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EFFECT OF COOKING ON ORGANIC AND CONVENTIONAL SRI LANKAN GREEN VEGETABLE LEAVES; DETERMINING THE ANTIOXIDANT, ANTIMICROBIAL AND NITRATE CONTENT

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ABSTRACT

Consumption of organic and conventional green leaves in Sri Lanka is greatly highlighted towards its economical demand but the awareness of both the cultivation methods is still not clear. Organic green leaves provide a positive significance towards health and safety as there is no usage of pesticides and inorganic fertilizers whereas, conventional samples occupy a higher usage of inorganic nitrogen fertilizers. This brings out a major outbreak of food borne diseases. Cooking green leaves is popular in Sri Lanka, as it is consumed after cooking in different methods but the nutritional content could change as it gets treated by heat and eventually loses the positive impact of consuming green leaves. Moreover, organic green leaves occupy a greater content of phytophenols leading to a higher content of antioxidants and a stronger antimicrobial activity against bacterial strains whereas, conventional samples do show an antioxidant capacity and antimicrobial activity. Yet vegetable sellers could indeed sell fake organic products to increase the demand. This research is carried out to distinguish organic and conventional vegetable leaves by using Nitrate content assay and to identify the effect of cooking treatment on their antioxidant and antimicrobial capacity. Five organic and conventional green leaves such as mint (*Mentha*), parsley (*Petroselinum crispum*), gotukola (*Centella asiatica*), kankung (*Ipomoea aquatica*) and muhunuwenna (*Alternanthera sessilis*) were chosen from the local market in Colombo, Sri Lanka for this study. Antioxidant activity were assessed by total phenolic content (TPC), total flavonoid content (TFC), total antioxidant content (TAC) and radical scavenging activity were determined by assessing 1,1-Diphenyl-2-picrylhydrazyl Assay (DPPH), Ferric reducing antioxidant power assay (FRAP) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and Inhibitory concentration assay (IC 50). Antimicrobial activity was carried out by well diffusion technique against *Staphylococcus aureus* and *Escherichia coli.* As the results of this research proved that, raw mint had higher antioxidant levels compared to the heat treated samples.

Keywords: Antioxidant, organic, conventional, antimicrobial, phytophenols and cooking.

INTRODUCTION

Green leafy vegetables are inexpensive and thereby occupy micronutrients to mankind which also provides higher content of essential day to day required nutrients. Nutritional capacity of a particular green leafy vegetable relies on the methods of cultivation. This is where the cultivation of organic and conventional green leaves comes into play. Organic has its own highest demand in consumption where, 164 countries world-wide is producing organic green products and a value of $64 billion has been sold out. This Proves that the economic impact of bringing out organic substances. Organic products are synthetic products which are produced without the usage of pesticides and also with the absence of ultra violet light. Organic producers tend to use cultural practices which bring out awareness towards a safer use in consumption [1]. Organic fertilizers are used here in order to provide a usable form of the green vegetable at the correct time. Manure and chemical fertilizers achieve this goal as they influence the

phytochemical nutritional quality in crops [2]. Organic manures such as, poultry manure and farmyard protects the nutritional capacity of soil. Concerned consumers are much more particular in health by consuming safer products.

Regarding conventional green leafy vegetables, the products are produced *via* the usage of chemical pesticides which in turn increases the quantity production, quality and also increases the resistance towards pests and pathogens [1]. Impact of toxic product consumption in humans is literally higher in conventional plants and also contaminates the environment. Not only nutritional usage, but indeed medicinal as well. For a plant to cope up with its medicinal activity, the phytochemicals have to be perfect in order to carry out its function. It has been studied that the antioxidant content drastically reduces in inorganic fertilizers used for conventional plants [3]. Whereas, the antioxidant capacity is influenced highly in organic plants due to the organic fertilizers. The main constituent of inorganic products is nitrogen which is only found at a lesser amount of 5% of the total nitrogen in soil. This is the essential compound needed for a nourishing plant growth. Nitrogen fertilizers are absorbed by the plants where it is converted to products by the process of oxidation [3]. This increases nitrate content and thereby Demands a higher nitrate content in plants.

Organic and conventional green leafy vegetable consumption is a highly contradictory debate topic. It is said that, organic products are much more expensive than conventional. As organic producers continued producing organic products at a larger amount, the cost of organic reduced drastically. Yet, the cost can be cut down further by a large scale production. Organic vegetables undergo labelling process in order to never cheat the society and thereby induce environmental awareness for consumers to increase the demand of consumption.

As it is already mentioned above, organic products occupy higher levels of antioxidants than conventional products. Roghelia and Patel's study in 2015, concluded that the flavonoid and antioxidant potential is at its highest in products produced without fertilizers [1]. Further studies have to be carried out in order to comply organic and conventional products clearly and prove to the environment that the awareness created was correct.

Local markets could sell organic products by stating the absence of pesticides and inorganic fertilizers in order to increase the demand of consumption and economical values. But in reality, the concept of organic and conventional is still not clear. These green leaves are cooked before consumption in order to provide a better taste and aroma as it indeed impacts the demand of green leaf in Sri Lanka. In addition to this it is not yet clear on the antioxidant capacity towards heat treatment of the green leaves. As cooking could affect the antioxidant content and there by the consumption of cooked green leaves could decrease [4]. Rural development in Sri Lanka should be well maintained in order to reach the consumers demand.

This century is popular due to the major occurrence of cancer and cardiovascular diseases that plays a huge role in mortality of an individual. It has been experimented that the changes of oxygen usage and also the higher formation of reactive oxygen species (ROS) is the main cause for such chronic diseases. ROS and free radicals are produced in the human body due to cellular metabolism which includes the electron transport chain, phagocytosis

and other such major mechanisms as well. They carry out their mechanism of cellular degeneration and DNA damage by reacting with every single molecule present nearby a cell [5].

Free radicals are molecular species that can exist independently and tend to contain an unpaired electron. Most of the radicals are unstable and are highly reactive. Free radical targets tend to be included all over the world. Such as; protein, lipids and nucleic acids. Free radical reactions influence with ageing in every individual. Oxidative stress is a product of free radicals and ROS. It plays a concept when the balance between free radicals and antioxidant shielding effect is unfavourable (Fig 1) [6]. It can in turn cause injury to all cellular mechanisms thereby leading to cell death [7].

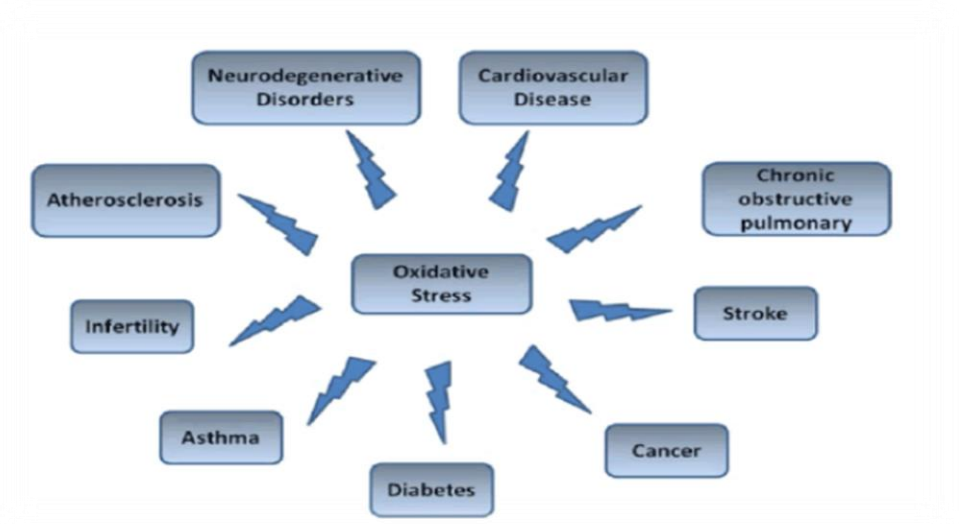


Fig.1. Oxidative stress has been involved in various physiological diseases following with the above mentioned illustration [7]

Oxidative stress can be fought against only by the help of antioxidants. Natural antioxidant shielding mechanisms occur in an individual's body which has the ability to work against free radicals. Antioxidants are present in the form of enzymes in the body as well as nutrients in food, which can scavenge or neutralize the reactive oxygen species and conduct a repair mechanism for the injured cell damage by oxidative stress. These molecules carry out the job by donating an electron to the unpaired atomic orbital present in the free radical thereby doing the free radical scavenging activity. Natural antioxidants produced in body are: glutathione, uric acid and ubiquinol [8]. Yet most of the antioxidants for instance, ascorbic acid cannot be built by the body therefore such should be supplemented via a healthy diet.

Antioxidants are mainly divided into two major categories: the synthetic and natural antioxidants. Synthetic antioxidants also called as phenolic compounds (butylated hydroxyanisol and propyl gallate) are food industry based produced to maintain the oxidation of food quality. Whereas, natural antioxidants; polyphenols (for example: flavonoids, ascorbic acid and carotenoids) are found naturally in food products. These antioxidants are

constituently used in medicine and foods. Moreover, in contrast to both types of antioxidants, people prefer natural antioxidants: plant derived as it is much more safe and cost effective. Among plants, leafy vegetables grasp the attention of inhibiting reactive oxygen species by antioxidants. This can be an alternative for the upcoming future to produce synthetic antioxidants *via* the maximum usage of natural antioxidants, which could be a better and safer economical method. Green leafy vegetables are mainly cooked before consumption especially over Asia. This could thereby change the components and special characteristics of compounds responsible for an effective function [9].

Considering green leafy vegetables and the antioxidant content, it is the active polyphenolic compounds which play a role in inhibiting free radicals. Such are, secondary metabolites produced by plants as it helps out in defensing pathogens and Ultraviolet light. The end of 20th century has clearly proven that, consumption of polyphenolic compounds in turn provides a medication to prevent the risk of cancer, diabetes and osteoporosis (Fig 2) [10].

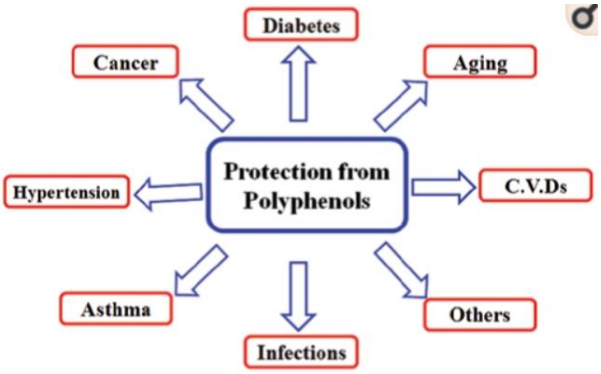


Fig. 2. Action of polyphenols towards certain diseases [11].

It has been identified that, more than 8000 polyphenolic compounds are present in plants, whereas they are produced *via* a common precursor, shikimic acid. Polyphenols are also named as, phenolic rings as it possesses one and thereby the function is related to the number of phenolic rings and also on the basic structure where these rings bind to. Such types of phenolic rings are, flavonoids, lignans and stilbenes and phenolic acids (Fig 3) [11].

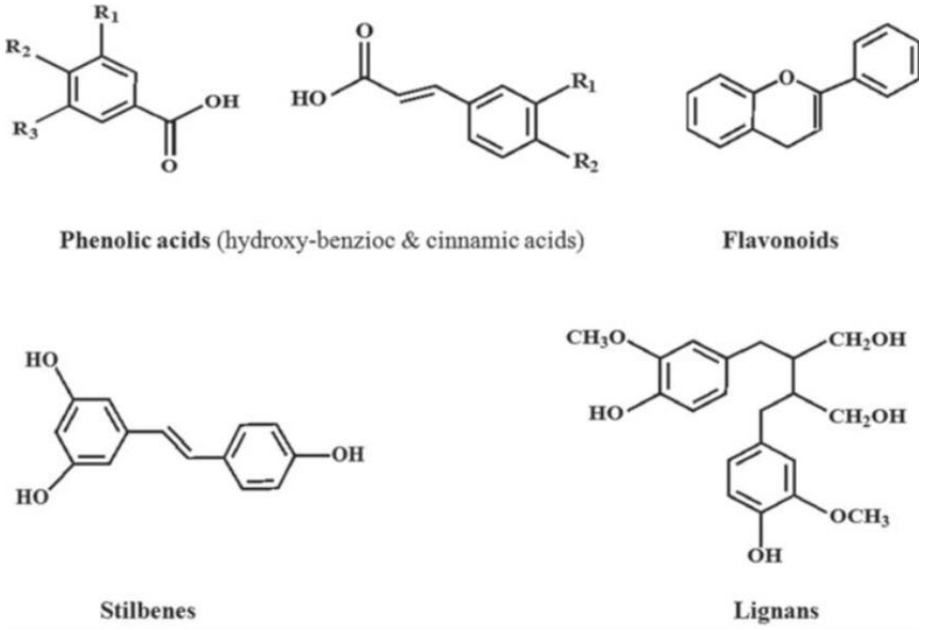


Fig. 3. **Detailed chemical structure of the classes of polyphenols [11].**

Polyphenols contain two aromatic rings in its structure which is bound together by three carbon atom. It has been found that, more than 4,000 types of flavonoids are present where each has its own role. Flavonoids can be divided into 6 other subclasses. Such are, Flavonols, flavones, flavanones, iso-flavones and anthocyanins. The most commonly found flavonoids are Quercetin and catechins (Fig 4) [10].

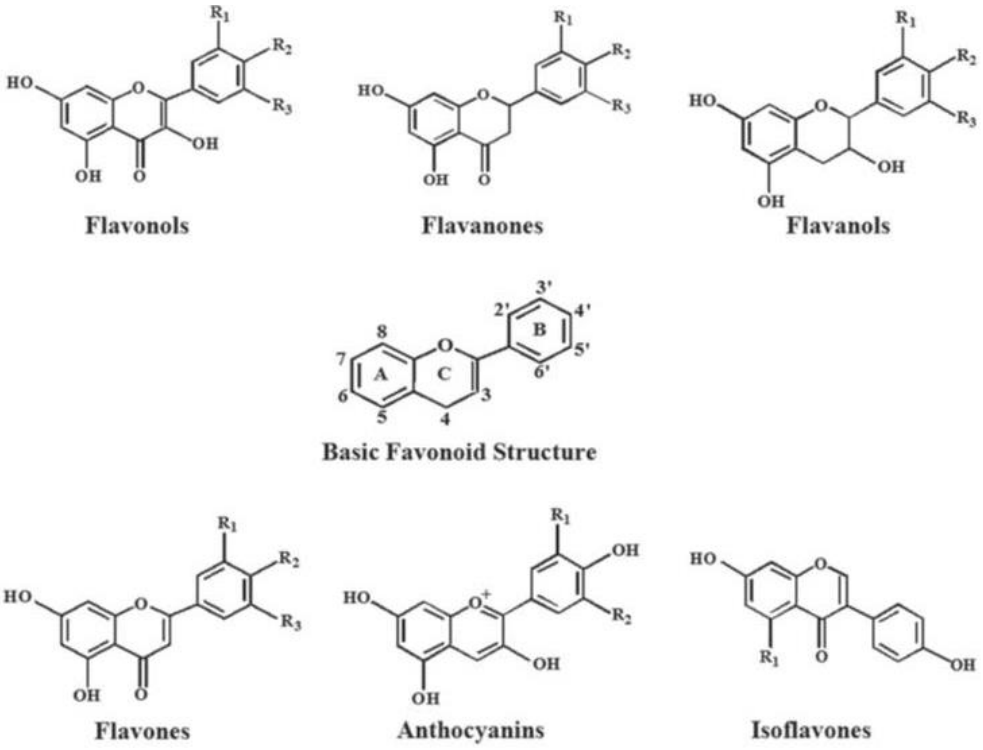


Fig. 4. Subclasses of flavonoids [11].

