

## Phytochemical screening, antioxidant and antimicrobial activities of the leaves from Parsley (Apiaceae) family plants grown in Sri Lanka

Ayesha Shazna Shehan<sup>1</sup>, Ahamed Imthikab<sup>1</sup> and Gayani Madara Senanayake<sup>1\*</sup>

<sup>1</sup>School of Science, Business Management School (BMS), Sri Lanka

\*madara.senanayake.ms@gmail.com

### Abstract

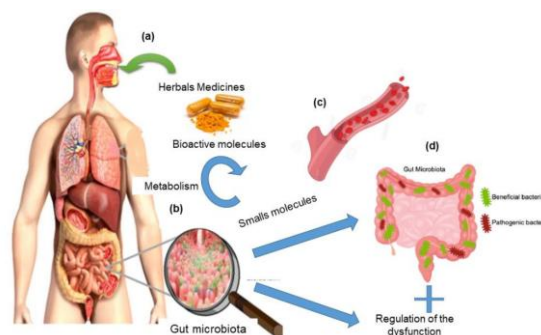
Plants that are classified as part of the Parsley (Apiaceae) family are aromatic and flavorful culinary herbs. They are distributed worldwide as good sources of raw materials for the development of pharmaceuticals. Some pharmaceutical properties of plants in Apiaceae family include antioxidant, anti-inflammatory and antitumor activities. In this investigation, the phytochemical profile was assessed, and the antioxidant and antimicrobial activities of leaf extracts were evaluated from five plant species of the Parsley family: *Anethum graveolens* (Dill), *Apium graveolens* (Celery), *Coriandrum sativum* (Coriander), *Foeniculum vulgare* (Fennel), and *Petroselinum crispum* (Parsley). Leaves were extracted by the maceration technique using water as the solvent. Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and Total Antioxidant Capacity (TAC) were quantified using Folin-Ciocalteu, Aluminum Chloride colorimetric and Phosphomolybdenum methods respectively. The free radical scavenging activity was analyzed using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay while the antimicrobial activity was determined by well diffusion technique using *Escherichia coli* and *Staphylococcus aureus* as test micro-organisms. Dill showed the highest content of phenols (0.016 mg GAE/g), flavonoids (0.0034 mg QE/g), antioxidants (0.039 mg AAE/g) as well as the highest free radical scavenging activity ( $IC_{50} = 1.18 \mu\text{g/mL}$ ). All samples exhibited no antimicrobial activity at a concentration of 0.2mg/mL. Thereby it can be concluded that, besides its nutritional value the intake of plant-based food such as leaves from the Apiaceae family can play a beneficial role in the prevention of certain infections and also provide sources of natural antioxidants.

**Keywords:** Phytochemicals, Antioxidant activity, Antimicrobial activity, Parsley leaves

### 1. Introduction

Since immemorial times, nature has been explored for several sources of medical agents for the development of drugs.<sup>1</sup> Herbal medicine plays a major role in the synthesis of alternative drugs as well as to treat various diseases. Similarly, drugs originated from natural resources has resulted in the success of modern medical science due to the ability of natural medicines to boost health.<sup>2</sup> Phytochemicals are bioactive, non-nutritive components found in plants that has health curative and disease preventive properties (Figure 1).<sup>3</sup> Thereby, plants are considered to be rich sources of natural phytochemical classes for the discovery of pharmaceutical compounds and medicines. Moreover, these natural compounds are used for commercial purposes in medicine, cosmetics as well as in food products.<sup>4</sup>

In addition, edible plants that are rich in secondary metabolites have been paid special attention recently due to the presence of phytochemicals in diet.<sup>5</sup>



**Figure 1.** Oral administration of bioactive molecules.<sup>6</sup>

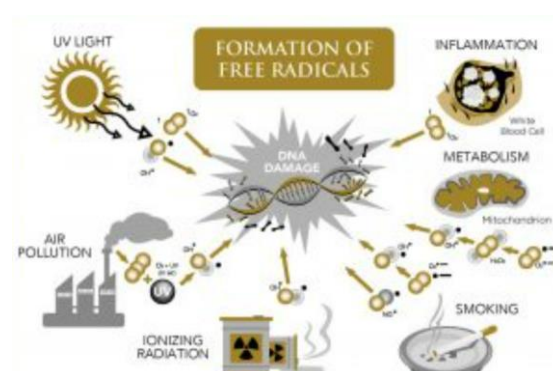
Phytochemicals are phytonutrients with biological activity that are naturally produced

by plants. Some of the natural phytochemicals include alkaloids, phenols, flavonoids, saponins and tannins.<sup>7</sup> The availability of these compounds, especially phenolic acids and flavonoids in plants indicate the presence of antioxidant and pharmacological properties which are recognized to exhibit many health benefits such as antitumor, anti-inflammatory, antihepatotoxic, antioxidant, and antimicrobial activities.<sup>8,9</sup> The properties of these natural compounds also play an important role in plants as a defense mechanism against the UV radiation, temperature, mechanical damage, as well as a chemical defense against insects.<sup>10</sup> Furthermore, phytochemicals show a major impact in exhibiting microbial activity by interfering with the transport of nutrients that are important to their function. In which the interference as a result causes the inhibition of microbial growth.<sup>10</sup>

