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Phytochemical analysis and the evaluation of antioxidant and antimicrobial activities of five Sri Lankan Cucurbitaceae varieties

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Abstract

Medicinal plants of the Cucurbitaceae family include a variety of edible fruits and vegetables. Various parts of these plants show different pharmacological activities such as anticancer, antidiabetic, hypolipidemic, immunomodulatory, anti-inflammatory, and antimicrobial activities. Five Sri Lankan Cucurbitaceae varieties: Cucumber (*Cucumis sativus*), Pumpkin (*Cucurbita maxima*), Watermelon (*Citrullus lanatus)*, Snake gourd (*Trichosanthes cucumerina*), and bitter gourd (*Momordica charantia*) were chosen and the leaves of the selected varieties were analyzed for their phytochemicals, antioxidant and antimicrobial activities, after extraction using the maceration technique. Total phenolic content, total flavonoid content, total antioxidant capacity and the free radical scavenging activity were determined by the Folin-Ciocalteau method, Aluminum Chloride colourimetric assay, Phosphomolybdenum method, and the DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay respectively. Antibacterial susceptibility tests were also done to find the antibacterial activity of the extracts using the disc diffusion and well diffusion methods. Qualitative analysis showed the presence of flavonoids, terpenoids, phenols, proteins, saponins, and reducing sugars in all the extracts. Snake gourd had the highest phenolic content and antioxidant capacity, while watermelon had the greatest flavonoid content and the highest free radical scavenging activity among the rest of the samples. This study was useful in identifying the antioxidant and antimicrobial activities in the selected Cucurbitaceae leaf extracts and using them as potential antioxidants.

Keywords: Cucurbitaceae, phytochemicals, antioxidant, antimicrobial, plant leaves

1. Introduction

The use of plants as a source of remedies for the treatment of diseases dates back to prehistory, and people from all continents still follow this ancient practice. Since they play such a significant role in community safety nowadays, medicinal plants are receiving more attention from research institutions.[1] Research on medicinal plants, including toxicological and pharmacological evaluations, is key for drug research and development. Some of the drugs that are, obtained from plants are morphine, colchicine, quinidine, aspirin, tubocurarine, artemisinin, digoxin, ephedrine, physostigmine, reserpine, pilocarpine, quinine, paclitaxel, vincristine, atropine, and vinblastine.[2] Because of their easy availability, affordability, and accessibility, as well as their promising potency in contrast to the general high cost and negative side effects of general synthetic drug agents, their use is currently on the rise.[3] According to Future Market Insights (FMI), a "trend shift from conventional medicines to traditional medicines," along with a more simpler regulatory environment and growing global productive capacity, will result in a progressing compound annual growth rate (CAGR) of 7.6% over the reported 10year

forecast span (2017–2027) in the global market for plant based medicinal medicines.[4]

Herbal medicines have a solid traditional or conceptual foundation and the ability to be effective drugs in terms of safety and efficacy, leading to the treatment of numerous diseases. The present research was on a family of plants known as the Cucurbitaceae also called the gourd family, figure 1 shows a classified list of the genera and tribes of the Cucurbitaceae family food plants including the five plants used in this research. This family includes both wild and domesticated species and is consumed in different ways,[5] many different species with medicinal value belong to the family Cucurbitaceae. It is a family of about 130 genera and about 800 different species. They are one of the most significant plant families that provide useful fibers and edible products to people.[6]They are climbers with stores in their roots that are widely distributed in the tropics, Africa, Madagascar and central South America, in addition to warm temperate areas of South, Southeast, and East Asia. Traditional medicine uses all parts of the Cucurbitaceae plant (leaf, stem, root or tuber, fruit, and seeds).[7]

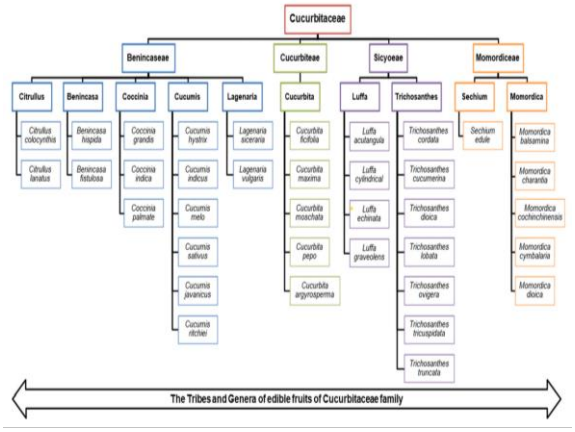


Figure 1. Cucurbitaceae family[8]

Many studies have been conducted on various Cucurbitaceae plants by researchers around the globe. Table 1 shows data from research done across the globe, on potential pharmacological effects of some plants of the Cucurbitaceae family.

