GLOBAL ACADEMIC RESEARCH INSTITUTE

COLOMBO, SRI LANKA

GARI International Journal of Multidisciplinary Research

ISSN 2659-2193

Volume: 09 | Issue: 03

On 30th September 2023

http://www.research.lk

Author: L. V. Chathura Nilupul Perera

Faculty of Health and Life Sciences, Northumbria University

GARI Publisher | Biomedical Sciences | Volume: 09 | Issue: 03

Article ID: IN/GARI/JOU/2022/160A | Pages: 201-222 (22)

ISSN 2659-2193 | Edit: GARI Editorial Team

Received: 14.07.2023 | Publish: 30.09.2023

PHYTOCHEMICAL ANALYSIS AND DETERMINATION OF ANTIOXIDANT ACTIVITY OF DIFFERENT SPECIES OF CITRUS, EXTRACTED USING SELECTED SOLVENTS

Liyanarathna Vidanage Chathura Nilupul Perera

Faculty of Health and Life Sciences, Northumbria University

ABSTRACT

Phytochemicals are naturally occurring compounds found in plants, and scientific studies have shown that they possess strong antioxidant and anti-inflammatory properties. This study aimed to investigating the impact of water and methanol for the phytochemical profile and the antioxidant activity of leaves of selected citrus species (Citrus aurantifolia, Citrus lemonicious, Citrus aurantium, Citrus sinensis, and Citrus reticulate). The qualitative analysis of phytochemicals was carried out initially. Total Phenolic Content (TPC), Total Antioxidant Capacity (TAC) and Total Flavonoid Content (TFC) were tested using Folin-Ciocalteu assay, Phosphomolybdenum assay, and aluminum chloride colorimetric method respectively. The free radical scavenging ability was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Antibacterial activity was determined against Escherichia coli (E.coli) and Staphylococcus aureus (S.aureus). Statistical analysis was carried out. Carbohydrates, phenols, flavonoids, terpenoids, alkaloids and tannins were present in all water and methanolic extracts except for steroids and saponin in both water and methanolic extracts of C.aurauntium, C.sinensis, and all methanolic extracts respectively. The highest TPC, TAC, and TFC were found in water extract of C. reticulate (66.25 µg GAE/g) C.sinensis (20.8411 µg AAE/mL) and C. lemonicious (80.58 µg/mL QE) respectively. C. reticulate had the highest DPPH free radical scavenging activity (32.11 mg/mL) among water extracts, while C. lemonicious (117.04 mg/mL) had the highest free radical activity in methanolic extracts. Water extract of C. aurantium demonstrated strong sensitivity (2.20 cm) against S. aureus, among both extracts. Citrus water extracts presented significantly higher values of TPC, TAC, and TFC when compared to their corresponding methanol extracts ($p < 0.05$). Overall, the water extracts have high amounts of phenols, flavonoids, and antioxidants furthermore methanol extracts have high potential for scavenging DPPH free radicals. In conclusion, this study provides valuable insights into the impact of water and methanol solvents on the phytochemical profile and antioxidant activity of the selected Citrus family.

INTRODUCTION

The use of plant-originated components has established an increased interest due to the numerous medical advantages and its role in herbal medicine, which is generally observed in developing countries (Ekor, M.2014). The defense mechanism of the plant consists of empirical knowledge which is then utilized for synthetic purposes. Studies show that multiple pathological conditions such as autoimmune diseases and degenerative diseases are affected by the cell damage caused by oxidative stress (Brambilla et

al., 2008). The use of natural oxidants is seemed significant due to the prevention, stabilization, and properties such as disabling free radicals before the biological targets in cells are attacked in order for the plant-based components to be beneficial, it is essential to confirm that there is no potential toxicity associated with these components, if disregarded, this can cause harm to human health. Therefore, verifying the absence of adverse. Oxygen is a crucial element for the existence of life. ATP (adenosine triphosphate) is used for the production of energy by mitochondria in the presence of oxygen and glucose. Free radicals are formed as a byproduct during this process. The byproducts of reactive oxygen species (ROS) and reactive nitrogen species (RNS) resulting from the cellular redox process consist of opposite effects. They must exist in a delicate balance since the effects produced by these products can either be beneficial or detrimental according to the prevailing concentration levels (Pham. H et al., 2008). These molecules can be generated through a variety of environmental and biological processes, such as air pollution from automobiles and exhaust fumes, smoking, forest fires, and volcanic activities, as well as water pollution, alcohol consumption, and exposure to UV, microwave, X-ray, or gamma radiation (Figure 1. 1.) (Tvrdá. E et al., 2020)

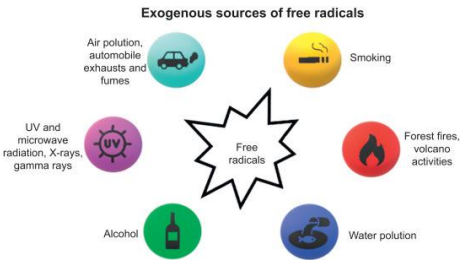


Figure 1. 1: Exogenous sources of free radicals (Tvrdá. E et al., 2020)

The accumulation of ROS leads to oxidative stress, which is characterized by damage to DNA, lipids, and proteins (Juan, C.A. et al., 2021). This oxidative stress results in decreased mitochondrial function and has been implicated in the aging process as well as the development of age-related diseases (Tan. B et al., 2018). Contribute to the overall concentration of free radicals, oxidation stress is generated at high concentrations causing damage to the overall cell structure (resulting in cell death/ tissue damage) due to excess free radicals. Studies show that the range of human diseases induced by oxidation stress includes cancer, neurodegenerative diseases, diabetes, inflammatory joint diseases, and cardiovascular dysfunction is directly related to oxidative stress (Figure 1.2) (Aruoma, G et al., 2006). The balance must yet be maintained by the intervention of antioxidants in order for the damage caused by free radicals to discontinue. As recent studies suggest that synthetic antioxidants produce hazardous effects and are reported to be dangerous and the natural antioxidants are preferred (Lobo et al., 2010). The natural antioxidant activity of Citrus is determined in this study as antioxidants are able to stabilize the effects prior to the attack on cells.

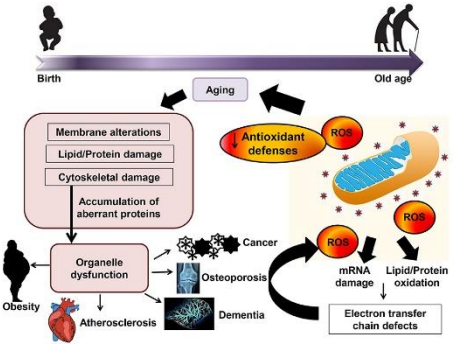


Figure 1.2: The Impact of Reactive Oxygen Species (ROS) on oxidative stress and age-related Diseases (Tan. B et al., 2018)

The development of tissue damage and pathological events is determined by free radicals according to their concentration levels. The oxidation lipids containing polyunsaturated fatty acids oxidize readily thus originating a free radical chain mechanism (Aruoma, G et al., 2006), (Adenaike et al., 2021). The ability of a bioactive compound to sustain the structure and function of the cell by successfully clearing the free radicals, along with the prevention of other damages caused by oxidation including lipid peroxidation reactions is known as "antioxidant activity" (Ranjan et al., 2019). Primary antioxidant activity refers to the ability of natural or synthetic compounds to scavenge ROS and prevent oxidative damage. These antioxidants can act directly by neutralizing ROS or indirectly by activating antioxidant enzymes (Figure 1.3) (Tan. B et al., 2018). Since the use of natural antioxidants are preferred for their contribution to health and disease. (Lourenço et al., 2019) Apart from providing endogenous protection, studies show that natural antioxidants with high antioxidant activity have possibility to delay damage caused by oxidation to tissues thereby increasing defenses (Schinella et al.,2002).

