entomology and Zoology Studies*, *7*(1), 545–547.

Om, A.-S., & Jeong, J. (2017). Effects of cooking time , temperature , and salt concentration on the phenolic content and antioxidant activity of selected edible mushrooms. *Cancer Prevention Research*, *24*(October), 2028–2032.

Om, A., & Jeong, J. (2007). Bamboo Salts Have Antioxidant Activity and Inhibit ROS Formation in Human Astrocyte U373MG Cells. *Cancer Prevention Research*, *12*(3), 225–230.

Onreal, N. E. Z., & Arc, L. G. (2009). Influence of Cooking Methods on Antioxidant Activity of Vegetables. *Journal of Food Science*, *74*(3), 97–103. https://doi.org/10.1111/j.1750-3841.2009.01091.x

Oz, A. T., & Kafkas, E. (2017). Phytochemicals in Fruits and Vegetables Phytochemicals in Fruits and Vegetables. *Chapter from the Book Superfood and Functional Foof-An Overview of Their Processing and Utilization*, *Chapter 8*, 176–181. https://doi.org/10.5772/65787

Patel, V., & Patel, R. (2016). *Journal of Chemical and Pharmaceutical Research , 2016 , 8 (4): 1423-1443 Review Article The active constituents of herbs and their plant chemistry , extraction and identification methods*. *8*(4), 1423–1443.

Pattanaik, B., & Lindberg, P. (2015). Terpenoids and Their Biosynthesis in Cyanobacteria. *Journal of Life*, *5*, 269–293. https://doi.org/10.3390/life5010269

Pavia, D. L., Lampman, G. M., Kriz, G. S., & Vyvyan, J. R. (2013). *Introduction to Spectroscopy* (Fifth Edit; MPS Limited, ed.). Cengage Learning.

Pietta, P. (2000). Flavonoids as Antioxidants. *Journal of Natural Products*, *63*, 1035–1042. https://doi.org/10.1021/np9904509

Pongrakhananon, V. (2013). Anticancer Properties of Cardiac Glycosides. In *Cancer Treatment - Conventional and Innovative Approaches* (pp. 65–79). https://doi.org/org/10.5772/55381

Prati, P., Henrique, C. M., Souza, A. S. De, Sônia, V., Teresa, M., & Pacheco, B. (2014). Evaluation of allicin stability in processed garlic of different cultivars. *Food Science and Technology*, *34*(3), 623–628. https://doi.org/http://dx.doi.org/10.1590/1678-457X.6397

Ra, L., Daniela, Č. K., Álová, K. O. Š. Ť., Bezáková, L., & Fialová, S. (2009). *Comparative study of two natural antioxidants , curcumin and Curcuma longa extract*. *48*(3), 148–152.

Rajauria, G., Foley, B., & Abu-ghannam, N. (2016). Identification and characterization of phenolic antioxidant compounds from Brown Irish Seaweed Himanthalia elongata using LC-DAD-ESI-MS/MS. *Innovative Food Science and Emerging Technologies*. https://doi.org/10.1016/j.ifset.2016.02.005

Rathod, B. H., Rani, S. S., Kartheek, N., & Kumar, A. A. (2014). *UV Spectrophotometric Method Development and Validation for the Quantitative Estimation of Indinavir Sulphate in Capsules*. *6*(6).

Release, P. (2018). *Latest global cancer data : Cancer burden rises to 18 . 1 million new cases and 9 . 6 million cancer deaths in 2018 Latest global cancer data : Cancer burden rises to 18 . 1 million new cases and 9 . 6 million cancer deaths in 2018*. (September), 13–15.

S, R. J. R., Msa, M. N., & Selvakumar, P. M. (2018). *Phytochemicals as a potential source for anti- microbial , anti-oxidant and wound healing - a review*. *2*(2), 61–70. https://doi.org/10.15406/mojboc.2018.02.00058

Serafini, M., Peluso, I., & Raguzzini, A. (2019). *3rd International Immunonutrition*

Workshop Session 1 : Antioxidants and the immune system Flavonoids as anti-inflammatory agents Proceedings of the Nutrition Society Proceedings of the Nutrition Society. (October 2009), 273–278. https://doi.org/10.1017/S002966511000162X

Shaima GA, Mahmoud MS, Mohamed MR, E. A. (2016). Natural Products Chemistry & Research Effect of Heat Treatment on Phenolic and Flavonoid Compounds and Antioxidant Activities of Some Egyptian Sweet and Chilli Pepper. *Natural Products Chemistry and Research*, *4*(3), 1–6. https://doi.org/10.4172/2329-6836.1000218

Shimada, K., Yahara, K., Nakamurat, T., Science, L., & Corporation, H. (1992). *Antioxidative Properties of Xanthan*. (1990), 945–948. https://doi.org/10.1021/jf00018a005

Silva, S. D., Rosa, N. F., & Ferreira, A. E. (2014). phenolic and flavonoid contents and antioxidant capacity Rapid Determination of α -Tocopherol in Vegetable Oils by Fourier Transform Infrared Spectroscopy. *Food Chemistry*, (January 2015). https://doi.org/10.1007/s12161-008-9047-y

Stenocarpa, S., Ex, H., Harms, A. R., Onyeike, E. N., & Uzogara, S. G. (2016). Effect of Soaking in Salt Solutions on Water Absorption , pH and Cooking Time of African Yam Bean Seeds. *Global Journal of Pure and Applied Sciences*, *6*(January 2000), 67–73. https://doi.org/10.4314/gjpas.v6i1.16079

Street, L. A. (2010). *Guidance on Food Additives*.

Tahir, A., Kalkotwar, R. S., Naikwade, J., & Shaikh, A. (2013). Development and Validation of UV / Visible Spectrophotometric Method for the Estimation of Rifapentine in Bulk and Pharmaceutical Formulations. *American Journal of Phytomedicine and Clinical Therapeutics*, *4*, 401–407.

Tanvir, E. M., Hossen, S., Hossain, F., Afroz, R., Gan, S. H., Khalil, I., & Karim, N. (2017). *Antioxidant Properties of Popular Turmeric (Curcuma longa) Varieties from Bangladesh*. *2017*.

Taylor, P., Memnune, Ş., Yildiz, H., & Kavaz, A. (2014). International Journal of Food Properties The Effect of Cooking on Total Polyphenolic Content and Antioxidant Activity of Selected Vegetables. *International Journal of Food Properties*, 37–41. https://doi.org/10.1080/10942912.2011.619292

Taylor, P., Scotter, M. J., & Castle, L. (2007). *Chemical interactions between additives in foodstuffs : a review Chemical interactions between additives in foodstuffs : a review*. https://doi.org/10.1080/02652030310001636912

The, I., & Asia, M. (2018). *Characterization of Active Compounds of Different Garlic (*. *68*(1), 73–81. https://doi.org/10.1515/pjfns-2017-0005

Tiwari, V., Shanker, R., Srivastava, J., & Vankar, P. S. (2006). Change in Antioxidant Activity of Spices – Turmeric and Ginger on Heat Treatment. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 1313–1317.