Table 1. Worldwide research data on potential pharmacological effects of some plants of the Cucurbitaceae family

Plant	Pharmacological Activity
Cucumis sativus	Anticancer potential against HepG2 cell.[9]
Cucurbita moschata	Anticancer potential and increased ribosomeinactivating protein activity.[10]
Citrullus lanatus	Antidiabetics.[11]
Cucurbita pepo	Anti-inflammatory, antioxidant, anticarcinogenic, antiviral, anti-proliferative antimicrobial, and analgesic properties.[12]
Iberis amara	Anti-inflammatory.[13]
Cucumis melo	Anticancer, analgesic, antimicrobial, antiinflammatory, antidiabetic, hepatoprotective, antioxidant, antiulcer, diuretic and immunomodulatory properties.[10]
C. Grandis	Antidiabetic and antioxidant properties.[14]
Coutarea hexandra	Anticancer, anti-inflammatory, antimalarial and antidiabetic properties .[15]
Rubus chingii	Anticancer, anti-inflammatory, antioxidant, antidiabetic, and anti-ageing properties.[16]
T. cucumerina	Antidiabetic, and antioxidant properties.[14]
Kageneckia oblonga	Anti-inflammatory, analgesic, and antipyretic properties.[17]

Plants from this family are widely used as traditional herbal treatments for a range of illnesses. They have shown anti-inflammatory, anti-fungal, anticancer, antiviral, anti-bacterial, antidiabetic, cardiovascular, hepatoprotective and immunoregulatory properties. The fact that members of this family are rich sources of proteins and have a variety of biological properties, has generally led to their consideration as research subjects. Several phytochemicals, including flavonoids, sterols, phenols and alkaloids, are also known to be present in this family.[18]

Most of the protective effects of these plants has been linked to phytochemicals, which are the non-nutrient plant compounds

such as alkaloids, carotenoids, phenolic acids, flavonoids and isoflavonoids. Although many phytochemicals found in food have been discovered, many more remain unidentified. Numerous phytochemicals have been discovered to possess a range of activities.[19] Due to their widespread presence in the diet and apparent minimal toxicity, phytochemicals can influence disease risk and human health at the population level.[21] Many phytochemicals function as antioxidants, stabilizing free radicals and removing their ability to cause damage.[22] Figure 2 shows the classification of the main phytochemicals, the phytochemical families are shown and examples of phytochemicals are shown in light grey.

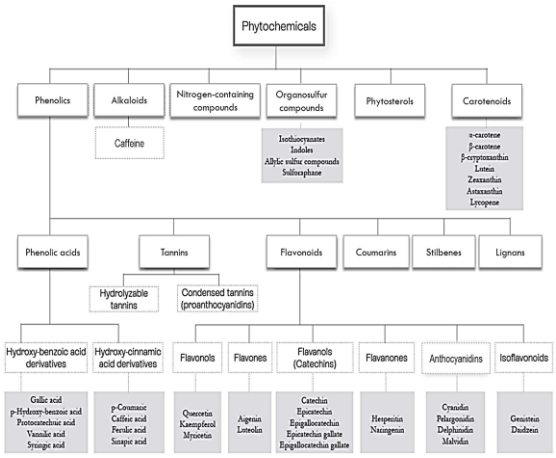


Figure 2. Classification of the main families of phytochemicals[23]

An antioxidant can be defined as "any substance that, when presented at a less amount compared to an oxidizable substrate (carbohydrates, lipids, proteins and DNA), prevents or significantly delays the oxidation of that substrate". Antioxidants' primary job is to shield the body from the damage that free radicals can cause. Free radicals might be produced in cells and tissues as a result of weakened protective capacity or from internal (metabolism, diseases or inflammation) or external (pollution, drugs, food or irradiation) sources as shown in figure 3. In any event, an increase in the production of free radicals can cause oxidative damage.[24] Due to the numerous negative side effects of synthetic antioxidants, natural forms of antioxidants are now being focused upon. Additionally, there is a need to look for novel antimicrobial agents due to the rise in antibiotic resistance.[25]

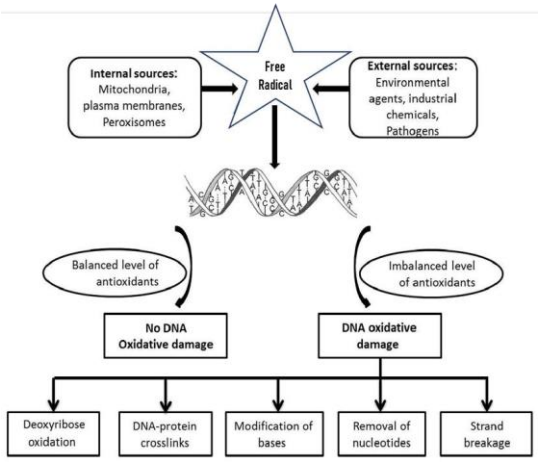


Figure 3. Free radical formation[26]

