**GLOBAL ACADEMIC RESEARCH INSTITUTE**

COLOMBO, SRI LANKA


**GARI International Journal of Multidisciplinary Research**

ISSN 2659-2193

**Volume: 02 | Issue: 02**

On 30th June 2016

http://www.research.lk

Author: L.G.P. Perera, Dr. Mathivathani Kandiah

BMS, Sri Lanka

GARI Publisher | Food Science | Volume: 02 | Issue: 02

Article ID: IN/GARI/ICHM/2016/256 | Pages: 23-52 (29)

ISSN 2659-2193 | Edit: GARI Editorial Team

Received: 20.03.2016 | Publish: 30.06.2016

# ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF CASHEW APPLES AND CASHEW NUTS IN SRI LANKA

[1] Perera L.G.P. , [2] Dr. Mathivathani Kandiah

[1,2] *School of Science, BMS, 591, Galle Road, Colombo 06. Sri Lanka*

[2]*mkandiah@gmail.com*

***ABSTRACT***

*Fruits are known to be rich sources of antioxidants that ail in the inhibition of radical mediated pathologic conditions while acting in the defense mechanisms against pathogenic microorganisms. The study was conducted to determine the antioxidant and antimicrobial potential of cashew apples and cashew nuts in Sri Lanka. Six samples of cashew including two cashew apple (early stage and late stage) extracts and four cashew nut (early stage, late stage, roasted and raw) extracts were subjected to five assays, total antioxidant capacity assay (TAC), total phenol content (TPC), total flavonoid content (TFC), Ferric Reducing Antioxidant Power (FRAP) assay and ABTS free radical scavenging assay in order to determine the antioxidant capacity. Hence the samples were introduced to two bacteria, Staphylococcus aureus and Escherichia coli in order to analyze the zone of inhibitions using well diffusion technique. Results from the five antioxidant assays exhibited high values for early and late cashew apple extracts when low values were obtained for cashew nut extracts. Both of bacteria strains displayed zone of inhibitions to all samples thus large zone of inhibitions were observed when the bacteria exposed to cashew apple extracts. All the results from antioxidant assays and antibacterial experiment were analyzed using single factor ANOVA to determine the significant differences between the results. Based on the results of the antioxidant and antimicrobial experiments it was concluded that cashew apples are more effective as antimicrobial activities consisting a higher antioxidant potential which can be used as a therapeutic component in the radically induced pathogenesis.Keywords: Cashew, Antioxidant, Antimicrobial, Nuts, Reducing Power*

## INTRODUCTION

Antioxidants are bioactive compounds which act as protective factors against most of deleterious effects and oxidative stress induced diseases (Pineli et al., 2011). The substances react with free radicals such as peroxide radicals (ROO•), superoxide radicals (O2•−) and hydroxyl radicals (•OH) in chain reaction mechanisms and convert them to non-reactive compounds under normal conditions (Jan et al., 2013). Therefore the propagation of the chain reaction mechanism is inhibited minimizing cellular damage (Hamad and Mubofu, 2015).

Plant based foods, vegetables and fruits supply vitamins and minerals to the diet while it possess high amounts of natural phytochemicals in different capacities that act as antioxidants, anti inflammatory agents and phytoestrogens in protective mechanisms (Slavin and Lloyd, 2012 and Wang et al., 2011). Moreover numerous studies based on the analysis of bioactive components in natural products have revealed the high levels of antioxidants present in fruits. Therefore the consumption of antioxidant rich foods have shown to enhance health benefits while reducing risk of having diseases such as cancer, cardiovascular diseases and liver diseases. Antioxidant activity has been identified to be associated with the mechanisms and reactions of phenolics, flavonoids, and anthocyanins in vegetables and fruits (Sun et al., 2002).

Cashew apples and cashew kernels are known to be rich sources of antioxidants, vitamins and minerals (Desai et al., 2010). The cashew kernals are known to be mostly consumed apart from other products of cashew including cashew apple and cashew nut shell liquid (CNSL).

The cashew apple (the peduncle) is commercially available as the whole fruit, juice, nectar and frozen pulp. Cashew apple contain high amounts of carbohydrates, minerals, organic acids, ascorbic acids and phenolic compounds while it has been identified that fresh juice is highly rich in ascorbic acid 5x higher than orange, carotenoids, flavonoids, phenolic acids, anacardic acids and tannins (Assunção and Mercadante, 2003; Kubo et al., 2006; Trevisan et al., 2006 and Michodjehoun-Mestres et al., 2009).

Harvested cashew nuts are processed using several steps in order to be available for the general consumption. Firstly cashew nut is dried in sun to remove excess water and roasted. The nut shell is removed by cutting and remaining kernel is heated. Then the kernel is blanched and graded based on the appearance and size. Different grades of cashew nuts are commercially available. The cashew nut consists of oil (47.0 %), carbohydrates (22.0 %), protein (21.0 %), moisture (5.9 %), vitamins (pyridoxine B-6, E) and minerals (Desai et al., 2010). Furthermore the cashew nut possesses fatty acids in the ratio of saturated: monosaturated :polystaurated to 1:2:1 which is ideal for normal humans beneficial for patients with low HDL (High Density Lipoprotein) (Athukorale, 2004).

CNSL is derived from cashew nut shell, which is eliminated as a waste product during cashew processing. CNSL is commercially available as a viscous, dark liquid which includes saturated and unsaturated long-chain phenols: cardanols, cardols, phytosterol, triacontanes and anacardic acids (Andrade et al., 2011). Moreover several studies on the chemical

properties and effects of the CNSL have reported the effectiveness of the extract as an antioxidant and antimicrobial agent.

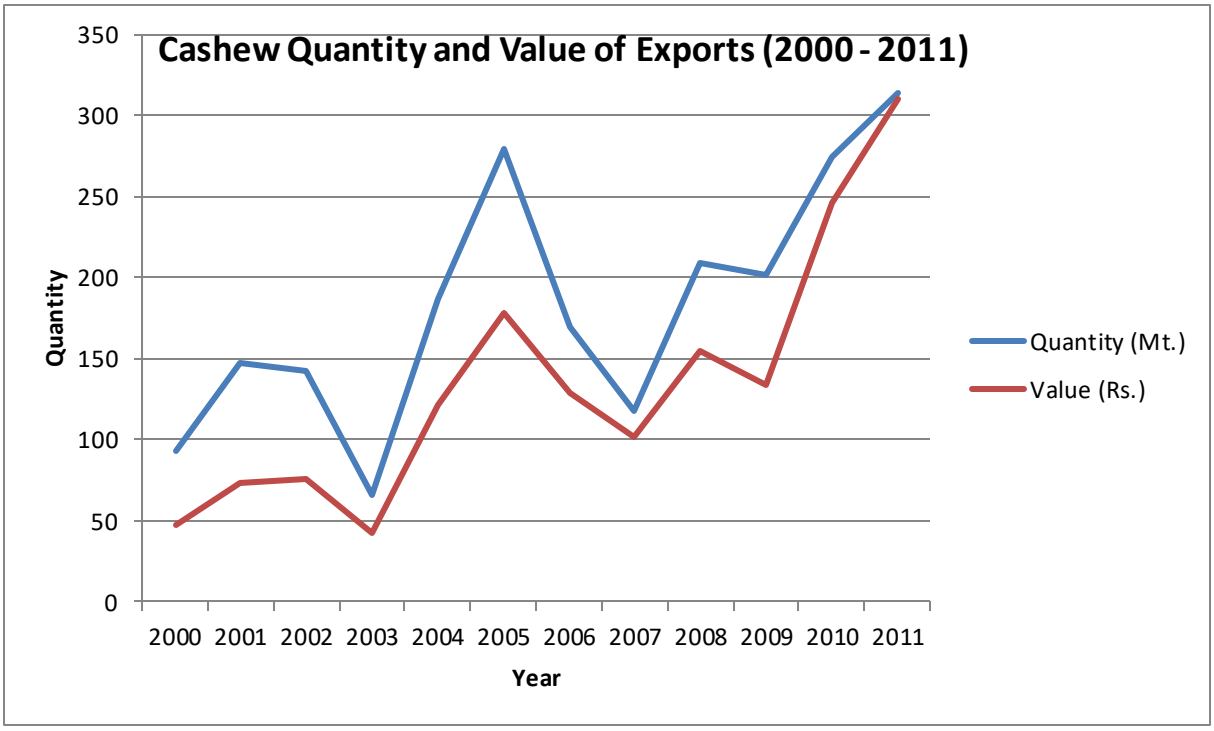
Cashew trees (Anacardium occidentale L.) are originally native from Brazil thus grows in the tropical and subtropical countries in Central America, Africa, South Asia and South East Asia. The A. occidentale plant has many applications apart from use as a food source. As an example leaves, roots, bark and stem are used for the treatments of skin infections, allergy, diarrhea, cough and stomach ache while resins are used to produce natural insectisides (Cavalcante et al., 2003 and Chabi Sika et al., 2013).

Brazil, India, Tanzania, Nigeria and Vietnam are known to be the main processing areas of cashew when the cashew industry was ranked as the 3rd largest edible nut producing industry in the world after almonds and hazelnuts (Queiroz et al., 2011).

According to the available data from the International Nut Council (INC), the global cashew consumption of the year 2014 was more than 710,000 tonnes thus India becoming the top consumer, by consuming more than 240000 tonnes that year covering more than 25 percent of the global consumption (Nair, 2016).