Upadhyay, S., & Dixit, M. (2015). Role of Polyphenols and Other Phytochemicals on Molecular Signaling. *Oxidative Medicine and Cellular Longetivity, Volume 201*, 1–15. https://doi.org/org/10.1155/2015/504253

Value, S. J. R. I., Sharma, K., Agrawal, S. S., & Gupta, M. (2012). *Available online http://www.ijddr.in Covered in Official Product of Elsevier , The Netherlands Development and Validation of UV spectrophotometric method for the estimation of Curcumin in Bulk Drug and Pharmaceutical Dosage Forms*. *4*(2), 375–380.

Wangcharoen, W. (2009). Effect of heat treatment on the antioxidant capacity of garlic. *Maejo International Journal of Science and Technology*, *3*(01 June), 60–70.

Wei, E., Chan, C., Tan, Y. P., Chin, S. J., & Gan, L. Y. (2014). *Original Article Antioxidant properties of selected fresh and processed herbs and vegetables*. *4*(1), 39–46. https://doi.org/10.5530/fra.2014.1.7

Yadav, N., Yadav, R., & Goyal, A. (2014). Chemistry of Terpenoids. *International Journal of Pharmaceutical Sciences Review and Research*, *27*(45), 271–278.

Zhang, X., Li, N., Lu, X., Liu, P., & Qiao, X. (2018). *Effects of temperature on the quality of black garlic*. (July 2015). https://doi.org/10.1002/jsfa.7351

Zhang, Y., & Liu, X. (2020). Biomedicine & Pharmacotherapy Phytochemicals of garlic : Promising candidates for cancer therapy. *Biomedicine & Pharmacotherapy*, *123*(December 2019), 109730. https://doi.org/10.1016/j.biopha.2019.109730

Zhong, L., & Hodgson, J. M. (2019). Characterization of polyphenols in Australian sweet lupin (Lupinus angustifolius) seed coat by HPLC-DAD-ESI-MS / MS. *Food Reasearch International*, 1153–1162. https://doi.org/10.1016/j.foodres.2018.09.061

Zingiber, G. (2010). The effects of type and time of thermal processing on ginger (Zingiber officinale Roscoe) rhizome antioxidant compounds and its quality. *International Food Research Journal*, *347*(17), 335–347.

APPENDICES

APPENDIX 1: LC-MS/MS data

a. LC-MS/MS data for fresh garlic aqueous extract

No.	Compound Name	Retention time	m/z	Fragments
1	Isoferrullic acid	4.036	180.1	163.1
2	Catechin	17.465	261.2	176.1, 55.2
3	Gentisic acid	17.516	153	108,109
4	Chlorogenic acid	17.595	177.1	80.1, 98.1
5	Caffeic acid	17.681	179	134. 135
6	Gallic acid	17.692	169	125, 79
7	Quercetin	17.829	290.1	166,77
8	Rutin	17.843	235.2	58.2, 86.1
9	Hesperidin	25.367	220.1	84.1

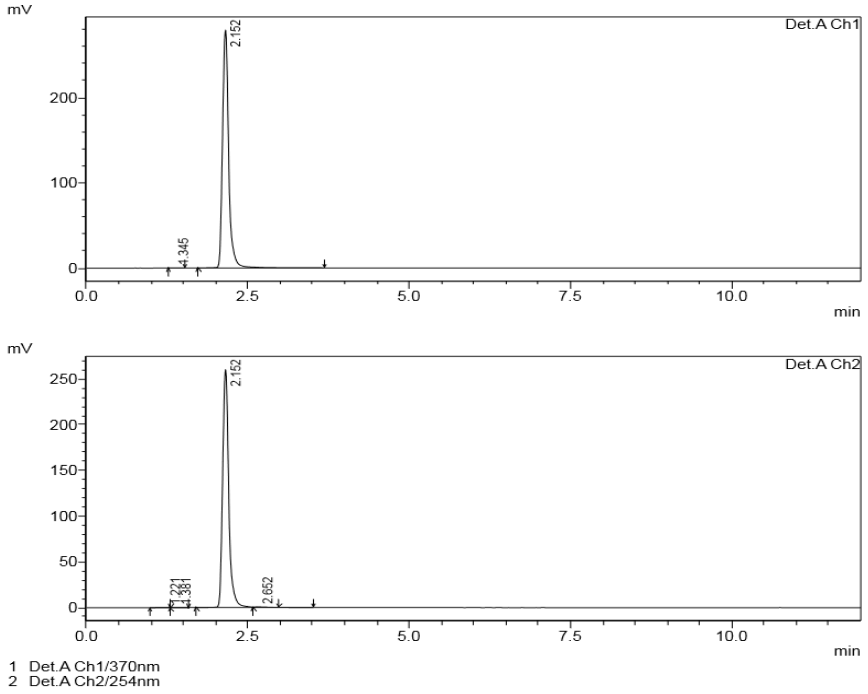
b. LC-MS/MS data for fresh garlic ethanolic extract

No.	Compound Name	Retention Time	m/z	Fragments
1	Catechin	9.483	261.2	55.2, 176.1
2	Quercetin	11.147	290.1	77, 168
3	Caffeic acid	17.589	179	134, 135
4	Coumarin	18.829	147	91.1,103.1
5	Chlorogenic acid	18.987	177.1	80.1, 98.1
6	Gentisic acid	19.001	153	108, 109
7	Naringin	19.053	234.1	84.1
8	Gallic acid	19.084	169	79, 125
9	Rutin	19.142	235.2	58.2, 86.1
10	Hesperidin	22.304	220.1	84.1
11	Hyperin	33.609	272.2	171.1, 215.1