Researchers are now considering the use of other natural products with antibiotic actions, such as medicinal plants, due to the global concern over the rapid development of bacterial resistance to synthetic antibiotics.[27] Higher plants have also been a source of antibiotics, even though the majority of the clinically used antibiotics are made by soil microorganisms or fungi. Antimicrobials derived from plants are a vast untapped supply of potential medicines. Research on plant-based antimicrobials must continue and be expanded upon. Antimicrobials derived from plants have a great deal of therapeutic potential. They effectively cure infectious diseases while also minimizing a number of the side effects frequently connected to synthetic antimicrobials.[28]

The aim of this research was to determine the phytochemicals, antioxidant and antimicrobial activity in the leaf extracts of cucumber, bitter gourd, watermelon, pumpkin and snake gourd.

2. Materials and Methodology

2.1. Materials

2.1.1. Plant Materials. Leaves of *Cucumis sativus* (cucumber) (C), *Momordica charantia* (bitter gourd) (B), *Citrullus lanatus*

(watermelon) (W), *Trichosanthes cucumerina* (snake gourd) (S) and *Cucurbita maxima* (pumpkin) (P) plants were used in this study.

2.2. Methodology

2.2.1. Preparation of plant materials and extracts. Leaves (50g) from each species of Cucurbitaceae were collected in February from the western and central province of Sri Lanka. They were then cleaned and left to shade dry for a week (Figure 4), the dried leaves were then powdered using a mortar and pestle, after which 2g of the powder was measured using an analytical balance and mixed with 100mL of distilled water (DW), this was placed into a roller mixer and left for 48 hours for extraction, after which the mixture was filtered using muslin cloth and filter paper to get the filtrate which was stored at 4°C.[29]

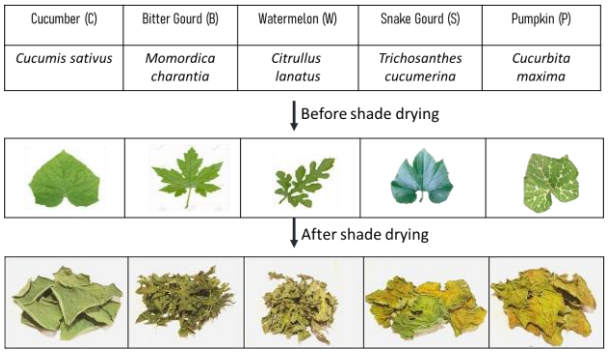


Figure 4. Sample collection and shade drying

2.2.2. Qualitative tests for phytochemicals. The below tests were carried out for all five samples.

2.2.2.1. Test for flavonoids. A few drops of NaOH were added to the aqueous extract (AE) (1mL), followed by the addition of a few drops of sulfuric acid.[30]

2.2.2.2. Test for terpenoids. Chloroform (2mL) and conc H_2SO_4 (3mL) was carefully added to the AE (1mL).[31]

2.2.2.3. Test for phenols. A few drops of 5% ferric chloride solution were added to the AE (1mL).[32]

2.2.2.4. Test for proteins. Millon's reagent (1mL) was mixed with the AE (2mL), and gently heated.[33]

2.2.2.5. Test for saponin. DW (2mL) was added to the AE(1mL) and shaken vigorously.[33]

2.2.2.6. Test for reducing sugars. Benedict's solution (2mL) was added to the AE (1mL) and boiled.[33]

2.2.3. Quantitative analysis of phenolics, flavonoids and antioxidants. The following tests were carried out for each sample in triplicates.

2.2.3.1. Determination of the Total Phenolic Content (TPC). 1mL of 1:10 diluted Folin-Ciocalteu reagent was mixed with 200 µL of AE. 800 mL of saturated sodium carbonate solution (75 g/L) was added after 4 minutes. The absorbance of the AE and standard solutions at 765 nm was measured in triplicates after 2 hours of incubation at room temperature, shielded from light. The standard curve was constructed using gallic acid (0–150 µg/mL) following said procedure. The TPC was expressed as mg gallic acid equivalent (mg GAE)/g dry weight of plant extract.[34]

2.2.3.2. Determination of the Total Flavonoid Content (TFC). 2mL of DW and 0.15mL of 5 % sodium nitrite solution was added to 0.5mL of AE, after 5 minutes, 0.15mL of 10% aluminum chloride was added, at the 6th minute,1mL of 1 M sodium hydroxide was added and mixed well. The absorbance of the AE and standard solutions at 510 nm was measured in triplicates against a blank containing DW. The standard curve was constructed using quercetin (100-1000 µg/mL) following said procedure. The TFC was expressed as milligram quercetin equivalent (mg QE)/g dry weight of plant extract.[35]

2.2.3.3. Evaluation of the Total Antioxidant Capacity (TAC). 1mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was added to 100 µL of AE. Tubes were sealed and incubated for 90 minutes in a water bath at 95°C. The tubes were allowed to cool at room temperature. The absorbance of the AE and standard solutions at 695 nm was measured in triplicates against a blank containing 1 mL of reagent and 100 µL of DW. The standard curve was constructed

using ascorbic acid (20-200 µg/mL) following said procedure. The TAC was expressed as milligram ascorbic acid equivalent (mg AAE/g) dry weight of plant extract.[36]

2.2.4. Quantitative evaluation of the DPPH free radical scavenging activity. The following tests were carried out for different concentrations of each sample in triplicates.

