GLOBAL ACADEMIC RESEARCH INSTITUTE

COLOMBO, SRI LANKA

GARI International Journal of Multidisciplinary Research

ISSN 2659-2193

Volume: 10 | Issue: 02

On 30th June 2024

http://www.research.lk

Author: Haleem F.H, Senanayake S.A.M.A.G.M

BMS School of Science, Sri Lanka

GARI Publisher | Medicinal Plants | Volume: 10 | Issue: 02

Article ID: IN/GARI/ICHM/SL/2023/107 | Pages: 26-41 (15)

ISSN 2659-2193 | Edit: GARI Editorial Team

Received: 26.02.2024 | Publish: 30.06.2024

INVESTIGATION OF FREE RADICAL SCAVENGING ACTIVITY AND ANTIBACTERIAL ACTIVITY OF FIVE SPECIES OF SYZYGIUM

Haleem F.H, Senanayake S.A.M.A.G.M

BMS School of Science,

Sri Lanka

ABSTRACT

Many herbal plants in Sri Lanka have been traditionally used in the treatment of various diseases including diabetes and cancer. Plants from the Syzygium genus are widely used as medicinal plants due to possessing pharmacological properties as traditionally reported. The aim of this study was to investigate the free radical scavenging activity and antibacterial activity of the aqueous leaf extracts of five selected Syzygium species (S.mrytifolium, S.samarangense, S.aromaticum, S.cumini and S.zeylanicum), and determine their Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Total Antioxidant Capacity (TAC). TPC, TFC and TAC were assessed using the Folin Ciocalteu method, Aluminum Chloride colorimetric assay and Phosphomolybdate method respectively. The free radical scavenging activity was assessed using DPPH free radical and antibacterial activity was determined by agar well diffusion method against E.coli and S.aureus. Results showed that S.cumini contained the highest TPC (13.68%±0.47 w/w) and TAC (22.83±0.78 AAE mg/mL), while S.mrytifolium expressed the highest TFC (20.56%±2.56 w/w). The strongest free radical scavenging activity with an IC50 of 35±2.31µg/mL was expressed by S.aromaticum. At 20mg/mL, S.mrytifolium, S.cumini and S.aromaticum depicted antibacterial activity against S.aureus, however none of the extracts exhibited activity against E.coli. The findings of the study unfold the potential of selected Syzygium species to act as sources of antioxidants and antibacterial agents that can be incorporated in drug formulations in future after the isolation of their active compounds.

Keywords: Syzygium, Free radical scavenging, Antibacterial activity, Phytochemicals

INTRODUCTION

For years, medicinal plants have possessed more acceptability into society and healthcare industries due to having a better adaptability in the human body with less side effects, compared to synthetic drugs (Uddin et al., 2022). As a tropical country, Sri Lanka owns a variety of herbal plant species that have been used in the treatment and control of various diseases, such as diabetes and cancer (Waisundara and Watawana, 2014). The medicinal property of the plants is reflected on compounds called phytochemicals, which are secondary plant metabolites acting as the primary source of pharmacological actions of a plant acting on the body (Yoo et al., 2018). These are classified based on their chemical structures and characteristics, which includes carbohydrates, polyphenols, terpenoids, alkaloids and others (Huang et al., 2016). These compounds play important roles in herbal medicine. Phytochemicals can be obtained from various parts of the plant, which include leaves, flowers, roots, stems or seeds. And each part could provide a

particular therapeutic response, which differs from another part of the same plant (Sindhia and Bairwa, 2010). Research by Napagoda et al (2019) shows that leaves are frequently used for medicinal preparation in Sri Lanka, followed by seeds or fruits (figure 1).

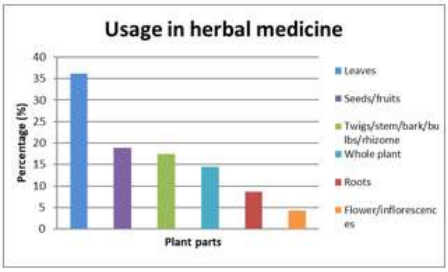

Figure 1: The diversity of plant parts used in medicinal preparations in Sri Lanka (Napagoda et al., 2019)

Phytochemicals from plants possess various pharmacological properties that have been used in the treatment of numerous conditions. Some of these properties include antioxidant activity, which is the ability to scavenge toxic free radical species (Michalak, 2022). The overproduction of such radicals causes oxidative stress in the body, which leads to the progression of many metabolic disorders, cardiovascular diseases and cancer (Ahmad et al., 2022). Free radical scavengers are able to interact with free radicals, preventing them from binding and damaging biomolecules such as DNA and proteins by inhibiting the oxidation reaction (Mfotie, 2021). Examples of phytochemicals with good antioxidant potential include quercetin, catechin, myricetin and more (Michalak, 2022). Another therapeutic property of most phytochemicals is antimicrobial activity, which involves mechanisms of actions such as inhibiting bacterial protein and cell wall synthesis, and metabolic pathways (Alvarez-Martinez et al., 2021). It has been shown that extracts of plants from the Myrtacea family prevented the formation of E.coli biofilms and S.aureus attachments (Famuyide et al., 2019). Antibacterial agents extracted from plants are viewed as an eco-friendly method to inhibit undesirable bacterial growth (Gonelimali et al., 2018), and could act as a solution to the rise in antibiotic resistance (Chassagne et al., 2021).