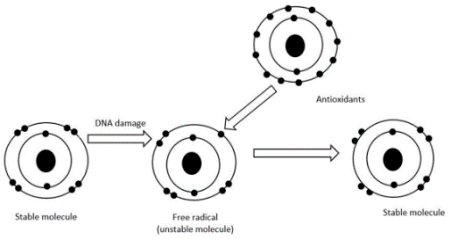


Figure 1.3: The mechanism of primary antioxidant activity

Diets rich in fruit and vegetables provide protective effects against cancer and thus theoretically concluding possible biologically active plants may exert anti-carcinogenic activity. The compounds are collectively known as "phytochemicals". (Lobo et al., 2010) Phytochemicals such as Benzylv isothiocynate, Capsaicin, Cucurbitacin B, Phenythyl isothiocynate, Genistein, Piperlongumine, Lycopene, Catechins and Isoflavones can produce biological activity (Figure 1.4) The bioactive compounds may be classified according to the effects of pharmacology or toxicology. Botanical categorization is essential based on families and genera of the plant since chemically similar bioactive compounds are released from closely related plant species such as citrus, blueberry, lettuce, sorghum, olive, and many more plants (Ranjan et al., 2019)

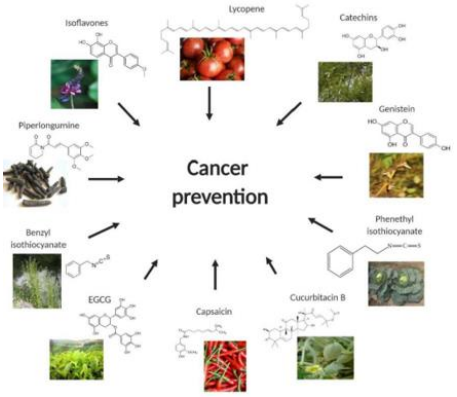


Figure 1.4: Potential of phytochemicals to prevent the growth of cancer cells (Ranjan et al., 2019)

Table 1.1 Different species of Citrus family (a) Citrus aurantifolia (b) Citrus lemonicious (c) Citrus sinensis (d) Citrus reticulate (e) Citrus aurantium

 (a)	Scientific name: Citrus aurantifolia English name: Lime Local name: Dehi
 (b)	Scientific name: Citrus lemonicious English name: Lemon Local name: Wal dehi
 (c)	Scientific name: Citrus sinensis English name: sweet orange Local name: Peni dodam
 (d)	Scientific name: Citrus reticulate English name: Caldamansi Local name: Narang
 (e)	Scientific name: Citrus aurantium Scientific name: sour orange Local name: Ambul dodam

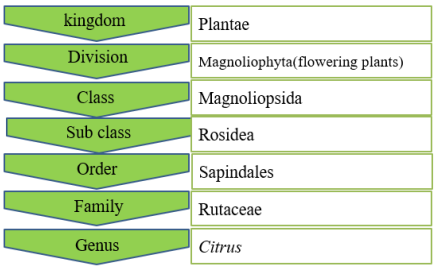


Figure 1.5: Taxonomy of Citrus (Inglese, 2019)

In all continents of the world, Citrus is one of the vital commercial fruits that is grown (Inglese, 2019) Recent studies have indicated that Citrus is a part of the Rutaceae family. The Citrus genus is the most cultivated species of Citrus, the classification based on the taxonomists is known identified to be between 16 and 156 species (Gastauer and Meira-neto, 2017). This study includes Citrus aurantifolia, Citrus lemonicious, Citrus aurantium, Citrus sinensis, and Citrus reticulate. In this study, water and methanol were selected as solvents for the extraction of bioactive compounds from different Citrus species (Table 1.1.) Water is a widely available and eco-friendly solvent (Lajoie et al., 2022), while methanol is known for its ability to extract a diverse range of bioactive compounds (Sasidharan et al., 2011). The health benefits observed from the consumption of citrus are mainly due to the natural phytochemicals contained within, these include multiple secondary metabolites; flavonoids, limonoids, furocoumarins, and pectins. The secondary metabolites provide numerous health benefits such as anti-oxidative, anti-cancer, neuroprotection, etc. (Patil, 2006). Citrus Flavonoids are considered to be excellent antioxidants that are potent free radical scavengers that contribute to the prevention of diseases which occur due to oxidation (Ashraf et al., 2017).

The main objective of this study was to determine the impact of different solvents (water and methanol), on the phytochemical profile and antioxidant activity of selected species from the Citrus family. To achieve this objective, several specific objectives were outlined. Firstly, the total antioxidant capacity of the extracts was examined using Molybdate reagent, while the total phenolic content of the extracts was examined using Folin ciocalteu reagent. Qualitative assays were carried out to recognize the presence of phytochemicals in the extracts, and the flavonoid content was determined using an aluminum chloride colorimetric method. The antimicrobial properties of the extracts were evaluated using E. coli and S. auers Finally, the free radical scavenging ability of the extracts was examined using DPPH. By achieving these specific objectives, this study provided valuable insights into the impact of different solvents on the phytochemical profile and antioxidant activity of Citrus species. The results of this study will enhance our knowledge of the effect of different solvents on the phytochemical composition and antioxidant activity of Citrus extracts and may have important implications for the development of novel plant-based antioxidant products and mainly focusing on making them promising candidates for drug development. In order to develop effective drugs, it is important to determine the safety and efficacy of these compounds through rigorous scientific investigation. In addition, understanding the mechanism of the activity of these bio-compounds is crucial for the development of effective and safe drugs.

MATERIALS & METHODOLOGY

Materials

Chemicals and reagent

Aluminium chloride ($AlCl_3$) (CAS no. 7446-70-0), Ammonium hydroxide (NH_4OH), Ammonium molybdate ($[NH_4]_6Mo_7O_{24}.4H_2O$), Chloroform ($CHCl_3$) (CAS no. 67-66-3), Copper

sulphate ($CuSO_4$) (CAS no. 7758-98-7), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (CAS no. 1898-66-4), Ethanol (C_2H_5OH) (CAS no. 64-17-5), Ferric chloride ($FeCl_3$) (CAS no. 7705-08-0), Folin-Ciocalteu phenol reagent, Methanol (CH_3OH) (CAS no. 67-56-1), Hydrochloric acid (HCl) (CAS no. 7647-01-01-0), McFarland solution, Molisch's reagent, Mueller-Hinton agar powder, Silver nitrate ($AgNO_3$) (CAS no. 7761-888), Sodium borohydride ($NaBH_4$), Sodium carbonate (Na_2CO_3) (CAS no. 497-19-8), Sodium hydroxide (NaOH) (CAS no. 1310-73-2), Sodium nitrate ($NaNO_3$) (CAS no. 7631-99-4), Sodium sulphate (Na_2SO_4) (CAS no. 775782-6), Sulphuric acid (H_2SO_4) (CAS no. 7664-93-9)

Apparatus

Analytical balance (Ohaus PA2141), Autoclave (Meditry LS-B35L-1), Biological safety cabinet (Gemmy PLC-025), Fume hood (Biobases FH1000), Incubator (Thermo scientific BB 15), Mortar and pestle, Refrigerator, Roller mixer, UV visible spectrophotometer (JENWAY 6305)

Consumables and glass-wear

Beakers, Cuvettes, Falcon tubes, Filter funnel, Filter paper, Measuring cylinder, Micropipettes, Parafilm, Spatula, Stand, Test tubes

Plant materials

Leaves from five species of Citrus were used for the study are given in Figure 2.1

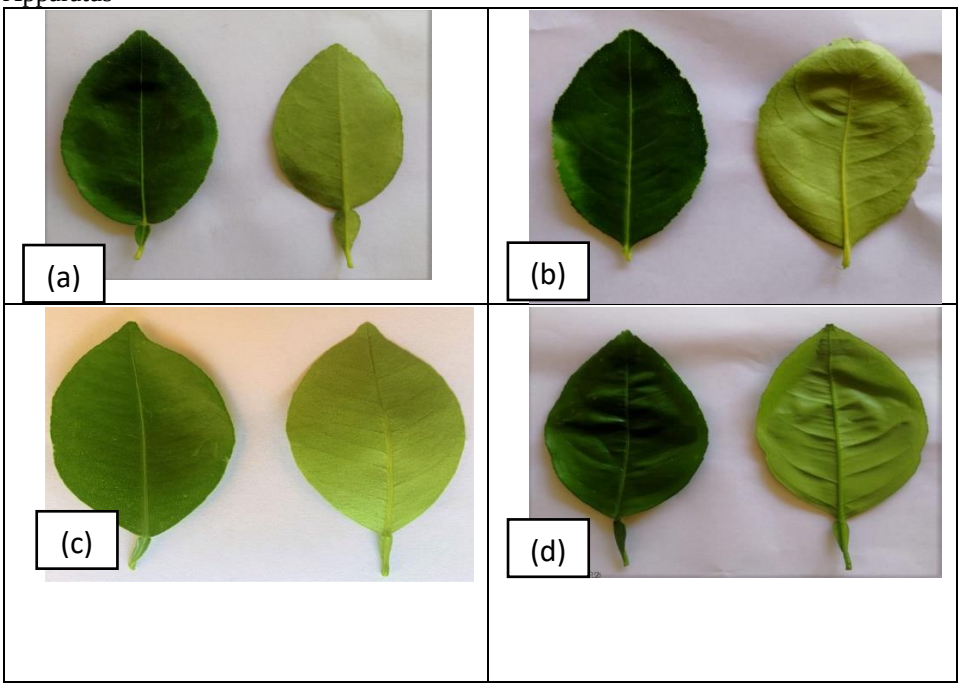


Figure 2.1: Selected leaves of Citrus family plants (a) Citrus aurantifolia (b) Citrus lemonicious (c) Citrus sinensis (d) Citrus reticulate (e) Citrus aurantium

Sample collection

Leaves of selected species (Figure 2.1) were collected from the Rathnapura district in Sri Lanka from various locations within the district in the month of September 2022. The collected leaves were then washed using distilled water.