Polyphenols can indeed act as an anti- allergic and antimicrobial agent. It has been investigated that, the action towards pathogens and microorganisms is due to the structure-activity relationship [12].

If microbial medicines from such natural products are synthesized, it would increase the economical impact and patients' desire too.

The aim of this research is carried out to determine the antioxidant activity by boiling organic and conventional green leaves in order to support and prove the environment with better evidence on safer consumption of green leaves. As this research is not well established in Sri Lanka with a better sufficient data, this research could be awareness towards the society by reducing the occurrence of free radical mediated diseases.

This research is carried out to determine the antioxidant capacity in boiled and raw of organic and conventional green leaves by conducting assays such as, total flavonoid content, total phenolic content, total antioxidant content and radical scavenging activity assays which are 1,1-Diphenyl-2-picrylhydrazyl Assay (DPPH), Ferric reducing antioxidant power assay (FRAP) and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) and Inhibitory concentration assay (IC 50). Antimicrobial activity of both raw and boiled organic and conventional green leafy vegetables was carried out against two bacterial strains such as, *Staphylococcus aureus* and *Escherchia coli*. In addition to this, to differentiate both organic and conventional green leaves, nitrate content will be determined by spectrophotometer method.

MATERIALS AND METHODOLOGY

Sample collection

The samples used in this research are of both organic and conventional green leafy vegetables (Parsley, Gotukola, Kankung, Muhunuwenna and Mint leaves) which were collected from the local market (Keels supermarket – Kotahena and Wellawathe – Colombo 06). Prior to research work; Short and Long Control of Substances Hazardous to Health (COSHH) assessment forms were filled for all the reagents and chemicals which were used. Moreover, safety precautions were carried out during the research assessment.

Reagents

All other chemicals were purchased from Analytical Reagents Co. Colombo Sri Lanka, of analytical grade. Chemicals used were of Anlytical grade and double distilled water used throughout the experiment.

Sample collection

The sample extracts were washed thoroughly in running water and was dried for two days. The samples were first cut and grinded separately, whereas a mass of 2 g was collected for extraction.

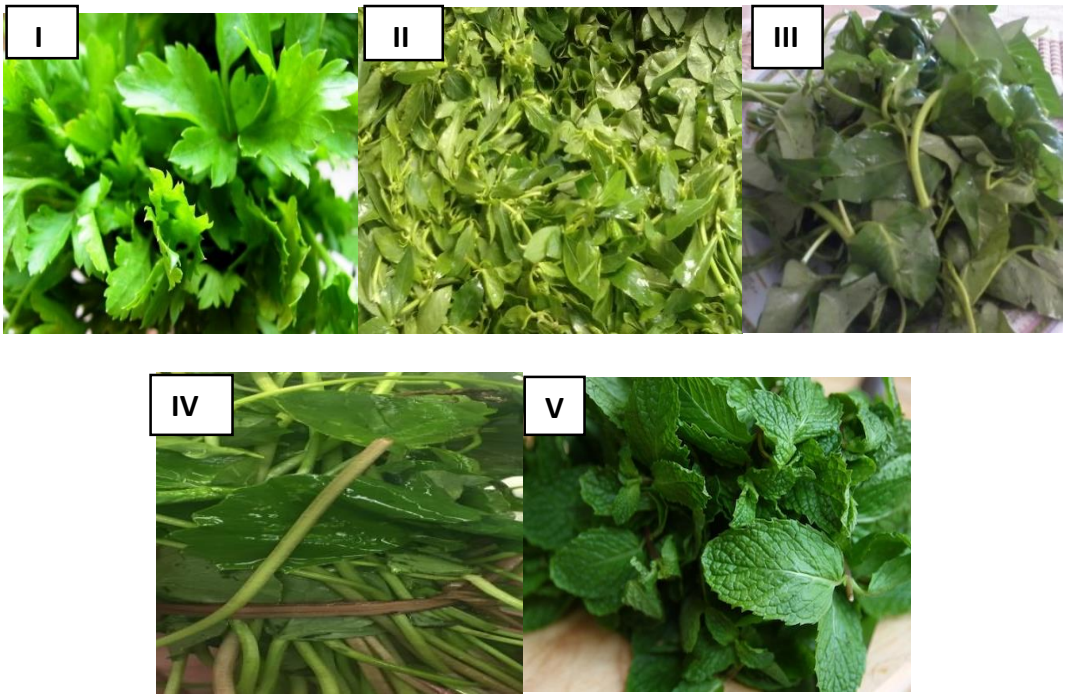


Fig. 5. Five different green leafy vegetables were collected for the study. I- Parsley (Par), II- Muhunuwenna (MuH), III-Kankung (Kan), IV- Gotukola (Got) and V. Mint (Min)

Water extraction preparation

A mass of 2 g of each sample was topped up with 40 ml of distilled water and was boiled under 90 °C for one hour. In parallel, a mass of 2 g of grinded samples were topped up with 40 ml of distilled water and was let on a roller mixer for 48 hours.

The boiled and raw samples (non-boiled) were filtered using a Whatman filter paper No. 1 to derive the supernatant which is the stock solution. The water dilution wad carried out by the addition of 1 ml of the stock solution which was topped up with 14 ml of distilled water.

Antioxidant activity

Determination of total phenolic content (TPC):

Total phenolic content was assayed by the use of Folin- Ciocalteu reagent. A volume of 0.5 ml of the diluted sample was mixed with 2.5 ml of Folin- Ciocalteu reagent. After 2 minutes, a volume of 2.5 ml of 7 % sodium carbonate was added to the solution. The mixture was allowed for incubation in the dark for 30 minutes and the absorbance was measured in 765 nm against distilled water which is the blank. The assay was carried out in triplicates for each sample extract.

The total phenolic content was calculated by a standard gallic acid graph, whereas the results are expressed in mg of gallic acid equivalents per 100 g of dry weight of extract (mg GAE/100 g).

Determination of total flavonoid content (TFC):

Total flavonoid content was carried out by the addition of 1.5 ml of the diluted sample extract with an equal amount of 1.5 ml of the 2 % $AlCl_3$ solution (1:1). The solution was incubated for 10 minutes at room temperature. Following which the absorbance was measured against distilled water by using a spectrophotometer. The assay was performed in triplicates for each extract.

The total phenolic content was calculated by a standard quercetin graph, whereas the results are expressed in mg of quercetin equivalents per 100 g of dry weight of extract (mg QE/100 g).

Determination of total antioxidant content (TAC):

The TAC reagent was prepared, by using 1 ml of 0.6 M sulphuric acid, 28 mM of sodium sulphate and 4mM of ammonium molybdate at 1:1 ratio.

Total antioxidant content assay was carried out by the addition of 3 ml of diluted sample extract with a volume of 1 ml of the TAC solution. The mixture was incubated at 90 °C for 90 minutes. The absorbance was measured at 695 nm against distilled water by using a spectrophotometer. The assay was performed in triplicates for each extract.

The total antioxidant content was calculated by a standard Ascorbic acid graph, whereas the results are expressed in mg of Ascorbic acid equivalents per 100 g of dry weight of extract (mg AAE/100 g).

1,1-diphenyl-2-picrylhydrazyl assay (DPPH):

The free radical scavenging activity for the plant samples was carried out using 1,1- Diphenyl-2-picrylhydrazyl (DPPH) solution. (0.1 mM) of DPPH was mixed in 95 % ethanol. The absorbance of the stock solution was measured at 517 nm using a spectrophotometer. A volume of 1 ml of the diluted sample was added in 2 ml of the stock solution which was later incubated in the dark, at room temperature for 45 minutes. Following which the absorbance was measured at 517 nm against 95 % ethanol using a spectrophotometer. Experiment was performed with time.

The scavenging activity was determined by calculating the percentage of DPPH radicals being scavenged by the sample extract. The calculation as follows:

$$\textbf{Scavenging activity \% = 100 X Abs}_{\textbf{(Control)}} \textbf{ – Abs}_{\textbf{(Sample)}} \textbf{ / Abs}_{\textbf{(Control)}}$$

Ferric reducing antioxidant power assay (FRAP):

The FRAP reagent was prepared with (10 mM) TPTZ in 1 M HCl, (300 mM) acetate buffer and (20 mM) ferric chloride hexahydrate. The FRAP reagent was readily prepared by mixing 25 ml (300 mM) acetate buffer, 2.5 ml of (20 mM) ferric chloride and 2.5 ml (10 mM) of TPTZ solution in 1 M HCl. Subsequently, a volume of 0.27 ml of distilled water was added with 2.70 ml of FRAP reagent and then 0.09 ml of the diluted sample extract. The absorbance was measured at 595 nm per minute. Distilled water was used as the blank.

Determination of ABTS scavenging activity:

ABTS solution was prepared by the addition 5 ml of 7 mM ABTS with 5 ml of 2.45 mM of ammonium persulphate. The solution was incubated for 16 hours at room temperature. The working solution was prepared using 3 ml of the prepared ABTS solution in 100 ml of methanol.

Initial absorbance of the working solution was measured at 734 nm using a spectrophotometer. Methanol was used as the blank. A volume of 150 µl of the diluted sample was mixed with 2850 µl of ABTS solution and the absorbance was measured at 734 nm with time.

Inhibitory concentration assay (ic50) assay:

The IC50 assay was carried out with the addition of 15 µl of the diluted sample with 135 µl of distilled water. A volume of 2850 µl of ABTS was later added to the mixture and the absorbance was measured at 734 nm in 6 different concentrations (10 %, 20 %, 40 %, 60 %, 80 % and 100 %) of the original stock solution.

Determination of antimicrobial activity: well diffusion

The antimicrobial activity of the sample extracts was determined by the procedure of well diffusion which was carried out against two bacterial strains *Staphylococcus aureus* and *Escherichia coli*. Following with an even spread of an inoculum using sterile cotton swabs of *Staphylococcus aureus* and *Escherichia coli* on the Mueller Hinton agar plates was carried out. Subsequently, three wells were punctured aseptically with a diameter of 6 to 8 mm with a sterilized cut micropipette tip for: negative control and two wells for similar sample (S1 and S2). Gentamycin discs were used for the positive control. Saline solution was added for the negative control well, and gentamycin discs were placed on the Mueller Hinton agar plate. Sample was added into the wells respective for sample (S1 and S2). The plates were later incubated at 37 °C for 24 hours. The zones of inhibition were measured by a ruler (cm).

Extraction of organic phase

The extraction was conducted by adding 5 ml of toluene solution for 10 minutes with frequent shaking. The lower aqueous layer was discarded. Eventually, the organic phase was washed twice with 10 ml of distilled water and thereby discarding the aqueous phase. A volume of 10 ml of 10 % sodium carbonate solution was added to the resulting mixture for further extraction of the organic phase. The absorbance was measured using a spectrophotometer at 407 nm. Toluene was used as the blank.

Statistical analysis

ANOVA tables were constructed by the use of Microsoft Excel and the Pearson correlation graphs were put up by the IBM SPSS Statistics viewer software.

RESULTS AND DISCUSSION

Environmental awareness is brought out due to consumption of organic and conventional green leafy vegetables. Therefore, evidence of proving this concept of organic and conventional green leafy vegetables in the local market of Sri Lanka should be elaborated and practiced further. The best practice is to determine the nitrate content in both the farming products in order to bring out an evident to the society between organic and conventional as it is carcinogenic in consumers and also leads to various severe diseases. Conventional leafy vegetables occupy the usage of nitrate fertilizers to increase the nutritional content but whereas, organic leafy vegetables do not occupy nitrate but contains vitamins and higher levels of antioxidants [13]. Nitrate consumption is toxic towards consumer, because the mammalian metabolic pathways convert nitrate to nitrite and thereby higher levels of nitrite is dangerous. Sri Lanka uses a higher amount of fertilizers and pesticides in farming leafy vegetables which has become a risk in consumption in this country. Yet, research sources to prove this concept is insufficient [14].

One of the aims of this study was to determine the difference between organic and conventional green leaves by evaluating the nitrate content. To determine nitrates in plants, sulphuric acid was used because; as the concentration of acid increases the nitrate level increases too. The absorbance was measured for the formation of sodium nitrophenoxide by the reaction of phenol present in samples and along with sulphuric acid and the addition of silver sulphate solution (Fig 6) [14].

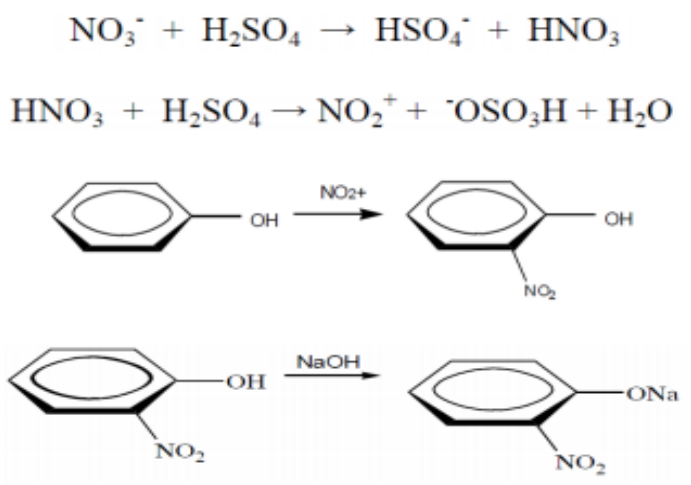


Fig. 6. The mechanism on evaluating nitrate [14].

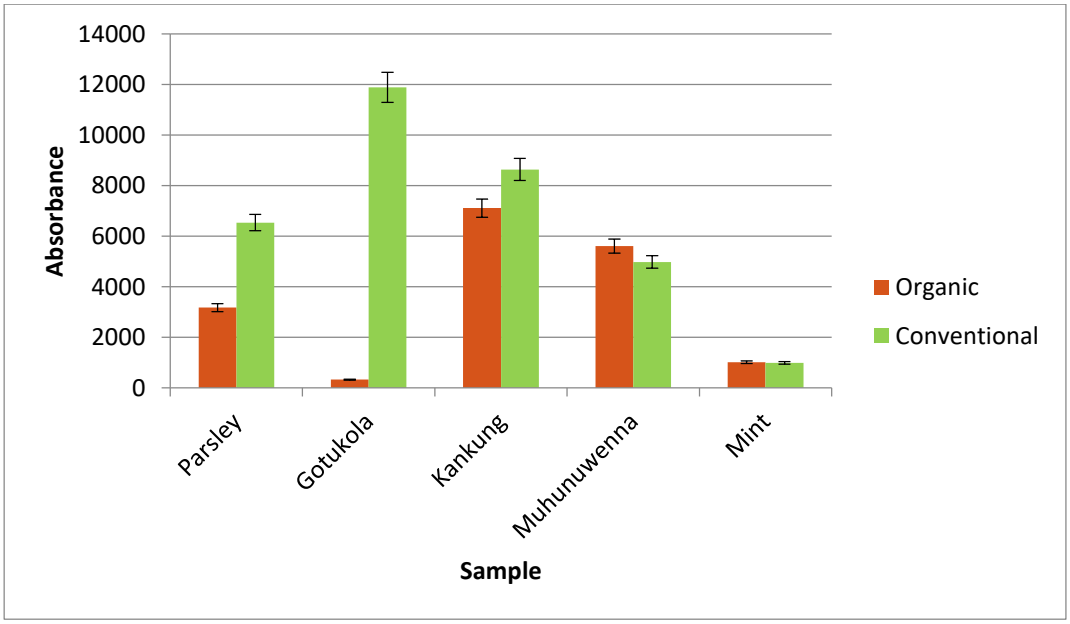


Fig. 7. Evaluation of Nitrate content of organic and conventional samples.