2mL of DPPH solution (0.1mM in methanol) was added to 1mL of AE of different concentrations (0.2 – 6 µg/mL), mixed well and incubated at room temperature for 30 minutes in the dark. The absorbance at 510 nm was measured in triplicates against methanol as a blank and DPPH as a control.[37] The percentage inhibition (PI)was calculated using the following formula:[36]

% Inhibition = [(Control absorbance - Sample absorbance) / (Control absorbance)] × 100

The dose response curve was constructed with PI against the concentration, and IC_{50} was calculated for each sample.[38]

2.2.5. Evaluation of antimicrobial activity

2.2.5.1. Well Diffusion Method. Muller-Hinton-agar was cultured with a standard 0.5 McFarland (1.5 × 108 CFU/mL; Absorbance at 625 nm: 0.08–0.13)[39] inoculum of each microorganism (*E. coli*, *S. aureus*) in two different plates for each sample. Wells were made in the agar (9 mm in diameter), one standard (gentamycin) antibiotic disk (positive control), DW (100 µL) (negative control) and sample (100 µL x 3 for triplicates) was placed onto 5 wells in the inoculated plate (Figure 5), the plates were then incubated in 37 °C for 24 h. The diameter of inhibition zone against the tested organisms was measured by a calliper to find the antimicrobial activity.[40] This procedure was repeated for each sample.

2.2.6. Statistical Analysis

All tests were done in triplicates, the results were expressed as mean ± standard deviation (SD). One-way ANOVA on mean values were used to analyse the significance of differences between means and Pearson's correlation analysis was done using SPSS and a $p< 0.05$ was considered to be statistically significant.[41]

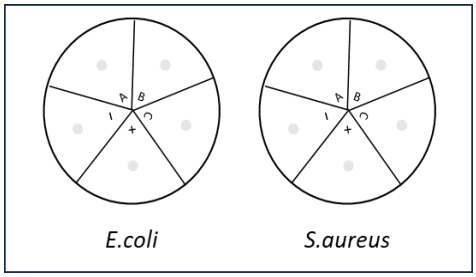


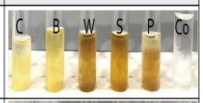
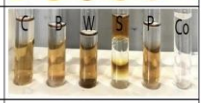
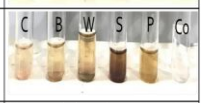
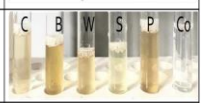
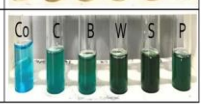
Figure 5. Plate separation and wells made for both the microorganisms for each sample

A: Sample, B: Sample, C: Sample, -: Negative control, +: Positive control

3. Results

3.1 Qualitative tests for phytochemicals. Phytochemical analysis of the extracts revealed that flavonoids, terpenoids, phenols, proteins, saponins, and reducing sugars were present in all the extracts.

Table 2. Qualitative phytochemical analysis of leaf extracts from selected plants.

	Cucumber(C)	Bitter Gourd(B)	Watermelon(W)	Snake Gourd(S)	Pumpkin(P)	Results
Flavonoid	++	++	+++	+++	+++	
Terpenoid	+	+	++	+++	+	
Phenol	+	+	++	+++	++	
Protein	+	+	+	+++	++	
Saponin	++	++	+	+	+++	
Reducing Sugars	+	+	++	++	+++	

Key = + Slightly positive, ++ moderately positive, +++ Strongly positive; Co- Control

The results are shown in Table 2**,** snake gourd was found strongly positive for flavonoids, terpenoids, phenols and proteins, while pumpkin was found strongly positive for

flavonoids, saponins and reducing sugars and watermelon was found strongly positive for flavonoids.

3.2 Total Phenolic Content. TPC was measured using the Folin–Ciocalteu Reagent (FCR) in each extract. The results were calculated from a calibration curve of gallic acid and expressed as gallic acid equivalents (GAE) per gram dry extract weight (Figure 6) mg GAE/g ± Standard Deviation (SD).