Sample preparation and extraction

The cleaned leaves were shade dried for two weeks and dried leaves were ground to a fine powder using a pestle and mortar, 2g of grounded powder was added to 100mL of water and methanol solvents separately and placed in a roller mixer for 48 hours and then filtered using the Whatman filter paper no: 42 (Maceration technique). The extracts were stored at 4°C (Ehiobu, 2021)

Qualitative analysis of phytochemicals

All five water and methanolic extracts were analyzed qualitatively for the presence of phenols, tannins, terpenoids, flavonoids, saponins, steroids, alkaloids, and carbohydrates.

Phenols

To 1mL of the extract added an equal volume of distilled water and added 1-2 drops of 5% iron (III) chloride (Mumtaz et al.,2014)

Tannins

To 0.5mL of the extract added 0.5mL of 5% iron (III)chloride (Khong, 2019)

Terpenoids

To 0.5mL of the extract added 2mL of chloroform and added 3mL of concentrated sulphuric acid (Rao, 2016)

Flavonoids

To 0.5mL of the extract added 1mL of 20% sodium hydroxide and added diluted hydrochloride acid (Panchal, 2019)

Saponin

To 0.5mL of the extract added 1mL of distilled water and shake thoroughly (Mumtaz et al.,2014)

Steroids

To 0.5mL of the extract added 1mL of chloroform and added one or two drops of concentrated sulphuric acid (Mumtaz et al.,2014)

Alkaloids

To 1mL of the extract added 0.2mL of hydrochloric acid and added 1mL of prepared Meyers reagent (Rao, 2016)

Carbohydrates

To 1mL of the extract added equal volume of molishs reagent and added 2mL of concentrated sulphuric acid (Panchal, 2019)

Quantitative analysis of antioxidants

Citrus extracts were analyzed quantitatively to determine the total phenolic content, total antioxidant capacity and total flavonoid content.

Determination of the Total Phenolic Content (TPC)

This method was adapted from a study conducted by Shirazi et al., 2014 with slight modifications. The Folin-Ciocalteu assay was used to examined the total

phenolic content of Citrus extracts. A calibration curve was created using different concentrations of gallic acid (2-14 µg/mL). 100 µL of the Citrus extract (20 mg/mL) and 500 µL of 0.5N Folin-Ciocalteu reagent were combined with 500 µL of distilled water, and all samples were prepared in triplicate. After incubating for 6 minutes at room temperature, 1 mL of 3.5% Na_2CO_3 solution was added along with 500 µL of distilled water. The solution was left for 60 minutes at room temperature, and the absorbance was measured at 760 nm using a UV-Visible spectrophotometer. The TPC was determined as gallic acid equivalent (µg GAE/g) using the gallic acid standard curve.

Determination of the Total Antioxidant Capacity (TAC)

This method was adapted from a study conducted by Shirazi et al., 2014 with slight modifications. The concentration series of levo ascorbic acid (L-ascorbic acid) was prepared (2-20 µg/mL). 300 µL of the L-ascorbic acid solution was mixed with 3 mL of prepared Phosphomolybdenum reagent, which was composed of 1 mL of 28 mM Sodium Phosphate monobasic, 1 mL of 4 mM Ammonium Molybdate, and 1 mL of 0.6 mM H_2SO_4. The resulting mixture was then incubated at 95°C for 90 minutes. The absorbance of the samples was measured at 695 nm using a spectrophotometer. The same procedure was repeated for all the Citrus extracts in triplicate. The TAC was determined as L-ascorbic acid equivalent (µg AAE/mL) using the L-ascorbic acid standard curve.

Total Flavonoid Content (TFC)

This method was adapted from a study conducted by Shirazi et al., in 2014 with slight modifications. For the determination of TFC 100µL of each extract was diluted with 500µL of distilled water and 100µL of 5% $NaNO_3$. After incubation for 6 minutes, 150µL of 10% $AlCl_3$ was added, followed by another 5-minute incubation period. 200µL of 1M NaOH was added, and the absorbance was measured at 510nm. Triplicates were analysedfor each sample. The TFC was determined as quercetin equivalent (µg/mL QE) using the quercetin standard curve.

Determination of the free radical scavenging activity:

DPPH free radical scavenging assay was carried out to determine the ability of extracts to scavenge the DPPH free radicals.

2,2 Diphenyl-1-picrylhydrazyl (DPPH)

The DPPH radical scavenging assay was carried out with slight modifications based on the method described by HeimLer et al., 2005. 2.0 mL volume of a diluted Citrus extract with a concentration range of 0.2-14mg/mL was mixed with 2.0mL of a methanol solution containing 0.025% DPPH. Incubated in dark at room temperature for 20 minutes. The decrease in absorbance of each sample was measured at 517nm using a UV-Visible spectrophotometer and compared. To determine the half-maximal Inhibitory Concentration (IC50) values for the samples, the percentage inhibition was calculated using this formula:

$$\text{DPPH inhibition (\%)} = \left[\frac{(A_{\text{control}} - A_{\text{sample}}) \times 100}{A_{\text{control}}} \right]$$

A control = Absorbance of control

A Sample = Absorbance of Citrus sample

To determine the IC50 value, a dose responsive graph was generated by plotting the percentage of inhibition against the concentration of the extracts. The analysis was done in triplicates.

Antibacterial susceptibility test

Antibacterial susceptibility test was carried out to determine the antibacterial

activity of extracts for E. coli and S. aureus.

This method was adapted from a study conducted by Sati, 2011. Muller Hinton agar was prepared and autoclaved. Macfarland standard was prepared and absorbance was measured at 525nm to ensure it fell within the range of 0.08 Macfarlnd forming units (MFU) to 0.10 MFU. A loopful of E. coli and S. aureus cultures were collected using a sterilized inoculation loop and suspended in 10 mL of sterile distilled water. The turbidity of the bacterial suspensions was compared to the MacFarland standard using a wickerham card to ensure standardization. The bacteria were inoculated on the agar plates using the spread plate technique. Wells were made on the agar surface, 50µL of sterile distilled water (negative control), and 50µL Citrus extract were added to the wells. Gentamicin (positive control) discs were also placed on the agar plate. The plates were then incubated at 37°C for 24 hours, after which the zone of inhibition around the wells was measured and the strains' susceptibility was determined.

Statistical analysis

Obtained results were subjected to one way ANOVA analysis. The mean and standard deviation of the results were calculated. The data were further analyzed for correlations and regression by SPSS and t-tests at a significance level of $p<0.05$. Statistical and linear regression evaluations were performed using IBM SPSS 28 and Microsoft Excel software, as described by Dahiru T. 2008.