Referring to fig 7, it is clearly proven that conventional samples contain higher nitrate than organic samples. Specifically, gotukola has a higher nitrate content in conventional samples whereas, kankung has higher nitrate content in organic samples. The standard error bars among conventional and organic showed no overlap in kankung, gotukola and parsley whereas, muhunuwenna and mint showed an overlap. The single factor ANOVA was constructed to evaluate the significance of nitrate content in organic and conventional samples. ANOVA showed an F-value < Fcrit (F- 2.447633, Fcrit-5.317655) and p value > 0.05 (0.156332) showing no significant difference of nitrate content in organic and conventional samples (Table 1).

Table. 1. Single factor ANOVA evaluation on nitrate content among conventional and organic samples.

Anova: Single Factor

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Organic	5	16590	3318	7877070		
Conventional	5	33690	6738	16016220		
ANOVA						
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	29241000	1	29241000	2.447633	0.156332	5.317655
Within Groups	95573160	8	11946645			
Total	1.25E+08	9				

Yet, all the samples showed a detectable value of nitrate. In comparison to conventional samples, gotukola had the highest nitrate content followed by, kankung>parsley>muhunuwenna>mint. Organic samples were observed to acquire nitrate content but relatively lesser than in conventional. Organic kankung had the highest nitrate content followed by muhunuwenna>parsley>mint>gotukola. In the study of Nifras and Riyas in 2017 [14], it was also evident that nitrate content was higher in conventional samples than in organic. The capacity of nitrate carries from one sample to the other. The major reason behind the varied nitrate content in Sri Lankan organic and conventional samples was proved by Gunathilake and Iwao in 2010[15] that the heterogeneity changes are due to ecological destructions and environmental factors. It does not have to be the conventional samples occupying nitrate content, but if organic samples are developed by the use of nitrogen-rich fertilizer it could still bring up lower content of nitrate. Climate and heavy rainfall can indeed affect the nitrate content. Moreover, the location of nitrate in these samples matters for the level of nitrate content [14].

Increase in organic production is higher than the production of conventional due to its past decade concerns on food borne diseases. This greater interest on organic consumption than in conventional was brought out due to the higher content of phytochemicals and the nutrient content. Yet to claim this evidence, studies are still under consideration. Even though researches proved the theory behind antioxidants and polyphenolic content, only a minor amount of practices is carried out to compare the impact on green leafy vegetables grown organically and conventionally [16].

As green vegetables contain a great amount of antioxidants; therefore determining antioxidants in green leafy vegetables is a major establishment scientifically in realism. Moreover, cooking methods and the use of different temperatures highly affects the change of antioxidants, flavonoids and phenols. Researches based on radical

scavenging activity and the phytochemical activity evaluation to analyze the antioxidant content in these leafy extracts in Sri Lanka is still in process [7,17].

Antioxidants and radical scavenging assays were determined by various assays such as, Total phenolic content (TPC), Total flavonoid content (TFC), Total antioxidant content (TAC) and 1,1-Diphenyl-2-picrylhydrazyl Assay (DPPH), Ferric reducing antioxidant power assay (FRAP), ABTS scavenging activity and IC50 assay respectively.

Total phenolic content (TPC) was estimated by the use of Folin-Ciocalteu method to determine the total phenolic content of green leafy samples. The samples are being oxidized with the Folin-Ciocalteu's (F-C) reagent. TPC is a colorimetric technique which is based on the electron transfer reaction between phenolics and F-C reagent [18]. The molybdates from the F-C reagent are reduced and it is studied as the most prominent electron transfer reaction. As a phenolic proton dissociates it forms a phenolate ion and is capable of reducing F-C. The color changes from yellow to blue (figure 8).

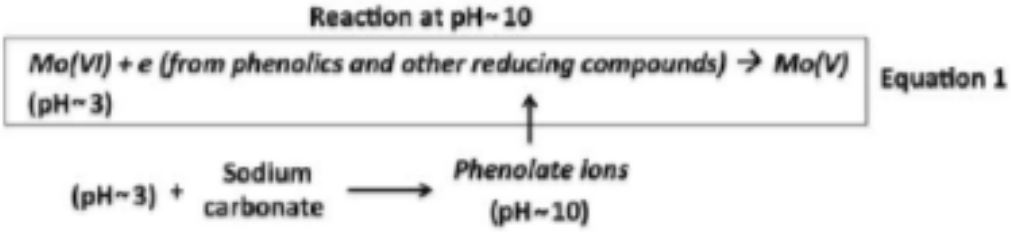


Fig. 8. The reaction between F-C and phenolic compounds [18].

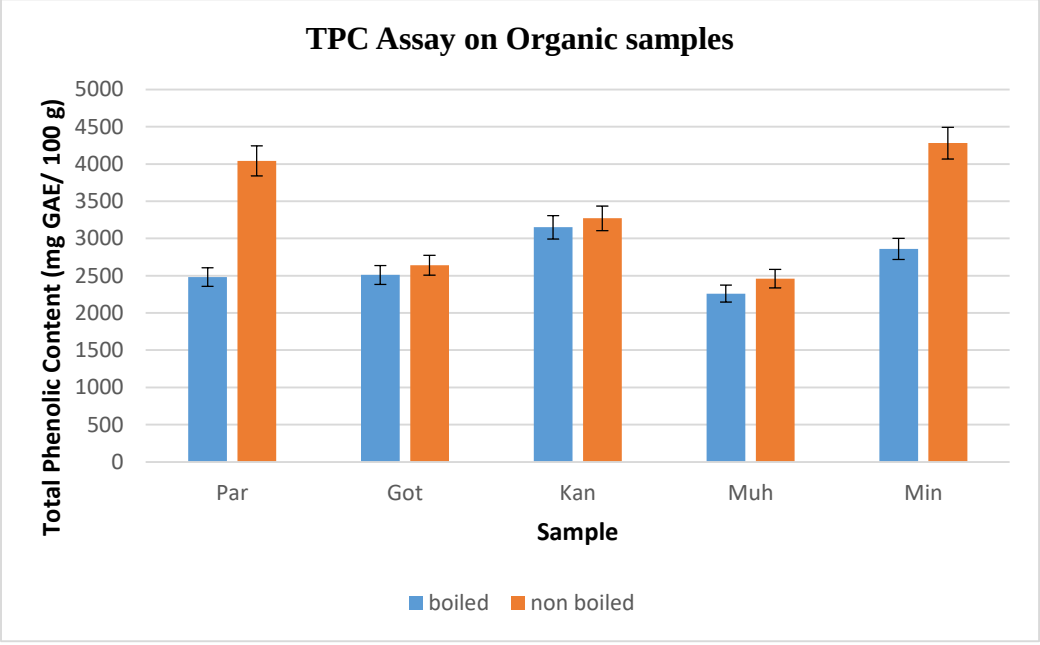


Fig. 9. Total Phenolic Content for both boiled and raw organic samples expressed in Gallic acid equivalents (GAE).

The above data in fig 9 shows that the raw samples had higher TPC than boiled samples. Specifically, raw mint samples had higher TPC and boiled kankung had higher TPC.

The standard error bars for organic parsley, and mint showed no overlapping however gotukola, kankung and muhunuwenna showed overlapping between sample. The single factor ANOVA was constructed for the respective sample to evaluate the significance in TPC between boiled and raw organic samples. ANOVA showed an F-value < Fcrit (F-2.999758, Fcrit-5.317655) and p value > 0.05 (0.121516) showing no significant difference of TPC between raw and boiled organic samples (table-2).

Table. 2. Single factor ANOVA evaluation for TPC between 5 different boiled and raw organic samples.

Anova: Single Factor

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Boiled	5	13260	2652	123670		
Raw	5	16690	3338	660720		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1176490	1	1176490	2.999758	0.121516	5.317655
Within Groups	3137560	8	392195			
Total	4314050	9				

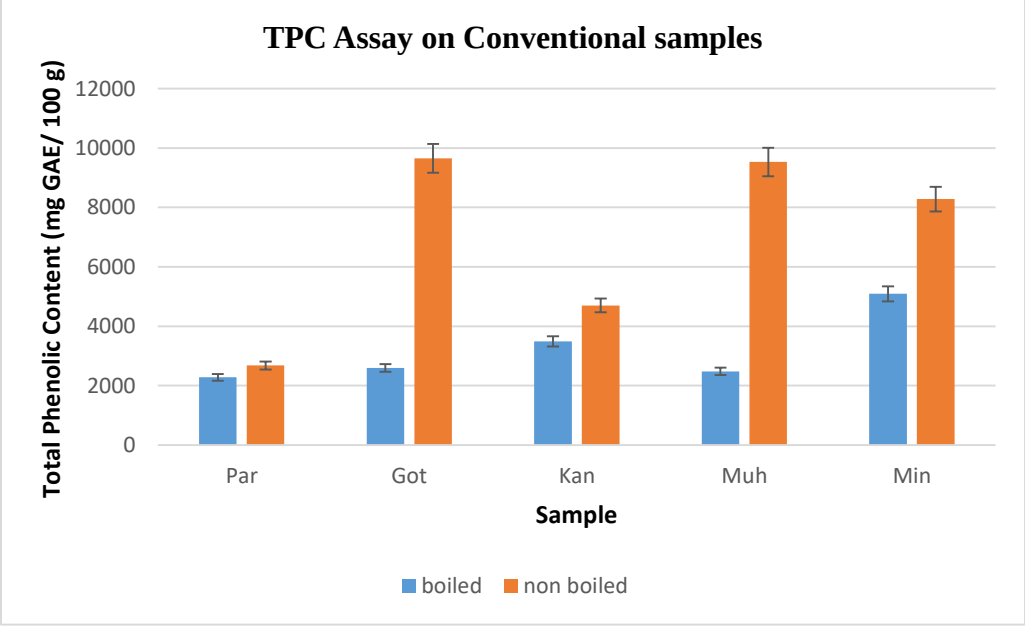


Fig. 10. Total Phenolic Content on boiled and raw conventional samples expressed in GAE.

The above data in fig 10 shows that the raw samples had higher TPC than boiled samples. Specifically, raw gotukola samples had higher TPC and boiled mint had higher TPC. The standard error bars for all conventional samples showed no overlapping except for parsley.

The single factor ANOVA was constructed for the respective sample to evaluate the significance in TPC between boiled and raw conventional samples. ANOVA showed an F-value > Fcrit (F-6.437759, Fcrit-5.317655) and p value < 0.05 (0.034858) showing a significant difference of TPC between raw and boiled conventional samples (table-3).

Table. 3. Single factor ANOVA evaluation for TPC between 5 different boiled and raw conventional samples.

Anova: Single Factor

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Boiled	5	15940	3188	1345070		
Raw	5	34840	6968	9752270		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	35721000	1	35721000	6.437759	0.034858	5.317655
Within Groups	44389360	8	5548670			
Total	80110360	9				

More over in organic raw samples, mint had the highest TPC followed by parsley>kankung>gotukola>muhunuwenna. Whereas when boiled, kankung had the highest TPC followed by Mint>gotukola>parsley>muhunwenna. Regarding conventional samples, gotukola had the highest TPC followed by muhunuwenna>mint>kankung>parsley when raw and mint had the highest TPC when boiled followed by kankung>gotukola>muhunwenna>parsley. Mohankumar *et al* in 2018 [19] study proved that, there is no significant difference of TPC in organic and conventional samples. This study shows the TPC levels are high when it was not heat treated. Kao, Chiu and Chiang in 2014 proved this concept by revealing that, most of the phenolics are water soluble and sensitive, thereby result in loss during boiling [20].

This drastic change was observed because; boiling leads to a loss of total phenolics as it enters the cooking water and thereby is lost during the consumption of these boiled samples [17]. It was stated in the study of Hwang *et al* in 2012 [21] (Fig 11) that raw fresh samples provided higher TPC, but yet the sample type varied in Hwang's study. The concept of higher antioxidant in raw sample is easily tackled in this case study.

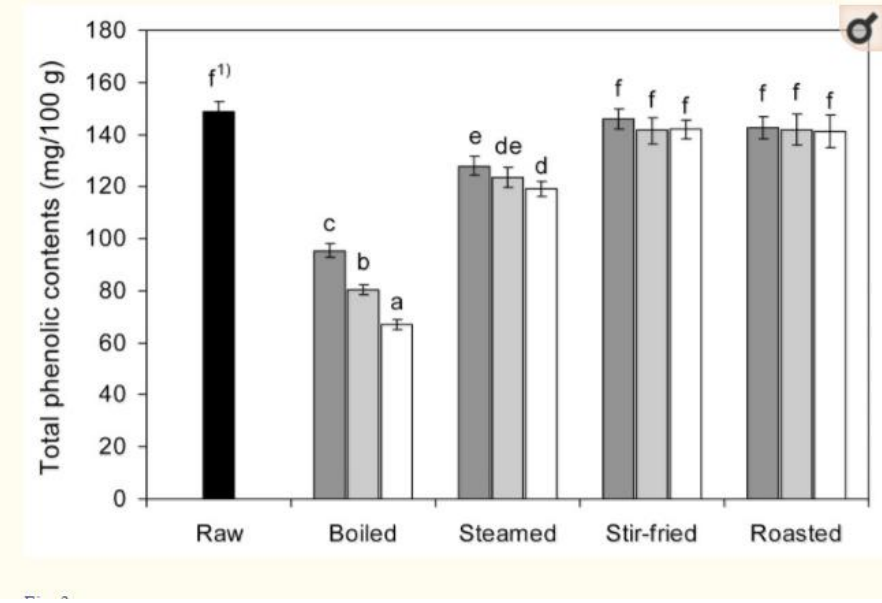


Fig. 11. Evaluation of TPC among different cooking methods [21]

In regards to organic and conventional samples, organic samples contained the highest TPC than in conventional samples. Studies showed that organic and conventional mint has higher TPC [19]. Organic green leafy vegetables are rich in antioxidant activity than in conventional and this could be a reason to cover up this result [19]. By relating this information on phenolic content of raw organic, it is practically clear that when raw antioxidant content in organic is still the same and there are no chances for a loss to occur. In the study of Chawla and Thakur in 2014[22], fresh raw mint has been proven to occupy higher phenolic content (Fig 12).