Sri Lanka has an increasing market for cashew nuts internationally due to the quality of the cashew kernel. (Figure 1) Cashew was introduced in to Sri Lanka from Brazil during the 16th century and mostly found in the dry regions of Sri Lanka including Puttalam, Hambanthota, Kurunegala and Mannar. Additionally, 8 varieties of cashews: WUCC1, WUCC5, WUCC8, WUCC9, WUCC13, WUCC19, WUCC21, WUCC23 can be found in Sri Lanka which have distinguished focusing on the size, colour and shelling percentage (Surendra, 2011).

**Table 1**: Export quantity of Cashew nuts with the elevation of kernel value of one kg during year 2000-2011 (Surendra, 2011).




Moreover a study based on the alkyl phenols in cashew products and their antioxidant capacity has revealed that the highest amount of main alkyl phenols and anacardic acids were found in CNSL while least amounts were found in roasted cashew nut (Trevisan et al., 2006).

The most common assays used to detect the antioxidant contents and activity of the natural products include2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, FRAP (ferric reducing antioxidant power) assay, 2,20-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS) assay and Total antioxidant activity assays. Moreover total phenolic assays and flavonoids assays can be carried out to measure the available antioxidant compounds.

Antimicrobial agents (antibiotic, antiviral, antifungal, and antiparasitic agents) act inhibitive chemical compounds against growth and survival of microorganisms. Natural products of fruits and vegetables are long known to contain chemical compounds which act as antimicrobial agents against pathogenic microorganisms and are known to expand the shelf life of the product during the natural state. Additionally many studies are being carried out to determine the effects of antimicrobials in natural products in order to develop drugs which are effective at low concentrations and non-toxic (Davidson, Critzer and Taylor, 2013). Furthermore, number of studies based on the effects of the cashew derivatives has reported the antimicrobial effects of cashew (Gonçalves and Gobbo, 2012).

This study was based on the determination of the effects of antioxidants and antimicrobials in cashew apples and cashew nuts in Sri Lanka.

## 2. MATERIALS

### 2.1 Sample selection

Early and late stage cashew apples and nuts were harvested from a selected cashew tree in Mahawewa, Sri Lanka and roasted and raw cashews were purchased at a local grocery store in Wennappuwa, Sri Lanka which used for analysis.

### 2.2 Chemicals and reagents

Methanol (CH3OH) (CAS-67-56-1), Hydrochloric acid, Sodium carbonate (Na2CO3) (CAS-497-19-8), Sulfuric acid (H2SO4) (CAS-7664-93-9), Sodium sulfate (Na2SO4) (CAS- 7757-82-6)., Ammonium molybdate ([NH4]6Mo7O24.4H2O) (CAS-12054-85-2), Ferric chloride, Sodium acetate, Glacial acid, Sodium nitrate, Aluminum Chloride (AlCl3) (CAS-7446-70-0), Sodium hydroxide, Ammonium persulfate ((NH4)2S2O8) (CAS-7727-54-0), Folin-Ciocalteu phenol reagent, 2,4,6,-tri(2-pycridyl)-s-triazine reagent, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) (C18H18N4O6S4) (CAS-30931-67-0), Mueller-hinton agar powder, nutrient agar powder, saline, McFarland

### 2.3 Standard Curves

Ascorbic acid standard, Gallic acid standard, Quercetin standard (See Appendix).

### 2.4 Apparatus

Consumables: Micropipettes (100µl, 1000µl) Glass beakers, measuring cylinders, tea filter, filter funnels, centrifuge tubes (15 ml and 50 ml), watch glasses, mortar and pestle, tripod, spatula, gauze, Whatman no.1 filter paper, sterile pipette tips, sterile eppendorf tubes, cuvettes, aluminium foil, inoculation loop, petri plates (Glass and platic), test tubes, conical flasks (250 ml).

Equipment: Spectrophotometer (JENWAY 6305), fume hood (BIOBASE FH1000), hot air oven (meditry DHA-9053A), food processor, bench top centrifuge, electronic balance, refrigerator, autoclave

## 3. METHODOLOGY

### 3.1 Sample extraction

#### 3.1.1 Extraction of the cashew apples

A weight of 40 g of cashew apples were cut into small pieces and blended for two minutes. The pulp was further grinded by motor and pestle. The pulp was filtered using a tea filter into a beaker. The solution was kept overnight at 40 0C to evaporate excess water. 5 g of the cashew apple dry powder was added into a 50 ml falcon tube followed by adding 50 ml of 80% ethanol. The solution was kept in the roller mixer at a medium rolling speed for 24 hours to mix the contents with ethanol. The mixed solution was centrifuged at 4000 rpm for 15 minutes. The supernatant was separated and filtered using a Whatman No.1 filter paper and the filtered solution was stored in the refrigerator at -20 0C for further use.

#### 3.1.2 Extraction of the cashew nuts

The nut testa was removed by cutting and the kernels were allowed to air dry for two days. The weights of the cashew kernels were measured and grinded to obtain cashew powder. The powder was added to a 50 ml falcon tube and topped it up to 50 ml by adding 80% ethanol. The mixture was kept in the roller mixer for 24 hours and then centrifuged at 4000 rpm for 15 minutes. The supernatant was carefully separated and filtered using a Whatman No.1 filter paper. Then the filtered solution was stored in the refrigerator at -200C for further use.

### 3.2 Sample preparation for assays

Volumes of 10 ml from each extracted samples were transferred into 50 ml falcon tubes and equal amount of 80% ethanol was added. The prepared solutions were used for the assays.

### 3.3 Determination of Total Phenolic Content (TPC)

Volumes of 1 ml of deionized water and 0.5 ml of FC reagent were added to 50 µl of sample and kept at room temperature for 3 minutes. Then 2.5 ml of 20% sodium carbonate was added to the mixture and incubated for 1 hour at room temperature in the dark. Absorbance was measured at 765 nm with a methanol blank. The phenolic concentration was denoted in mgGAE/100 g.

### 3.4 Determination of Total Flavonoid Content (TFC)

Aluminum chloride colorimetric technique was used to determine the flavonoid content. A solution was prepared by adding 3.4 ml of 30% ethanol, 150µl of 0.5 M sodium nitrate and 150 µl of 0.3 M aluminum chloride followed by adding 300 µl of sample extract. The solution was incubated at room temperature in the dark for 5 minutes. A volume of 1 ml of 1M sodium hydroxide was added to the solution and the absorbance was measured at 506 nm. A methanol blank was used for calibration. The concentration was expressed in mgQE/100g.

### 3.5 Determination of Total Antioxidant Capacity (TAC)

The protocol used for TAC was adapted from Ahmed, Khan and Saeed (2015). A volume of 3ml from the sample was added to 1 ml of reagent solution prepared by adding equal volumes of 0.6 M sulfuric acid: 28 mM sodium sulfate: 4 mM ammonium molybdate, in 1:1:1 ratio. The mixture was covered in aluminum foil and incubated at 95 0C for 90 minutes in a hot air oven. A methanol blank was used for calibration and the absorbance was measure at 765 nm. The TPC concentration was denoted in mgAAE/100g based on the calibration curve.

### 3.6 Determination of Ferric Reducing Antioxidant Potential (FRAP)

The protocol for the determination of FRAP for cashew samples were adapted from Benzie and Strain (1996). A volume of 25 ml of 300 mM acetate buffer at pH 3.6 was mixed with 2.5 ml of 30mM ferric chloride and 2.5 ml of 10 mM TPTZ solution in 40 mM HCl in order to prepare the FRAP reagent. To 100µl of distilled water 1.5 ml of FRAP reagent and 100 µl of extracted sample was added. The sample mixture was kept at room temperature for 4 minutes and absorbance was measured at 593 nm with a methanol blank.

### 3.7 Determination of ABTS radical scavenging activity

Volumes of 5 ml of 7 mM ABTS solution and 5 ml of 2.45 mM ammonium persulfate were mixed in order to prepare the solution. The solution was incubated in the dark at room temperature for 16 hours and diluted with 100 ml of 3% methanol in order to prepare a working solution. An initial absorbance of the solution was measured without adding the sample extract. Then 150 µl of sample was mixed with 2850 µl of above prepared working solution. The absorbance values were recorded with time. The ABTS inhibition activity was calculated using the formula below %ABTS Antioxidant inhibition activity =

[(Absorbance of solution – Absorbance of sample)/Absorbance of solution] × 100

### 3.8 Determination of Antimicrobial activity (Well diffusion technique)

The bacteria from the prepared subculture were introduced to freshly prepared petri plates with Mueller-Hinton agar by streaking uniformly using a prepared inoculum. The prepared petri plates were separated into four quadrants by labeling and three wells were punched in three separate quadrants using a pipetted tip. Three drops of distilled water was added into a well, labeled as the negative control and three drops of each sample was added to the other two wells. A Gentamycin disk was placed on the remaining quadrant as the positive control. The prepared petri plates were incubated at 37 0C for 24 hours and the zones of inhibition (ZOI) were measured.