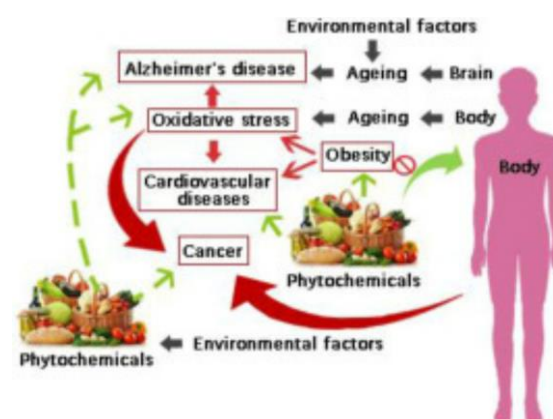
Globally, resistance to antibiotics has become a huge concern, and in recent years the occurrence of multiple resistances in humans has been growing significantly due to indiscriminate consumption of pharmaceutical antibiotics which are commonly administered for the management of infectious diseases. Thereby, this has drawn increased attention to scientists in search for new antimicrobial substances from different sources like the medicinal plants.<sup>1</sup> For instance, plants with alternative mechanisms of action that have been searched for bioactive compounds has also been triggered due to the increasing occurrence of antibiotic-resistant microorganisms and several chronic and degenerative pathologies of humans caused by Reactive Oxygen Species (ROS). These bioactive compounds help neutralize harmful microorganisms and natural antioxidants which are capable of defending the body from oxidative stress and free radical-induced damage. In addition to that, any disruption to the natural phytochemical compounds that contributes to various antimicrobial properties in plant extracts can result in causing disruption to the cell membrane, resulting in cell death.<sup>5</sup>

As a vital part of normal physiology, free radicals are naturally produced in the human body and food systems.<sup>11</sup> However, due to external factors such as UV radiation, ionizing radiation, or air pollution (Figure 2), it can result in the event of numerous degenerative

and chronic diseases such as respiratory, cancer, neurodegenerative, and digestive diseases that is caused due to the overproduction of ROS leading to oxidative stress.<sup>5</sup> Moreover, the pathogenesis of many diseases including heart disease, cancer, atherosclerosis, Alzheimer's disease and in the aging process was found to be implicated by the free radical action thus can be blocked by the natural antioxidants found in plants (Figure 3).<sup>11</sup> Furthermore, due to health risks and toxicity caused by synthetic phenolic antioxidants, they have been replaced with natural antioxidants which can neutralize the free radicals.<sup>12</sup>



**Figure 2.** Formation of free radicals.<sup>13</sup>



**Figure 3.** How phytochemicals help to mitigate oxidative stress and human diseases.<sup>14</sup>

The Parsley (Apiaceae) family is widely distributed throughout the world and is said to be one of the most significant groups of flowering plants, which consists of 3780 species in 434 genera.<sup>15</sup> The Parsley family consists of aromatic and flavorful flowering plants that has drawn attention to researchers as good sources of raw materials to industries such as the food, cosmetic, perfumery and most

importantly the pharmaceutical industry where it has been used as a treatment to cure different illnesses associated with digestive, endocrine, reproductive, and respiratory systems.<sup>15-17</sup>

*Anethum graveolens* (Dill), *Apium graveolens* (Celery), *Coriandrum sativum* (Coriander), *Foeniculum vulgare* (Fennel), and *Petroselinum crispum* (Parsley) are members of the parsley family that possess certain medicinal properties. These plants have hypoglycaemic and hypolipidemic activities as well as anticancer properties thereby are used for the prevention and treatment of many disorders via food supplements.<sup>18</sup> Moreover, it has also been documented that its value as a potential source of natural agrochemicals and biological activities such as anti-inflammatory, antimicrobial, anti-tumour, diuretic, analgesic, radical scavenging, gastrointestinal and anti-obesity properties was revealed from previous studies on the Apiaceae family plant materials.<sup>15</sup> In addition, the most frequently researched plant parts were bark and seeds followed by leaves and aerial parts.<sup>19</sup>





The current study was conducted to evaluate the phytochemical profile and to determine the antioxidant and antimicrobial activities of leaf extracts from the Parsley family plants made using the maceration extraction technique.

## 2. Methodology

**2.1 Plant material collection.** The selected five plant species of the Apiaceae family were purchased from a local market from Western province, Sri Lanka in the month of March in 2023. The leaves of each fresh plant samples were cleaned, segregated and, shade dried for 18 days. Then they were finely powdered (Table 1).<sup>21</sup>

**2.2 Preparation of extracts.** The extraction was conducted by the maceration technique using water as the solvent, by mixing 2g of each finely powdered sample with 100mL distilled water.<sup>22</sup> The samples were left to run in the roller mixer for 48 hours, then filtered using the Whatman No1 filter paper, and the filtrates were collected and stored at 4°C for further analysis.<sup>23</sup>

**Table 1.** Sample collection and preparation

Sample	Shade Dried (18 days)	Powdered sample
Dill		
Celery		
Coriander		
Fennel		
Parsley		

## 2.3 Analysis of phytochemical content

### 2.3.1 Qualitative assays

Qualitative tests were executed to detect the presence of phytochemicals based on colour or precipitation reactions. Froth test, Alkaline reagent test, Salkowski's test, Ferric Chloride test, and Millon's test were conducted to assess the phytochemicals: saponins, flavonoids, terpenoids, phenols and proteins respectively.