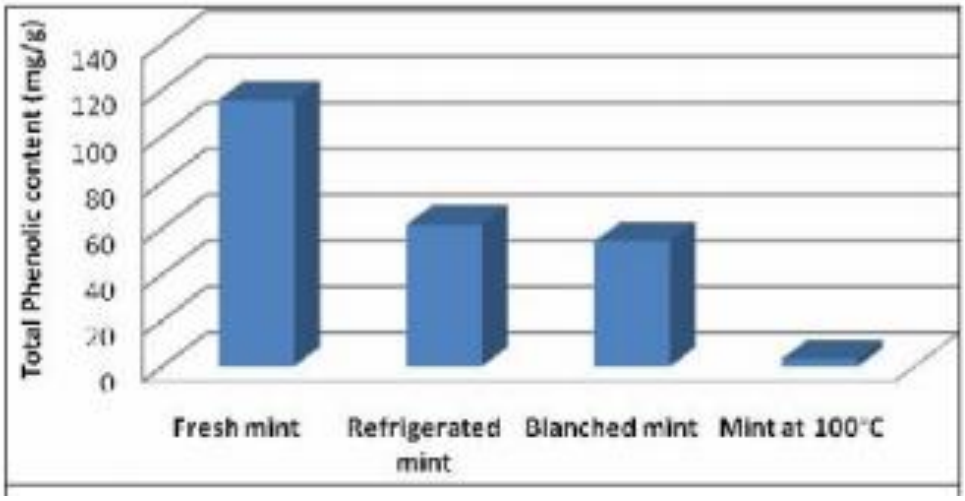


Fig. 12. Thermal treatment determination on mint leaves [22]

The most probable reasons for this heterogeneity change in TPC are due to various reasons.

Phenolic compounds vary from one plant to another. The change of TPC is suggested that it is influenced by environmental factors and fertilizers as it plays a role in adaptations of plants. Indeed, association of an increased attack plants in conventional plots by insects affects the TPC levels [1]. Inaccurate results on TPC could be due to weather and the chemical composition of the fertilizers which could thereby affect the TPC levels.

The FC reagent is also a reason for an inaccurate level of TPC. As FC reagent is not specific to phenols, it could have been reacted with other substrates such as, ascorbic acid [16].

The flavonoid content (TFC) was assessed by the analysis of total flavonoid content (TFC). This helps in forming stable acid complexes with the C-3 or C-5 hydroxyl groups of flavanols due to the addition of aluminium chloride. Aluminium chloride has the capability to form acid labile complexes with the groups of ortho-dihydroxyl in the A or either the B-rings of flavonoids [23]. The final color denoted will be yellow due to the reaction between aluminium chloride and the flavonoids [24]. The generated ANOVA in table 4 and table 5 of TFC showed that, there is a significant difference in organic samples p value < 0.05 (p value = 0.016252) and showed no significant difference in conventional samples p value > 0.05 (p value = 0.315038) when boiled and raw respectively.

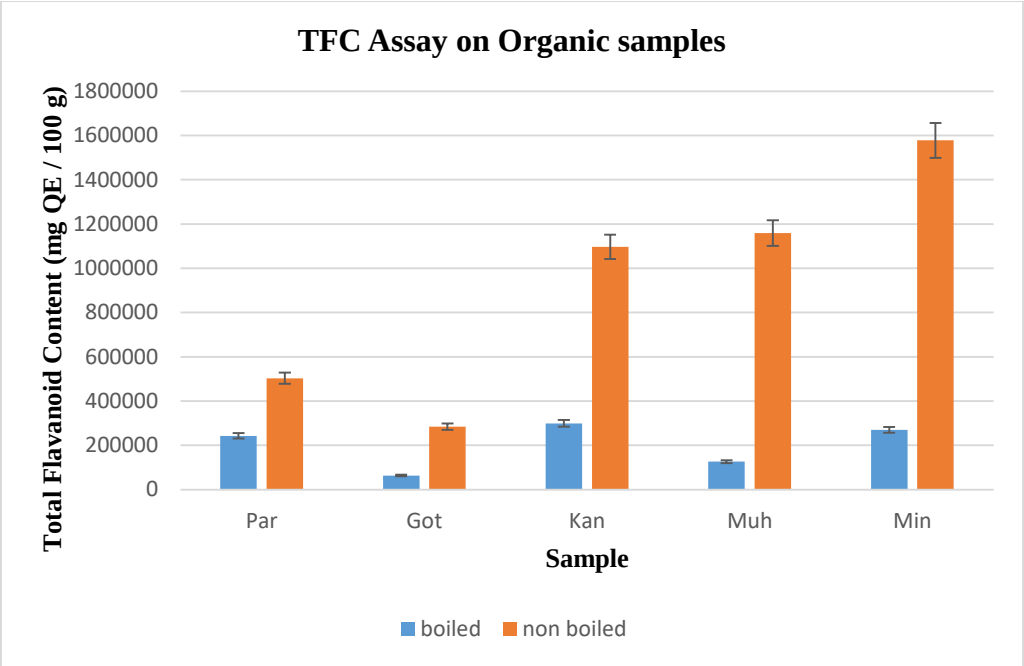


Fig. 13. Total Flavonoid Content (TFC) on boiled and raw organic samples expressed in quercetin equivalents(QE).

The raw samples had a higher TFC than in boiled samples. Mint had the highest TFC when raw and kankung had the highest TFC when boiled (figure-13). The standard error bars for all organic samples showed no overlapping. The single factor ANOVA was constructed for the respective sample to evaluate the significance in TFC between boiled and raw organic samples. ANOVA showed an F-value > Fcrit (F-9.195011, Fcrit-5.317655) and p value < 0.05 (0.016252) showing a significant difference of TPC between raw and boiled organic samples.

Table. 4. Single factor ANOVA evaluation for TFC between 5 different boiled and raw organic samples.

Anova: Single Factor

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Boiled	5	1002083	200416.7	1.01E+10		
Raw	5	4621875	924375	2.75E+11		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.31E+12	1	1.31E+12	9.195011	0.016252	5.317655
Within Groups	1.14E+12	8	1.43E+11			
Total	2.45E+12	9				

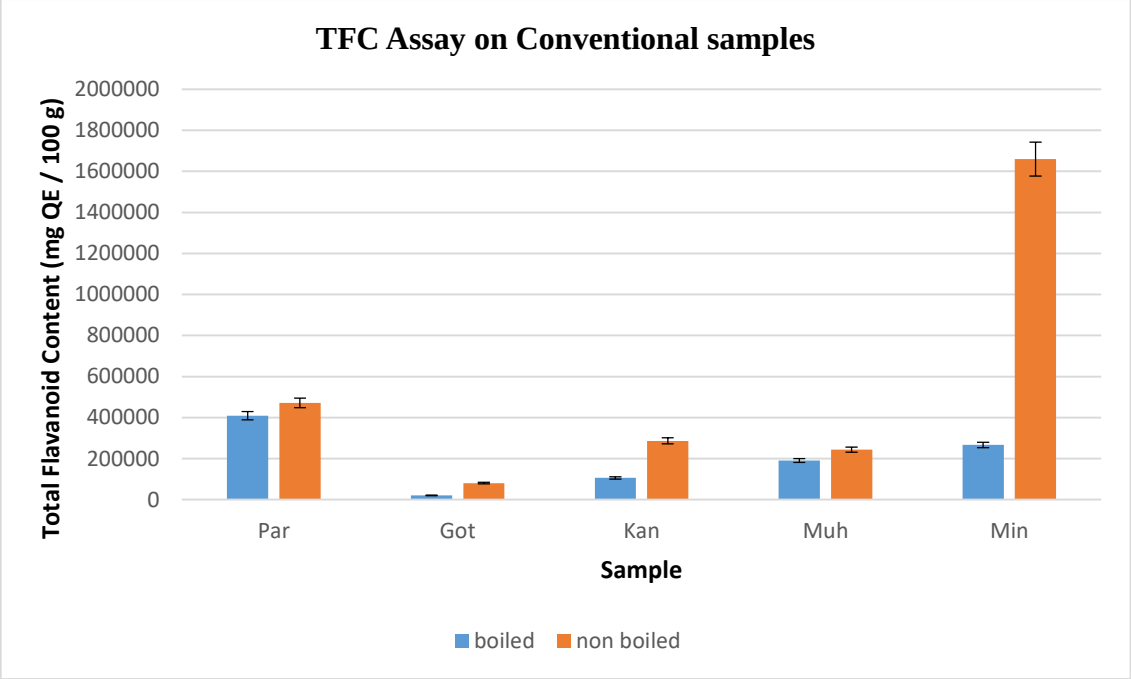


Fig. 14. Total Flavonoid Content (TFC) on boiled and raw conventional samples expressed in QE.

The raw samples had a higher TFC than in boiled samples. Mint had the highest TFC when raw and parsley had the highest TFC when boiled. The standard error bars in conventional gotukola, kankung and mint showed no overlap whereas, parsley and muhunuwenna overlapped. The single factor ANOVA was constructed for the respective sample to evaluate the significance in TFC between boiled and raw conventional samples. ANOVA showed an F-value < Fcrit (F-1.428607, Fcrit-5.317655) and p value > 0.05 (0.266219) showing no significant difference of TFC between raw and boiled organic samples (table-5).

Table. 5. Single factor ANOVA evaluation for TFC between 5 different boiled and raw conventional samples.

Anova: Single Factor

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Boiled	5	994166.7	198833.3	2.23E+10		
Raw	5	2741458	548291.7	4.05E+11		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.05E+11	1	3.05E+11	1.428607	0.266219	5.317655
Within Groups	1.71E+12	8	2.14E+11			
Total	2.01E+12	9				

Organic samples had higher TFC than in conventional samples in this study. Luckily in organic samples, mint when raw had the highest TFC followed by muhunuwenna>kankung>parsley>gotukola. Whereas the boiled samples, kankung had the highest TFC followed by Mint>parsley>muhunuwenna>gotukola. Regarding conventional samples, mint had the highest TFC followed by parsley>kankung>muhunwenna>gotukola when raw and parsley had the highest TFC when boiled followed by mint>muhunwenna>kankung>gotukola. Yet, heat treated samples contain lower TFC levels compared to raw samples. The reason follows the same as the above mentioned reason for TPC.

It is literally practical enough to understand that as TPC levels are high in samples, it is acceptable that TFC levels are indeed high as they ascend from the same phytochemical category.

As mint occupied higher levels of flavonoid content in organic samples when raw it is sensible that, as TPC results in organic samples were similar to the TFC results for organic samples. Indeed, the correlation of TFC follows the trend of TPC [25].

The change of TFC levels in conventional samples could be due to geographical practices, temperature and climate as well. For an increase of TFC is also due to light, which can increase the TFC synthesis as the amount of light increases [16]. The reason behind an inaccurate result of higher TFC could be also be due to aluminium chloride. Aluminium chloride could have bonded with other compounds and not with flavonoids. This could be a reason for a very high TFC.

Total antioxidant content (TAC) assay was carried out to evaluate the total antioxidant activity present in the samples. In this phosphomolybdate system, Molybdate (Mo) (VI) was converted to Mo (V) by the potential of the antioxidants present in the samples (Shabbir, Khan and Saeed, 2013). The absorbance was measured at 765 nm.

Regarding this study, one-way ANOVA from table 6 and 7 showed there is a significant difference in organic samples p value < 0.05 (p value = 0.007036) and also in conventional samples p value < 0.05 (0.035279) when boiled and raw respectively Organic samples showed higher levels of TAC, especially in raw kankung.

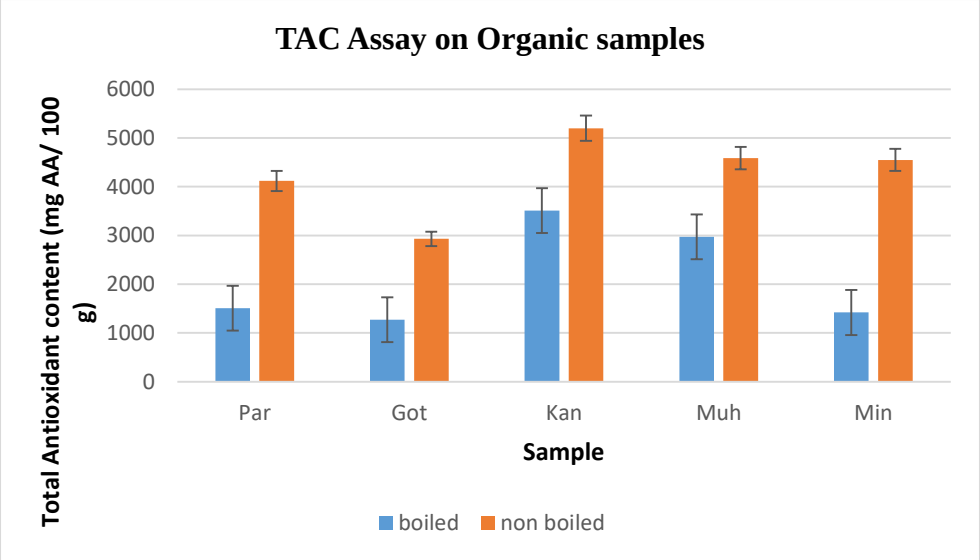


Fig. 15. Total Antioxidant Content (TAC) on boiled and raw organic samples expressed in Ascorbic acid equivalents (AAE).

The raw samples had a higher TAC than in boiled samples. Kankung had the highest TAC when raw and kankung had the highest TAC when boiled (figure 15). The standard error bars showed no overlap among the organic samples of raw and boiled. The single factor ANOVA was constructed for the respective sample to evaluate the significance in TAC between boiled and raw organic samples. ANOVA showed an F-value > Fcrit (F-12.92186, Fcrit-5.317655) and p value < 0.05 (0.007036) showing a significant difference of TAC between raw and boiled organic samples.

Table. 6. Single factor ANOVA evaluation for TAC between 5 different boiled and raw organic samples

Anova: Single Factor

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Boiled	5	10680	2136	1059480		
Raw	5	21390	4278	715870		
ANOVA						
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11470410	1	11470410	12.92186	0.007036	5.317655
Within Groups	7101400	8	887675			
Total	18571810	9				

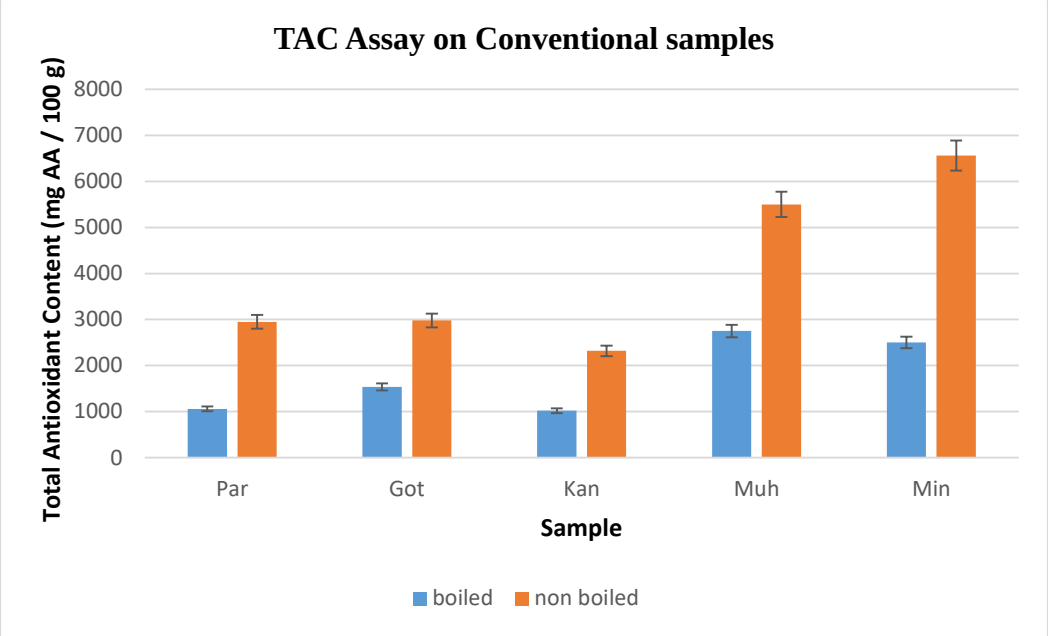


Fig. 16. Total Antioxidant Content (TAC) on boiled and raw conventional samples expressed in AAE.