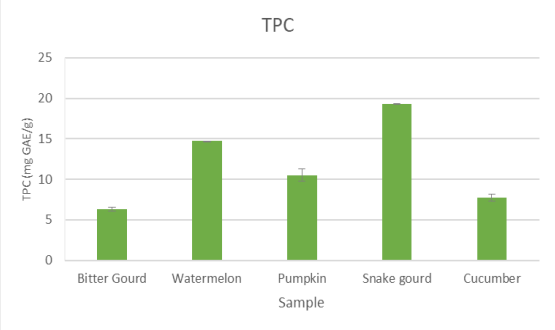


Figure 6. Total phenolic content of plant leaf extracts. Values are the mean ±SD

The content of phenolic compounds in AE ranged from 6.3±0.2 to 19.3mg GAE/g. Snake gourd had the greatest phenolic content and bitter gourd had the least. The TPC of all the samples were significantly different from each other (p<0.05).

3.3 Total Flavonoid Content (TFC). TFC was determined using the aluminium chloride colorimetric assay. The results were calculated from a calibration curve of quercetin and expressed as quercetin equivalents (QE) per gram dry extract weight (Figure 7) QE± SD.

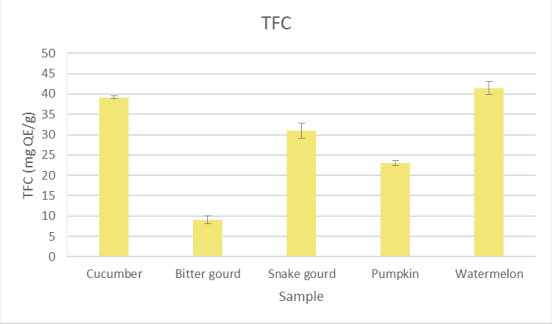


Figure 7. Total flavonoid content of plant leaf extracts. Values are the mean ±SD

The content of flavonoids in AE ranged from 9.1±1.0 to 41.4±1.6 mg QE/g. Watermelon had the greatest flavonoid content and bitter gourd had the least. The TFC of all the samples were significantly different from each other (p<0.05).

3.4 Total Antioxidant Capacity (TAC). TAC was evaluated using the Phosphomolybdenum method in each extract. The results were calculated from a calibration curve of ascorbic acid and expressed as ascorbic acid equivalents (AAE) per gram dry extract weight (Figure 8) AAE± SD.

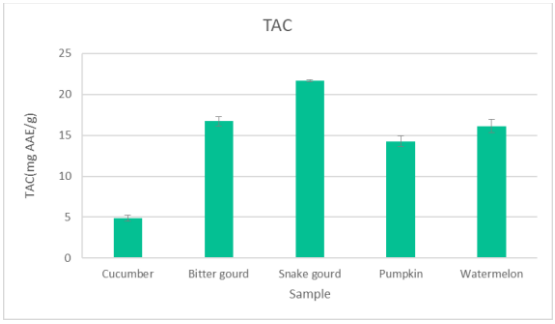


Figure 8. Total antioxidant capacity of plant leaf extracts. Values are the mean ±SD

The content of the TAC in AE ranged from 4.9±0.4 to 21.7± 0.1 mg AAE/g. Snake gourd had the greatest TAC and cucumber had the least. The TAC of all the samples were significantly different from each other (P<0.05).

3.5 DPPH Assay. The DPPH free radical scavenging activities of the selected plants are presented in Figure 9.

All the AE showed concentration-dependent increases in free radical scavenging capacity.

The greatest DPPH radical scavenging potency, with a minimum IC_{50} value was recorded for watermelon (0.6±0.1µg/mL), and the least, with a maximum IC_{50} value was recorded for cucumber (3.1±0.1µg/mL). The free radical scavenging activity of all the samples were

significantly different from each other (p<0.05).

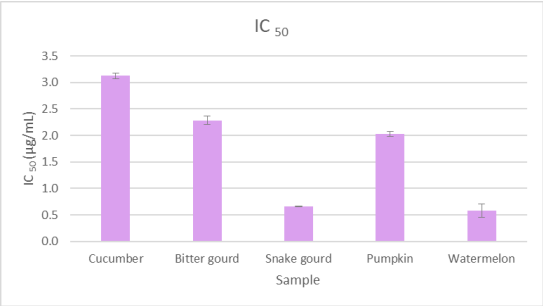


Figure 9. IC_{50} of each plant leaf extract. Values are the mean ±SD

3.6 ABST. ABST was done to find the antimicrobial activity of the AE against *E. coli* and *S. aureus.*

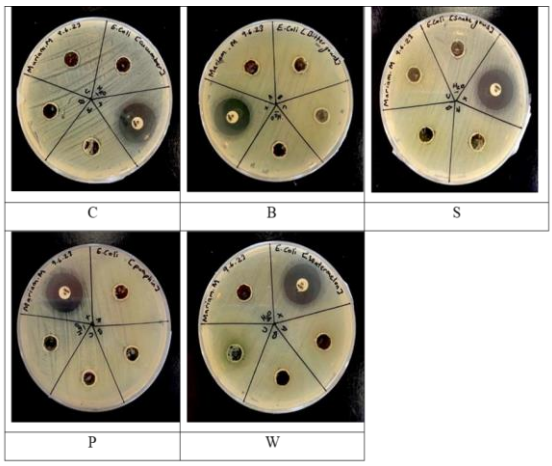


Figure 10. Antimicrobial activity of AE against *E. coli* (C- *Cucumis sativus*, B- *Momordica charantia*, *S- Trichosanthes cucumerina*, *P- Cucurbita maxima*, W-*Citrullus lanatus)*