## 4. RESULTS

### 4.1 Results of the antioxidant assays

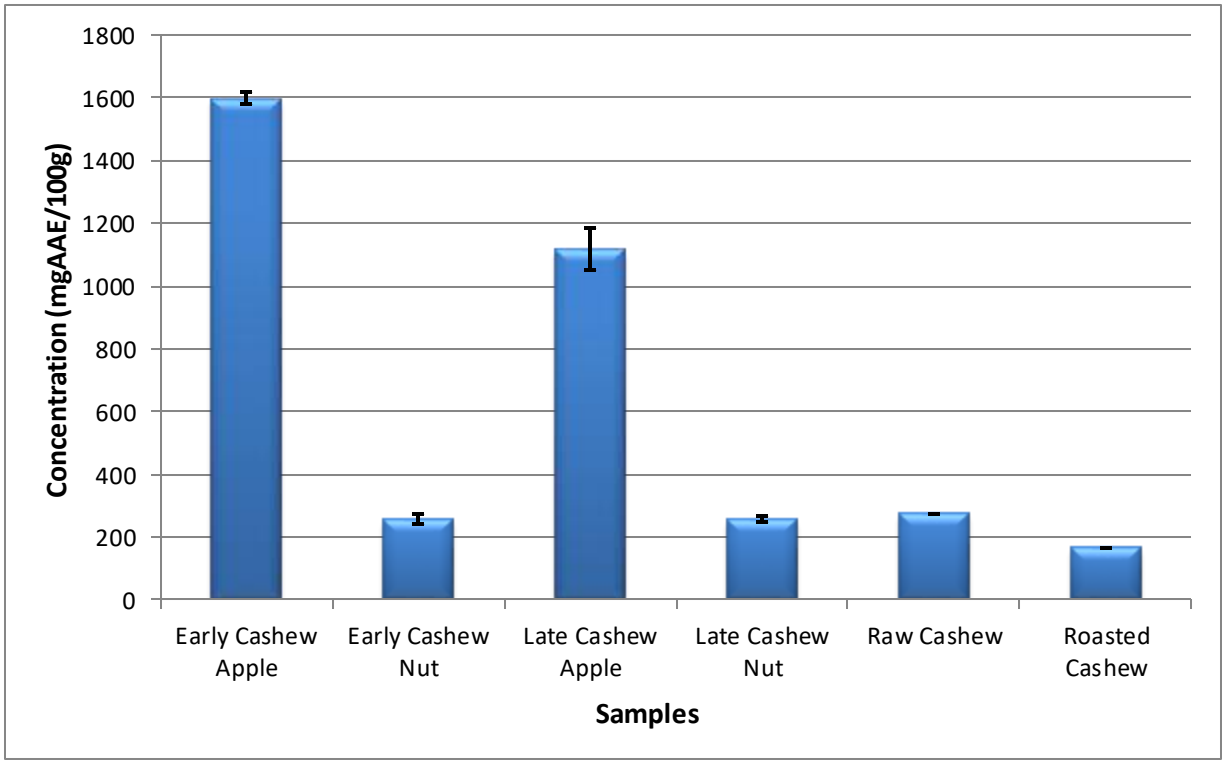



**Figure 1**: Concentrations of cashew samples from Total antioxidant capacity assay

**Table 2:** One way ANOVA analysis of TAC concentration values of cashew samples

| Groups | Count | Sum | Average | Variance |
|---|---|---|---|---|
| Apples | 6 | 7.143 | 1.1905 | 0.39637 |
| Nuts | 6 | 10.619 | 1.769833 | 0.007617 |

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 1.006881 | 1 | 1.006881 | 4.984728 | 0.049619 | 4.964603 |
| Within Groups | 2.019932 | 10 | 0.201993 | | | |
| | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Total** | 3.026814 | 11 | | | | |

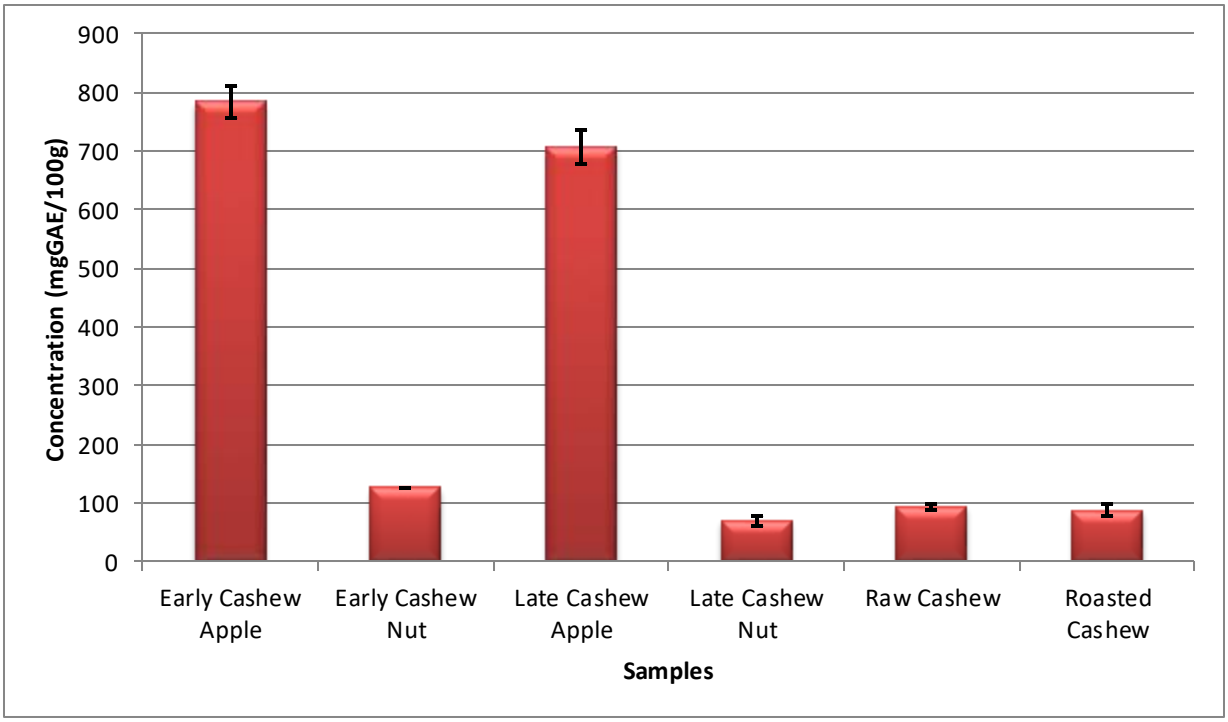



**Figure 2:** Concentrations of cashew samples from TPC assay

**Table 3**: One way ANOVA analysis of TPC concentration values of cashew samples

| **Groups** | **Count** | **Sum** | **Average** | **Variance** |
|---|---|---|---|---|
| **Apples** | 6 | 5.98 | 0.996667 | 0.172019 |
| **Nuts** | 6 | 1.294 | 0.215667 | 0.004547 |

| **ANOVA** | | | | | | |
|---|---|---|---|---|---|---|
| **Source of Variation** | **SS** | **df** | **MS** | **F** | **P-value** | **F crit** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Between Groups** | 1.829883 | 1 | 1.829883 | 20.72736 | 0.001054 | 4.964603 |
| **Within Groups** | 0.882835 | 10 | 0.088283 | | | |
| **Total** | 2.712718 | 11 | | | | |

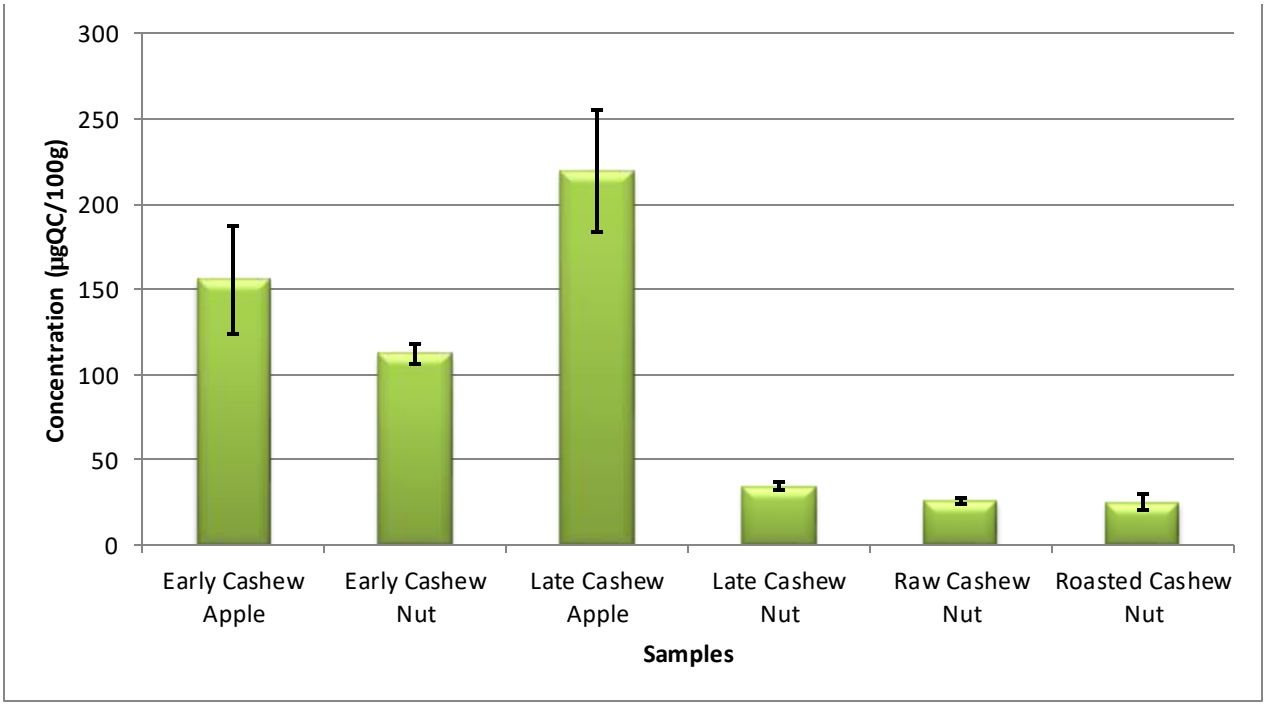



**Figure 3:** Concentrations of cashew samples from TFC assay

**Table 4:** One way ANOVA analysis of TFC concentration values of cashew samples

| Groups | Count | Sum | Average | Variance |
|---|---|---|---|---|
| Apples | 6 | 0.382 | 0.063667 | 0.000265 |
| Nuts | 6 | 0.264 | 0.044 | 0.000659 |

| ANOVA | | | | | | |
|---|---|---|---|---|---|---|

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 0.00116 | 1 | 0.00116 | 2.509733 | 0.144228 | 4.964603 |
| Within Groups | 0.004623 | 10 | 0.000462 | | | |
| | | | | | | |
| Total | 0.005784 | 11 | | | | |