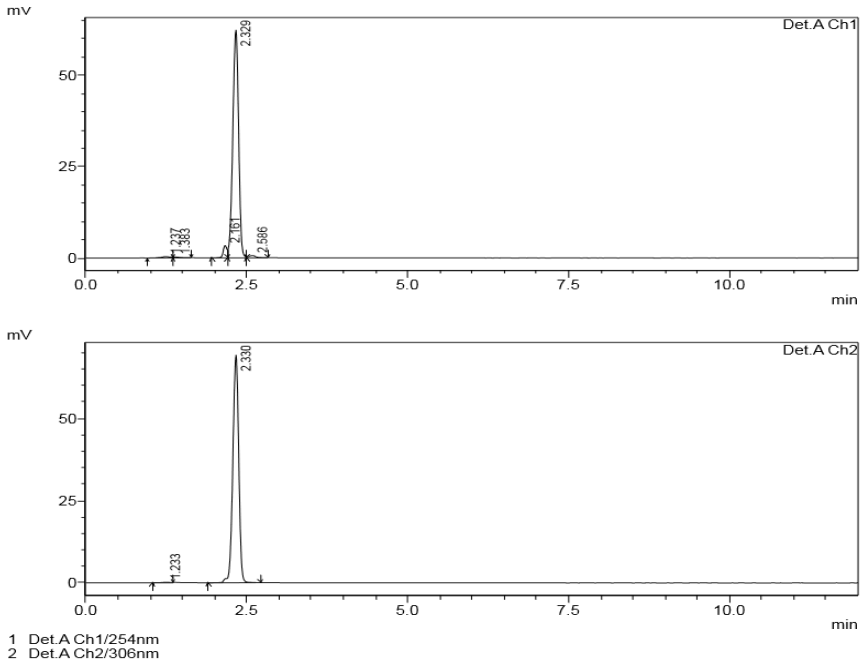
c. LC-MS/MS data for cooked garlic at 150 ºC aqueous extract

No.	Compound Name	Retention Time	m/z	Fragments
1	Gallic acid	0.337	169	79,125
2	Chlorgenic acid	2.747	177.1	80.1, 98.1
3	Isoferrulic acid	8.305	180.1	163.1
4	Caffeic acid	10.814	179	134, 135
5	Ferrulic acid	12.756	194.1	163.1,105.1
6	Quercetin	26.645	290.1	77, 168
7	Catechin	26.652	261.2	55.2, 176.1
8	Coumarin	28.388	147	91.1, 103.1
9	Ellargic acid	28.808	195.1	83
10	Rutin	29.351	235.2	58.2, 86.1
11	Tannic acid	29.828	195.1	138
12	Gentisic acid	30.416	153	108, 109
13	Hyperin	34.723	272.2	171.1, 215.1

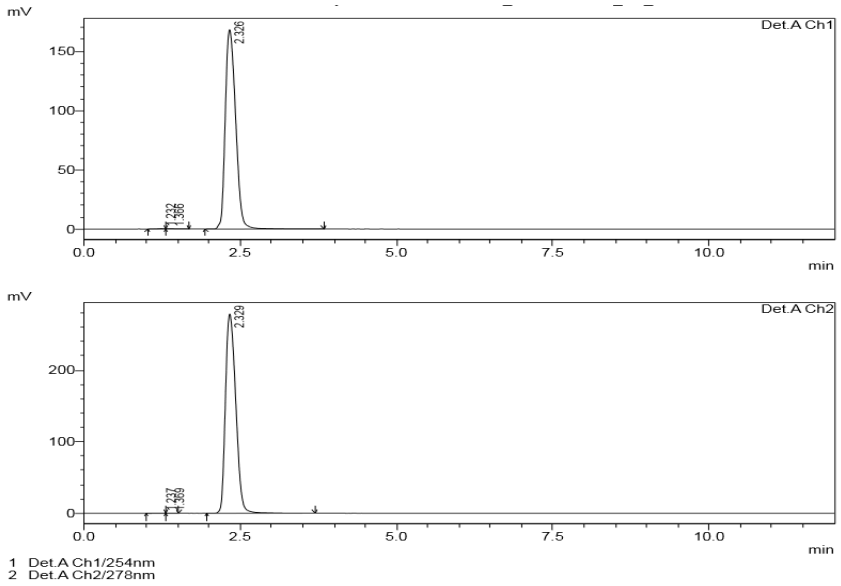
APPENDIX 2: HPLC-UV chromatograms for standards



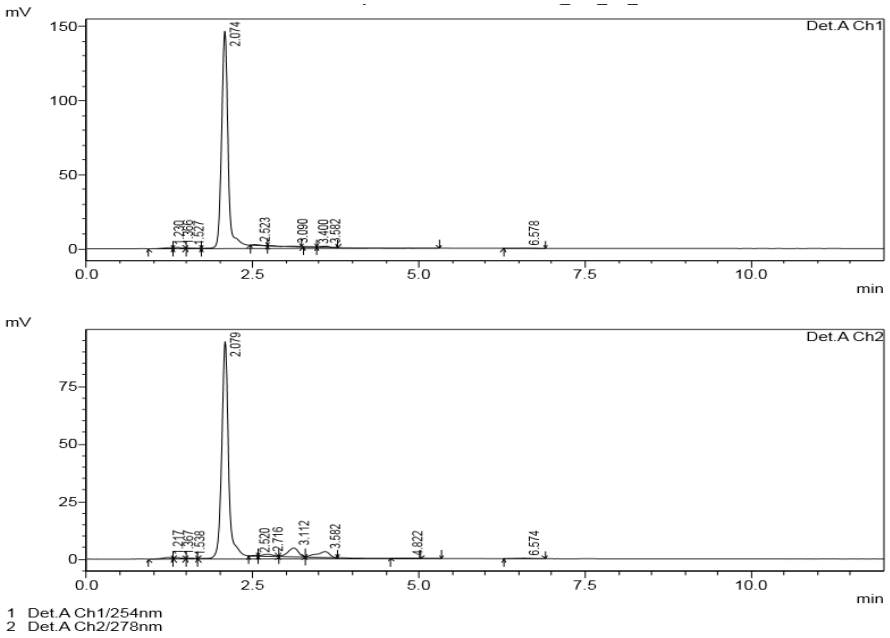
a. HPLC-UV chromatogram for quercetin standard