RESULTS

Qualitative analysis of phytochemicals

Phytochemical analysis of five selected citrus in water and methanol were interpreted in table 3.1 and table 3.2

Table 3.1: Qualitative analysis of Citrus

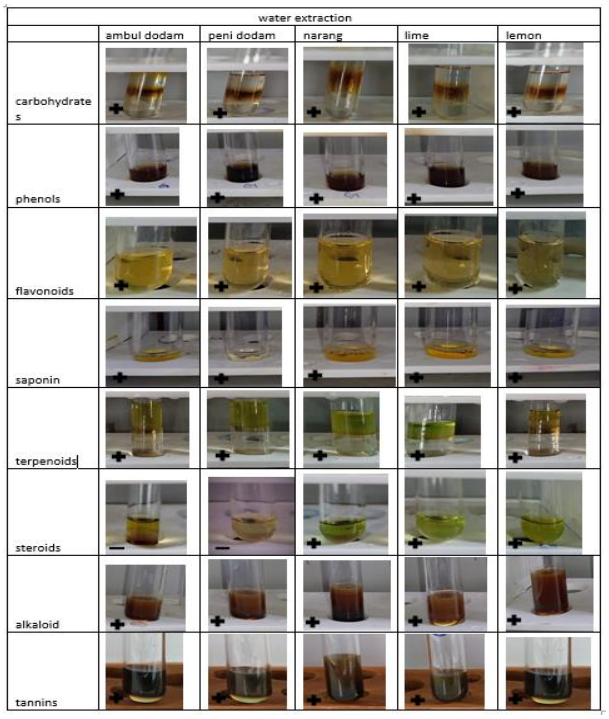
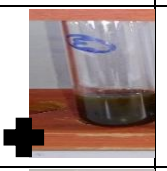
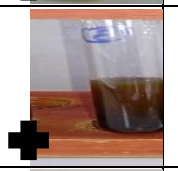
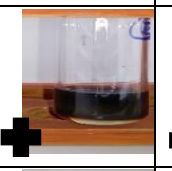
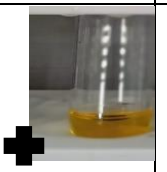
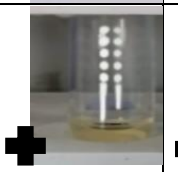
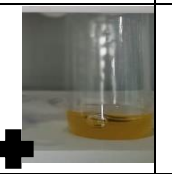
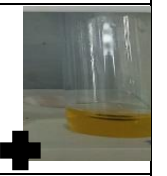
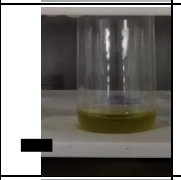
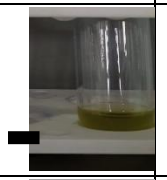
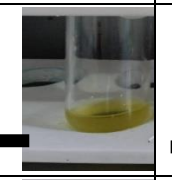
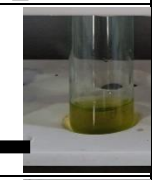
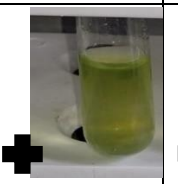
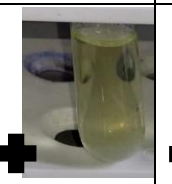
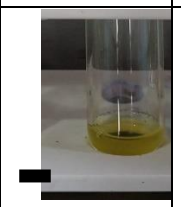
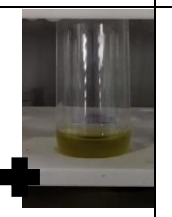
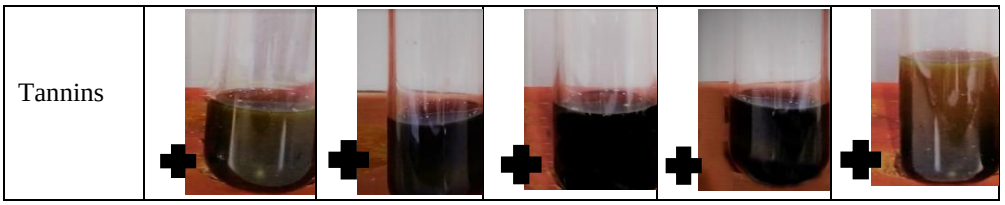


Table 3.2: Qualitative analysis of Citrus

	Methanol extracts				
	C.auranti	C.sinesi	C.aurantif	C.lemoni	C.reticulat
Carbohydrates					
Phenols					
Flavonoids					
Saponin					
Terpenoids					
Steroids					
Alkaloid					



No steroids were found in the water extracts of C. aurantium and C. sinensis, whereas extracts of C. aurantifolia, C. lemonicious, and C. reticulate contained steroid (Table 3.1).

Saponin was absent in all found methanol extracts and no steroids were found in the methanol extracts of C. aurantium and C. sinensis, whereas extracts of C. aurantifolia, C. lemonicious, and C. reticulate contained steroid content (Table 3.2)

Quantitative analysis of antioxidants

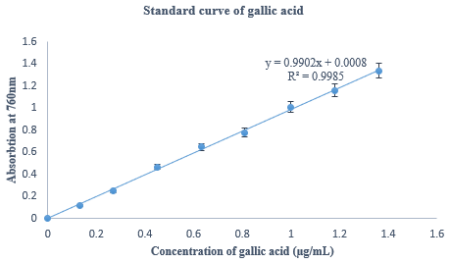


Figure 3.1: The standard curve of gallic acid. The mean absorbance values were reported as mean ± Standard deviation (SD)

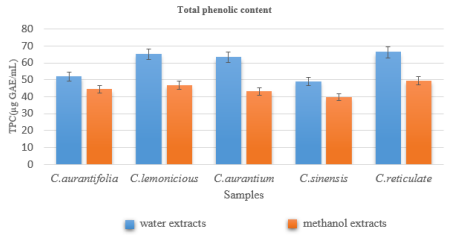


Figure 3.2: Results of total phenolic content. The mean of TPC values were reported as mean ± SD

Total Antioxidant Capacity

Citrus extracts were analyzed quantitatively to determine the total phenolic content, total antioxidant capacity and total flavonoid content.

Total Phenolic Content

Among the water extracts, (66.25 µg GAE/g) C. reticulate had the highest TPC value, while (49 µg GAE/g) C. sinensis had the lowest TPC value. Among the methanolic extracts, (49.3 µg GAE/g) C. reticulate had the highest TPC value, while (39.6 µg GAE/g) C. sinensis had the lowest TPC value.

Among the water extracts, C.sinensis showed the highest TAC (20.8411 µg AAE/mL), followed by C.reticulate (19.7287 µg AAE/mL) and C.aurantifolia (19.9974 µg AAE/mL). C.aurantifolia displayed the highest TAC (14.6263 µg AAE/mL) in methanolic extracts, followed by C.sinensis (10.5106 µg AAE/mL) and C.reticulate (6.2069 µg AAE/mL). The TAC values for C.lemonicious and C.aurantium were found to be moderate in both water and methanolic extracts.

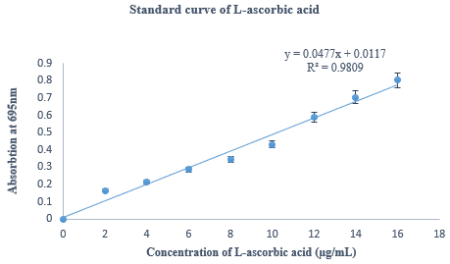


Figure 3.3: The standard curve of L-ascorbic acid. The mean absorbance values were reported as mean ± SD

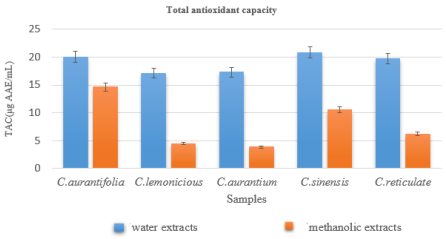


Figure 3.4: Results of total antioxidant capacity. The mean of TAC values was reported as mean ± SD

Total flavonoid content

The highest TFC value was obtained from the water extract of C. lemonicious (80.58 µg/mL QE), followed by C. reticulate (65.78 µg/mL QE) and C. aurantium (65.48 µg/mL QE). The lowest TFC value was obtained from the methanolic extract of C. sinensis (14.01 µg/mL QE), followed by C. aurantium (28.24 µg/mL QE) and C. lemonicious (24.21 µg/mL QE).

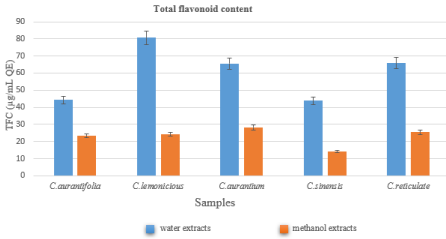


Figure 3.5: Results of total flavonoid content. The mean of TFC values were reported as mean ± SD

Total flavonoid content

The highest TFC value was obtained from the water extract of C. lemonicious (80.58 µg/mL QE), followed by C. reticulate (65.78 µg/mL QE) and C. aurantium (65.48 µg/mL QE). The lowest TFC value was obtained from the methanolic extract of C. sinensis (14.01 µg/mL QE), followed by C. aurantium (28.24 µg/mL QE) and C. lemonicious (24.21 µg/mL QE).