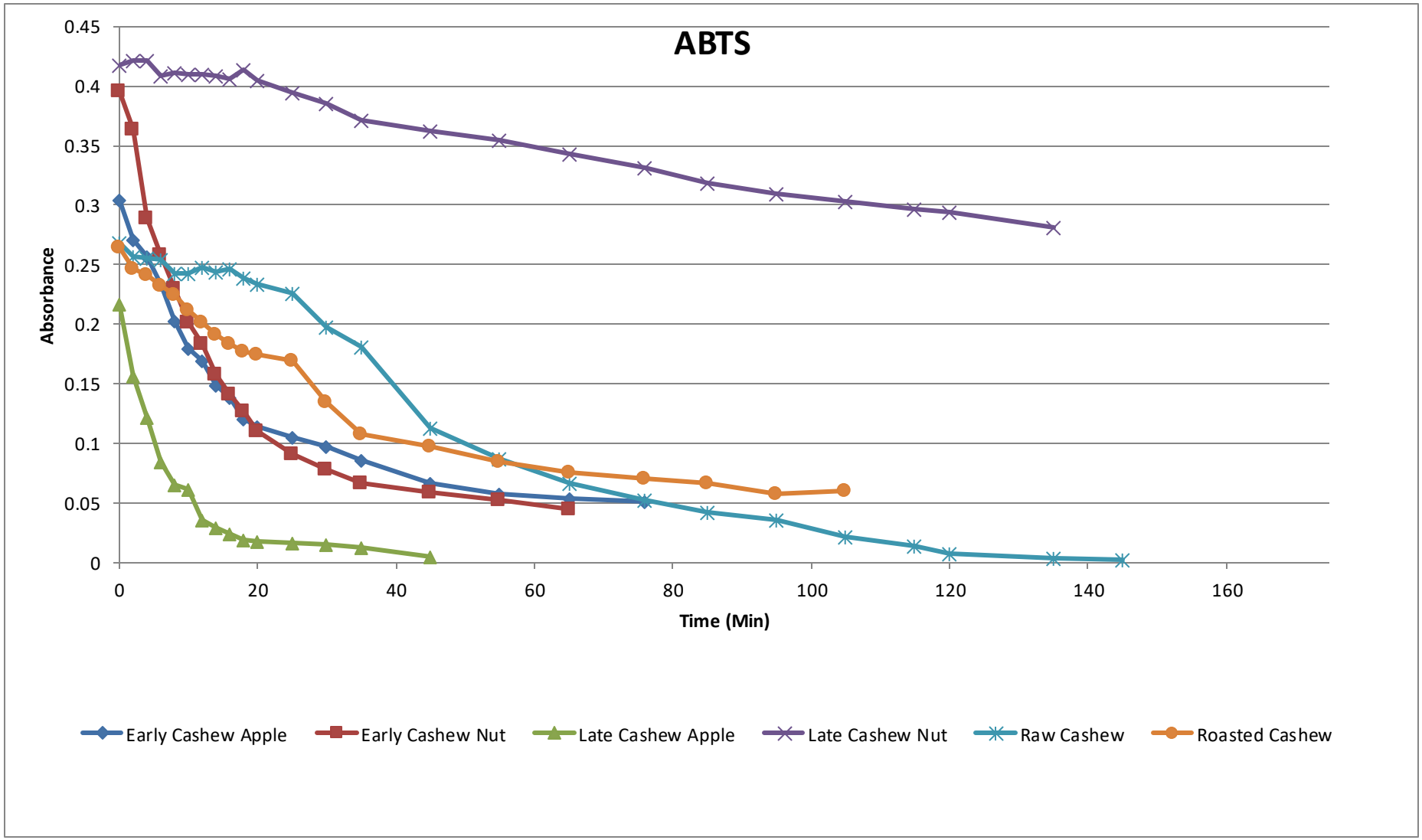



**Figure 4** ABTS absorbance vs time of cashew samples


ICHM

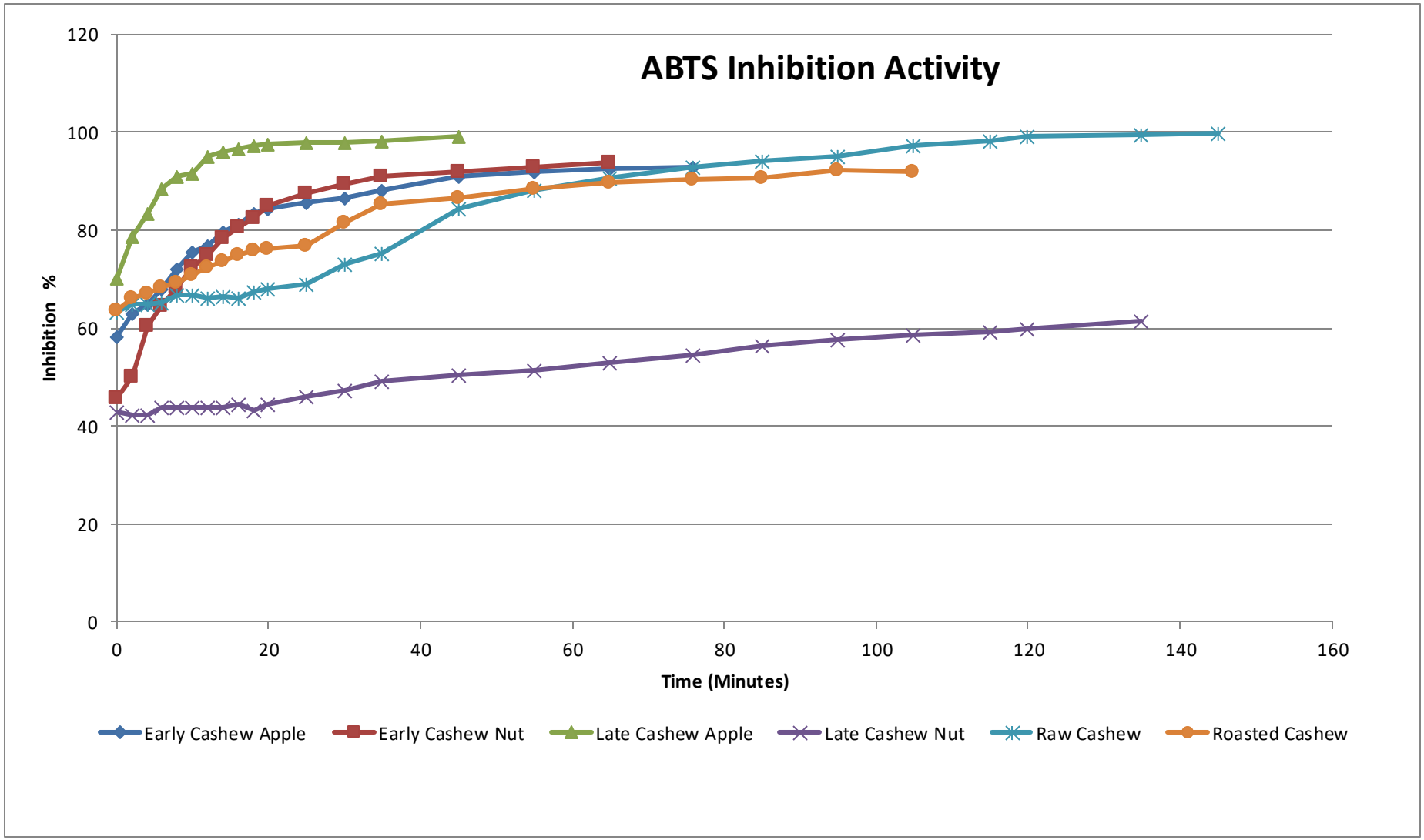



**Figure 5**: Percentage of ABTS inhibition activity vs Time of cashew samples

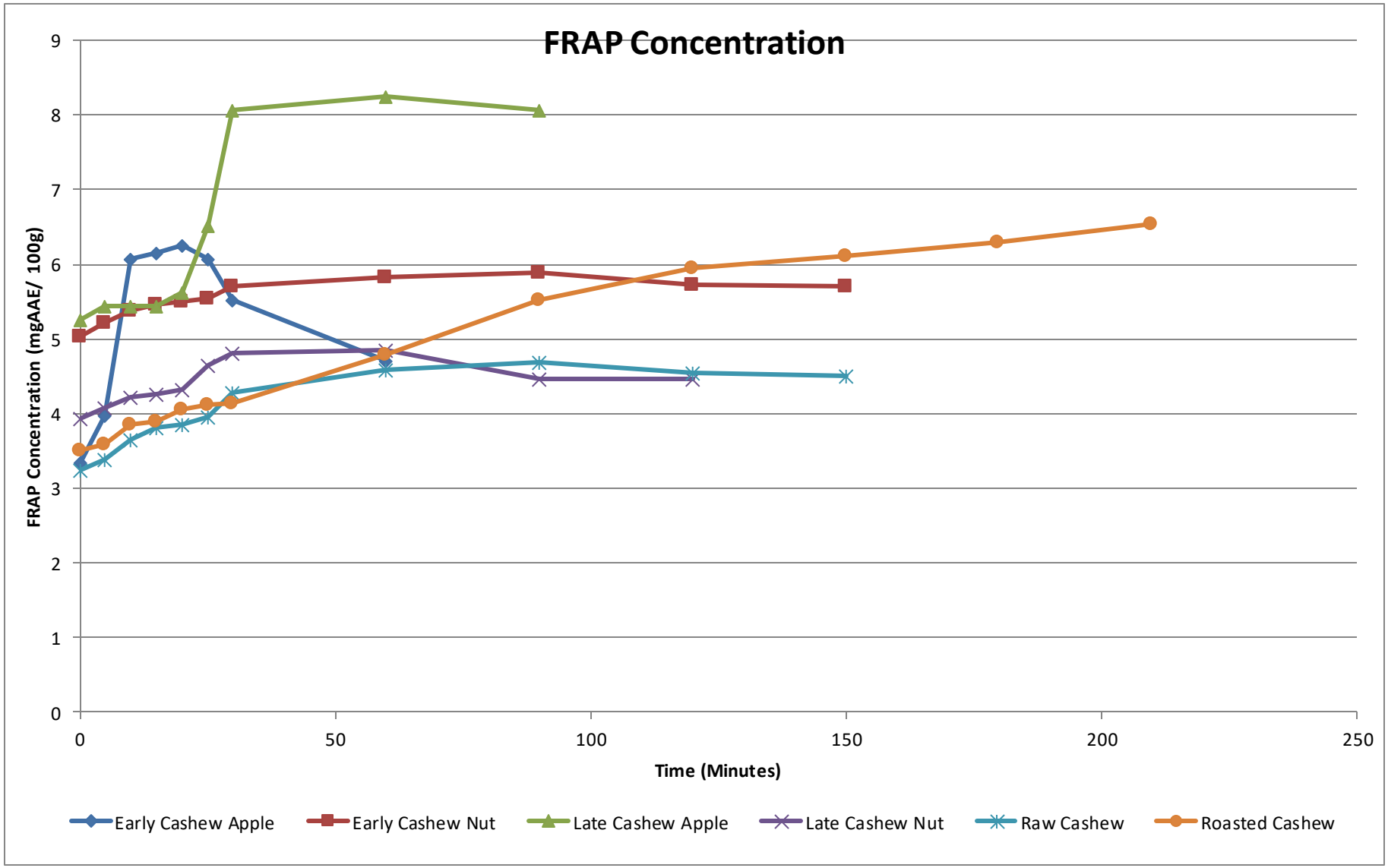

FRAP Concentration
FRAP Concentration (mgAAE/ 100g)
Time (Minutes)
0
1
2
3
4
5
6
7
8
9
0
50
100
150
200
250
Early Cashew Apple
Early Cashew Nut
Late Cashew Apple
Late Cashew Nut
Raw Cashew
Roasted Cashew

**Figure 6**: Reducing power of FRAP in cashew samples

4.2 Results of well diffusion method

**Table 5**: Inhibition zone values of *Staphylococcus aureus*

| **Sample** | **Well 1 (cm)** | **Well 2 (cm)** | **Mean value (cm)** |
|---|---|---|---|
| Early Cashew Nut (EN) | 1.1 | 1.0 | 1.05 |
| Early Cashew Apple (EA) | 1.5 | 1.4 | 1.45 |
| Late Cashew Apple (LA) | 1.2 | 1.4 | 1.30 |
| Late Cashew Nut (LN) | 0.8 | 0.7 | 0.75 |
| Raw Cashew (RW) | 0.9 | 0.9 | 0.90 |
| Roasted Cashew (RO) | 1.0 | 1.0 | 1.0 |

**Table 6:** Inhibition zone values of *E.coli*

| **Sample** | **Well 1 (cm)** | **Well 2 (cm)** | **Mean Value (cm)** |
|---|---|---|---|