The raw samples had a higher TAC than in boiled samples. Mint had the highest TAC when raw and muhunuwenna had the highest TAC when boiled (figure 16). The standard error bars showed no overlap among the conventional samples of raw and boiled. The single factor ANOVA was constructed for the respective sample to evaluate the significance in TAC between boiled and raw organic samples. ANOVA showed an F-value > Fcrit (F-6.398748, Fcrit-5.317655) and p value < 0.05 (0.035279) showing a significant difference of TAC between raw and boiled conventional samples.

Table. 7. Single factor ANOVA evaluation for TAC between 5 different boiled and raw conventional samples.

Anova: Single Factor

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Boiled	5	8870	1774	653180		
Raw	5	20310	4062	3437420		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	13087360	1	13087360	6.398748	0.035279	5.317655
Within Groups	16362400	8	2045300			
Total	29449760	9				

This was followed by muhunuwenna>mint>parsley>gotukola. Whereas, when the samples were boiled kankung had the highest TAC followed by, muhunuwenna>parsley>mint>gotukola. In regards to conventional samples with the absence of heat treatment, mint had the highest TAC followed by, muhunuwenna>gotukola>parsley>kankung. Moreover, from the samples that underwent boiling, Muhunwenna contained higher TAC followed by, mint>gotukola>parsley>kankung.

Kankung; water spinach was studied that, it contains higher levels of antioxidants and is still unclear on what components are responsible for a very high antioxidant content in kankung [26]. As organic boiled kankung had higher levels of TPC and TFC, it is sensible that this is the reason behind higher levels of TAC in boiled organic kankung. Conventional raw mint had shown higher levels of TAC similar to conventional raw mint on TFC. This is evident to prove that there is a positive relationship between TFC and TAC. The study from Benabdallah in 2016 [27] determined that mint has a greater correlation with phenols and antioxidants. Yet, it is difficult to analyze TAC on mint, as antioxidants rely on the chemical structure and thereby further studies should be carried out [27]. Raw samples occupied higher TAC, luckily the study of Hwang *et al* in 2012 [21] proved it but yet the samples were different (figure 17).

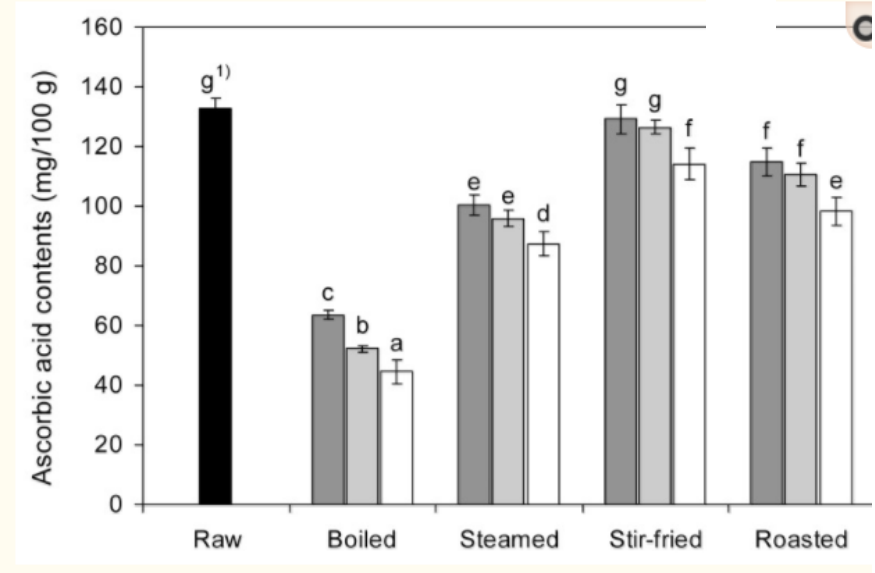


Fig. 17. TAC in raw samples compared to boiled samples [21]

Numerous methods are brought out to assess the radical scavenging effects towards antioxidants.

ABTS assay, also known as the cation radical, was carried out to assess the antioxidant activity in terms of equivalents of ascorbic acid which was absorbed at 734 nm. This was due to the loss of an electron via the nitrogen atom of ABTS. It can be oxidized by either potassium persulphate or manganese dioxide which forms a cation

radical. The bluish green color denotes a successful scavenging activity by ABTS [28]. The following formula was used to calculate the scavenging activity %:

Inhibition% = [(control – sample) / control] x 100%

The results in this study for ABTS were carried out for 50 minutes. In fig 16, boiled Muhunuwenna showed the highest scavenging activity of 86.49 % in 50 minutes and raw Muhunuwenna showed a least scavenging activity of 13.01 % in 50 minutes for conventional samples. Interestingly, boiled mint showed the highest scavenging activity of 98.76 % in 50 minutes and raw Gotukola showed the lowest scavenging activity of 21.93 % in organic samples.

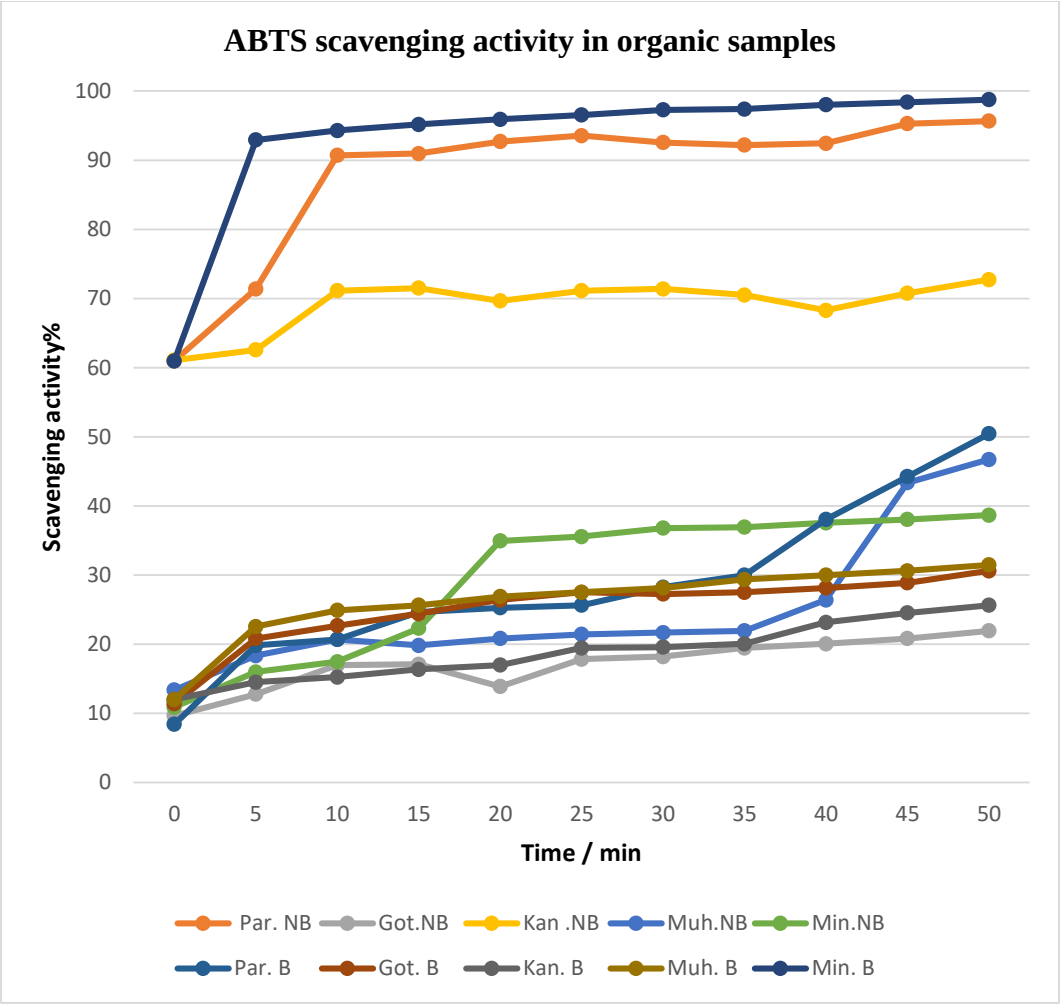


Fig. 18. ABTS scavenging activity among boiled and raw organic samples.

Conferring figure 18, boiled mint leaf extract had the highest ABTS free radical scavenging activity whereas, it took the shortest time to reach the maximum inhibition activity.

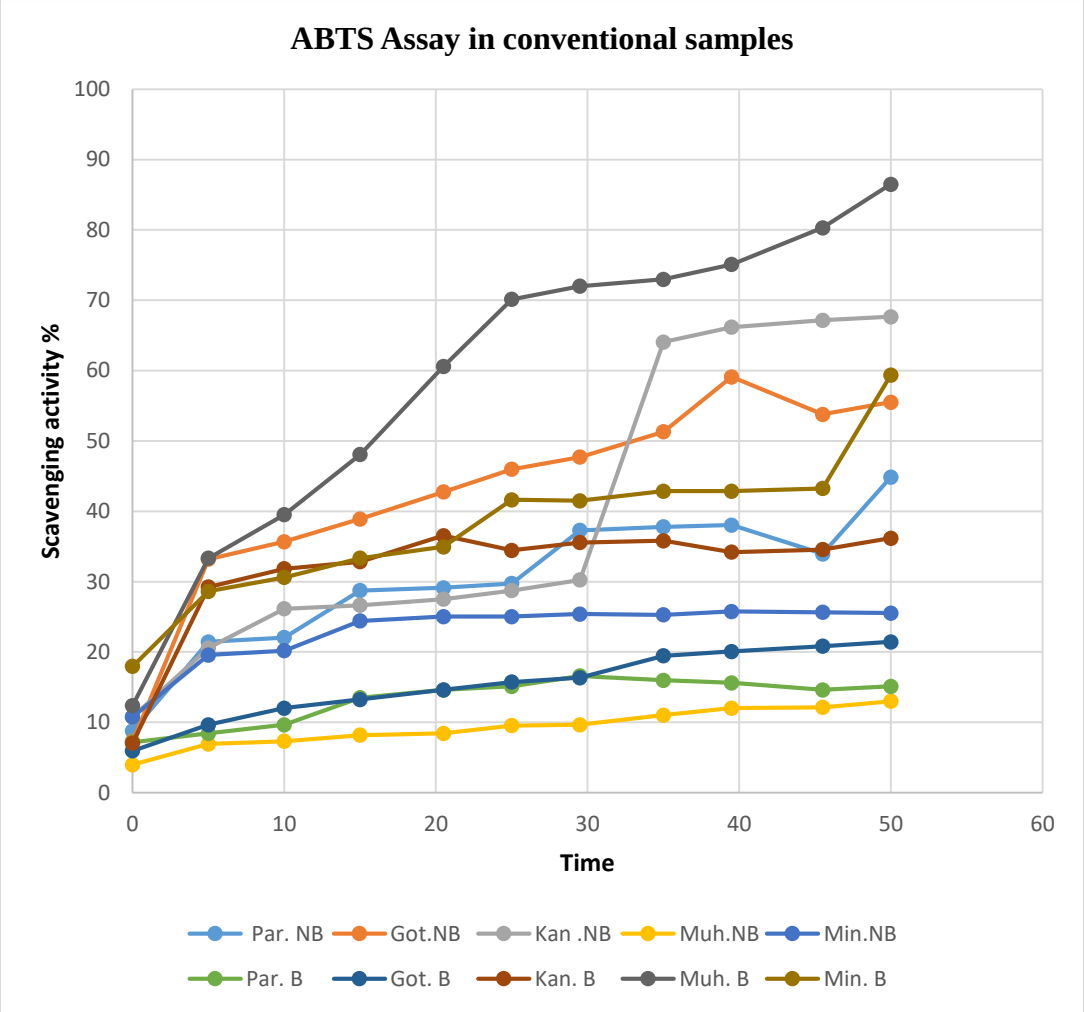


Fig. 19. ABTS scavenging activity among boiled and raw conventional samples.

Conferring fig 19, boiled muhunuwenna had the highest ABTS scavenging activity which took the shortest time period to reach the maximum inhibition activity.

The color change from dark green to colorless proved that the samples had faster scavenging activity of ABTS radicals. This is much more sensible that, as organic mint has higher TFC, TPC and TAC then it is literally acceptable for a higher scavenging activity. Organic samples contain a higher scavenging activity than in conventional samples comparing both figure 18 and 19. Reasons for the inaccurate lower scavenging activity in both organic and conventional could be due to environmental factors and miss practices of geographical studies on these samples. In addition, the reason behind the least scavenging activity could also be due to the compounds present in antioxidants [29].

Inhibitory concentration assay (IC50), is the inhibitory concentration at which ABTS free radicals are being scavenged at 50 % [30]. Lower the IC 50 value indicates a greater capacity to neutralize free radicals [24]. The IC 50 derived from ABTS should inhibit the radical formation by 50 %. In organic samples, raw mint had the highest

antioxidant capacity as it acquired the lowest IC 50 values of 1.7 %, whereas in conventional samples, non -boiled mint had the highest antioxidant capacity as it had the lowest IC50 values of 3.2 % (figure 20 and 21). This proves how strong antioxidant capacity is towards mint [30].

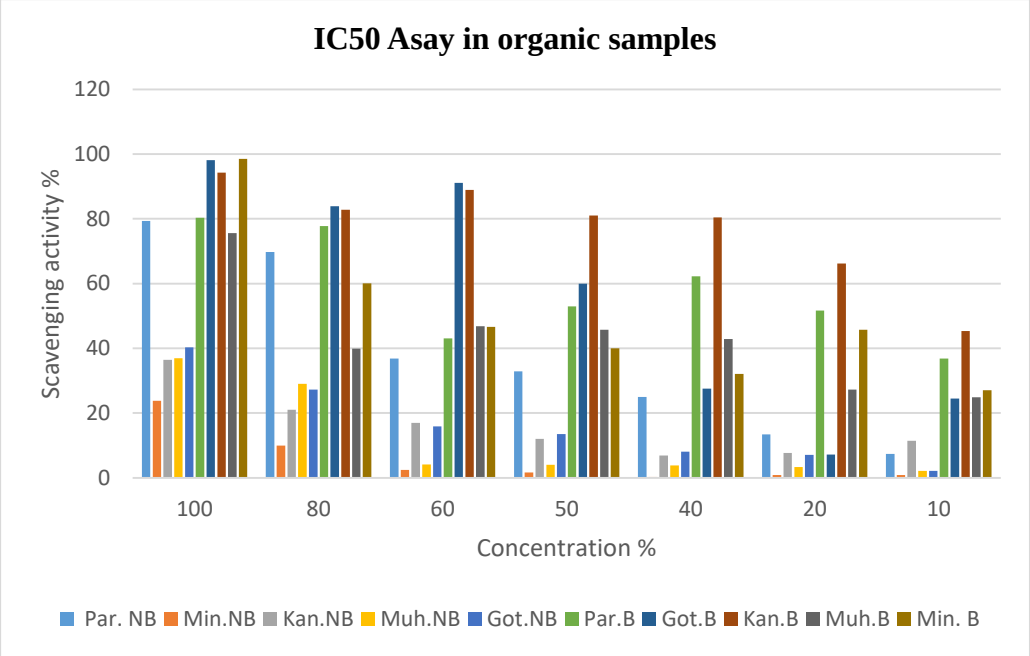


Fig. 20. Inhibitory concentration among boiled and raw organic samples.