**2.3.1.1 Froth test.** 1mL extract was mixed with 1mL distilled water and shaken for few minutes.<sup>24</sup>

**2.3.1.2 Alkaline reagent test.** 1mL of 2N NaOH was added to 1mL extract.<sup>25</sup>

**2.3.1.3 Salkowski's test.** 2mL chloroform was added to 1mL extract, followed by 3mL concentrated H<sub>2</sub>SO<sub>4</sub>.<sup>24</sup>

**2.3.1.4 Ferric Chloride test.** 1mL extract was mixed with few drops of 5% FeCl<sub>3</sub>.<sup>26</sup>

**2.3.1.5 Millon's test.** 1mL extract was mixed with few drops of Millon's reagent and heated.<sup>27</sup>

### 2.3.2 Quantitative assays

**2.3.2.1 Determination of TPC (Total Phenolic Content).** The TPC of samples were analysed using the Folin-Ciocalteu method carried out by

El-Sayed *et al* (2018)<sup>28</sup> with slight modifications. 200µL of sample was mixed with 1000µL Folin-Ciocalteu (diluted 10 times). After 5 minutes, 7.5% Na<sub>2</sub>CO<sub>3</sub> (800µL) was added followed by a 1-hour incubation at room temperature. Gallic acid was used as the standard with dilutions (20-140µg/mL) prepared from 1mg/mL stock solution.

Absorbance was measured at 765nm for each sample and gallic acid concentrations in triplicates. The calibration curve was plotted using mean absorbance of gallic acid concentrations, and TPC of samples were expressed as Gallic Acid Equivalent (mg GAE/g).<sup>15</sup>

**2.3.2.2 Determination of TFC (Total Flavonoid Content).** TFC of extracts were determined using the Aluminum Chloride colorimetric method.<sup>29</sup> 500µL of extract was mixed with 5% Sodium Nitrite (150µL). After five minutes, 10% aluminium chloride (300µL), 1M sodium hydroxide (1000µL), and 2000µL of distilled water were added and shaken vigorously. The solution was incubated for 15 minutes at room temperature and the absorbance were measured at 510nm in triplicates. Lastly, the TFC of each sample was calculated and expressed as Quercetin Equivalents (mg QE/g) using the Quercetin standard.<sup>30</sup>

**2.3.2.3 TAC (Total Antioxidant Capacity) Assay.** Based on the Phosphomolybdenum method, the TAC for each sample was evaluated.<sup>31</sup> L-ascorbic acid was used as the standard (20-140µg/mL). 200µL of the sample was mixed with 4000µL Phosphomolybdenum reagent (0.6M sulfuric acid, 28mM Sodium Phosphate, and 4mM ammonium molybdate) followed by 90 minutes of incubation in a water bath at 95 °C.

Samples were cooled and the absorbance of each sample in triplicates were measured at 695nm.<sup>32</sup> The calibration curve was used to express TAC of each sample as Ascorbic Acid Equivalents (mg AAE/g).

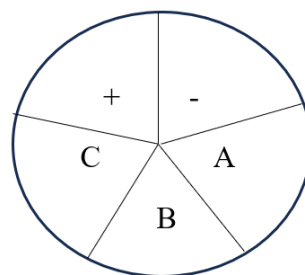
**2.3.2.4 DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) Free Radical Scavenging Activity.** DPPH radical scavenging assay is a rapid, reliable assay that was used to assess the antiradical activities in the plant samples.<sup>33</sup> Firstly, a range of concentrations (0-10µg/mL) of each extract was prepared and to each, 500µL of sample extract, 0.1mM DPPH

solution (2500µL) was added and stored in the dark for 30 minutes at room temperature. Absorbance was measured at 517nm for different concentrations of each sample triplicates.

$$\text{Radical scavenging activity (\%)} = [(A_C - A_S) / A_C] \times 100$$

Where, A<sub>C</sub>= absorbance of control and A<sub>S</sub>= absorbance of sample. From the above-mentioned formula, the radical scavenging activity (%) was calculated. Sample concentration required to scavenge 50% of DPPH free radical (IC<sub>50</sub>) was calculated from the dose response graph of radical scavenging activity against the concentration of extracts.<sup>34</sup>

**2.4 Evaluation of Antimicrobial activity.** The antimicrobial activity of extracts was evaluated using the well diffusion technique. Each petri plate was sectioned to five equal parts labelled: positive control gentamycin (+), negative control autoclaved distilled water (-), and A, B, C as triplicates for each sample (Figure 4).



**Figure 4.** Petri plate sectioned to 5 parts

0.5% McFarland turbidity standard was prepared and visually compared using the test suspension turbidity to standardize the approximate number of bacteria in the suspension. The spread plate technique of test organisms was performed on the Muller Hinton agar plates prepared under aseptic conditions.<sup>35</sup> 50µL of an extract was dispensed into the wells (1cm in diameter) labelled A, B, and C. 50µL of distilled water was used as the negative control (-) and gentamycin disc (10mcg) as positive control (+). The plates were left for 15 minutes for pre-diffusion followed by incubation at 37°C for 24 hours. Lastly, the diameter of zones was measured.<sup>36</sup> Above procedure was repeated for all extracts.