| Early Cashew Nut (EN) | 1.0 | 1.1 | 1.05 |
| Early Cashew Apple (EA) | 1.5 | 1.4 | 1.45 |
| Late Cashew Apple (LA) | 1.2 | 1.3 | 1.25 |
| Late Cashew Nut (LN) | 0.9 | 0.8 | 0.85 |
| Raw Cashew (RW) | 1.0 | 1.0 | 1.0 |
| Roasted Cashew (RO) | 1.0 | 1.0 | 1.0 |

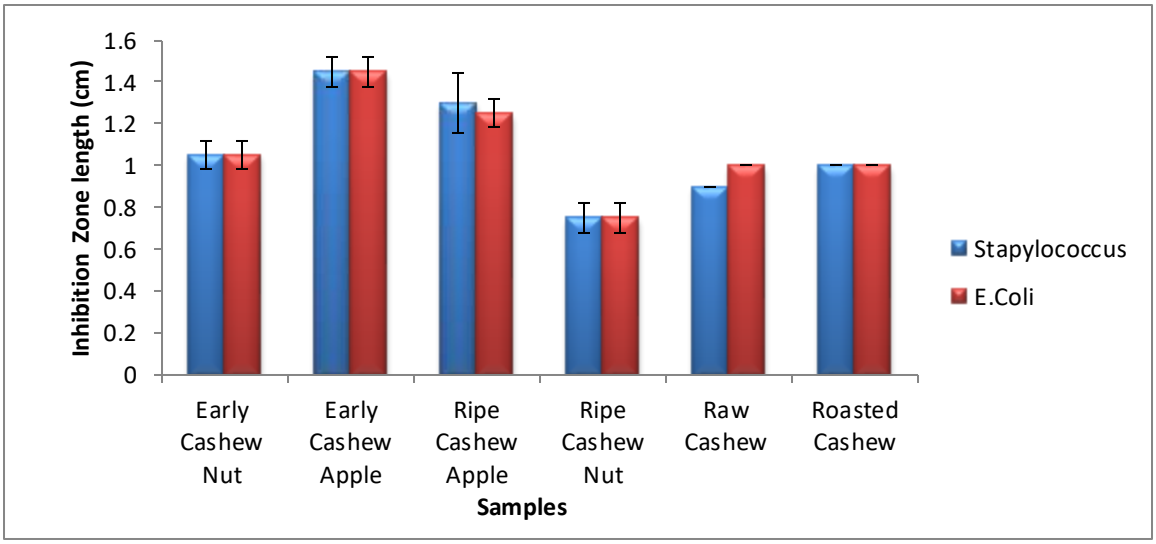



**Figure 7:** Zone of inhibition values of bacteria in the presence of cashew

**Table 7**: One way ANOVA analysis between two bacterial inhibition values

| SUMMARY | | | | |
|---|---|---|---|---|
| **Groups** | **Count** | **Sum** | **Average** | **Variance** |
| **Early Cashew Apple** | 2 | 2.1 | 1.05 | 0 |
| **Early Cashew Nut** | 2 | 2.9 | 1.45 | 0 |
| **Late Cashew Apple** | 2 | 2.55 | 1.275 | 0.00125 |
| **Late Cashew Nut** | 2 | 1.5 | 0.75 | 0 |
| **Raw Cashew Nut** | 2 | 1.9 | 0.95 | 0.005 |
| **Roasted Cashew Nut** | 2 | 2 | 1 | 0 |

| ANOVA | | | | | | |
|---|---|---|---|---|---|---|
| **Source of Variation** | **SS** | **df** | **MS** | **F** | **P-value** | **F crit** |
| **Between Groups** | 0.616042 | 5 | 0.123208 | 118.28 | 6.57E-06 | 4.387374 |
| **Within Groups** | 0.00625 | 6 | 0.001042 | | | |
| **Total** | 0.622292 | 11 | | | | |