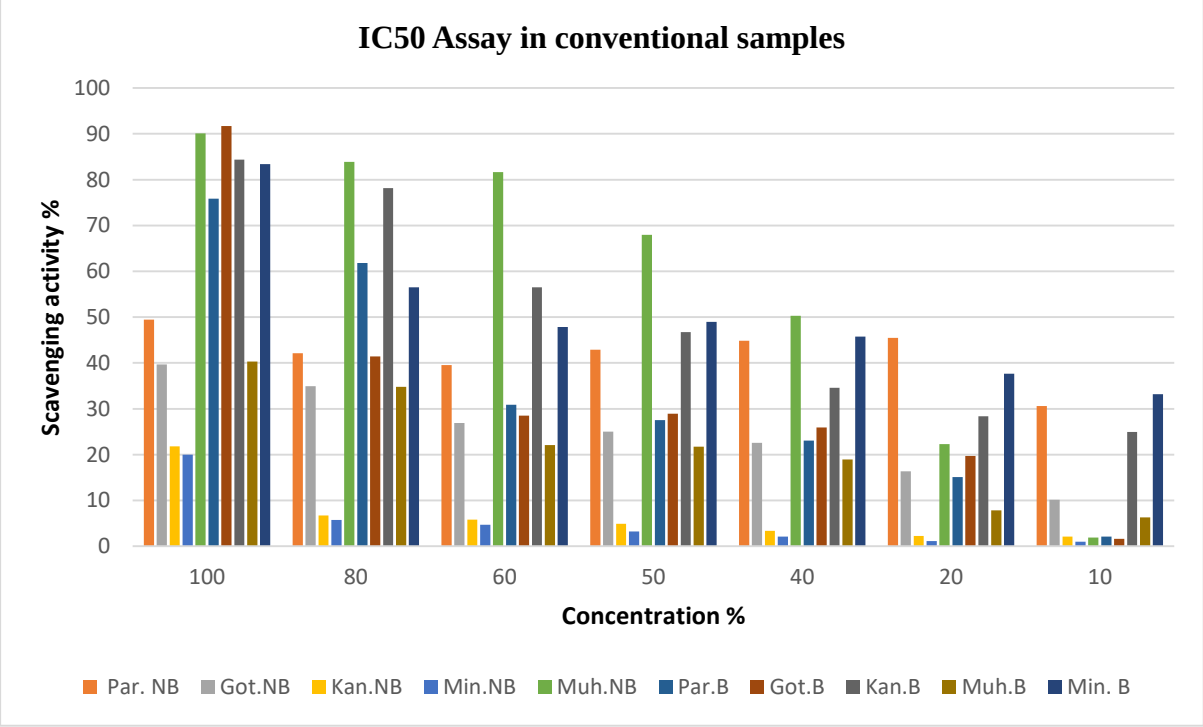


Fig. 21. Inhibitory concentration among boiled and raw conventional samples.

DPPH is one of the most prominent methods used, as it is fast and reliable, there by does not require a reaction device in order to carry out the assay [24]. DPPH; stable radical which never disintegrates in water or ethanol. The DPPH radical is at its highest wavelength of 517 nm, which can easily receive an electron or hydrogen from an antioxidant to stabilize and become a diamagnetic molecule. As it can bind to Hydrogen it is proved that it occupies the capacity to scavenge radicals. The discoloration of DPPH denotes the decrement of DPPH radical quantity [31]. The color transformation is from purple to yellow (Pallab *et al*., 2013). Regarding the results in organic samples, raw parsley had highest scavenging activity of 60.5 % in 5 minutes, whereas, boiled gotukola showed a least scavenging activity of 13.6 % in 5 minutes (figure 22 and 23). Conferring the conventional samples used in this study, raw parsley showed a higher scavenging activity of 62.9 % in 5 minutes, whereas boiled parsley showed least scavenging activity of 17.93 % in 5 minutes.

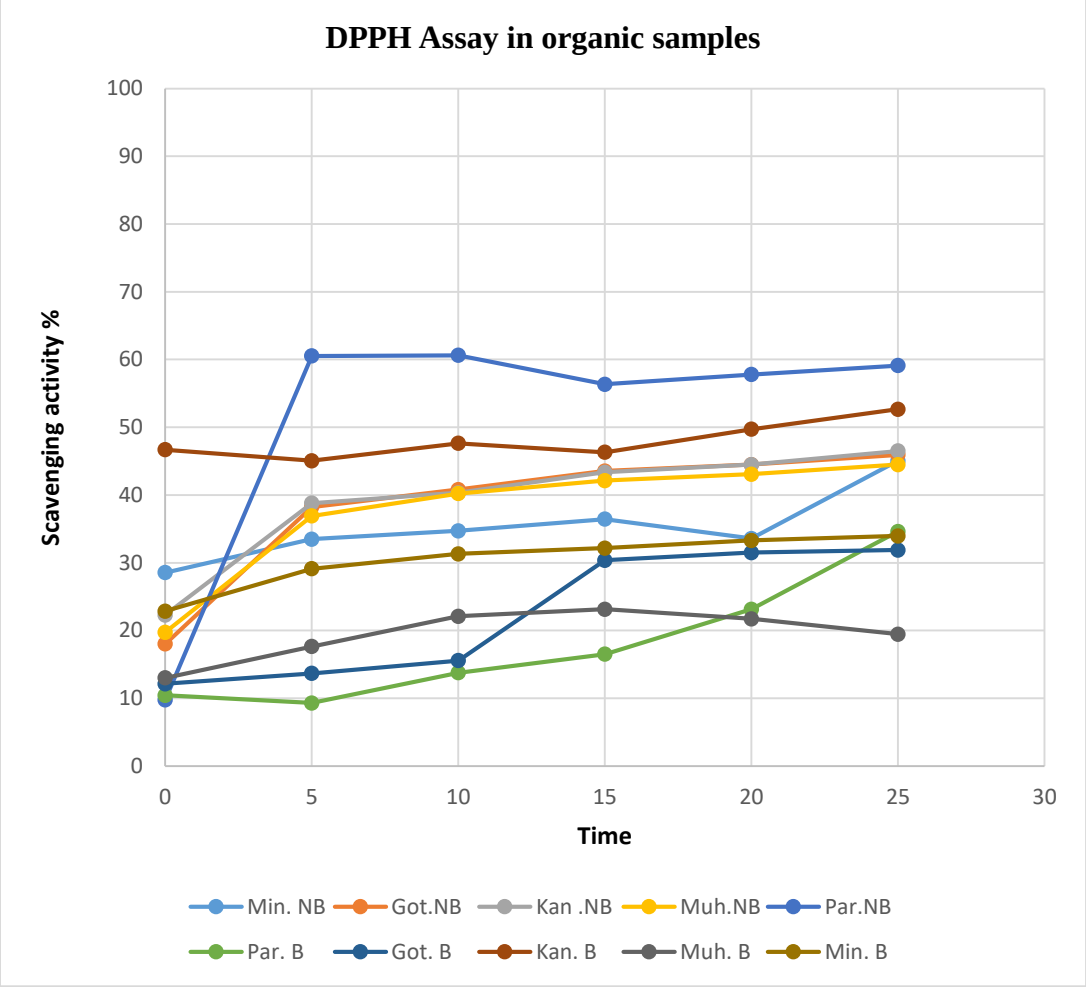


Fig. 22. DPPH scavenging activity among boiled and raw organic samples.

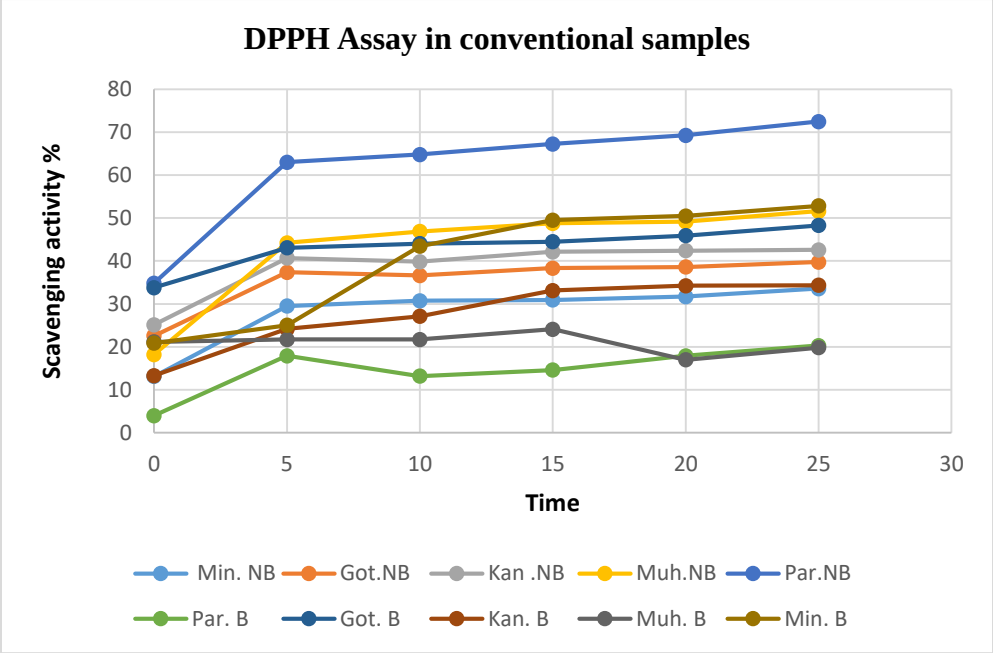


Fig. 23. DPPH scavenging activity among boiled and raw conventional samples.

It was proved in the study of Marin in 2016 [32] that the organic parsley had higher DPPH radical ability.

Overall, this study shows that the raw samples have higher scavenging activity when compared with boiled samples. This is also proved by the study of Hwang *et al*., 2012 [21] in figure 24 that the scavenging activity is literally high in raw samples compared to boiled samples.

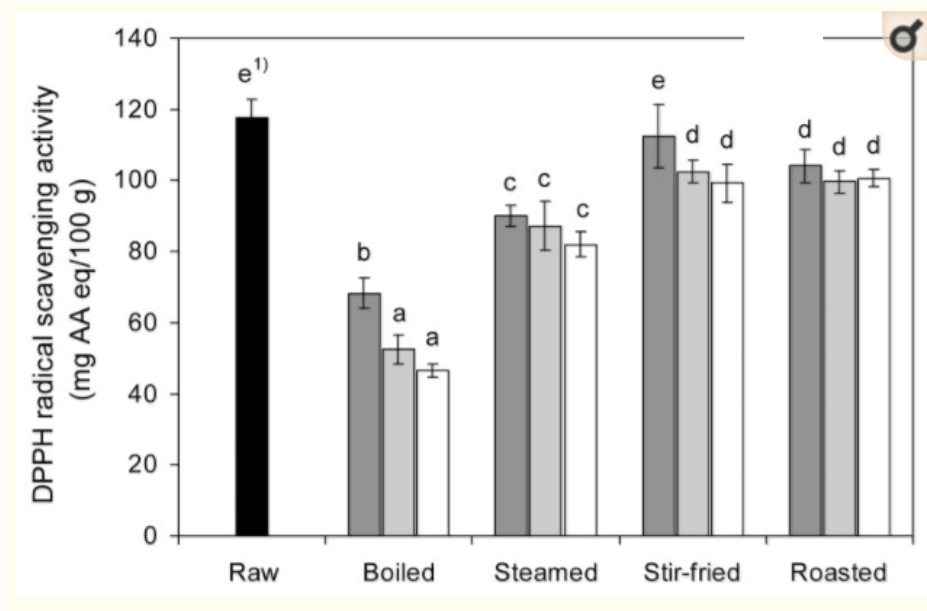


Fig. 24. Determination of DPPH assay on different cooking methods [21].

Ferric reducing antioxidant power method (FRAP), depends on the reduction of the ferric ion-TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine) by antioxidants. As the Fe^{2+} ions bind to the ligand, an extreme navy blue color will be denoted [28]. The above observed results denote that, the highest absorbance activity in 5 minutes for organic samples were, parsley, gotukola, kankung, mint and muhunuwenna, whereas the highest absorbance which reached in 5 minutes for conventional samples were; parsley, gotukola, muhunuwenna, kankung and mint. Yet, the highest reducing power activity in organic samples was evaluated from raw parsley at a frap value of 177.02 mg AAE/100 g at 15 minutes. It was also observed that the lowest ferric reducing power activity was from boiled muhunuwenna with a frap value of 120.8 mg AAE /100 g in 5 minutes. The highest reducing power activity in conventional samples was from raw mint at a frap value of 196.3 mg AAE / 100 g in 15 minutes, whereas the lowest ferric reducing power activity in conventional samples was observed from 100.6 mg AAE/ 100 g in 5 minutes (figure 25 and 26).

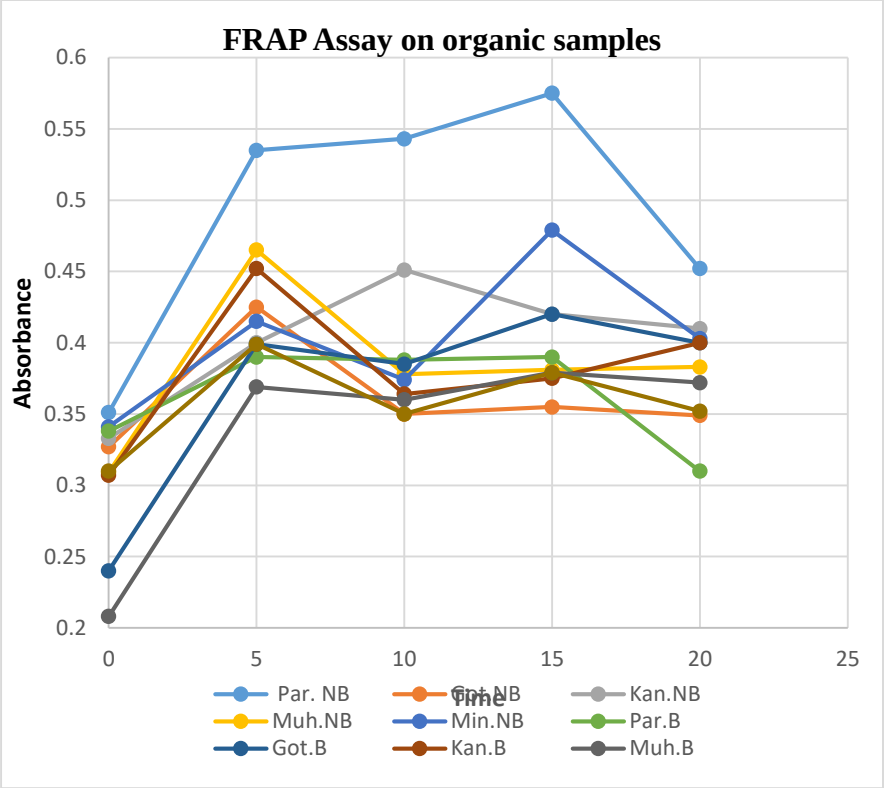


Fig. 25. FRAP for five different boiled and raw organic samples.