**2.5 Statistical Analysis.** Statistical analysis was performed using the SPSS software. All the analysis were performed in triplicates with results expressed as mean  $\pm$  SD. Data was analyzed using t-test and Analysis of Variance (ANOVA) at confidence level  $p < 0.05$ . Pearson's test was used to find the correlation between TPC, TAC, TFC, and DPPH from the studied plant extracts.

### 3. Results

Results obtained for the qualitative analysis of the phytochemicals: Saponins, Flavonoids, Terpenoids, Phenols and Proteins are shown in Table 2 below.

**Table 2.** Results of qualitative analysis of phytochemicals

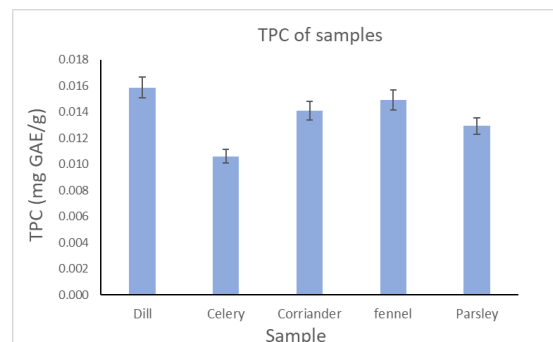
Sample \ Test	Dill	Celery	Coriander	Fennel	Parsley	Results
Froth test (Saponins)	++	+	++++	+++	+	
Alkaline reagent test (Flavonoids)	++++	++	+	+++	+	
Salkowski's test (Terpenoids)	+++	++	++++	+	++	
Ferric Chloride test (Phenols)	+++	+	++++	++	++	
Millon's test (Proteins)	++	+	++++	+	+++	

  
 [ + < ++ < +++ < ++++ ]  
 least positive                      most positive

As shown in Table 2, all samples revealed positive results for each qualitative tests indicating the presence of Saponins, Flavonoids, Terpenoids, Phenols and Proteins. However, both Dill and Coriander showed the most positive results for the qualitative phytochemical tests, whereas Celery showed the least.

### 3.1 TPC of samples

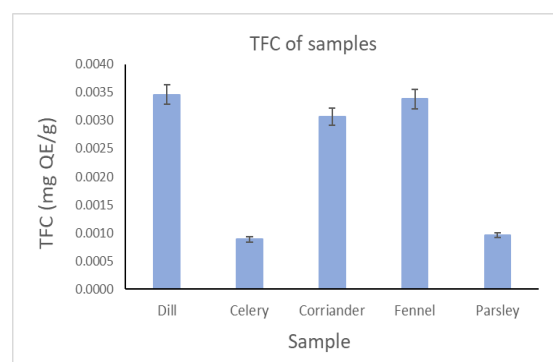
The TPC of each sample was quantitatively evaluated and expressed as GAE that was executed using the Folin-Ciocalteu method.



**Figure 5.** TPC of samples

The results obtained from the quantitative analysis of TPC for each plant extract (Figure 5), showed that Dill (0.016 mg GAE/g) expressed the highest TPC while Celery (0.011 mg GAE/g) showed the least. TPC results expressed a significant difference ( $p < 0.05$ ) between all samples.

The TFC of each sample was quantitatively evaluated and expressed as QE using the Aluminum Chloride colorimetric method.



**Figure 6.** TFC of samples

The results obtained from the quantitative analysis of TFC (Figure 6) for each plant extract, showed that Dill (0.0034 mg QE/g) expressed the highest TFC while Celery (0.0009 mg QE/g) showed the least. TFC results expressed a significant difference ( $p < 0.05$ ) between all samples.

3.3 TAC of samples

The TAC for each sample was quantitatively evaluated and expressed as AAE that was executed using the Phosphomolybdenum method.

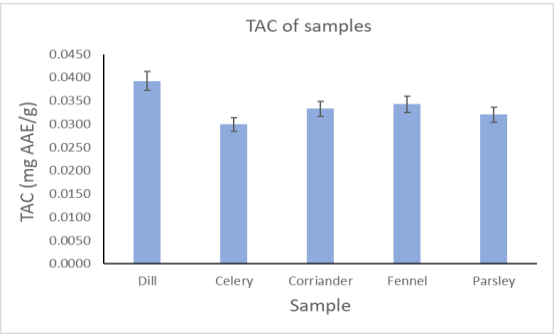


Figure 7. TAC of samples

The results obtained from the quantitative analysis of TAC (Figure 7) for each plant extract, showed that Dill (0.039 mg AAE/g) expressed the highest TAC while Celery (0.030 mg AAE/g) showed the least. TAC results expressed a significant difference ( $p < 0.05$ ) between all samples.

3.4 DPPH of samples

The free radical scavenging activity of each sample was expressed as IC<sub>50</sub>, that was executed using the DPPH assay.