4.3 Correlation graphs

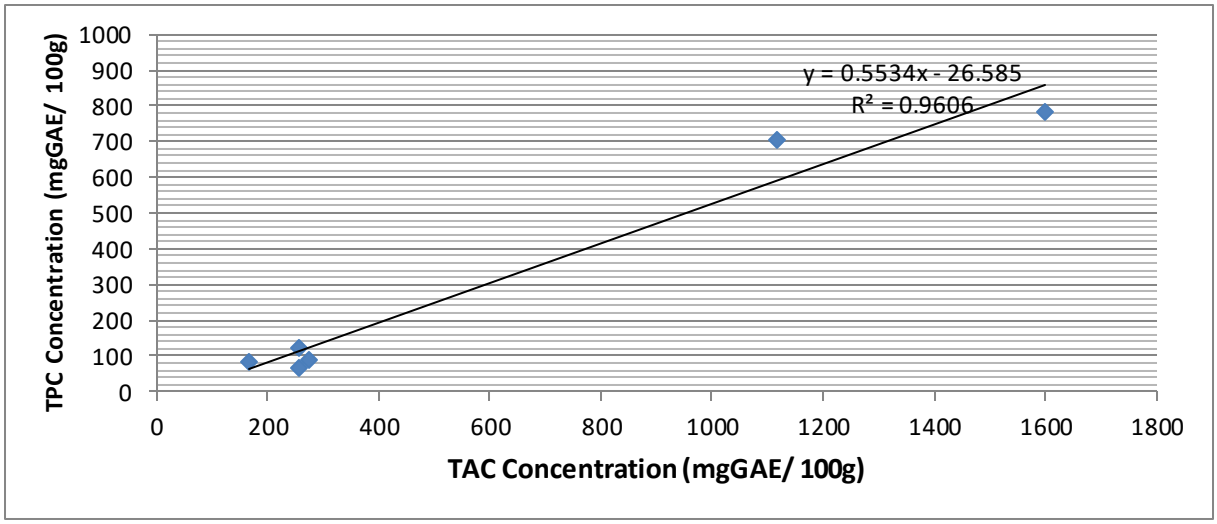



**Figure 8**: Correlation between total antioxidant capacity and total phenolic content of cashew

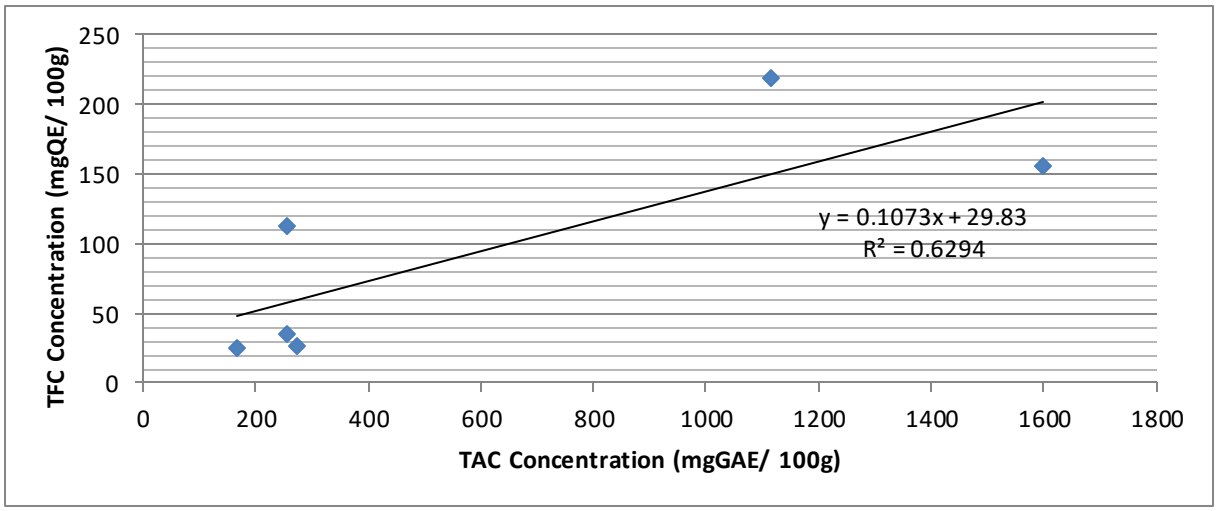



**Figure 9**: Correlation between total antioxidant capacity and total flavonoid content of cashew

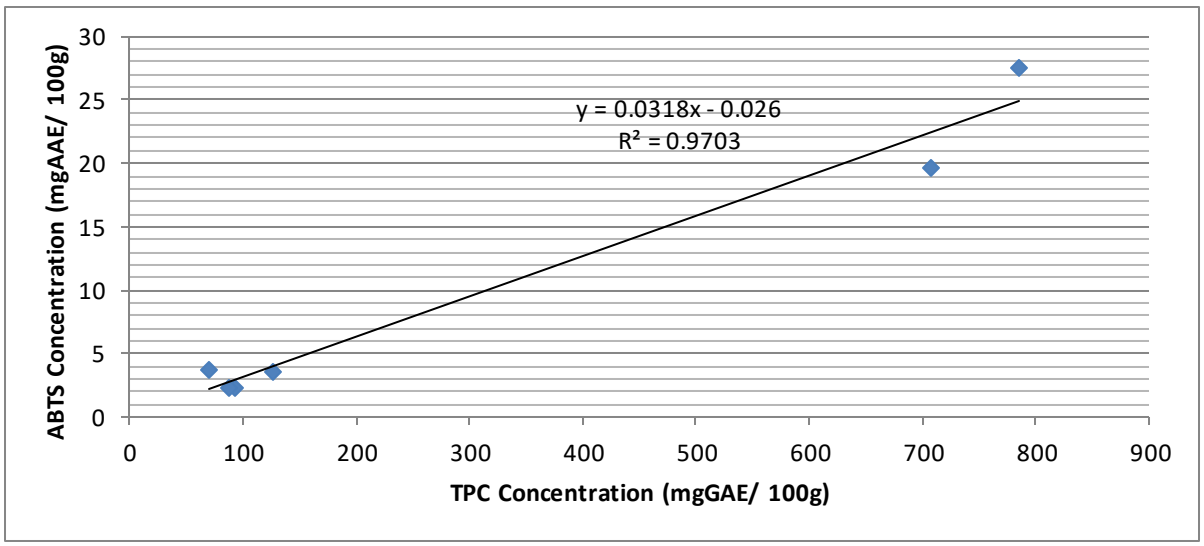



**Figure 10**: Correlation between ABTS concentration in ascorbic acid equivalence and TPC concentration of cashew

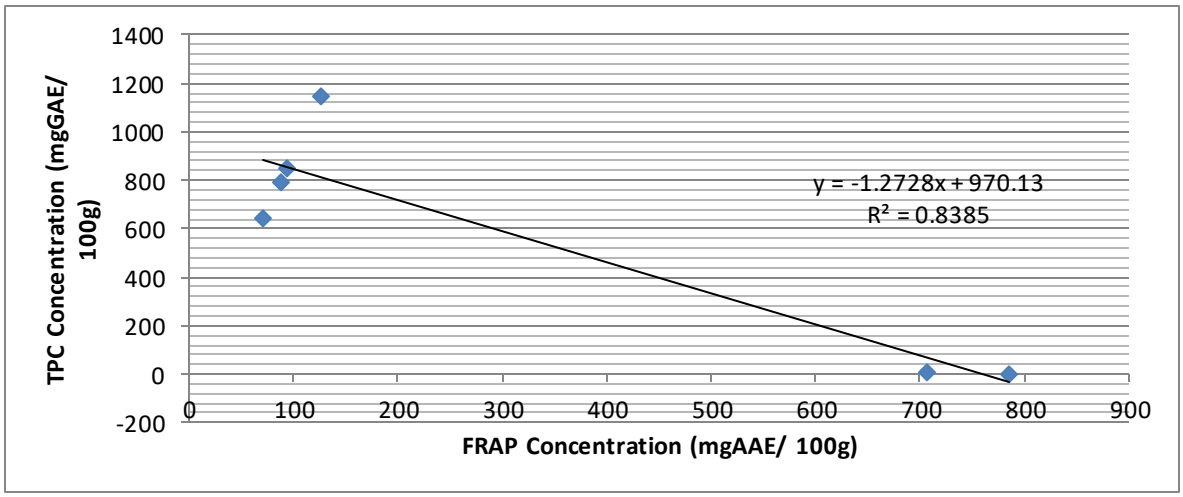



**Figure 11**: Correlation between TPC concentration and FRAP concentration in ascorbic acid equivalence

## 5. DISCUSSION AND CONCLUSION

The study was based on the determination of the antioxidant and antimicrobial activities of early stage cashew apples and nuts, late stage cashew apples and nuts, raw cashew and roasted cashew which was harvested by a selected cashew tree.

The cashew apples used in the project were directly harvested from the tree and transported safely without causing any physical damage in order to maintain the quality since physical damages and cutting of the apple causes metabolic changes, by increasing respiration rate, ethylene production and polyphenol oxidase activity that can affects the contents of the cashew

apple (Hodges and Toivonen, 2008 and Queiroz *et al.*, 2011).

In order to enhance the accuracy of the test at least three values were obtained from each sample and the mean values were calculated to compare the results. Moreover a one way ANOVA was carried out to determine the significance of the statistics in multiple comparisons.

Several studies focused on the variations of phytochemicals during different drying methods, has reported that air drying, vacuum microwave-drying and combination of air and microwave-vaccum drying has reduced phenolic contents, anthocyanin contents, and antioxidant activities compared with freeze drying method (Kwok *et al.*, 2004). Therefore air drying and sun drying process which was carried out as a step of the extraction of cashew nut samples can be a factor to further reduce the antioxidant activities of cashew samples.

Ascorbic acid (Vitamin C) is a known antioxidant which is rich in fruits and vegetables. A study based on the determination of antioxidant capacities and ascorbic acid contents of 24 exotic fruits of Colombia has found that the edible cashew apple contain the highest ascorbic acid content among the 24 fruits which was selected (Contreras-Calderón *et al.*, 2011).

Determination of total antioxidant capacity of the cashew samples was based on the phosphomolybdate assay, which is able to form a green coloured phosphomolybdate compound by reducing Mo VI) to Mo(V) in the presence of an antioxidant at a maximum wavelength of 765 nm (Jan *et al.*, 2013). Regarding the results from the TAC assay, the highest absorbance values were obtained (Figure 1) from the early stage cashew apple sample (1598 mgAAE in 100g of sample) followed by late stage cashew apples (1116.54 mgAAE/ 100g) > raw cashew nuts > late stage cashew nuts > early cashew nuts and roasted cashew. The one way ANOVA analysis (Table 2) resulted a slightly high F value (4.98) compared with F crit value (4.96) and a p value of almost 0.05 (0.0496). Therefore, an indistinct statistical significance between the TPC values of cashew apples and cashew nuts cannot be detected which is possible due to overlapping of the error bars of cashew apple values.