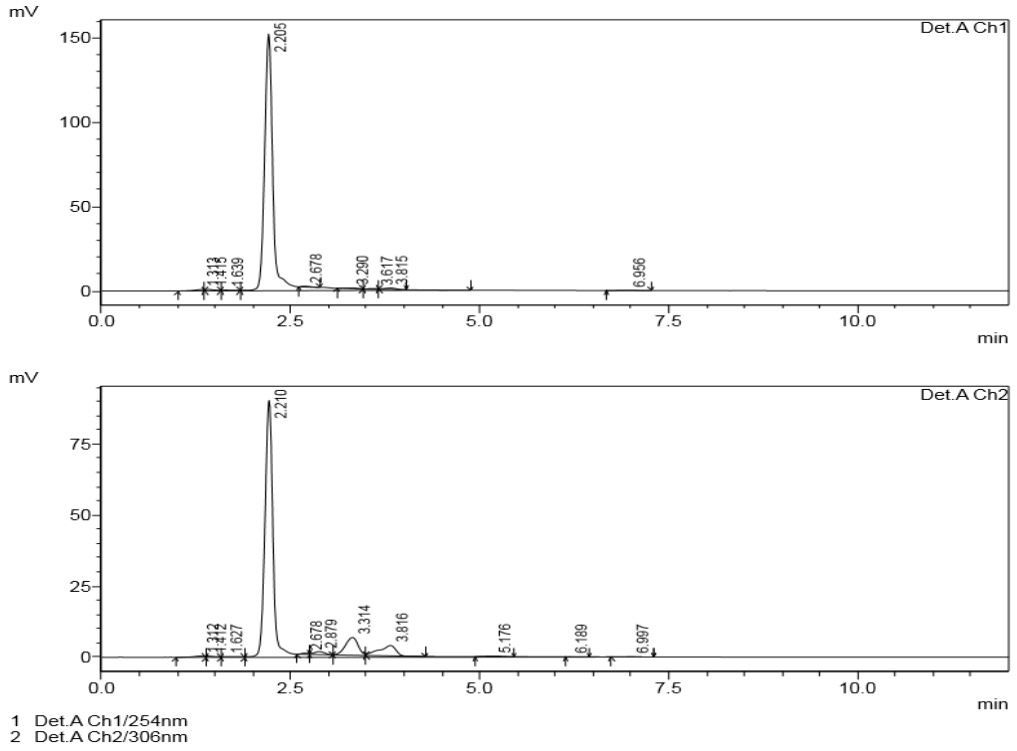
b. HPLC-UV chromatogram for coumarin standard



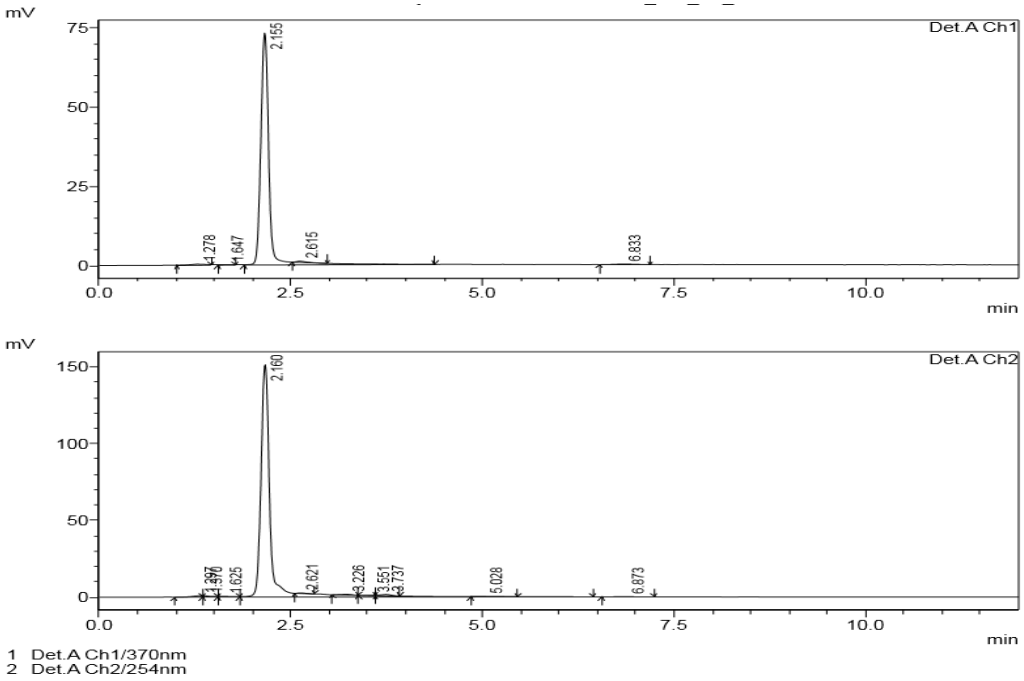
c. HPLC-UV chromatogram for Cinnamic acid standard



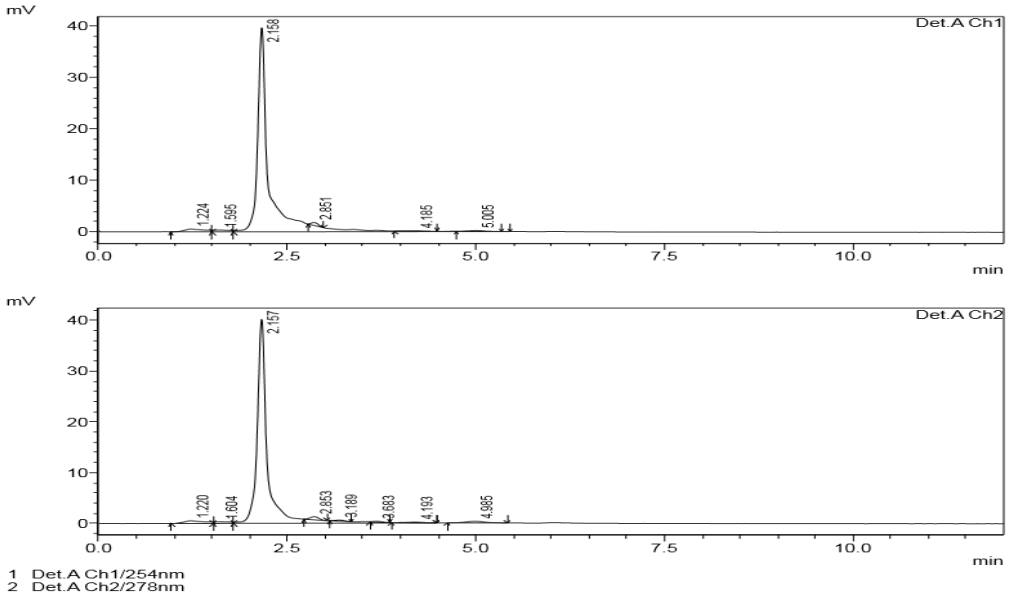
d. Cinnamic acid HPLC-UV chromatogram for fresh garlic of ethanolic extract