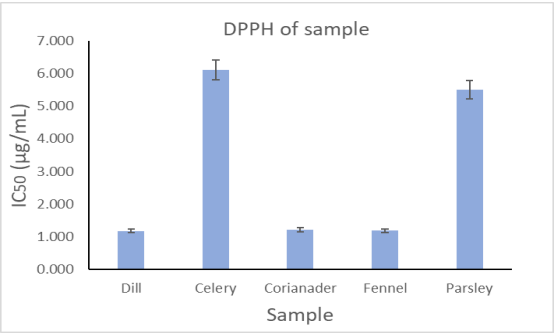


Figure 8. DPPH free radical scavenging activity

The results obtained from the analysis of DPPH for each plant extract (Figure 8), showed that Dill (IC<sub>50</sub>= 1.18 µg/mL) expressed the highest free radical scavenging activity while Celery (IC<sub>50</sub>= 6.10 µg/mL) showed the least. DPPH results expressed a significant difference ( $p < 0.05$ ) between all samples. The colour changes

of DPPH free radical scavenging activity for each plant extract of the Apiaceae family is as shown in Table 3.

Table 3. Free radical scavenging activity for each plant extracts

Sample	Result
Dill	0.4µg/mL 0.7µg/mL 1µg/mL 1.3µg/mL 1.6µg/mL
Celery	2µg/mL 4µg/mL 6µg/mL 8µg/mL 10µg/mL
Coriander	0.4µg/mL 0.7µg/mL 1µg/mL 1.3µg/mL 1.6µg/mL
Fennel	0.4µg/mL 0.7µg/mL 1µg/mL 1.3µg/mL 1.6µg/mL
Parsley	2µg/mL 4µg/mL 6µg/mL 8µg/mL 10µg/mL

3.5 Antimicrobial activity of samples

Table 4. Results of Antimicrobial activity using well diffusion.

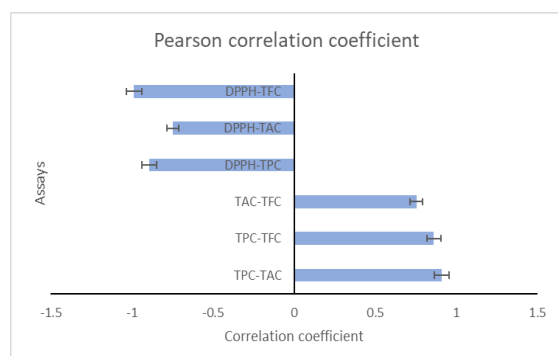
Sample	Test-microorganism	
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Dill		
Celery		
Coriander		
Fennel		
Parsley		

The results for the antimicrobial activity of the plant samples were assessed from the well diffusion method using *Escherichia coli* and *Staphylococcus aureus* as shown in Table 4. As the positive control, gentamycin showed a zone

of inhibition, however no zones of inhibition were observed in any of the samples that were tested with both the test microorganisms at a concentration of 0.2mg/mL.

### 3.6. Data Analysis

The Pearson correlation coefficient results obtained between the assays, TPC, TAC, TFC, and DPPH are shown below (Figure 9).



**Figure 9.** Correlation coefficient between assays

The correlation coefficient between TPC and TAC was positive and highly significant ( $p < 0.05$ ), while TAC and TFC was insignificant ( $p > 0.05$ ). Similarly, DPPH and TFC presented a strong, negative correlation that was highly significant ( $p < 0.05$ ) compared to DPPH and TAC ( $p > 0.05$ ).

## 4. Discussion

In this study, leaves from the Parsley (Apiaceae) family were chosen for the evaluation of phytochemical screening and the determination of antioxidant and antimicrobial activity. These culinary herbs are readily available as consumables and have been widely used as a garnish due to its great aroma and flavour. In addition, the plants of the Apiaceae family are believed to have health beneficial properties that can help heal and treat various diseases.<sup>8</sup> Once the samples were air dried in room temperature and powdered using the mortar and pestle, they were extracted using the maceration extraction technique. Water was used as the solvent due to its beneficial properties such as: highly polar, soluble, inexpensive, nontoxic, and non-flammable.<sup>22</sup> This investigation was also executed using plant leaves as they were found to have a

greater abundance of phytochemicals than other parts of the plant.<sup>37</sup>

Despite the use of various non-conventional methods recently, which are much costly, conventional methods such as the maceration extraction was used due to its inexpensiveness, simple procedure, and lower solvent consumption.<sup>38</sup> Furthermore, during the maceration extraction, the sample solution was ensured to run in the roller mixer for 48 hours until a homogenized sample solution was obtained.

Phytochemicals are natural bioactive components produced by plants via primary or secondary metabolites that is used as a remedy for various diseases. A wide range of medicinal properties such as providing protection against various diseases was found to be possessed in different phytochemicals.<sup>39</sup> Some important primary bioactive components include phenolic compounds, flavonoids, terpenoids, tannins, steroids and carbohydrates.<sup>40</sup> The aqueous plant extracts of the Parsley family were analyzed qualitatively for the presence of phytochemicals using phytochemical tests such as: Froth test, Alkaline reagent test, Salkowski's test, Ferric Chloride test, and Millon's test. In this investigation the phytochemicals: saponins, flavonoids, terpenoids, phenols and proteins showed presence in all sample extracts. Moreover, these phytochemicals found to have beneficial properties such as antioxidant, antiviral, anti-inflammatory, and anticancer effects.<sup>41</sup> Dill and Coriander showed the most positive results while celery showed the least for the qualitative tests. However, celery also showed presence of more flavonoids and terpenoids than other phytochemicals. Similarly, the presence of flavonoids and terpenoids in celery was found to exhibit pharmacological effects including anti-microbial, anti-oxidant, and cardiovascular protective effects.<sup>42</sup>