Determination of the free radical scavenging activity:

DPPH free radical scavenging assay was carried out to determine the ability of extracts to scavenge the DPPH free radicals.

2, 2 Diphenyl-1-picrylhydrazyl

C. reticulate had the lowest IC50 value for water extracts (32.11 mg/mL), indicating the highest radical scavenging activity. C. sinensis had the highest IC50 value for water extracts (395.46 mg/mL), indicating the lowest radical scavenging activity. For methanolic extracts, C. lemonicious had the lowest IC50 value (117.04 mg/mL), while C.sinensis had the highest IC50 value (244.21 mg/mL).

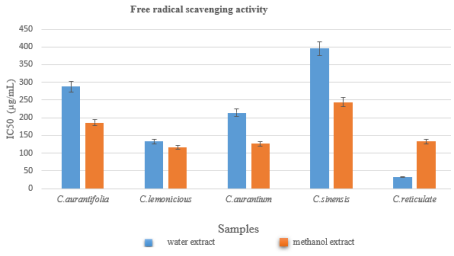
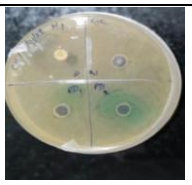
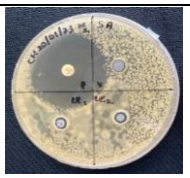
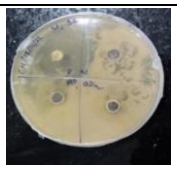
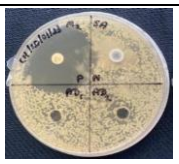
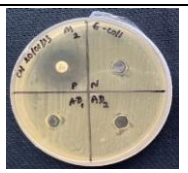
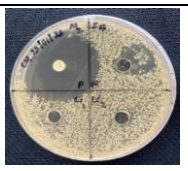
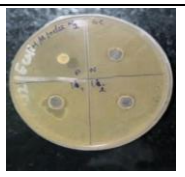
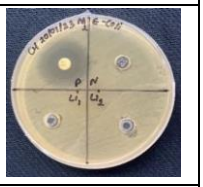
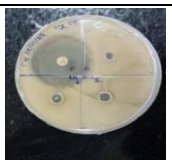
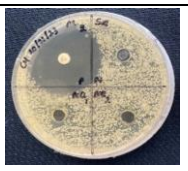
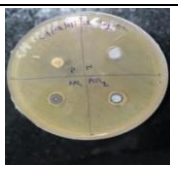
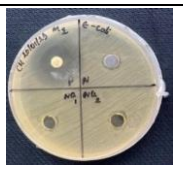


Figure 3.6: Results of free radical scavenging activity. The mean of IC50 values were reported as mean ± SD

Antibacterial susceptibility test

ABST carried out for both methanol and water extracts of Citrus sample using E.coli and S.aureus for 20mg/mL concentration.

Table 3.3: Efficacy on antibacterial activity against E. coli and S. aureus in Citrus water and methanol extracts at 20mg/mL

	S. aureus		E. coli	
	Water extracts	Methanol extracts	Water extracts	Methanol extracts
C. sinensis				
C. lemonicious				
C.aurantium				
C.aurantifolia				
C. reticulate				

In water extracts C. aurantifolia and C. lemonicious exhibited moderate sensitivity against E. coli, with Zone of inhibition (ZOI) of 1.15 cm and 0.9 cm, respectively. C. aurantium and C. sinensis demonstrated moderate to strong sensitivity against S. aureus, with ZOI of 2.20 cm and 1.75 cm, respectively.

In methanolic extracts C. aurantifolia ZOI of 1.225 cm against E. coli, indicating moderate sensitivity. In contrast, C. lemonicious and C. sinensis did not show any ZOI against E. coli. C. reticulate had ZOI of 1.1 cm, indicating moderate sensitivity against E. coli.

Regarding S. aureus, none of the Citrus methanol extracts showed any ZOI.

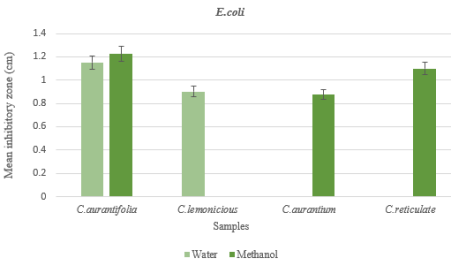


Figure 3.7: Antibacterial susceptibility test for Citrus water and methanol extracts against E.coli at 20mg/mL. The mean of ZOI were reported as mean ± SD

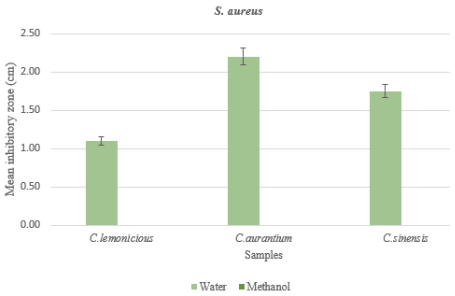


Figure 3.8: Antibacterial susceptibility test for Citrus water and methanol extracts against S.aureus at 20mg/mL. The mean of ZOI were reported as mean ± SD

Statistical analysis

The Pearson correlation analysis showed that there were strong positive correlations between TPC and TFC in both citrus methanol (0.643) and water (0.916) extracts, while negative correlations were observed between TAC and TPC (methanol: -0.372, water: -0.719) as well as TAC and TFC (methanol: -0.522, water: -0.84) in both extracts. Additionally, a strong positive correlation was observed between TAC and DPPH in citrus methanol extract (0.743).

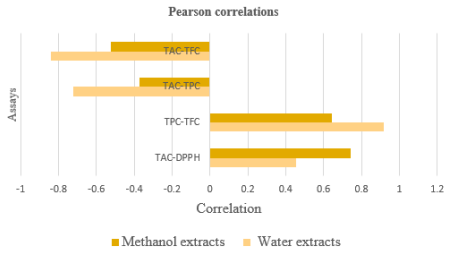


Figure 3.9: Pearson correlations among TAC-TFC, TAC-TPC, TPC-TFC, and TAC-DPPH for water and methanol extracts

DISCUSSION

The Citrus is phytochemicals rich source that have been shown to possess a range of potential health benefits, including antioxidant and antimicrobial properties (Okwu, D.E. 2007). The composition and bioactivity of Citrus extracts can be influenced by a number of factors, including the type of solvent used for extraction. This study investigated the impact of water and methanol solvents on the phytochemical composition and antioxidant activity of selected Citrus species. TAC was determined using the MoO_4^{2-} reagent, TPC was determined using the $C_{10}H_5NaO_5S$ reagent, and TFC was determined using an aluminum chloride colorimetric method. Qualitative assays were carried out to identify the presence of certain phytochemicals in the extracts, and the antimicrobial properties of the extracts were evaluated against E. coli and S. aureus. The free radical scavenging ability of the extracts was determined using the DPPH. The results of this study provide valuable insights into the effect of solvent type on the phytochemical composition and antioxidant activity of Citrus extracts, with potential implications for the development of novel plant-based antioxidant products and drugs. This qualitative analysis is based on the principle that certain phytochemicals have characteristic colors or reactions with specific reagents. The present study revealed that the water extracts of C. aurantium and C. sinensis lacked the presence of steroids, while extracts of C. aurantifolia, C. lemonicious, and C. reticulate were found to contain steroid content. Furthermore, the water extracts of all Citrus samples examined in the study demonstrated the presence of carbohydrates, phenols, flavonoids, saponin, terpenoids, alkaloids, and tannins, with saponin being absent in all methanolic extracts. The results are consistent with previous findings by

Irdeena, N.I. et al., 2019, who reported alkaloids, reducing sugars, and carbohydrates in the crude extracts of C. aurantifolia, C. hystrix, and C. microcarpa. Additionally, Tamboli.A et al., 2020 research revealed a diverse range of phytochemicals, including alkaloids, saponins, tannins, phenols, flavonoids, glycosides, steroids, terpenoids, and flavonoid, present in the water extracts of Citrus peel. Roghini, R. and Vijayalakshmi, K. 2018 study confirmed the presence of alkaloids, flavonoids, reducing sugars, phenols, amino acids, saponins, tannins, terpenoids, and glycosides in Citrus paradisi, with anthraquinone and steroids being absent in all extracts.