All the AE showed negative antimicrobial activity against *E. coli* (Figure 10) and *S. aureus* (Figure 11). A larger zone of inhibition was observed for the positive control (gentamycin) against *S. aureus* (3.1cm) compared to *E. coli* (2.6cm).

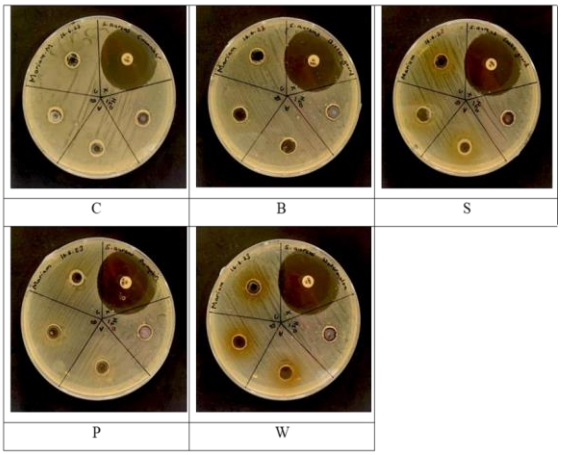


Figure 11. Antimicrobial activity of AE against *S. aureus* (C- *Cucumis sativus*, B- *Momordica charantia*, *S- Trichosanthes cucumerina*, *P- Cucurbita maxima*, W-*Citrullus lanatus*)

3.7 Statistical Analysis. Different assays were compared to find the linear association between them.

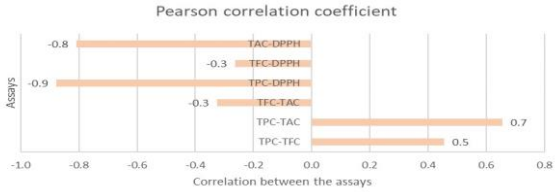


Figure 12. Pearson correlation between the different assays

A strong negative correlation was seen between TPC-DPPH and a strong positive correlation was seen between TPC-TAC (Figure 12).

4. Discussion

The Cucurbitaceae family of plants is a great source of bioactive functional elements with a range of therapeutic applications. The exploration of new biomolecules to be used directly by the pharmaceutical and agrochemical industries or to serve as a lead molecule to synthesis more potent molecules requires the extraction of bioactive components from plants and their quantitative and qualitative measurement.[42] The discovered metabolites are extremely beneficial due to their wide biological activity.[8] In this research a conventional extraction technique called maceration was performed, this involves

soaking the powdered leaf in a solvent at room conditions for at least three days with intermittent agitation,[43] the critical factor influencing extraction efficiency is the solvent's polarity,[44] water was used as the solvent as it is the most polar solvent and it dissolves a wide range of substances, it is also cheap, non-flammable and nontoxic.[45] After the extraction was completed, the mixture was filtered through filter paper and muslin cloth, a stock concentration of 0.02 g/mL was obtained and the extract was left at 4°C, as Cheng et al., (2022) showed that storage at temperatures below 5°C remarkably improved the retention of the major constituents of the extracts.[46]

It is interesting to note that the Cucurbitaceae leaf extracts showed the presence of many phytochemicals through qualitative tests, flavonoids, terpenoids, phenols, proteins, saponins and reducing sugars, these were found in various amounts through the qualitative phytochemical tests. Similar results were seen in AE's of bitter gourd, ethanolic extracts (EE) of snake gourd and methanolic extracts (ME) of cucumber, watermelon and pumpkin.[47-51]

Flavonoids play a crucial role in a wide scope of pharmaceutical, nutraceutical, and medicinal applications because they have a wide range of therapeutic effects due to their ability to influence important cellular enzyme activities as well as their anti-inflammatory, antioxidative, anti-carcinogenic, and anti-mutagenic capabilities.[52] The majority of terpenoids, despite structural variations, are biologically active and are utilized worldwide for the treatment of many ailments. Many terpenoids suppress various human cancer cells and are used as anticancer treatments, such as Taxol and its variants.[53] Plant phenols are recognized as powerful natural antioxidants that play a significant role in a variety of biological and pharmacological properties, such as anti-inflammatory, anti-cancer, antiviral, antimicrobial, antithrombotic, antiallergic, hepatoprotective, and many more.[54] The health benefits of plant-based proteins include anticancer, antioxidant, hypoglycemic, antibacterial, and hypolipidemic effects, according to numerous publications.[55] Saponins are naturally occurring sugar-conjugated compounds that have a variety of biological properties, such as therapeutic effects and antibacterial and antiviral activity.[56] Against this background, our work on Cucurbitaceae proves to be quite interesting due to the presence of all the above-mentioned important classes of bioactive phytochemicals in the selected leaves.