As the most abundant secondary metabolites, phenolic compounds are ubiquitous constituents in plants that play a crucial factor in promoting growth and protection against the harmful effects of pathogens, parasites, and UV rays.<sup>15</sup> In addition, Folin-Ciocalteu assay being a fast and rapid assay was used for the assessment of phenolic content. In the Folin-Ciocalteu assay, after the samples were left for incubation, a

colour change from yellow to blue was observed. This is due to the presence of phenolic compounds in the aqueous plant extract that caused the Folin-Ciocalteu reagent to be reduced and resulting in the formation of a blue coloured complex.<sup>43</sup> Thus, darker the intensity of the blue coloured complex, greater the phenolic compound present in the sample. Therefore, based on the colour intensity observed and the calculated results, Dill was found to have the highest TPC (0.016 mg GAE/g) indicating high amounts of phenolic compounds while celery showed the least (0.011 mg GAE/g). The TPC results in the current study were shown to be varied when compared with the results obtained from Derouich et al (2020)<sup>44</sup>, who measured the highest TPC in parsley ( $21.63 \pm 1.81$  mg GAE/g DW) and the least TPC in Coriander ( $13.72 \pm 1.13$  mg GAE/g DW). Thus, the occurrence of these variations in total polyphenols level may be due to certain genetic differences, conditions of cultivation, extraction time or type of solvent used for extracting solvent. Moreover, TPC of Dill was highly significant ( $p < 0.05$ ) to Parsley, however showed insignificance ( $p > 0.05$ ) to Celery, Coriander and Fennel.

Flavonoids are natural secondary metabolites that are most abundant in plants, fruits, vegetables, seeds as well as in wine and tea.<sup>45</sup> The TFC assay was carried out using Aluminium Chloride colorimetric method which resulted in the formation of a yellow-coloured complex.<sup>29</sup> In the presence of Aluminium Chloride stable complexes with keto group and hydroxyl group of flavones were formed.<sup>46</sup> Thus, based on the colour intensity observed and the calculated results obtained from TFC, Dill displayed the highest concentration (0.0034 mg QE/g) at 510 nm while Celery (0.0009 mg QE/g) showed the least.

The TAC assay was conducted using the phosphomolybdenum method, which resulted in the formation of a yellow-green complex due to the reduction of molybdate ions.<sup>31</sup> Therefore, based on the results, Dill was found to have the highest TAC (0.039 mg AAE/g) indicating high antioxidant capacity while Celery showed the least (0.030 mg AAE/g). Dill showed a significance ( $p < 0.05$ ) between Coriander and

Parsley, however, was highly insignificant between the other plant extracts.

The DPPH assay was carried out in order to determine the scavenging activity of each plant extract. From a range of concentrations of each plant extract, the dose response curves were plotted and  $IC_{50}$  values for each sample extract was calculated. From the results obtained, Dill (1.18  $\mu$ g/mL) showed the least  $IC_{50}$  value while Celery (6.10  $\mu$ g/mL) showed the highest. This indicates that, lower the  $IC_{50}$  value the higher free radical scavenging activity and vice versa.<sup>34</sup>

Results from the Pearson correlation coefficient showed strong positive correlations between TPC and TAC, TPC and TFC, as well as TAC and TFC. While strong negative correlations were observed between DPPH and TPC, DPPH and TAC, as well as DPPH and TFC. This is because plant extracts with increasing TPC, TAC, or TFC show reduced DPPH and vice versa. In addition, a previous study on medicinal herbs such as *Adhatoda vasica* Nees, *Bergenia ciliata* (Haw) Sternb, *Phyllanthus emblica* Linnaeus, *Terminalia bellirica* (Gaerth) Roxb, *Terminalia chebula* Retzius and *Vitex negundo* Linnaeus demonstrated a positive correlation between radical scavenging activity and total phenolic content, in which the extract having the highest phenolic content showed the lowest  $IC_{50}$ .<sup>34</sup>

In recent years, bacterial resistance to antibiotics and antimicrobials has increased due to changes in major mechanisms that allow bacteria to resist them. Some of the changes include: changes to active drug efflux systems, mutations that alter cell permeability, cellular degradation of antimicrobials, and changes in cellular targets. Thereby, this has resulted in the rise of bacterial resistance to antibiotics and antimicrobials in recent years.<sup>47</sup>

In this current study all five of the plant extracts were tested for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* using the well diffusion technique. From the results obtained from each of the samples, the positive control (Gentamicin) was found to be more sensitive against *Staphylococcus aureus* than compared to *Escherichia coli*. Thus, large, and clear zones of inhibition were observed for the positive control of each plant extracts against *Staphylococcus aureus* (3.4 cm



in diameter) while clear and smaller zones of inhibition was observed for *Escherichia coli* (2.2 cm in diameter). In fact, *Escherichia coli* being a gram-negative anaerobic bacterium, there has been a rise in the rates of resistance among *Escherichia coli* around the world.<sup>48</sup> Thereby, this has confirmed that the gram-positive bacteria (*Staphylococcus aureus*) were more sensitive to gentamicin than the gram-negative bacteria (*Escherichia coli*). The reason for this susceptibility difference between the two test microorganisms is because of the Gram-negative cell wall acting as a barrier to many compounds, such as antibiotics, due to changes in their outer membrane structure.<sup>49</sup>