The variability in the composition of phytochemicals among different Citrus species revealed by these studies is significant and may have important implications for their potential bioactivity and health benefits. The determination of TPC is based on the deduction of the Folin-Ciocalteu reagent by phenolic compounds present in the Citrus extracts. Comparing these results to the literature, it can be observed that the TPC values of the Citrus extracts in this study are relatively low compared to those of other Citrus species and different fruit varieties reported in previous studies. For example, Gurung, R 2022 reported that C. aurantifolia and C. maxima contain higher phenol content, 12.48 - 0.400 GAE/gm and Abeysuriya, H. et al., 2020 found that Psidium guajava (white) showed the highest TPC of 180.6 mg/100 g, which is significantly higher than the TPC values obtained in this study. However, it is important to note that the TPC values of the Citrus leave extracts in this study are within the range reported by Ben Rejeb, I. 2020 for phenolic contents of juices, which varied according to ranged and species between 319.27 and 786.85 GAE/g. The TPC values of the water extracts of the Citrus extracts in this study fall within this range, with the highest TPC value of 66.25 µg GAE/g observed for C. reticulate. In this assay all the water extracts showed significant higher value than the corresponding methanol extracts ($p < 0.05$) and there is a significant difference between the mean of water extracts and mean of methanolic extracts (F-critical < F-statistic).

Overall, the results suggest that the water and methanolic extracts of the Citrus extracts studied in this research contain moderate levels of phenolic compounds. However, the TPC values obtained are lower than those reported for some other Citrus fruits. The determination of TAC is based on the reduction of MoO_4^{2-} to Mo^{5+} by antioxidants present in the Citrus extracts. The results showed that C.sinensis had the highest TAC in water extracts, followed by C.reticulate and C.aurantifolia. In contrast, C. aurantifolia displayed the highest TAC in methanolic extracts, followed by C.sinensis and C.reticulate. These findings are consistent with the study conducted by Azman, et al., 2019, which found that frozen Citrus peels had higher TFC than fresh Citrus peels. The TFC of fresh lemon peel and frozen lemon peel was significantly different, and frozen lemon peel had a 54.7% increment in TFC compared with the fresh lemon peel. The determination of TFC is based on the formation of a complex between flavonoids and $AlCl_3$. The TFC values obtained in the present study ranged from 43.91 µg/mL QE C. sinensis to 80.58 µg/mL QE C. lemonicious in water extracts and from 14.01 µg/mL QE C. sinensis to 28.24 µg/mL QE C. aurantium in methanolic extracts. The highest TFC value was obtained from the water extract of C. lemonicious, followed by C.reticulate and C.aurantifolia. These results are compatible with the study conducted by Zhang H, 2018 which found that the number of flavonoids present in fruit peels was higher than in pulp residues, seeds, and juices. The results

showed that, on average, the flavonoid content in different fruit tissues ranked in the following order from highest to lowest: peels > juices > pulp residues and seeds.

Furthermore, the present study found that C. aurantifolia had the highest TAC in methanolic extracts concurrences with the findings of Jerang, A, 2022 who reported that the value of total antioxidant capacity was recorded as 150±0.333 (µg AAE/mL) in peel extract in Citrus aurantium. Additionally, Hunlun, C. 2017 found that the juice extracted from various cultivars cultivated in the same geographic area exhibited variations in terms of their TPC and TFC, while the impact of the seasons on this difference was found to be insignificant. The present study provides evidence that Citrus species have significant variations in their TAC and TFC values in water and methanolic extracts ($p < 0.05$). These findings are consistent with previous research on Citrus peels, juices, and extracts. These variations may be attributed to differences in genetic makeup, environmental conditions, and processing methods. Expressing TAC and TFC values, all the water extracts showed significant higher values than the corresponding methanol extracts values ($p < 0.05$) and there is a significant difference between the mean of water extracts and mean of methanolic extracts (F-critical < F-statistic) of both assays.

Determination of free Radical Scavenging activity is based on the ability of the Citrus extracts to scavenge free radicals. The results found that among the tested Citrus species, C. reticulate had the lowest IC50 value for water extracts, indicating the highest radical scavenging activity (32.11 mg/mL). This is consistent with the findings of the study conducted by Jerang, A. 2022 which reported that C. reticulate showed 85% scavenging activity and had the lowest IC50 value among the samples tested using hexane extracts.

The results showed that C. sinensis had the highest IC50 value for water extracts (395.46 mg/mL), indicating the lowest radical scavenging activity. This is different from the findings of the studies conducted by Azman, et al., 2019 and Abeysuriya, H et al., 2020, which reported that lemon and lime peels had lower EC50 values and higher radical scavenging activities compared to other fruits. In terms of methanolic extracts, the results showed that C.lemonicious had the lowest IC50 value (117.04 mg/mL), indicating high radical scavenging activity, which is different with the findings of the study conducted by Abeysuriya, H et al., 2020, which reported that C. aurantifolia had low IC50 values compared to other fruits. Conversely, C. sinensis had the highest IC50 value (244.21 mg/mL), indicating low radical scavenging activity, which is also different from the findings of the study conducted by Azman, et al., 2019, which reported that lemon peel had a low EC50 value and high radical scavenging activity. Except C.reticulate, all the water extracts showed significant higher IC50 values than the corresponding methanol extracts values ($p < 0.05$)

Overall, the results align with some of the findings from previous studies, but there are also differences. It is important to consider the methods and conditions used in each study and the specific plant genotypes and extracts used, as these factors can influence the results. The ABST is based on the ability of the Citrus extracts to inhibit the growth of E. coli and S. aureus bacterial strains in 20mg/mL concentration. The extracts are tested using the agar well diffusion method, and the ZOI are measured. In terms of antibacterial activity against E. coli, the study found that C. aurantifolia had a moderate sensitivity with ZOI of 1.15 cm for water extraction and 1.225 cm for methanol extraction. This is comparable to the results found in the Hasan MM 2022 study, which showed that methanolic

extract of Citrus macroptera peel had the highest antibacterial activity of 2.2 cm and 2.6 cm against Bacillus spp. and E. coli, respectively. However, the studies by Shetty et al., 2016 and Ekawati et al., 2019 did not use C. aurantifolia in their experiments. For C. lemonicious, the study found a weak sensitivity with ZOI of 0.9 cm for water extraction and no ZOI for methanol extraction against E. coli. This is consistent with the Ekawati et al., 2019 study, which found that lemon juice did not inhibit Enterotoxigenic Escherichia coli (ETEC) bacterial growth at concentrations of 100 mg/mL to 400 mg/mL, and showed only intermediate results with diameters of ETEC bacteria growth ZOI of 11 mm, 12 mm, 13 mm and 15 mm at concentrations of 500 mg/mL to 800 mg/mL.

Regarding S. aureus, the study found that C. lemonicious had a moderate sensitivity with an ZOI of 1.10 cm for water extraction, while none of the Citrus methanol extracts showed any ZOI against this strain. This is similar to the finding from the Hasan MM 2022 study, where the methanolic extract of Citrus assamensis had the lowest antibacterial activity of 1.7cm and 2.1 cm as ZOI against Bacillus spp. and E. coli, respectively, and C. reticulate water extract did not show any ZOI against E. coli. without C. aurantifolia and C. sinensis, Only the C. lemonicious water extract was showed significant higher value than the corresponding methanol extract value ($p < 0.05$) against E. coli. without C. aurantifolia and C. reticulate, rest of the water extracts were showed significant higher values than the corresponding methanol extracts ($p < 0.05$) against S. aureus. Overall, the results suggest that different Citrus fruits have varying degrees of antibacterial activity against E. coli and S. aureus, and the extraction method and concentration can also affect the effectiveness of the extracts. The findings provide additional insights into the antibacterial activity of different Citrus fruits and their extracts.

In conclusion, the phytochemical composition, TPC, TAC, TFC of water and methanolic extracts from various Citrus species were analyzed. The presence of carbohydrates, phenols, flavonoids, terpenoids, alkaloids, and tannins was detected in all Citrus extracts, while steroid content were absent in water and methanol extracts of C. aurantium and C. sinensis. Compared to other Citrus species and fruit varieties reported in earlier research, the TPC values of Citrus extracts were relatively low, but the TAC values varied among extracts, with C. sinensis and C. aurantifolia displaying the highest TAC in water and methanolic extracts, respectively. C. lemonicious showed the highest TFC value in water extracts, while C. aurantium exhibited the highest TFC value in methanolic extracts. Moreover, the study evaluated the free radical scavenging activity and antibacterial potential of Citrus extracts using water and methanol extracts. The DPPH assay and ABST results indicated that C. reticulate had the highest radical scavenging activity for water extracts, while C.lemonicious had the highest activity for methanol extracts. Additionally, C. aurantifolia exhibited comparable moderate sensitivity against E. coli, while C. aurantium displayed great sensitivity against S. aureus. When comparing solvent effects on the phytochemical composition, the study showed that water extracts presented significantly higher values of TPC, TAC, and TFC when compared to their corresponding methanol extracts ($p < 0.05$). This study highlights the significance of solvent selection when extracting phytochemicals from Citrus species. Water extracts of Citrus species contain comparable levels of phenolic compounds and exhibit antioxidant activity, while methanolic extracts are rich in flavonoids and antioxidants.