Total phenolic content (TPC) assay is used to measure the amount of phenols in the solution by reducing Folic-Ciocalteu reagent due to the production of phenolate ions which is monitored spectrophotometrically (Ahmed, Khan and Saeed, 2015). Moreover the reaction of Folic-Ciocalteu reagent due to the presence of non-phenolic compounds apart from phenolics, flavonoids and anthocyanin can also enhance the sample results (Benvenuti *et al.*, 2004). Among the cashew apple and nut samples the total phenolic content (Figure 2) was found to be highest in early cashew apple with a concentration of 785.14 mgGAE in 100 g of sample followed by late stage cashew apple: 707.80 mgGAE/ 100 g, early cashew nut, roasted cashew, raw cashew and late stage cashew nut. Moreover the TPC values of cashew apples and cashew nuts are observed to be statistically significant according to the one way ANOVA analysis (Table 3) indicated by low p value ($0.001p < 0.05p$) and high F value than F crit value (F-20.73 > F-Crit 4.96). Therefore the late and early cashew apples were found to contain a higher phenolic content than late and early cashew nuts.

Flavonoid assay is based on the formation of the $Al^{3+}$ complexes with C-4 keto, C-3, or C-5 hydoxyl compounds (Ahmed, Khan and Saeed, 2015). The Aluminum ion mediates the formation of unstable complexes with the ortho – dihydroxyl groups in the A or B ring of flavonoids (Ibrahim *et al.*, 2017). The highest TFC value was obtained (Figure 3) from the late stage cashew apple (219.44µgQE/ 100 g) followed by early stage cashew apple (155.564 µgQE/ 100 g) > early cashew nut > late cashew nut > raw cashew> roasted cashew. The one way ANOVA analysis (Table 4) denoted a p value (0.1442) above than 0.05 and F crit value (4.964) more than F value (2.509) indicating no significant difference between the total flavonoid content of cashew apples and cashew nuts. It was found out that the cashew apple contains more flavonoids than cashew nuts and among cashew apples, the late cashew apple containing a higher amount than early cashew apple.

Moreover high TPC values of all the samples than the values of the TFC samples support the fact that most flavonoids are phenolics while the total antioxidant capacity concentrations of all the samples were significantly higher than the TPC values and TFC values supporting the fact that the phenol and flavonoids are components of antioxidants which are analyzed in TAC (Ahmed *et al.*, 2015).

The scavenging activity of the ABTS was determined using the percentage inhibition of the ABTS radical caption by antioxidants present in the cashew samples. In ABTS assay, the ABTS in the solution is converted to a bluish green ABTS radical cation which absorbs light at 734nm by the oxidation with potassium persulfate or sodium persulfate. The cation reacts with antioxidants such as phenolics, vitamins and thiols in the solution and decolorize into neutral form which is monitored spectrophotometrically with time (Zheng *et al.*, 2016). Moreover ABTS is useful to measure the antioxidant ability of lipophilic and hydrophilic compounds which are generally found in vegetables and fruits (Gan *et al.*, 2010).

The most efficient scavenging activity was displayed (Figure 5) by late cashew apple (97.94% inhibition) which was stabilized after 45 minutes against ABTS radical cation while early cashew nut and early cashew apples were also detected as efficient radical scavengers after late cashew apple. Subsequently the highly efficient activity of cashew apples can be correlated with high concentration values from TPC and TAC. Moreover the inhibition activities of the cashew nuts (roasted cashew, raw cashew) was less efficient as expected due to low total antioxidants while late cashew nut was the least efficient scavenger of ABTS radical resulting a 61.5% inhibition activity after 135 minutes from the start. Therefore considering all the samples, it can be reported that cashew apples are more efficient scavengers of ABTS radicals than cashew nut samples.

In FRAP assay, antioxidants acts as reducing agents in a redox-linked method which can be determined colorimetrically. The antioxidants reduces Ferric tripyridyl triazine (Fe III TPTZ) complex into a ferrous form complex, increasing the colour of blue in the solution which is monitored at 593 nm (Bordbar *et al.*, 2013). Furthermore, the amount of $Fe^{3+}$ ions reduced to $Fe^{2+}$ ions can differ according to the structural differences of phenols such as phenols with two hydroxyl groups (o-

pyrocatechuic acid) showing high and radical activity while phenols with two hydroxyl groups (pyrogallol) showing weak reducing power and antioxidant activity (Biskup *et al*., 2013). The highest reducing power activity was observed (Figure 6) in late cashew apples with a FRAP value of 8.24 mgAAE/ 100 g after 60 minutes of scavenging activity followed by a value 6.25 mgAAE/ 100 g after 20 minutes of scavenging activity. Moreover the least efficient reducing power was obtained in late cashew nuts with a value of 4.46 mgAAE/ 100 g after 90 minutes of scavenging activity. Therefore it was found out that the antioxidant compounds in the cashew apples have a higher reducing power than the compounds in the cashew nut samples.

Correlation between the antioxidant assays were determined by the analysis of correlation graphs. The correlation graph between the TAC and TPC (Figure 8) resulted with a $R^2$ value of 0.9606 indicating a significant correlation in the cashew samples between the phosphomolybdate total antioxidant capacity assay and total phenolic assay. The $R^2$ value of 0.6294 from the correlation graph between TAC and TFC (Figure 9) indicated a less correlation between the antioxidant capacity values and flavonoid values compared to the correlation between total antioxidant capacity and total phenolic content of the graph. Therefore the antioxidant capacities of the cashew samples are more dependent on phenolic content than flavonoid content. The radical scavenging activity of the antioxidants by ABTS (Figure 10) and the total phenolic content was determined resulting a $R^2$ value of 0.9703. This indicates a significant correlation in the samples between scavenging activity of the antioxidants determined by ABTS method and the total phenolic content in the samples. Furthermore the correlation graph between reducing power in FRAP by antioxidants and total phenolic content assay (Figure 11) resulted a $R^2$ value of 0.8385 indicating a correlation less than the radical scavenging activity of antioxidants in ABTS assay and total phenolic content in phosphomolybdate assay. Therefore the total phenolic content of the cashew samples are more dependent on radical scavenging activity by ABTS assay than FRAP assay and TAC phosphomolybdate assay. Moreover the antioxidant activity of polyphenols present in the natural systems is known to be altered due to the presence of hydroxyl groups in *ortho*- and *para*-positions (Jan *et al*., 2013).

In order to assess the antimicrobial activities of cashew samples by using two different bacteria strains such as *S.aureus* and *E.coli*, the well diffusion technique was performed. Staphylococcus was selected to observe the antimicrobial effect on gram positive bacteria while E.coli was selected as a gram negative bacterium. Thus Gentamicin was used as the positive control since it is an aminoglycosidic antibiotic which has a broad spectrum of activity against several gram negative bacteria including *E.Coli*, *Pseudomonas* and gram positive *Staphylococcus* (Wang *et al*., 2016). All of the cashew samples were able to result zone of inhibitions which were measured in centimeters using a ruler. There was no microbial growth in the negative control area.

Ethanolic extract of cashew apples exhibited (Figure 7) activity against *S.aureus* showed highest zone of inhibition for early cashew apples and lowest for late cashew apples. However the among the

cashew nut types the early cashew nut the showed the highest inhibition zone for *S.aureus* while late cashew nut showed the lowest zone of inhibition. Moreover the considering the cashew apple activity against *E.coli* the highest zone of inhibition was obtained by early cashew apple and the lowest zone of inhibition was obtained due to the antibacterial activity of late cashew apple. Furthermore, among the cashew nut types in the inhibition of *E.coli*, the early cashew nut showed the highest zone of inhibition while the late cashew nut showed the lowest zone of inhibition for *E.coli*. The one way ANOVA analysis (Table 7) which was generated to compare the two bacterial inhibition by the cashew samples showed a p value of 0.000006 which is less than 0.05 and a F value (118.28) higher than (4.38) indicating a significant difference between the inhibition activity against *S.aureus* and *E.coli*.

Finally it can be concluded that the TFC,TPC, and TAC assays signified elevated levels of flavonoids, phenols and total antioxidants hence the ABTS and FRAP assays indicated increased radical scavenging activity in both cashew apple types and all four cashew nut types. Additionally all the samples were proven to be active against the selected bacteria while early cashew apple being the highest effective, suggesting the uses of cashew for antimicrobial therapy. Moreover the cashew apples were found to contain more phenols, flavonoids and antioxidants with scavenging activities than cashew nut types which indicate the health benefits of consuming cashew apples that has to be more publically encouraged. Furthermore early cashew apple content can be used for the development of novel therapeutics against oxidative stress induced diseases. (1973 Words)

## 6. FURTHER WORK

The use of different varieties of cashew apples and cashew nuts from different areas of Sri Lanka to determine the effect of antioxidant potentials based on the climate, soil content, colour size and shape of cashew samples.