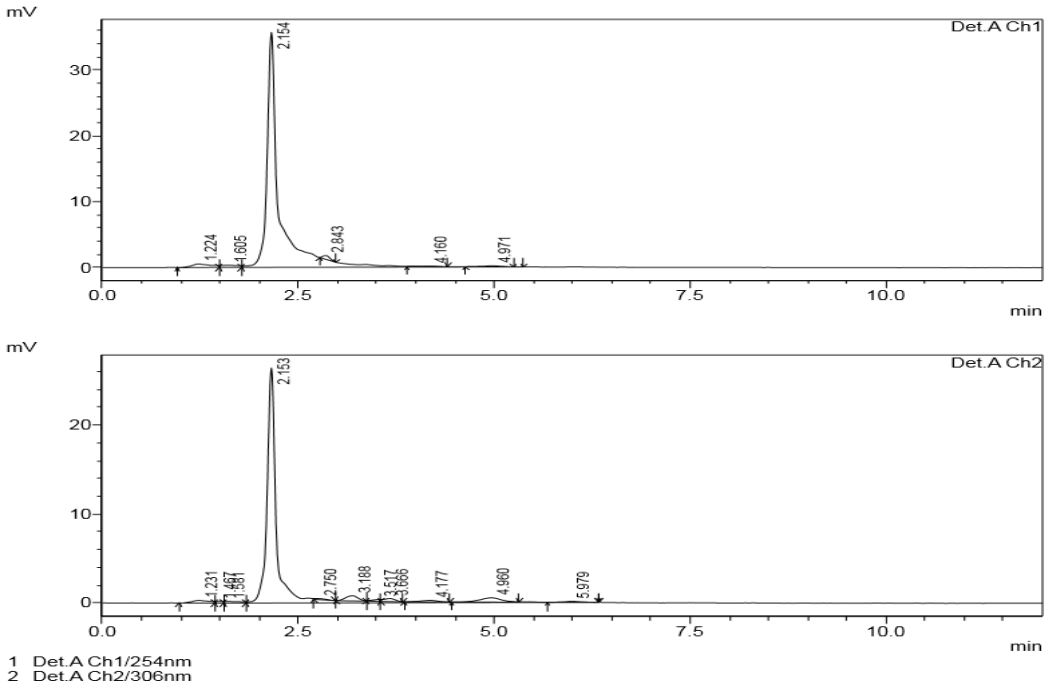
e. Coumarin HPLC-UV chromatogram for fresh garlic of ethanolic extract



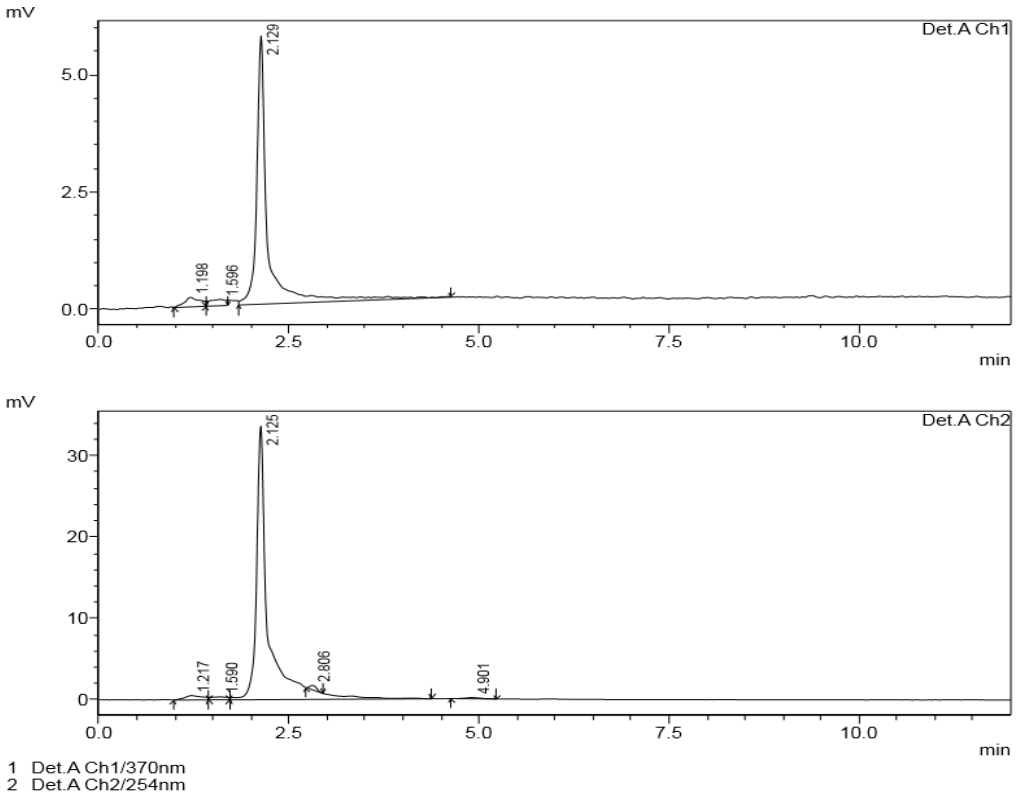
f. Quercetin HPLC-UV chromatogram for fresh garlic of ethanolic extracts



g. Cinnamic acid HPLC-UV chromatogram for fresh garlic of aqueous extract



h. Coumarin HPLC-UV chromatogram for fresh garlic of aqueous extract



i. Quercetin HPLC-UV chromatogram for fresh garlic of aqueous extract

APPENDIX 3: Statistical analysis output tables

1. Effect of cooking temperature on total phenolic content and total flavonoid content

a. ANOVA table for total phenolic content for aqueous extracts

ANOVA					
Total Phenolic Content					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1391275.00	5	278255.00	21.171	.000
Within Groups	236583.17	18	13143.51		
Total	1627858.17	23			

b. ANOVA table for total phenolic content for ethanolic extract

ANOVA					
Total Phenolic Content					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	84675.771	5	16935.154	12.919	.000
Within Groups	23594.917	18	1310.829		
Total	108270.688	23			

c. ANOVA table for total flavonoid content for aqueous extracts

ANOVA					
Total Flavonoid					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	227165.856	5	45433.171	18.629	.000
Within Groups	43898.458	18	2438.803		
Total	271064.314	23			

d. ANOVA table for total flavonoid content for ethanolic extracts

ANOVA					
Total Flavonoid					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	323581.968	5	64716.394	76.663	.000
Within Groups	15195.058	18	844.170		
Total	338777.026	23			