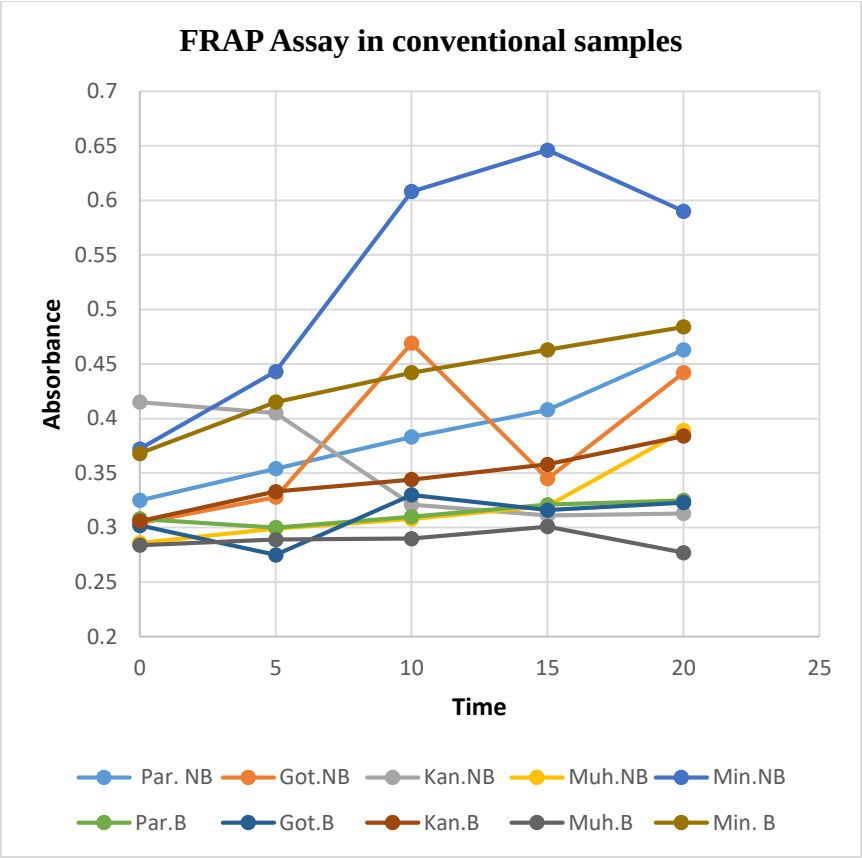


Fig. 26. FRAP for five different boiled and raw conventional samples.

Green leafy vegetables consist a greater amount of compounds which can prevent the occurrence of foodborne diseases by pathogens accumulated from green leafy vegetables [33]. These secondary metabolites with the antimicrobial capacity seek attention on consumer in regards to health [34]. Yet, to be more serious on whether conventional and organic farming vegetables will affect the antimicrobial property is still a question. The antimicrobial activity indeed has a relationship with the phytochemicals, as it is not clear on how they work.

The phenolic compounds occupy a greater ability to alter the microbial cell wall and allow the loss of macromolecules from the microbial cell. Studies have also brought out a theory on that, the phenolic compounds can interfere with the membrane proteins of the invaded microbe and thereby permit loss of functionalization of the cell [34]. The samples showed antimicrobial activity for both *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive).

The mechanism of antimicrobial on leafy vegetables is still not up to clear. Gram positive bacteria are much more susceptible than gram negative bacteria. The gram negative bacteria enclose around the cell wall where diffusion of hydrophobic compounds through the liposaccharide cover are restricted.

Regarding gram positive bacteria, as it lacks a cell wall; the direct allowance of the hydrophobic content of the phospholipid bilayer present in the cell membrane, causes an increase of intracellular leakage [35]. In this study the

antimicrobial capacity was evaluated by the measure of zone of inhibitions observed from each sample *against E.coli* and *Staphylococcus aureus*. The organic samples acquired higher antimicrobial activity against both *Staphylococcus aureus* and *E.coli* than in conventional samples. Mint had the strongest inhibiting capacity in this study against *Staphylococcus aureus* and *E.coli* in organic and conventional samples. Interestingly, as the antioxidant content is literally higher in mint, thereby there could be a greater antimicrobial activity [22]. Mint is highly resistant towards both the bacterial strains. It has the ability to inhibit the bacterial growth. The study of Al-Sum and Al-Arfaj in 2013 [36] proved that mint had the highest inhibitory effect on both *E.coli* and *Staphylococcus aureus.*

A single factor ANOVA was generated which demonstrated that organic samples had a significant difference against *Staphylococcus aureus* p value < 0.05 (0.03957) whereas, conventional samples showed no significant difference against *Staphylococcus aureus* p value <0.05 (0.01282) (table 8 and 9). Conferring the organic samples against *E.coli*, there is no significant difference between organic p value < 0.05 (0.071268) and conventional samples p value > 0.05 (0.204064) (table 10 and 11).

This clearly elaborates from fig 26, 27 and 28 that the raw samples had stronger inhibition ability towards *Staphylococcus aureus* whereas, the similar results were observed against *E.coli* in fig 29, 30 and 31.

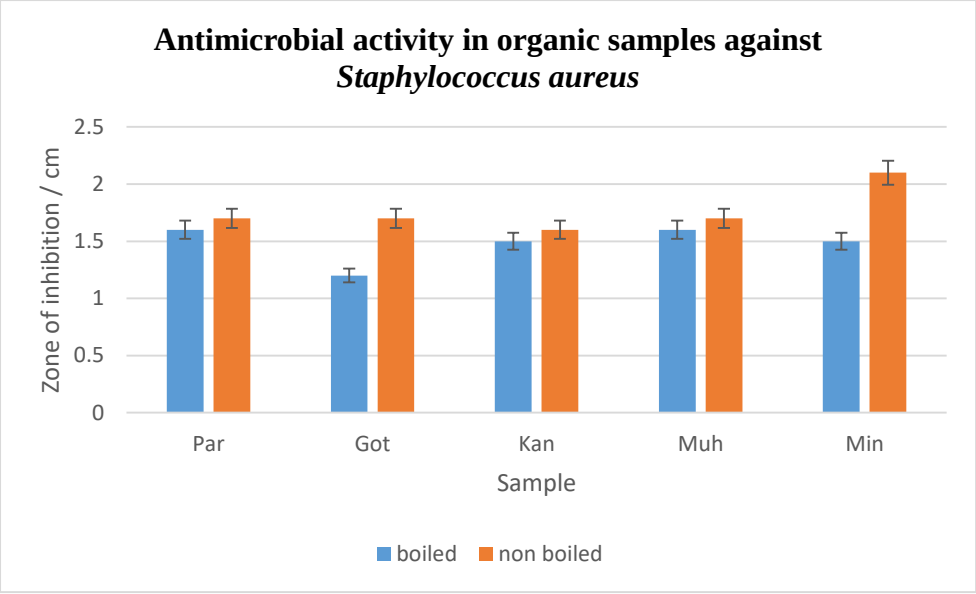


Fig. 26. Antimicrobial activity among boiled and raw organic samples against *Staphylococcus aureus.*

The raw samples showed a stronger ability to inhibit the growth of *Staphylococcus aureus* than the boiled samples. Raw organic mint had the highest zone of inhibition against *Staphylococcus aureus*. The standard error bars showed no overlap in organic gotukola and mint whereas, parsley, kankung and muhunuwenna showed an overlap. The single factor ANOVA was constructed for the respective sample to evaluate the significance of antimicrobial activity between boiled and raw organic samples. ANOVA showed an F-value > Fcrit (F-6.030769, Fcrit-

5.317655) and p value < 0.05 (0.039579) showing a significant difference of antimicrobial activity between raw and boiled organic samples.

Table. 8. Single factor ANOVA evaluation among boiled and raw organic samples against *Staphylococcus aureus.*

Anova: Single Factor

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Boiled	5	7.4	1.48	0.027		
Raw	5	8.8	1.76	0.038		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.196	1	0.196	6.030769	0.039579	5.317655
Within Groups	0.26	8	0.0325			
Total	0.456	9				

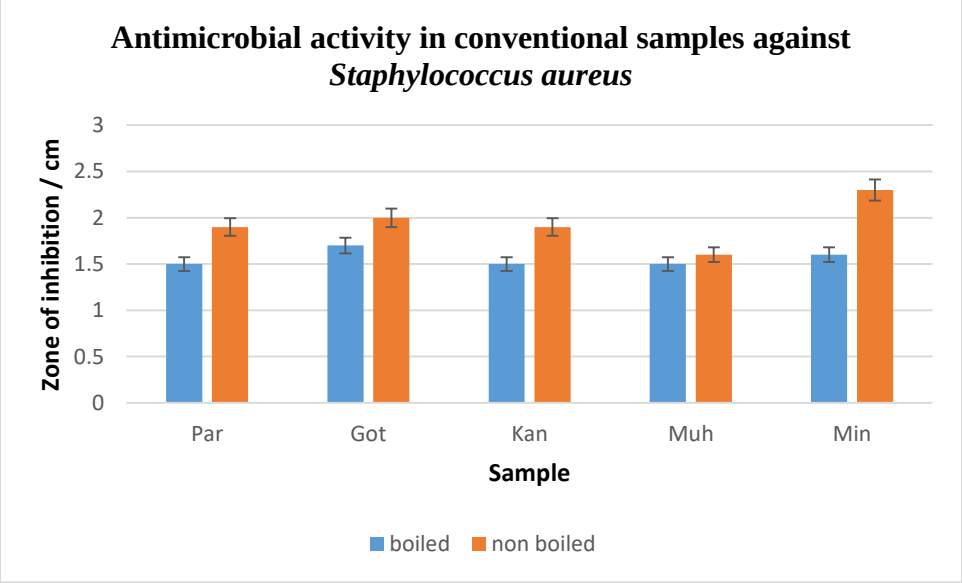


Fig. 26. Antimicrobial activity among boiled and raw conventional samples against *Staphylococcus aureus.*

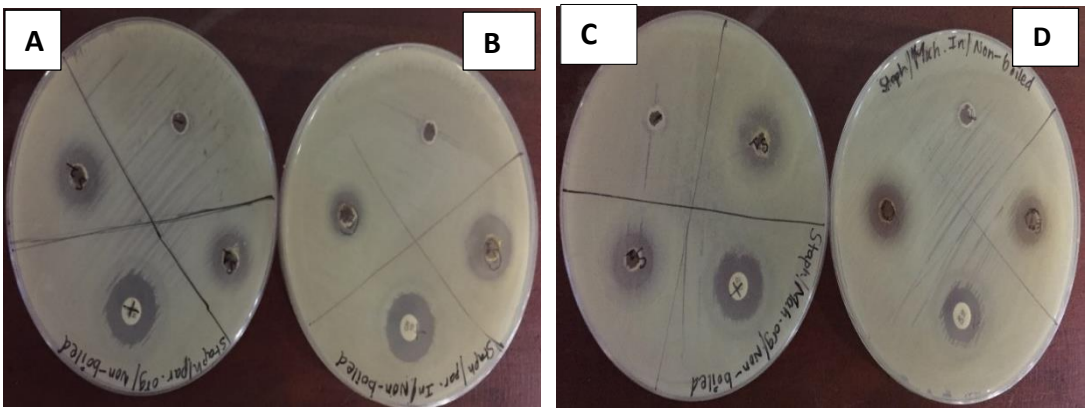
The raw samples showed a stronger ability to inhibit the growth of *Staphylococcus aureus* than the boiled samples. Raw conventional mint had the highest zone of inhibition against *Staphylococcus aureus*. The standard error bars showed no overlap in conventional mint, parsley, gotukola and kankung samples whereas, muhunuwenna showed

an overlap. The single factor ANOVA was constructed for the respective sample to evaluate the significance of antimicrobial activity between boiled and raw organic samples. ANOVA showed an F-value > Fcrit (F- 10.16901, Fcrit-5.317655) and p value < 0.05 (0.012825) showing a significant difference of antimicrobial activity between raw and boiled conventional samples.

Table. 9. Single factor ANOVA evaluation among boiled and raw conventional samples against Staphylococcus aureus.

Anova: Single Factor

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Boiled	5	7.8	1.56	0.008		
Raw	5	9.7	1.94	0.063		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.361	1	0.361	10.16901	0.012825	5.317655
Within Groups	0.284	8	0.0355			
Total	0.645	9				



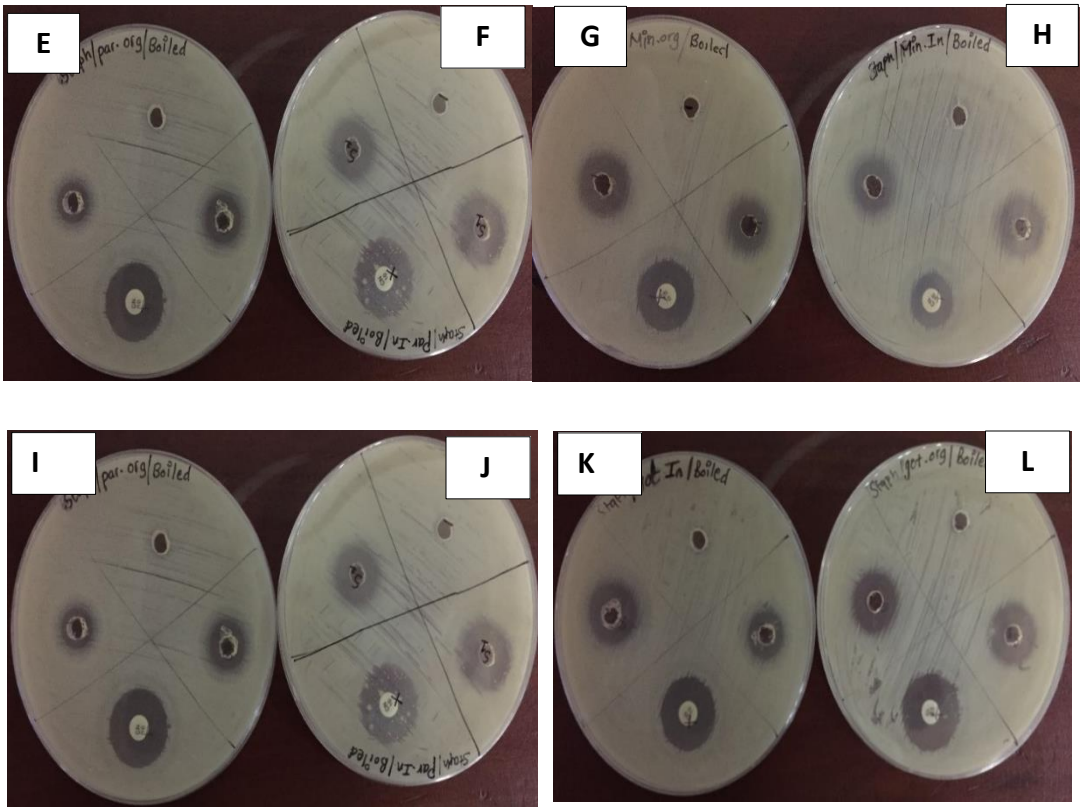


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Fig. 27. Zone of inhibition against *Staphylococcus aureus* for boiled and raw samples.