Quantification of phenols was done, the technique of measuring TPC in plant materials is based on the color change reaction between the FCR and polyphenols[57] leading to the formation of complex blue compounds that can be measured at a wavelength of 765 nm. The heteropoly acid (phosphomolybdate-phosphotungstate) in the FCR will be reduced into a molybdenum-tungsten complex by the oxidation of phenol or phenolic-hydroxy groups by FCR[58] (Figure 13). In this experiment gallic acid is used as the standard solution since it is a natural and stable phenol that is also relatively inexpensive compared to the others. It is also a constituent of the phenolic compound derived from hydroxybenzoic acid, classified as simple phenolic acid.[59]

The results showed snake gourd had the highest (19.3 mgGAE/g) TPC and bitter gourd had the least (6.3±0.2 mg GAE/g), the phenolic content of previous research differed from the present research. The present research had a lower TPC for bitter gourd and snake gourd compared to the previous research ,[60,61] while it had a higher TPC for cucumber compared to the previous research,[62] and a higher TPC for watermelon compared to the previous research on watermelon methanolic seed extract,[63] and a higher TPC for pumpkin compared to the previous research on pumpkin aqueous fruit extract.[64] Differences in the phenolic composition of the same species reported in different studies may be caused by the variance in growth conditions. This suggests that phenolic content may vary with variation in geographical location and climatic circumstances.

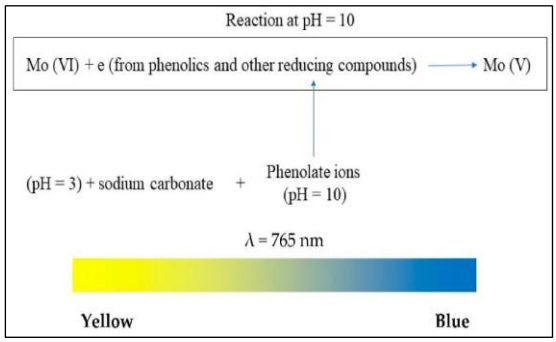


Figure 13. Folin–Ciocalteu method[65]

Quantification of flavonoids was done, the TFC content was determined using the $AlCl_3$ colorimetric assay. This method is based on the formation of chelates of Al(III)-flavonoids (Figure 14***)***. Due to their numerous hydroxyl and oxo groups, flavonoids show a strong affinity for binding metal ions like Al(III), typically at a 1:1 ratio, depending on experimental conditions. Experimentally, yellow-colored Al(III)-flavonoid complexes are formed, upon the addition of AlCl3, and their absorbance is subsequently measured at 510nm.[66]

The results showed watermelon had the highest (41.4±7.3 mg QE/g) TFC and bitter gourd had the least (9.1±1.8 mg QE/g), the flavonoid content of previous research's differed from the present research. The present research has a lower TFC for bitter gourd and snake gourd compared to the previous research, [61,67] while it had a higher TFC for cucumber compared to the previous research,[62] and also a higher TFC for watermelon compared to the previous research on watermelon methanolic seed extract,[63] and a lower TFC for pumpkin compared to the previous research on pumpkin fruit AE.[64] Differences in the flavonoid composition of the same species reported in different studies may be caused by the variance in growth conditions. This suggests that flavonoid content may vary with variation in geographical location and climatic circumstances.

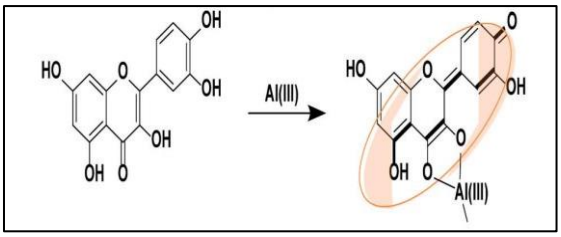


Figure 14. Al(III)-quercetin chelate[66]

Due to the abundance of phenolic and flavonoid components they contain, they are said to have strong antioxidant action. The antioxidant capacity of the sample was assessed using the phosphomolybdenum method with a spectrophotometer, which is based on the reduction of Mo (VI) to Mo (V) by the plant sample and the subsequent generation of blue-green phosphate/Mo (V) compound with a maximum absorption at 695 nm ***(***Figure 15***)***. In terms of the reduction of molybdate ions, the phosphomolybdenum technique provides a quantitative way to assess antioxidant activity. Since ascorbic acid is used to generate a standard curve, the antioxidant activity is expressed in terms of ascorbic acid equivalents.[68]