Furthermore, it is ensured that the antimicrobial susceptibility testing is carried out under aseptic conditions to avoid any contamination that would affect the results. In addition, a previous study on the seeds of 6 plants of the Apiaceae family: Caraway (*Carum carvi* L.), anise (*Pimpinella anisum* L.), coriander (*Coriandrum sativum* L.), dill (*Anethum graveolens* L.), fennel (*Foeniculum vulgare* Mill.) and cumin (*Cuminum cyminum* L.) showed a strong antimicrobial activity against a wide range of pathogens.<sup>18</sup> However, no zones of inhibition were observed in any of the plant extracts against both the test organisms in this present study. Moreover, it has been found that in various literature studies the antimicrobial activities have a direct relationship to the concentration of extract used.<sup>50</sup> Thus, by increasing the concentration of the plant extract placed in the wells, a positive result may be observed. Similarly, the use of ethanolic plant extracts showed a greater impact than the use of aqueous extracts.<sup>49</sup> Both *Escherichia coli* and *Staphylococcus aureus* are also considered normal intestinal flora that are harmless commensal, that plays a major role in preventing and fighting infections, therefore the intake of certain plant-based food such as leaves from the Apiaceae family that has no antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* can play a beneficial role in the prevention of certain infections.<sup>51</sup>

## 5. Conclusion

In the present study, Dill (*Anethum graveolens*) showed the highest total phenolic content, total flavonoid content, total antioxidant potential as well as the highest free radical scavenging

activity along with moderately high amounts of other phytochemicals from the qualitative tests. Thus, demonstrated potent anti-inflammatory properties along with other beneficial implications in human health such as the treatment and prevention of cancer, cardiovascular disease and other pathologies via inhibition of the oxidation of lipids and the propagation of oxidative chain reactions.

Further analysis can be conducted on leaf extracts of Apiaceae family of their pharmacognostic properties for the development of natural drugs.

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## References

1. J. Parekh and S. Chanda. *African Journal of Biomedical Research*, 2010;**10**(2).
2. T. Kebede, E. Gadisa and A. Tufa. *PLOS ONE*, 2021;**16**(3):e0249253.
3. A.N.M. Alamgir. *Progress in Drug Research*, 2018;1–24.
4. B.D. Bhatt and G.C. Parajuli. *Journal of Nepal Chemical Society*, 2017;**36**:68–73.
5. S.M. Kamel. *Journal of Food Processing & Technology*, 2013;**04**(06).
6. K. Djenadi, H. Khechfoud, M. Azouaou, M. Bachir Bey and D.E. Kati. *International Journal of Innovative Approaches in Science Research*, 2020;**4**(4):141–52.
7. N B. Ndezo Bisso, R. Njikang Epie Nkwelle, R. Tchuenguem Tchuenteu and J.P. Dzoyem. *Advances in Pharmacological and Pharmaceutical Sciences*, 2022;**2022**:1–8.
8. N.J. Miller and C.A. Rice-Evans. *Free Radical Research*, 1997;**26**(3):195–9.
9. J. Ramkissoon, M. Mahomoodally, N. Ahmed and A. Subratty. *Asian Pacific Journal of Tropical Medicine*, 2013;**6**(7):561–9.
10. M. Kozłowska, I. Ścibisz, J.L. Przybył, A.E. Laudy, E. Majewska, K. Tarnowska, J. Małajowicz and M.