2. Effect of cooking time on total phenolic content and total flavonoid content

e. ANOVA test for total phenolic content for aqueous extract

ANOVA					
Final Concentration					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	173524.566	3	57841.522	.795	.511
Within Groups	1454333.605	20	72716.680		
Total	1627858.171	23			

f. ANOVA test for total phenolic content for ethanolic extracts

ANOVA					
Concentration					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15111.435	3	5037.145	1.081	.380
Within Groups	93159.253	20	4657.963		
Total	108270.688	23			

g. ANOVA table for total flavonoid content for aqueous extract

ANOVA					
Total Flavonoid					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	30523.936	3	10174.645	.846	.485
Within Groups	240540.378	20	12027.019		
Total	271064.314	23			

h. ANOVA table for flavonoid content for ethanol extracts

ANOVA					
Total Flavonoid					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8176.909	3	2725.636	.165	.919
Within Groups	330600.117	20	16530.006		
Total	338777.026	23			

3. Effect of cooking temperature on antioxidant activity

i. ANOVA table for antioxidant activity of ethanolic extracts

Antioxidant					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6044.167	5	1208.833	26.001	.000
Within Groups	836.861	18	46.492		
Total	6881.028	23			

j. ANOVA table for antioxidant activity for aqueous extracts

Antioxidant					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2566.204	5	513.241	22.475	.000
Within Groups	411.057	18	22.837		
Total	2977.262	23			

4. Independent sample t-test for the effect of different types of salts on TPC and TFC

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Total Phenolic Content	Equal variances assumed	**6.330**	**.046**	**-7.603**	**6**	**.000**	**-1191.50**	**156.72**	**-1574.99**	**-808.01**
	Equal variances not assumed			**-7.603**	**3.413**	**.003**	**-1191.50**	**156.72**	**-1657.80**	**-725.21**
Total Flavonoid Content	Equal variances assumed	**.104**	**.758**	**-.460**	**6**	**.662**	**-22.395000**	**48.735123**	**-141.645550**	**96.855550**
	Equal variances not assumed			**-.460**	**5.926**	**.662**	**-22.3950**	**48.7351**	**-142.0087**	**97.2187**

APPENDIX 4: Post-Hoc LSD tables

a. Post-Hoc LSD table for differences between temperature groups for TPC of aqueous extracts

Multiple Comparisons

Dependent Variable: Final Conc

LSD

(I) Temp levels	(J) Temp levels	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
at 25 degrees c	at 50 degrees c	-315.924565*	81.066359	.001	-486.23867	-145.61046
	at 75 degrees c	-438.589555*	81.066359	.000	-608.90366	-268.27545
	at 100 degrees c	-443.438659*	81.066359	.000	-613.75276	-273.12456
	at 125 degrees c	-666.506238*	81.066359	.000	-836.82034	-496.19214
	at 150 degrees c	-736.247329*	81.066359	.000	-906.56143	-565.93323
at 50 degrees c	at 25 degrees c	315.924565*	81.066359	.001	145.61046	486.23867
	at 75 degrees c	-122.664989	81.066359	.148	-292.97909	47.64911
	at 100 degrees c	-127.514094	81.066359	.133	-297.82820	42.80001
	at 125 degrees c	-350.581672*	81.066359	.000	-520.89577	-180.26757
	at 150 degrees c	-420.322764*	81.066359	.000	-590.63686	-250.00866
at 75 degrees c	at 25 degrees c	438.589555*	81.066359	.000	268.27545	608.90366
	at 50 degrees c	122.664989	81.066359	.148	-47.64911	292.97909
	at 100 degrees c	-4.849105	81.066359	.953	-175.16321	165.46500
	at 125 degrees c	-227.916683*	81.066359	.012	-398.23078	-57.60258
	at 150 degrees c	-297.657774*	81.066359	.002	-467.97188	-127.34367
at 100 degrees c	at 25 degrees c	443.438659*	81.066359	.000	273.12456	613.75276
	at 50 degrees c	127.514094	81.066359	.133	-42.80001	297.82820
	at 75 degrees c	4.849105	81.066359	.953	-165.46500	175.16321
	at 125 degrees c	-223.067578*	81.066359	.013	-393.38168	-52.75348
	at 150 degrees c	-292.808670*	81.066359	.002	-463.12277	-122.49457
at 125 degrees c	at 25 degrees c	666.506238*	81.066359	.000	496.19214	836.82034
	at 50 degrees c	350.581672*	81.066359	.000	180.26757	520.89577
	at 75 degrees c	227.916683*	81.066359	.012	57.60258	398.23078
	at 100 degrees c	223.067578*	81.066359	.013	52.75348	393.38168
	at 150 degrees c	-69.741091	81.066359	.401	-240.05519	100.57301
at 150 degrees c	at 25 degrees c	736.247329*	81.066359	.000	565.93323	906.56143
	at 50 degrees c	420.322764*	81.066359	.000	250.00866	590.63686
	at 75 degrees c	297.657774*	81.066359	.002	127.34367	467.97188
	at 100 degrees c	292.808670*	81.066359	.002	122.49457	463.12277
	at 125 degrees c	69.741091	81.066359	.401	-100.57301	240.05519

*. The mean difference is significant at the 0.05 level.

b. Post-Hoc LSD table for differences between temperature groups for TPC of ethanolic extracts

Multiple Comparisons						
Dependent Variable: Final Conc LSD						
(I) Temp levels	(J) Temp levels	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
at 25 degrees c	at 50 degrees c	-.157995	25.601062	.995	-53.94383	53.62784
	at 75 degrees c	2.435606	25.601062	.925	-51.35023	56.22144
	at 100 degrees c	49.873824	25.601062	.067	-3.91201	103.65966
	at 125 degrees c	111.673285*	25.601062	.000	57.88745	165.45912
	at 150 degrees c	150.607411*	25.601062	.000	96.82158	204.39325
at 50 degrees c	at 25 degrees c	.157995	25.601062	.995	-53.62784	53.94383
	at 75 degrees c	2.593601	25.601062	.920	-51.19223	56.37944
	at 100 degrees c	50.031818	25.601062	.066	-3.75402	103.81765
	at 125 degrees c	111.831279*	25.601062	.000	58.04544	165.61711
	at 150 degrees c	150.765405*	25.601062	.000	96.97957	204.55124
at 75 degrees c	at 25 degrees c	-2.435606	25.601062	.925	-56.22144	51.35023
	at 50 degrees c	-2.593601	25.601062	.920	-56.37944	51.19223
	at 100 degrees c	47.438217	25.601062	.080	-6.34762	101.22405
	at 125 degrees c	109.237679*	25.601062	.000	55.45184	163.02351
	at 150 degrees c	148.171805*	25.601062	.000	94.38597	201.95764
at 100 degrees c	at 25 degrees c	-49.873824	25.601062	.067	-103.65966	3.91201
	at 50 degrees c	-50.031818	25.601062	.066	-103.81765	3.75402
	at 75 degrees c	-47.438217	25.601062	.080	-101.22405	6.34762
	at 125 degrees c	61.799461*	25.601062	.027	8.01363	115.58530
	at 150 degrees c	100.733587*	25.601062	.001	46.94775	154.51942
at 125 degrees c	at 25 degrees c	-111.673285*	25.601062	.000	-165.45912	-57.88745
	at 50 degrees c	-111.831279*	25.601062	.000	-165.61711	-58.04544
	at 75 degrees c	-109.237679*	25.601062	.000	-163.02351	-55.45184
	at 100 degrees c	-61.799461*	25.601062	.027	-115.58530	-8.01363
	at 150 degrees c	38.934126	25.601062	.146	-14.85171	92.71996
at 150 degrees c	at 25 degrees c	-150.607411*	25.601062	.000	-204.39325	-96.82158
	at 50 degrees c	-150.765405*	25.601062	.000	-204.55124	-96.97957
	at 75 degrees c	-148.171805*	25.601062	.000	-201.95764	-94.38597
	at 100 degrees c	-100.733587*	25.601062	.001	-154.51942	-46.94775
	at 125 degrees c	-38.934126	25.601062	.146	-92.71996	14.85171
*. The mean difference is significant at the 0.05 level.						