Consequently, water is deemed the optimal solvent for extracting TPC, TAC, and TFC from Citrus species. These findings hold crucial implications for the development of functional foods and natural antioxidant medicines with health-promoting properties.

REFERENCES

Abeysuriya, H.I., Bulugahapitiya, V.P. and Loku Pulukkuttige, J. 2020 Total vitamin C, ascorbic acid, dehydroascorbic acid, antioxidant properties, and iron content of underutilized and commonly consumed fruits in Sri Lanka, International Journal of Food Science. Hindawi. Available at: https://www.hindawi.com/journals/ijfs/2020/4783029/.

Academy, T. N. 2010 The Norwegian Academy of Science and Letters Bioactive compounds in plants – benefits and risks for man and animals Proceedings from a symposium held at.

Adenaike, O. and Abakpa, G. O. 2021 'Antioxidant Compounds and Health Benefits of Citrus Fruits', 13(2), pp. 65–74.

Aruoma, O. I., Grootveld, M. and Bahorun, T. 2006 'Free Radicals in Biology and Medicine :From Inflammation to Biotechnology', 27, pp. 1–3.

Ashraf, H., Butt, M. S. and Iqbal, M. J. 2017 'Asian Paci fi c Journal of Tropical Biomedicine', pp. 1–11.

Azman, N.F.I.N. 2019 Antioxidant properties of fresh and frozen peels of citrus species, Current Research in Nutrition and Food Science Journal. Available at: https://www.foodandnutritionjournal.org/volume7number2/antioxidant-properties-of-fresh-and-frozen-peels-of-citrus-species/.

Ben Rejeb, I. 2020 Quality Evaluation and functional properties of reduced sugar jellies formulated from citrus fruits, Journal of Chemistry. Hindawi. Available at: https://www.hindawi.com/journals/jchem/2020/5476872/.

Brambilla, D., Mancuso, C., Scuderi, M. R., Bosco, P., Cantarella, G., Lempereur, L., Di Benedetto, G., Pezzino, S., & Bernardini, R. 2008. The role of antioxidant supplement in immune system, neoplastic, and neurodegenerative disorders: a point of view for an assessment of the risk/benefit profile. Nutrition journal, 7, 29.

Caputo, L., Quintieri, L., Cavalluzzi, M. M., Lentini, G., & Habtemariam, S. 2018. Antimicrobial and Antibiofilm Activities of Citrus Water-Extracts Obtained by Microwave-Assisted and Conventional Methods. Biomedicines, 6(2), 70.

Dahiru T. 2008. P - value, a true test of statistical significance? A cautionary note. Annals of Ibadan postgraduate medicine, 6(1), 21–26.

Daniella HeimLer, P . V. M. G. D. A. R., 2005. Rapid Test to Assess the Antioxidant Activity of Phaseolus vulgaris L. Journal of agricultural and food chemistry, 53(8), pp.3053-3056.

Di Majo D, Giammanco M, La Guardia M, Tripoli E, Giammanco S, Finotti E 2005. Flavanones in Citrus fruit: Structure-antioxidant activity relationships. Food Res. Intern. 38:1161-1166.

Donno, D., Mellano, M., De Biaggi, M., Riondato, I., Rakotoniaina, E., & Beccaro, G. 2018. New Findings in Prunus padus L. Fruits as a Source of Natural Compounds: Characterization of Metabolite Profiles and Preliminary Evaluation of Antioxidant Activity. Molecules, 23(4), 725.

Ekawati, E.R. and Darmanto, W. 2019 Lemon (citrus limon) juice has antibacterial potential against diarrhea . IOP Conf. Ser.: Earth Environ. Sci. 217 012023. Available at: https://iopscience.iop.org/article/10.1088/1755-1315/217/1/012023.

Ekor, M. 2014 The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety, Frontiers in

pharmacology. U.S. National Library of Medicine. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3887317/

Fiorentini, C., Duserm Garrido, G., Bassani, A., Cortimiglia, C., Zaccone, M., Montalbano, L., Martinez-Nogues, V., Cocconcelli, P. S., & Spigno, G. 2021. Citrus Peel Extracts for Industrial-Scale Production of Bio-Based Active Food Packaging. Foods, 11(1), 30.

Gastauer, M. and Meira-neto, J. A. A. 2017 'Updated angiosperm family tree for analyzing phylogenetic diversity and community structure', 31(June), pp. 191–198.

Gloria Bobo-García, G. D.-P. C. A. P. V. M. R. M.-A. a. M. N., 2014. Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. J Sci Food Agric, pp. 204-209.

Gurung, R. 2022 A comparative study on ascorbic acid concentration, total phenol, and flavonoid content in citrus species grown in a different region of western Nepal, International Journal of Food Science. Hindawi. Available at: https://www.hindawi.com/journals/ijfs/2022/3012623/.

Hafiza Noreen, N. S. F. J. S. M., 2017. Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant Coronopus didymus. Asian Pacific Journal of Tropical Medicine, pp. 793-799.

Hasan MM 2022 Antimicrobial activity of peels and physicochemical properties of juice prepared from Indigenous Citrus Fruits of Sylhet region, Bangladesh, Heliyon. Elsevier. Available at: https://www.sciencedirect.com/science/article/pii/S2405844022012361.

Hlima, H. B., Bohli, T., Kraiem, M., Ouederni, A., Mellouli, L., Michaud, P., Abdelkafi, S., & Smaoui, S. 2019. Combined Effect of Spirulina Platensis and Punica Granatum Peel Extacts: Phytochemical Content and Antiphytophatogenic Activity. Applied Sciences, 9(24), 5475.

Hua Zhang, Yi fei Yang and Zhi qin Zhou 2018 Phenolic and flavonoid contents of Mandarin (citrus reticulate blanco) fruit tissues and their antioxidant capacity as evaluated by DPPH and Abts Methods, Journal of Integrative Agriculture. Elsevier. Available at: https://reader.elsevier.com/reader/sd/pii/S2095311917616642.

Hunlun, C. 2017 Characterisation of the flavonoid composition and total antioxidant capacity of juice from different citrus varieties from the Western Cape Region, Journal of Food Composition and Analysis. Academic Press. Available at: https://www.sciencedirect.com/science/article/abs/pii/S088915751730116 3.

Inglese, P. a. S. G., 2019. Citrus History, Taxonomy, Breeding, and Fruit Quality. Oxford Research Encyclopedia of Environmental Science, Issue University of Oxford, p. 23.

Inglese, P. S. G., 2019. Citrus history, Taxonomy, Breeding and fruit quality. Issue University of oxford.

Irdeena., N.I. 2019 Qualitative phytochemical screening and antioxidant activities from three different citrus leaves (Rutaceae), Preprints. Preprints. Available at: https://www.preprints.org/manuscript/201908.0293/v1.

J.M. Ehiobu, M. I. A. A., 2021. Phytochemical content and antioxidant potential of leaf extracts of Citrus limon (L.) Osbeck collected in the Eastern Cape. South African Journal of Botany, Issue https://www.sciencedirect.com/science/article/pii/S025462992100209, pp. 480-486.

Jerang, A. 2022 Anatomical and physiological responses of citrus megaloxycarpa lush.: A cryptic species of Northeast India - Applied Biochemistry and Biotechnology, SpringerLink. Springer US. Available at:

https://link.springer.com/article/10.1007/s12010-021-03786-4.

Juan, C.A. 2021 The chemistry of reactive oxygen species (ROS) revisited: Outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies, International journal of molecular sciences. U.S. National Library of Medicine. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8125527

Kathia L. Moreno-García, M. A.-R. M. M.-A. J. M.-. C. D. G.-F., 2022. Evaluation of the antioxidant, anti-inflammatory and antihyperglycemic activities of black bean (Phaseolus vulgaris L.) by-product extracts obtained by supercritical CO2. The Journal of Supercritical Fluids, 183(105560), pp. 2-6.