Determination of the antioxidant capacities and antimicrobial activity based on the heating and baking temperatures of the cashew nut kernels which are performed during the processing of cashew

Analysis of the antioxidant effects of the cashew nut shell liquid (CNSL) of different varieties of cashews found from different locations of Sri Lanka

The use of other radical scavenging activities assay such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) along with the current assays to compare and determine the most effective assays to detect the antioxidant activity of cashew samples

Performance of other novel assays to determine the contents of other chemicals present in the cashew apples and cashew nuts such as tannins, saponins and nitrogenous compounds

The use of novel extraction methods such as high performance liquid chromatography (HPLC) and Solid Phase Extraction (SPE) for an efficient extraction of the sample based on mobile phase separation which results a broad separation of components required for antioxidant assays.

Evaluation and comparison of the amounts of extraction concentrations using different polar solvents such as water, acetone, ethanol, acetyl acetate, *n*-hexane and in different concentrations such as water,

methanol, methanol: water, acetone, acetone: water etc.

## 7. ACKNOWLEDGEMENT

It has been a privilege to thank all the people who helped in any way to complete this project. Firstly I would like to thank Dr. Mathi Kandiah, my supervisor for the encouragement and continued guidance that had in order to get successful results and to finish the project and constant supervision and endless support throughout the project work. Moreover I would like to express my gratitude to BMS to giving me this opportunity and for the guidance and support through the academic staff. Furthermore I would like to thank all the academic staff members and Dr. Sajani Dias, the programme leader for guiding and supporting me to follow the best choices and the laboratory staff, Ms. Mathura and Ms. Namirah for the enormous support given during the lab time. Finally my thanks goes to my colleges who helped be by standing by my side and specially my parents who all this would have not been possible without.

## 8. REFERENCES

Ahmed, D., Khan, M. & Saeed, R. (2015) Comparative Analysis of Phenolics, Flavonoids, and Antioxidant and Antibacterial Potential of Methanolic, Hexanic and Aqueous Extracts from Adiantum caudatum Leaves, Antioxidants, 4, 394-409.

Andrade, T., Araújo, B., Citó, A., da Silva, J., Saffi, J., Richter, M. & Ferraz, A. (2011) Antioxidant properties and chemical composition of technical Cashew Nut Shell Liquid (tCNSL) Food Chemistry, 126, 1044-1048.

Assunção, R.B. & Mercadante, A.Z. (2003) Carotenoids and ascorbic acid from cashew apple (Anacardium occidentale, L.): A variety and geographic effects, Food Chemistry, 81, 495–502.

Athukorale, D.P. (2004) Nutrition and health aspects of cashew nuts, The Island,13

Benvenuti S., Pellati F., Melegari M. & Bertelli D. (2004) Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of Rubus, Ribes, and Aronia, Journal of Food Science, 69, 164-169.

Benzie, I. & Strain, J. (1996) The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay, Analytical Biochemistry, 239, 70-76.

Biskup, I., Golonka, I., Gamian, A. & Sroka, Z. (2013) Antioxidant activity of selected phenols estimated by ABTS and FRAP methods, Postepy Higieny Medycyny Doswiadczalnej,67, 957-963.

Bordbar, S., Ebrahimpour, A., Abdul Hamid, A., Abdul Manap, M., Anwar, F. & Saari, N. (2013) The Improvement of The Endogenous Antioxidant Property of Stone Fish (Actinopyga lecanora) Tissue Using Enzymatic Proteolysis, BioMed Research International, 2013, 1-9.

Cavalcante, A., Rubensam, G., Picada, J., Gomes da Silva, E., Fonseca Moreira, J. & Henriques, J. (2003) Mutagenicity, antioxidant potential, and antimutagenic activity against hydrogen peroxide of cashew

(Anacardium occidentale) apple juice and cajuina, Environmental and Molecular Mutagenesis, 41, 360-369.

Chabi Sika, K., Adoukonou-Sagbadja, H., Ahoton, L., Adebo, I., Adigoun, F.A., Saidou, A., Kotchoni, S. O., Ahanchede, A. & Baba-Moussa, L. (2013) Indigenous knowledge and traditional management of cashew (Anacardium occidentale L.) genetic resources in Benin, Journal of Experimental Biology and Agricultural Sciences, 376-382.

Contreras-Calderón, J., Calderón-Jaimes, L., Guerra-Hernández, E. & García-Villanova, B. (2011) Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia, Food Research International, 44, pp.2047-2053.

Davidson, P., Critzer, F. & Taylor, T. (2013) Naturally Occurring Antimicrobials for Minimally Processed Foods, Annual Review of Food Science and Technology, 4, 163-190.

Desai, A.R., Singh, S.P., Faleiro, J.R., Thangam, M., Priya Devi, S., Safeena S.A. & Singh N.P. (2010) Techniques And Practices For Cashew Production, ICAR Research Complex for Goa

Fu, L., Xu, B., Xu, X., Gan, R., Zhang, Y., Xia, E. & Li, H. (2011) Antioxidant capacities and total phenolic contents of 62 fruits, Food Chemistry, 129, 345-350.

Gan, R.Y., Kuang, L., Xu, X.R., Zhang, Y., Xia, E.Q. & Song, F.L. (2010) Screening of natural antioxidants from traditional Chinese medicinal plants associated with treatment of rheumatic disease, Molecules, 15, 5988–5997.

Gonçalves, G. & Gobbo, J. (2012) Antimicrobial effect of Anacardium Occidentale extract and cosmetic formulation development, Brazilian Archives of Biology and Technology, 55, 843-850.

Hamad, F. and Mubofu, E. (2015) Potential Biological Applications of Bio-Based Anacardic Acids and Their Derivatives, International Journal of Molecular Sciences, 16, 8569-8590.

Hodges, D.M. & Toivonen, P.M.A. (2008) Quality of fresh-cut fruits and vegetables as affected by exposure to abiotic stress, Postharvest Biology and Technology, 48, 155–162.

Ibrahim, S., Al-Ahdal, A., Khedr, A. & Mohamed, G. (2017) Antioxidant α-amylase inhibitors flavonoids from Iris germanica rhizomes, Revista Brasileira de Farmacognosia, 27, 170-174.

Jan, S., Khan, M., Rashid, U. & Bokhari, J. (2013) Assessment of Antioxidant Potential, Total Phenolics and Flavonoids of Different Solvent Fractions of Monotheca Buxifolia Fruit, Osong Public Health and Research Perspectives, 4, 246-254.

Kubo, I., Masuoka, N., Ha, T.J., & Tsujimoto, K. (2006) Antioxidant activity of anacardic acids, Food Chemistry, 99, 555–562.

Kwok, B. H. L., Hu, C., Durance, T., & Kitts, D. D. (2004) Dehydration techniques

affect phytochemical contents and free radical scavenging activities of Saskatoon berries (Amelanchier alnifolia Nutt.), Journal of Food Science, 69, S122-S126.

Michodjehoun-Mestres, Souquet, J.M., Fulcrand, H., Bouchut, C., Reynes, M., & Brillouet, J.M. (2009) Monomeric phenols of cashew apple (Anacardium occidentale L.), Food Chemistry, 112, 851–857.

Nair, G.K. (2016) Is India consuming bulk of the cashew it produces, The Hindu Business Line [Online].

Pineli, L., Moretti, C., dos Santos, M., Campos, A., Brasileiro, A., Córdova, A. & Chiarello, M. (2011) Antioxidants and other chemical and physical characteristics of two strawberry cultivars at different ripeness stages, Journal of Food Composition and Analysis, 24, 11-16.

Queiroz, C., Lopes, M., Fialho, E. & Valente-Mesquita, V.L. (2011) Changes in bioactive compounds and antioxidant capacity of fresh-cut cashew apple, Food Research International, 44, 1459-1462.

Queiroz, C., Silva, A.J.R., Lopes, M.L.M., Fialho, E. & Valente-Mesquita, V.L. (2011) Polyphenol oxidase activity, phenolic acid composition and browning in cashew apple (Anacardium occidentale, L.) after processing, Food Chemistry, 125, 128–132.

Slavin, J. and Lloyd, B. (2012) Health Benefits of Fruits and Vegetables, Advances in Nutrition: An International Review Journal, 3, 506-516.

Sun, J., Chu, Y.F., Wu, X., & Liu, R. H. (2002) Antioxidant and Antiproliferative Activities of Common Fruits, Journal of Agricultural and Food Chemistry, 50, 7449-7454.

Surendra, G.B.B. (2011) Research and development of cashew in Sri Lanka, Sri Lanka Cashew Corporation [Online]. Avalable at: http://www.cashew.lk/index.php?option=com_content&view=article&id=122&Itemid=127&lang=en

Trevisan, M.T.S., Pfundstein, B., Haubner, R., Würtele, G., Spiegelhalder, B. & Bartsch, H. (2006) Characterization of alkyl phenols in cashew (Anacardium occidentale) products and assay or their antioxidant capacity, Food and Chemical Toxicology, 44, 188–197.

Wang, S., Melnyk, J., Tsao, R. & Marcone, M. (2011) How natural dietary antioxidants in fruits, vegetables and legumes promote vascular health, Food Research International, 44, 14-22.

Wang, Y.E., Gonella, N., Devries, R., Witt, D. & Prasad, S., (2016) Gentamicin Induces The Formation And Alters The Composition Of Gram Negative Rod Biofilms. This Process Is Blocked By Erythromycin Co-Treatment. In B16. CYSTIC FIBROSIS (CF) AND NON-CF BRONCHIECTASIS, American Thoracic Society, 193, A2884-A2884

Zheng, L., Zhao, M., Xiao, C., Zhao, Q. & Su, G. (2016) Practical problems when using ABTS assay to assess the radical-scavenging activity of peptides: Importance of controlling reaction pH and time, Food Chemistry, 192, 288-294.


ICHM