A. Organic raw parsley **B.** Conventional raw parsley **C.** Raw organic muhunuwenna **D.** Raw conventional muhunuwenna **E**. Boiled organic kankung **F.** Boiled conventional kankung **G.** Boiled organic parsley **H**. Boiled conventional parsley **I.** Boiled organic mint **J.** conventional boiled mint **K**. Boiled conventional gotukola **L**. Boiled organic gotukola.

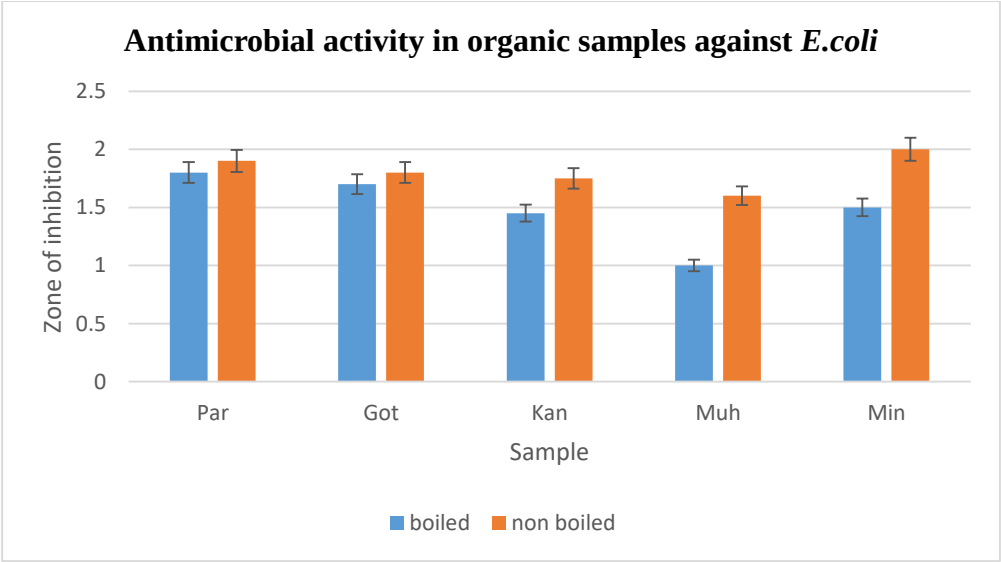


Fig. 29. Antimicrobial activity among boiled and raw organic samples against *Escherichia coli.*

The raw samples showed a stronger ability to inhibit the growth of *Escherichia coli* than the boiled samples. Raw organic mint had the highest zone of inhibition against *Escherichia coli*. The standard error bars for organic kankung, muhunuwenna and mint showed no overlapping however parsley and gotukola showed overlapping between samples. The single factor ANOVA was constructed to evaluate the significance of antimicrobial activity among boiled and raw organic samples against *Escherichia coli*. ANOVA showed an F-value < Fcrit (F- 4.320675, Fcrit-5.317655) and p value >0.05 (0.071268) showing a significant difference of antimicrobial activity between raw and boiled organic samples.

Table. 10. Single factor ANOVA evaluation among boiled and raw conventional samples against *Escherichia coli.*

Anova: Single Factor

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Boiled	5	7.45	1.49	0.0955		
Raw	5	9.05	1.81	0.023		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.256	1	0.256	4.320675	0.071268	5.317655
Within Groups	0.474	8	0.05925			
Total	0.73	9				

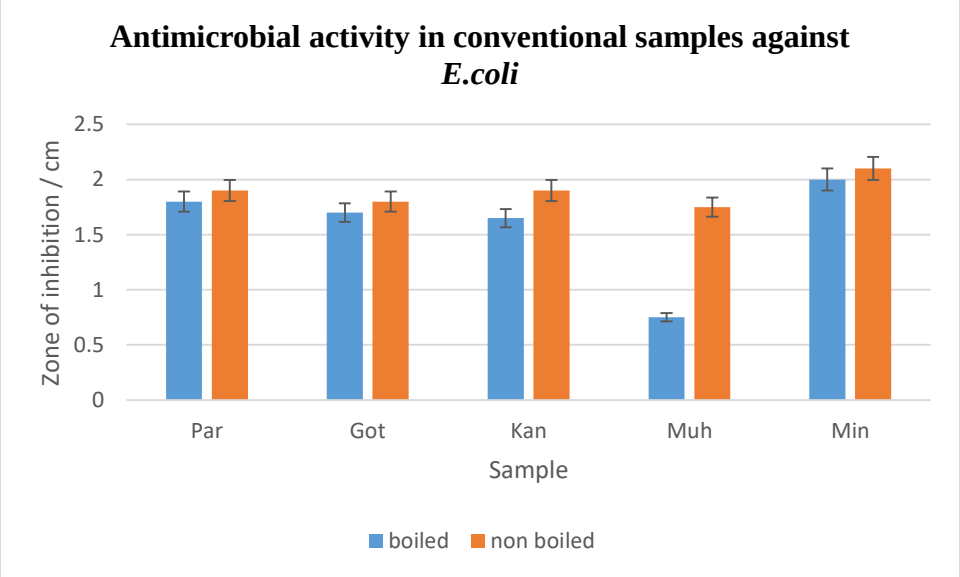


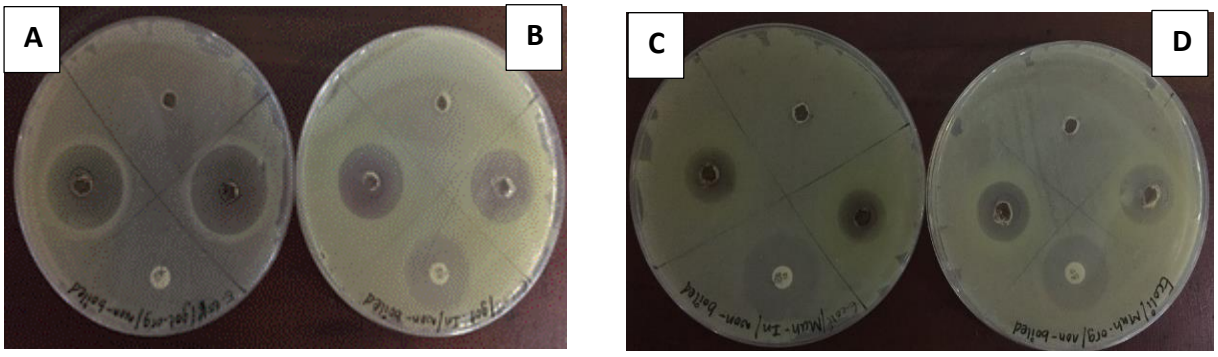
Fig. 30. Antimicrobial activity among boiled and raw conventional samples against *Escherichia coli.*

The raw samples showed a stronger ability to inhibit the growth of *Escherichia coli* than the boiled samples. Raw conventional mint had the highest zone of inhibition against *Escherichia coli.*

The standard error bars for conventional kankung and muhunuwenna showed no overlapping, however parsley gotukola and mint showed overlapping between samples. The single factor ANOVA was constructed to evaluate the significance of antimicrobial activity among boiled and raw conventional samples against *Escherichia coli.* ANOVA showed an F-value < Fcrit (F- 1.912438, Fcrit-5.317655) and p value >0.05 (0.204064) showing a significant difference of antimicrobial activity between raw and boiled conventional samples.

Table. 11. Single factor ANOVA evaluation among boiled and raw conventional samples against *Escherichia coli.*

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Boiled	5	7.9	1.58	0.23325		
Raw	5	9.45	1.89	0.018		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.24025	1	0.24025	1.912438	0.204064	5.317655
Within Groups	1.005	8	0.125625			
Total	1.24525	9				



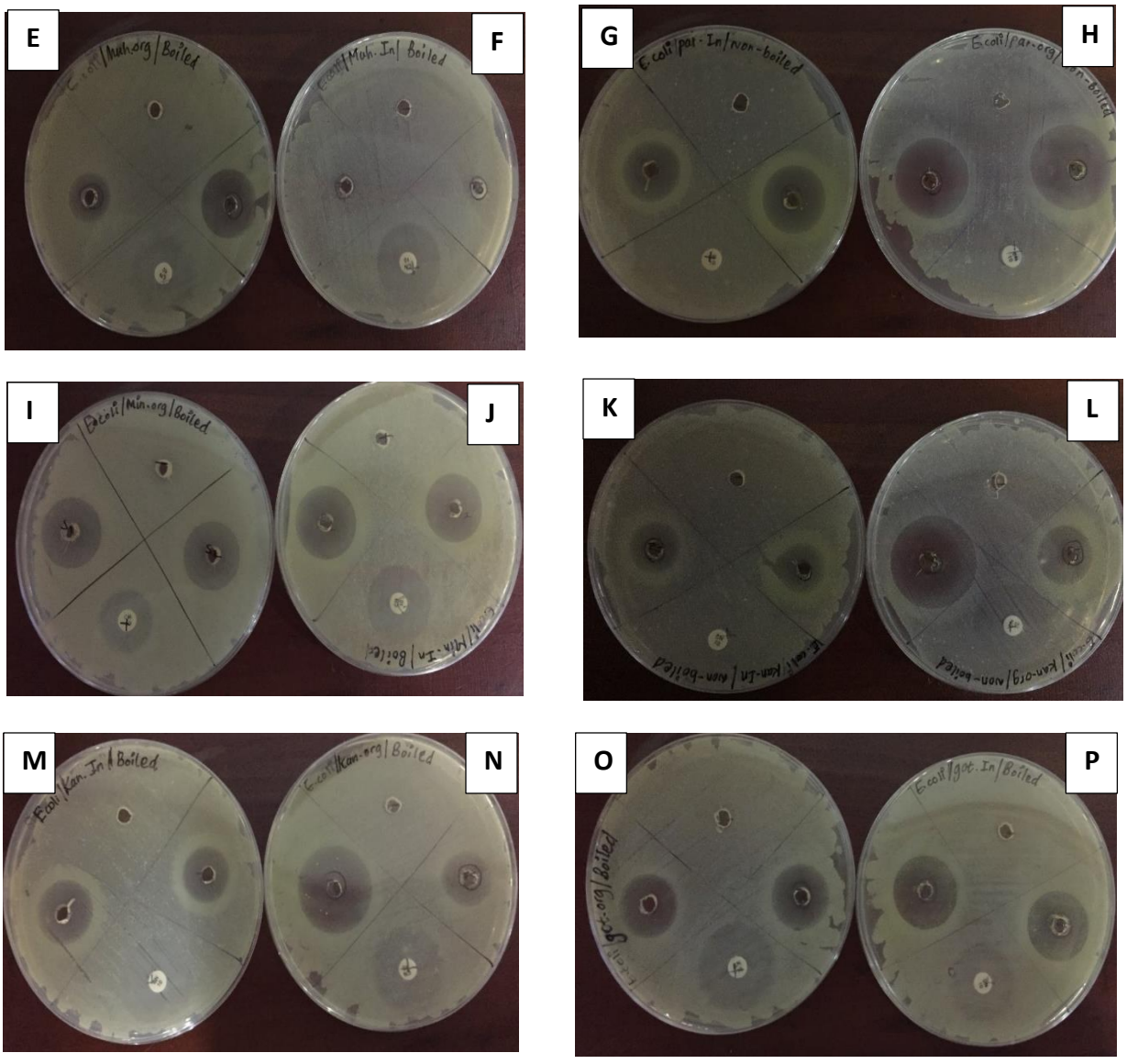


Fig. 31. Zone of inhibition against *Escherichia coli* for boiled and raw samples.

A. Conventional raw parsley **B**. Organic raw parsley **C.** Raw organic gotukola **D**. Raw conventional gotukola **E**. Raw conventional muhunuwenna **F**. Raw organic mhunuwenna **G**. Raw conventional kankung **H**. Raw organic kankung **I**. Boiled organic gotukola **J.** Boiled conventional gotukola **K**. Boiled conventional kankung **L**. Boiled organic kankung **M.** Boiled organic mint **N**. Boiled conventional mint **O.** Boiled organic muhunuwenna **P**. Boiled conventional Muhunuwenna.

The scatter plot showed a R^2 value of 0.549 in between TFC and TPC assay whereas; between TPC and TAC the R^2 value was 0.763. The R^2 value observed between TAC and TFC was 0.454. In addition to this, The R^2 value in

between ABTS and DPPH was 0.765. In between ABTS and FRAP, The R^2 value was 0.905. This has a higher correlation between both the assays. By comparing with DPPH and FRAP, the R^2 value was 0.831. The correlation between TPC and TFC, TPC and TAC, TAC and TFC, ABTS and DPPH, ABTS and FRAP and DPPH and FRAP were illustrated in fig 31.

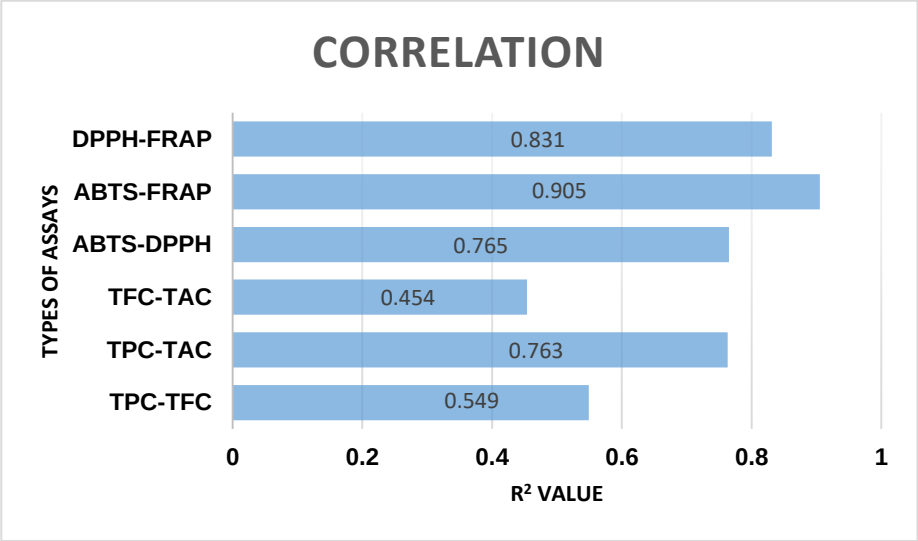


Fig. 32. Correlation between various assays.

CONCLUSION

It is concluded that observations towards the samples showed that the raw samples occupy a greater antioxidant content than boiled samples which underwent heat treatment. Regarding the samples, organic and conventional mint has the highest antioxidant capacity which was determined by all the antioxidant assays and indeed by the radical scavenging assays. In regards to nitrate content, it was observed that the conventional samples showed a higher nitrate content. As antioxidant content is relatively higher, that is much more sensible to prove that the samples should occupy an antimicrobial activity. Well diffusion technique provided a righteous result in between both *Staphylococcus aureus* and *Escherichia coli*. Comparison of graphical illustration on antimicrobial activity showed that, all the sample extracts could inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* but mint had the highest antimicrobial activity among all the sample extracts.

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