Khong, M. A. J. a. H. Y., 2019. Total Phenolic Content and Antioxidant and Antibacterial Activities of Pereskia bleo. Hindawi, 2019(7428593), pp. 1-4.

Kinpelu, DA and D.O. Kolawole, 2004. Phytochemical and antimicrobial activity of leaf extract of Piliostigma aaaathonningii (Schum). Sci. Focus J., 7: 64-70.

Lajoie, L., Fabiano-Tixier, A.-S., & Chemat, F. 2022. Water as Green Solvent: Methods of Solubilisation and Extraction of Natural Products—Past, Present and Future Solutions. Pharmaceuticals, 15(12), 1507.

Lobo, V. 2010 'Free radicals , antioxidants and functional foods : Impact on human health'.

Lourenço, S. C., Moldão-Martins, M., & Alves, V. D. 2019. Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications. Molecules (Basel, Switzerland), 24(22), 4132.

Lv, X. 2015 Citrus fruits as a treasure trove of active natural metabolites that potentially provide benefits for human health, Chemistry Central journal. U.S. National Library of Medicine. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4690266/.

Ma, Z., & Zhang, H. 2017. Phytochemical Constituents, Health Benefits, and Industrial Applications of Grape Seeds: A Mini-Review. Antioxidants, 6(3), 71.

Maria Castro-Puyana, M. L. M. a. M. P., 2017. Water as green extraction solvent: Principles and reasons for its use. ScienceDirect, Volume 5, pp. 31-36.

Md. Mahbubur Rahman, M. B. I., M. B. A. H. M. K. A., 2015. In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. BMC research notes, 8(621), pp. 2-6.

Meiyanto, E. and Hermawan, A. 2012 'Natural Products for Cancer-Targeted Therapy : Citrus Flavonoids as Potent Chemopreventive Agents', pp. 427–436.

Mumtaz, F. &. R. S. &. A. Z. &. A. A. &. M. H., 2014. Qualitative phytochemical analysis of some slected medicinal plants occuring in local area of Faisalabad, Pakistan. Journal of Pharmacy and Alternative Medicine, Volume 3, pp. 17-21.

Ndhlala, A., Moyo, M., & Van Staden, J. 2010. Natural Antioxidants: Fascinating or Mythical Biomolecules? Molecules, 15(10), 6905–6930.

Okwu, D.E. 2007 Citrus fruits: A rich source of phytochemicals and their roles in human health, Citrus fruits are the main fruit trees grown throughout the world and are well. research gate.

Ovais Ullah Shirazi, M. M. A. K. K. N. A. M. S. N. N. A., 2014. Determination of total phenolic, flavonoid content and free readical scavenging activities of common herbs and spices. research gate, 3(3), pp. 104-108.

Patil, B. J. E., 2006. Potential health benifits of citrus. ACS symposium series, Volume 936, pp. 1-16.

Patil, B. S. 2006 'Potential Health Benefits of Citrus : An Overview', pp. 1–16.

Pham-huy, L. A., He, H. and Pham-huy, C. 2008 'Free Radicals , Antioxidants in Disease and Health', 4(2), pp. 89–96.

Priya Panchal, N. P., 2019. Phytochemical ananlysis of medicinal herb (ocimum

sanctum). International Journal of Nanomaterial, Nanotechnoloy and Nanomedicine, 5(2), pp. 8-10.

R. Ganesan, M. V. L. R. S. P. S. a. S. C., 2017. hptlc study and in-vitro antioxidant assay of 'nellikkay thenural' a siddha drug. world journal of pharmaceutical and medical research, 3(10), pp. 162-167.

Ranjan, A., Ramachandran, S., Gupta, N., Kaushik, I., Wright, S., Srivastava, S., Das, H., Srivastava, S., Prasad, S., & Srivastava, S. K. 2019. Role of Phytochemicals in Cancer Prevention. International Journal of Molecular Sciences, 2020, 4981.

Roghini, R. and Vijayalakshmi, K. 2018 phytochemical screening, quantitative analysis of flavonoids and minerals in ethanolic extract of citrus paradisi, international journal of pharmaceutical science and research . international journal of pharmaceutical science and research . Available at: https://www.ijpsr.com/.

Samaniego, I., Brito, B., Viera, W., Cabrera, A., Llerena, W., Kannangara, T., Vilcacundo, R., Angós, I., & Carrillo, W. 2020. Influence of the Maturity Stage on the Phytochemical Composition and the Antioxidant Activity of Four Andean Blackberry Cultivars (Rubus glaucus Benth) from Ecuador. Plants, 9(8), 1027.

Samaniego, I., Espin, S., Cuesta, X., Arias, V., Rubio, A., Llerena, W., Angós, I., & Carrillo, W. 2020. Analysis of Environmental Conditions Effect in the Phytochemical Composition of Potato (Solanum tuberosum) Cultivars. Plants, 9(7), 815.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Yoga Latha, L. 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African journal of traditional, complementary, and alternative medicines : AJTCAM, 8(1), 1–10.

Sati, S. 2011. Antibacterial Activity of the Himalayan Lichen Parmotrema nilgherrense Extracts. British Microbioloy Research Journal, 1 (2), pp. 26-32.

Schinella, G. R., Tournier, H. A., Prieto, J. M., Mordujovich de Buschiazzo, P., & Ríos, J. L. 2002. Antioxidant activity of anti-inflammatory plant extracts. Life sciences, 70(9), 1023–1033.

Shetty, S.B. 2016 Antimicrobial effects of citrus sinensis peel extracts against dental caries bacteria: An in vitro study, Journal of clinical and experimental dentistry. U.S. National Library of Medicine. Available:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4739372/.

Siddeeg, Azhari & Alkehayez, Nora & Abu-Hiamed, Hind & Al-Sanea, Ekram & Al-Farga, Ammar, 2020. Journal Pre-proofs Review Mode of action and determination of antioxidant activity in the dietary sour- ces: An Overview Mode of action and determination of antioxidant activity in the dietary sources: An Overview. Saudi Journal of Biological Sciences, Volume 28, pp. 2-3.

Sombié, P., Compaoré, M., Coulibaly, A., Ouédraogo, J., Tignégré, J.-B., & Kiendrébéogo, M. 2018. Antioxidant and Phytochemical Studies of 31 Cowpeas (Vigna unguiculata (L. Walp.)) Genotypes from Burkina Faso. Foods, 7(9), 143.

Sun, Y., Li, M., Mitra, S., Hafiz Muhammad, R., Debnath, B., Lu, X., Jian, H., & Qiu, D. 2018. Comparative Phytochemical Profiles and Antioxidant Enzyme Activity Analyses of the Southern Highbush Blueberry (Vaccinium corymbosum) at Different Developmental Stages. Molecules, 23(9), 2209.

Tamboli, A., Karande, N.K.| P. and Chandanshive, A. 2020 Qualitative and quantitative phytochemical screening of Citrus Peel, International Journal of Trend in Scientific Research and Development. International Journal of Trend in Scientific

Tan Bee Ling, N. M. E. L. W.-P.-P. S. R. H., 2018. Antioxidant and Oxidative Stress: A Mutual Interplay in Age-

Related Diseases.Frontiers in Pharmacology.

Tvrdá, E. and Benko, F. 2020 Free radicals: What they are and what they do, Pathology. Academic Press. Available at: https://www.sciencedirect.com/science/article/pii/B978012815972900019

U.S. Mahadeva Rao, m. a. k. s. m., 2016. phytochemical screening, total flavonoid and phenolic content assays of various solvent extracts of tepal of Musa paradisiaca. Malaysian Journal of Analytical Sciences, 20(5), pp. 1181-1190.

W.M. Otang, A. A., 2016. Antimicrobial and antioxidant efficacy of Citrus limon L. peel extracts used for skin diseases by Xhosa tribe of Amathole District, Eastern Cape, South Africa,. s.l.:South African Journal of Botany pp 46-49.

Xu, D., Hu, M.-J., Wang, Y.-Q., & Cui, Y.-L. 2019. Antioxidant Activities of Quercetin and Its Complexes for Medicinal Application. Molecules, 24(6), 1123.

Zou, T.-B., He, T.-P., Li, H.-B., Tang, H.-W., & Xia, E.-Q. 2016. The Structure-Activity Relationship of the Antioxidant Peptides from Natural Proteins. Molecules, 21(1), 72.