- Ziarno. *Applied Sciences*, 2022;**12**(19):9871.
11. A. Shehata, A.E.E. Mahmoud and H.M. Abdou. *Research Journal of Pharmaceutical Biological and Chemical Sciences*, 2014;**5**(6):266-273.
  12. P. Thiviya, A. Gamage, D. Piumali, O. Merah and T. Madhujith. *Cosmetics*, 2021;**8**(4):111.
  13. G. Jacobs. *Health Partners*, 2019.
  14. R. Guan, Q. Van Le, H. Yang, D. Zhang, H. Gu, Y. Yang, C. Sonne, S.S. Lam, J. Zhong, Z. Jianguang, R. Liu and W. Peng. *Chemosphere*, 2021;**271**:129499.
  15. B. Sayed-Ahmad, T. Talou, Z. Saad, A. Hijazi and O. Merah. *Industrial Crops and Products*, 2017;**109**:661–71.
  16. İ. Gülçin, M. Oktay, E. Kireçci and Küfrevioğlu Öİrfan. *Food Chemistry*, 2003;**83**(3):371–82.
  17. H. Soliman, N. Eltablawy and M. Hamed. *Journal of Medicinal Plants Studies*, 2015;**3**(4):92–100.
  18. M. Acimovic, L. Kostadinovic, S. Popovic and N. Dojcinovic. *Journal of Agricultural Sciences, Belgrade*, 2015;**60**(3):237–46.
  19. M.W. Biavatti, V. Marensi, Silvana Nair Leite and A. Reis. *Revista Brasileira de Farmacognosia*, 2007;**17**(4):640–53.
  20. N. Rajurkar and S. Hande. *Indian Journal of Pharmaceutical Sciences*, 2011;**73**(2):146.
  21. Loarie S. *iNaturalist*, 2016.
  22. A. Abubakar and M. Haque. *Journal of Pharmacy and Bioallied Sciences*, 2020;**12**(1):1–10.
  23. S. Jan, M.R. Khan, U. Rashid and J. Bokhari. *Osong Public Health and Research Perspectives*, 2013;**4**(5):246–54.
  24. M.S. Auwal, S. Saka, I.A. Mairiga, K.A. Sanda, A. Shuaibu and A. Ibrahim. *Veterinary research forum: an international quarterly journal*, 2014;**5**(2):95–100.
  25. N. Kancharla, A. Dhakshinamoothi, K. Chitra and R.B. Komaram. *Mædica*, 2019;**14**(4):350–6.
  26. J.R. Shaikh and M. Patil. *International Journal of Chemical Studies*, 2020;**8**(2):603–8.
  27. A.H. Lanjwani, I.H. Ghanghro, A.B. Ghanghro and M.J. Channa. *Int. J. Pharm. Med. Res*, 2015; **3**(4):263-266.
  28. M.M. El-Sayed, N.H. Metwally, I.A. Ibrahim, H. Abdel-Hady and B.S.A. Abdel-Wahab. *Journal of Applied Life Sciences International*, 2018;**19**(2):1–7.
  29. N. Benchikha, I. Chelalba, H. Debbeche, M. Messaoudi, S. Begaa, I. Larkem, D.G. Amara, A. Rebiai, J. Simal-Gandara, B. Sawicka, M. Atanassova and F.S. Youssef. *Molecules*, 2022;**27**(12):3744.
  30. M.T.Nguyen, V.T. Nguyen, V.M. Le, L.H. Trieu, T.D. Lam, L.M. Bui, L.T.H. Nhan and V.T. Danh. *IOP Conference Series: Materials Science and Engineering*, 2020;**736**:062012.
  31. N. Bibi Sadeer, D. Montesano, S. Albrizio, G. Zengin and M.F. Mahomoodally. *Antioxidants*, 2020;**9**(8):709.
  32. A. Untea, A. Lupu, M.Saracila and T. Panaite. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca Animal Science and Biotechnologies*, 2018;**75**(2):110.
  33. F. Al-Juhaimi and K. Ghafoor. *Pakistan Journal of Botany*, 2011;**43**(4):2235-2237.
  34. G.R. Genwali, P.P. Acharya and M. Rajbhandari. *Nepal Journal of Science and Technology*, 2013;**14**(1):95–102.
  35. K. Wise. *American Society for Microbiology*, 2006.
  36. A.A. Adegoke, P.A. Iberi, D.A. Akinpelu, O.A. Aiyegoro and C.I. Mboto. *International Journal of Applied Research in Natural Products*, 2011;**3**(3):6–12.
  37. M. Awasthi, C. Pokhrel, Y.H. You, S. Balam, R. Kunwar, S. Thapa, E.J. Kim, J.W. Park, J.H. Park, J.M. Lee and Y.S. Kim. *Ethnobotany Research and Applications*, 2023;**25**:1–13.
  38. L. Ngamwonglumlert, S. Devahastin and N. Chiewchan. *Critical Reviews in Food Science and Nutrition*, 2017;**57**(15):3243–59.
  39. Suresh. *International Journal of Creative Research*, 2018;**6**(2):933.
  40. A.P.A. Jayasiri, S.P. Senanayake, P. Paranagama, A.P.G. Amarasinghe. *Ceylon Journal of Science*, 2016;**44**(2):85.
  41. D. Youssef, R. El-Bakatoushi, A. Elframawy, L. El-Sadek and G.E. Badan. *Journal of Plant Research*, 2023;**136**(3): 305–322.

42. M.Y. Li, K. Feng, X.L. Hou, Q. Jiang, Z.S. Xu, G.L. Wang, J.X. Liu, F. Wang and A.S. Xiong. *Horticulture Research*, 2020;**7**(1).
43. L. Ford, K. Theodoridou, G.N. Sheldrake and P.J. Walsh. *Phytochemical Analysis*, 2019;**30**(6):587–99.
44. M. Derouich, E.D.T. Bouhlali, A. Hmidani, M. Bammou, B. Bourkhis, K. Sellam and C. Alem. *Scientific African*, 2020;**9**:e00507.
45. A.N. Panche, A.D. Diwan and S.R. Chandra. *Journal of Nutritional Science*, 2016;**5**(e47).
46. F. Ahmed and M. Iqbal. *Organic & Medicinal Chemistry International Journal*, 2018;**5**(4).
47. B.F. Brehm-Stecher and E.A. Johnson. *Antimicrobial Agents and Chemotherapy*, 2003;**47**(10):3357–60.
48. M. Kibret and B. Abera. *African Health Sciences*, 2011;**11**(3).
49. E. Silva, S. Fernandes, E. Bacelar and A. Sampaio. *African Journal of Traditional, Complementary, and Alternative Medicines*, 2016;**13**(6):130–4.
50. F.D. Gonelimali, J. Lin, W. Miao, J. Xuan, F. Charles, M. Chen and S.R. Hatab. *Frontiers in Microbiology*, 2018;**9**.
51. C.S. Vimalkumar, V.B. Hosagaudar, Suja, Vilash, Krishnakumar, N.M. and P.G. Latha. *Journal of Pharmacognosy and Phytochemistry*, 2014;**3**(4):69–72.