The results showed snake gourd had the highest (21.7±0.1 mg AAE/g) TAC and cucumber had the least (4.9 ±0.4 mg AAE/g), the TAC of previous research's differed from the present research. The present research had a lower TAC for snake gourd compared to the previous research,[61] while it had a higher TAC for bitter gourd compared to the previous research,[60] the present research also had lower TAC for cucumber compared to the previous research on cucumber ME,[69] it also had a lower TAC for watermelon compared to the previous research on watermelon methanolic seed extract,[63] and a higher TAC for pumpkin compared to the previous research on pumpkin fruit EE.[70] The variation of the TAC between the studies may be because variable amounts of antioxidants are found in the same species when it is grown under diverse environmental conditions and in different regions.

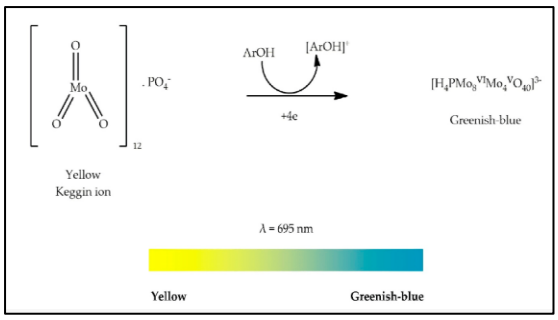


Figure 15. Phosphomolybdenum method[65]

The most rapid and popular approach used to assess the antioxidant activity of plant materials is DPPH radical scavenging. The free radical DPPH, which generates a violet solution in ethanol, is stable at room temperature. The IC_{50} value, which represents the concentration of each sample needed to scavenge 50% of DPPH free radicals, was used to express the DPPH radical scavenging activity. A lower IC_{50} value indicates a greater capacity to scavenge DPPH radicals.[71]

The results showed watermelon had the lowest (0.6 ±0.1 µg/mL) IC_{50} and cucumber had the highest (3.1 ±0.1 µg/mL). The IC_{50} values of previous research's differed from the present research. The present research had a lower IC_{50} for cucumber and bitter gourd , compared to the previous research on cucumber and bitter gourd ME.[72,73] It had a lower IC_{50} for snake gourd compared to the previous research, [61] it also had a lower IC_{50} for pumpkin compared to the previous research on pumpkin fruit AE[74] and a lower IC_{50} for watermelon compared to the previous research on watermelon seed ME .[63] This can be due to modifications to the measurement parameters, such as reaction time ,concentration, solvent, pH and the presence of inorganic salts or metal ions. These factors need to be closely monitored because they have an impact on the reaction's kinetics and equilibrium, thereby altering the IC_{50} values.[75]

The antimicrobial activity of the leaf extracts were evaluated against *S.aureus* and *E.coli* , no observations were seen for either of the bacteria in a concentration of 0.02 g/mL of the extract. Previous research done on cucumber ME (10 µg/mL) showed, a zone of inhibition of 10mm for *S.aureus* and 11mm for *E.coli* , and no zones of inhibition for acetone extract, this could be due to the various solvents used.[76] *E.coli* has a smaller zone of inhibition to gentamycin compared to *S.aureus*. In general, gram-negative bacteria are more resistant than gram-positive due to their phospholipidic membrane and lipopolysaccharide components.[20]

The Pearson correlation showed a strong negative relationship between TAC-DPPH and TPCDPPH, the higher the phenolic and antioxidant content the stronger the scavenging activity of the extract. A weak negative relationship was seen between TFC-DPPH and TFC-TAC, and a relatively strong relationship between TPC-TFC and TPC-TAC, the phenolic content had a significant effect on all antioxidant activities while the flavonoid content didn't contribute significantly.

5. Conclusion

In this research, the results of qualitative assays showed the presence of flavonoids, terpenoids, phenols, proteins, saponins, and reducing sugars in all tested samples. Snake gourd had the highest phenolic content of 19.3 mg GAE/g, and antioxidant capacity of 21.7 mg AAE/g, while watermelon had the greatest flavonoid content of 41.4 mg QE/g and the highest free radical scavenging activity (IC50 value of 0.6 µg/mL), among the rest of the samples. Among each variable, the correlation between TPC and TAC provided the strongest positive correlation ($r = 0.7$, $p < 0.05$) in aqueous leaf extracts.

This research suggested that cucumber, pumpkin, watermelon, snake gourd and bitter gourd leaves are a reliable source of phytochemicals and have shown significant antioxidant activities. Further, these results will support the curative claims of various ethnomedical uses of this plant family in Ayurvedic medicine of Sri Lanka.

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