c. Post-Hoc LSD table for differences between temperature groups for TFC of aqueous extracts

Multiple Comparisons

Dependent Variable: Total Flavonoid

LSD

(I) Temp levels	(J) Temp levels	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
at 25 degrees c	at 50 degrees c	-66.940000	34.919931	.071	-140.30405	6.42405
	at 75 degrees c	-112.112500*	34.919931	.005	-185.47655	-38.74845
	at 100 degrees c	-172.407500*	34.919931	.000	-245.77155	-99.04345
	at 125 degrees c	-236.127500*	34.919931	.000	-309.49155	-162.76345
	at 150 degrees c	-284.775000*	34.919931	.000	-358.13905	-211.41095
at 50 degrees c	at 25 degrees c	66.940000	34.919931	.071	-6.42405	140.30405
	at 75 degrees c	-45.172500	34.919931	.212	-118.53655	28.19155
	at 100 degrees c	-105.467500*	34.919931	.007	-178.83155	-32.10345
	at 125 degrees c	-169.187500*	34.919931	.000	-242.55155	-95.82345
	at 150 degrees c	-217.835000*	34.919931	.000	-291.19905	-144.47095
at 75 degrees c	at 25 degrees c	112.112500*	34.919931	.005	38.74845	185.47655
	at 50 degrees c	45.172500	34.919931	.212	-28.19155	118.53655
	at 100 degrees c	-60.295000	34.919931	.101	-133.65905	13.06905
	at 125 degrees c	-124.015000*	34.919931	.002	-197.37905	-50.65095
	at 150 degrees c	-172.662500*	34.919931	.000	-246.02655	-99.29845
at 100 degrees c	at 25 degrees c	172.407500*	34.919931	.000	99.04345	245.77155
	at 50 degrees c	105.467500*	34.919931	.007	32.10345	178.83155
	at 75 degrees c	60.295000	34.919931	.101	-13.06905	133.65905
	at 125 degrees c	-63.720000	34.919931	.085	-137.08405	9.64405
	at 150 degrees c	-112.367500*	34.919931	.005	-185.73155	-39.00345
at 125 degrees c	at 25 degrees c	236.127500*	34.919931	.000	162.76345	309.49155
	at 50 degrees c	169.187500*	34.919931	.000	95.82345	242.55155
	at 75 degrees c	124.015000*	34.919931	.002	50.65095	197.37905
	at 100 degrees c	63.720000	34.919931	.085	-9.64405	137.08405
	at 150 degrees c	-48.647500	34.919931	.181	-122.01155	24.71655
at 150 degrees c	at 25 degrees c	284.775000*	34.919931	.000	211.41095	358.13905
	at 50 degrees c	217.835000*	34.919931	.000	144.47095	291.19905
	at 75 degrees c	172.662500*	34.919931	.000	99.29845	246.02655
	at 100 degrees c	112.367500*	34.919931	.005	39.00345	185.73155
	at 125 degrees c	48.647500	34.919931	.181	-24.71655	122.01155

*. The mean difference is significant at the 0.05 level.

d. Post-Hoc LSD table for differences between temperature groups for TFC of ethanolic extracts

Multiple Comparisons						
Dependent Variable: Total Flavonoid LSD						
(I) Temp levels	(J) Temp levels	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
at 25 degrees c	at 50 degrees c	78.072500*	20.544706	.001	34.90967	121.23533
	at 75 degrees c	147.067500*	20.544706	.000	103.90467	190.23033
	at 100 degrees c	213.722500*	20.544706	.000	170.55967	256.88533
	at 125 degrees c	276.222500*	20.544706	.000	233.05967	319.38533
	at 150 degrees c	343.410000*	20.544706	.000	300.24717	386.57283
at 50 degrees c	at 25 degrees c	-78.072500*	20.544706	.001	-121.23533	-34.90967
	at 75 degrees c	68.995000*	20.544706	.004	25.83217	112.15783
	at 100 degrees c	135.650000*	20.544706	.000	92.48717	178.81283
	at 125 degrees c	198.150000*	20.544706	.000	154.98717	241.31283
	at 150 degrees c	265.337500*	20.544706	.000	222.17467	308.50033
at 75 degrees c	at 25 degrees c	-147.067500*	20.544706	.000	-190.23033	-103.90467
	at 50 degrees c	-68.995000*	20.544706	.004	-112.15783	-25.83217
	at 100 degrees c	66.655000*	20.544706	.005	23.49217	109.81783
	at 125 degrees c	129.155000*	20.544706	.000	85.99217	172.31783
	at 150 degrees c	196.342500*	20.544706	.000	153.17967	239.50533
at 100 degrees c	at 25 degrees c	-213.722500*	20.544706	.000	-256.88533	-170.55967
	at 50 degrees c	-135.650000*	20.544706	.000	-178.81283	-92.48717
	at 75 degrees c	-66.655000*	20.544706	.005	-109.81783	-23.49217
	at 125 degrees c	62.500000*	20.544706	.007	19.33717	105.66283
	at 150 degrees c	129.687500*	20.544706	.000	86.52467	172.85033
at 125 degrees c	at 25 degrees c	-276.222500*	20.544706	.000	-319.38533	-233.05967
	at 50 degrees c	-198.150000*	20.544706	.000	-241.31283	-154.98717
	at 75 degrees c	-129.155000*	20.544706	.000	-172.31783	-85.99217
	at 100 degrees c	-62.500000*	20.544706	.007	-105.66283	-19.33717
	at 150 degrees c	67.187500*	20.544706	.004	24.02467	110.35033
at 150 degrees c	at 25 degrees c	-343.410000*	20.544706	.000	-386.57283	-300.24717
	at 50 degrees c	-265.337500*	20.544706	.000	-308.50033	-222.17467
	at 75 degrees c	-196.342500*	20.544706	.000	-239.50533	-153.17967
	at 100 degrees c	-129.687500*	20.544706	.000	-172.85033	-86.52467
	at 125 degrees c	-67.187500*	20.544706	.004	-110.35033	-24.02467
*. The mean difference is significant at the 0.05 level.						

e. Post-Hoc LSD table for differences between temperature groups for AOA of aqueous extracts

Multiple Comparisons

Dependent Variable: Antioxidant

	(I) Temp levels	(J) Temp levels	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	at 25 degrees c	at 50 degrees c	-11.59250*	3.37909	.003	-18.6917	-4.4933
		at 75 degrees c	-17.75250*	3.37909	.000	-24.8517	-10.6533
		at 100 degrees c	-23.68000*	3.37909	.000	-30.7792	-16.5808
		at 125 degrees c	-27.88750*	3.37909	.000	-34.9867	-20.7883
		at 150 degrees c	-30.16500*	3.37909	.000	-37.2642	-23.0658
	at 50 degrees c	at 25 degrees c	11.59250*	3.37909	.003	4.4933	18.6917
		at 75 degrees c	-6.16000	3.37909	.085	-13.2592	.9392
		at 100 degrees c	-12.08750*	3.37909	.002	-19.1867	-4.9883
		at 125 degrees c	-16.29500*	3.37909	.000	-23.3942	-9.1958
		at 150 degrees c	-18.57250*	3.37909	.000	-25.6717	-11.4733
	at 75 degrees c	at 25 degrees c	17.75250*	3.37909	.000	10.6533	24.8517
		at 50 degrees c	6.16000	3.37909	.085	-.9392	13.2592
		at 100 degrees c	-5.92750	3.37909	.096	-13.0267	1.1717
		at 125 degrees c	-10.13500*	3.37909	.008	-17.2342	-3.0358
		at 150 degrees c	-12.41250*	3.37909	.002	-19.5117	-5.3133
	at 100 degrees c	at 25 degrees c	23.68000*	3.37909	.000	16.5808	30.7792
		at 50 degrees c	12.08750*	3.37909	.002	4.9883	19.1867
		at 75 degrees c	5.92750	3.37909	.096	-1.1717	13.0267
		at 125 degrees c	-4.20750	3.37909	.229	-11.3067	2.8917
		at 150 degrees c	-6.48500	3.37909	.071	-13.5842	.6142
	at 125 degrees c	at 25 degrees c	27.88750*	3.37909	.000	20.7883	34.9867
		at 50 degrees c	16.29500*	3.37909	.000	9.1958	23.3942
		at 75 degrees c	10.13500*	3.37909	.008	3.0358	17.2342
		at 100 degrees c	4.20750	3.37909	.229	-2.8917	11.3067
		at 150 degrees c	-2.27750	3.37909	.509	-9.3767	4.8217
	at 150 degrees c	at 25 degrees c	30.16500*	3.37909	.000	23.0658	37.2642
		at 50 degrees c	18.57250*	3.37909	.000	11.4733	25.6717
		at 75 degrees c	12.41250*	3.37909	.002	5.3133	19.5117
		at 100 degrees c	6.48500	3.37909	.071	-.6142	13.5842
		at 125 degrees c	2.27750	3.37909	.509	-4.8217	9.3767

*. The mean difference is significant at the 0.05 level.

f. Post-Hoc LSD table for differences between temperature groups for AOA of ethanolic extracts

Multiple Comparisons

Dependent Variable: Antioxidant

	(I) Temp levels	(J) Temp levels	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	at 25 degrees c	at 50 degrees c	-2.25500	4.82143	.646	-12.3844	7.8744
		at 75 degrees c	2.59250	4.82143	.597	-7.5369	12.7219
		at 100 degrees c	8.34750	4.82143	.100	-1.7819	18.4769
		at 125 degrees c	21.92500*	4.82143	.000	11.7956	32.0544
		at 150 degrees c	43.04750*	4.82143	.000	32.9181	53.1769
	at 50 degrees c	at 25 degrees c	2.25500	4.82143	.646	-7.8744	12.3844
		at 75 degrees c	4.84750	4.82143	.328	-5.2819	14.9769
		at 100 degrees c	10.60250*	4.82143	.041	.4731	20.7319
		at 125 degrees c	24.18000*	4.82143	.000	14.0506	34.3094
		at 150 degrees c	45.30250*	4.82143	.000	35.1731	55.4319
	at 75 degrees c	at 25 degrees c	-2.59250	4.82143	.597	-12.7219	7.5369
		at 50 degrees c	-4.84750	4.82143	.328	-14.9769	5.2819
		at 100 degrees c	5.75500	4.82143	.248	-4.3744	15.8844
		at 125 degrees c	19.33250*	4.82143	.001	9.2031	29.4619
		at 150 degrees c	40.45500*	4.82143	.000	30.3256	50.5844
	at 100 degrees c	at 25 degrees c	-8.34750	4.82143	.100	-18.4769	1.7819
		at 50 degrees c	-10.60250*	4.82143	.041	-20.7319	-.4731
		at 75 degrees c	-5.75500	4.82143	.248	-15.8844	4.3744
		at 125 degrees c	13.57750*	4.82143	.011	3.4481	23.7069
		at 150 degrees c	34.70000*	4.82143	.000	24.5706	44.8294
	at 125 degrees c	at 25 degrees c	-21.92500*	4.82143	.000	-32.0544	-11.7956
		at 50 degrees c	-24.18000*	4.82143	.000	-34.3094	-14.0506
		at 75 degrees c	-19.33250*	4.82143	.001	-29.4619	-9.2031
		at 100 degrees c	-13.57750*	4.82143	.011	-23.7069	-3.4481
		at 150 degrees c	21.12250*	4.82143	.000	10.9931	31.2519
	at 150 degrees c	at 25 degrees c	-43.04750*	4.82143	.000	-53.1769	-32.9181
		at 50 degrees c	-45.30250*	4.82143	.000	-55.4319	-35.1731
		at 75 degrees c	-40.45500*	4.82143	.000	-50.5844	-30.3256
		at 100 degrees c	-34.70000*	4.82143	.000	-44.8294	-24.5706
		at 125 degrees c	-21.12250*	4.82143	.000	-31.2519	-10.9931

*. The mean difference is significant at the 0.05 level.