Syzygium is a genus of the myrtle family, Myrtaceae, which comprises of more than 1000 species around the word (Uddin et al., 2022). These plants have been traditionally used for therapeutic purposes as their extracts are said to contain multiple phytochemicals, which possess antibacterial, antifungal, antioxidant activity, as well as influence inflammatory processes (Cock and Cheesman, 2019). It also has been confirmed that some of these species are able to exert neuroprotective agents against diseases like Alzheimer's disease (Amir Rawa et al., 2022). Plants from the Syzygium genus are commonly found in Sri Lanka as houseplants, wild plants and in botanical gardens. However, there are insufficient research carried out on the medicinal properties of these plants, and their potential in being used in the development of new drugs, formulations or alternative therapeutic options that are safer and healthier to use. Therefore, the aim of this study was to determine the free radical scavenging potential and antibacterial activity of the aqueous leaf extracts of five selected Syzygium plants growth in the western and central provinces of Sri Lanka.

METHODOLOGY

Chemicals and Reagents

2,2-diphenyl-picrylhydrazyl (DPPH) and Sodium phosphate were purchased from SRL. Folin Ciocalteu and Sodium Carbonate (Na2CO3) were purchased from Modern Industries. Aluminium Chloride (AlCl3) and Sodium hydroxide (NaOH) were manufactured by LOBA chemie. Gallic acid, L-ascorbic acid and methanol were purchased from Sigma. Ammonium molybdate and Sodium

nitrate (NaNO2) were manufactured by Techno Pharmchem and Fisher Scientific respectively. Sulfuric acid (H2SO4) and barium chloride (BaCl2) were purchased from Daytona limited. Gentamycin discs (10mcg) and Muller Hinton agar were purchased from Himedia.

Equipment and Instruments

The equipment included in this study are the Jenway 6305 spectrometer, OHAUS measuring balance, KJMR roller mixer, GEMMYCO water bath, Heal force type II biosafety cabinet and autoclave manufactured by Meditry. Miscellaneous items include micropipettes and sterile tips, beakers, conical flasks, measuring cylinders, sterile petri plates and cotton swabs, Whatman no.1 filter paper, test tubes, boiling tubes, forceps, mortar and pestle.

Test microorganisms

For assessing antimicrobial activity, Escherichia coli (E.coli) (ATCC 25922) and Staphylococcus aureus (S.aureus) (ATCC 25923) were selected as the test microorganisms.

Collection of Plant Material

The plant material used for the study were harvested leaves of species from the Syzygium genus as follows (figure 2); Syzygium mrytifolium (S.M), Syzygium samarangense (S.S), Syzygium aromaticum (S.A), Syzygium cumini (S.C) and Syzygium zeylanicum (S.Z). The fresh leaves were collected from the Western and Central provinces of Sri Lanka during the month of February 2023.

Figure 2: The leaves of five selected Syzygium species for the study: A-Syzygium myrtifolium; B-Syzygium samarangense; C- Syzygium aromaticum; D-Syzygium cumini; E-Syzygium zeylanicum

Preparation of aqueous leaf extract

The collected leaves (100g) were shade dried for a week, and then grinded into fine powder using a mortar and pestle. For the preparation of the extract, 2g of the powdered samples were mixed in 100mL of distilled water and left on a roller mixer for 48 hours. Afterwards, the extracts were filtered using Whatman no.1 filter paper, and the extracts were stored at -20°C till further analysis.

Determination of Total phenolic Content (TPC)

The TPC of the extracts was determined using the Folin Ciocalteu method (Sadef et al, 2022). 0.1 mL of each extract was mixed with 0.5 mL of 10% Folin C reagent. After 6 minutes in room temperature (rtp), 1mL of 7.5% Na2CO3 was added to the mixture. The solutions were then left standing for 2 hours at rtp. Afterwards, the absorbance of the samples was measured at 765 nm. The same procedure was carried out for the construction of a calibration curve, using Gallic acid as the standard (10 – 200 µg/mL). Using the curve, the TPC of the samples were expressed in terms of Gallic Acid Equivalent (GAE), and then presented as % weight to weight (%w/w) (Madaan et al., 2011).

Determination of Total Flavonoid Content (TFC)

The TFC of the extracts was determined using the Aluminum Chloride colorimetric assay (Kumla et al., 2021). 0.5 mL of the extract was mixed in 2 mL of distilled water, followed by 0.15 mL of NaNO2. The mixtures were left standing for 5 minutes, and then 0.15 mL of AlCl3 was added in. The reaction mixtures were then incubated for 15 minutes at rtp. The absorbances of the samples were measured

at 415 nm. The TFC was expressed in terms of Quercetin Equivalent (QE) using the standard curve obtained by Kumla et al (2021), and then presented as %w/w.

Determination of Total Antioxidant Capacity (TAC)

The determination of the TAC was carried out using the phosphomolybdate assay (Rajkumari et al., 2018). 1 mL of phosphomolybdate reagent (0.6M H2SO4, 28mM sodium phosphate and 4mM ammonium molybdate) was mixed with 0.1mL of the extract, and incubated for 90 minutes at 90°C. The absorbances of the mixtures were then recorded at 695 nm. A calibration curve was constructed using L-ascorbic acid (30 – 270 µg/mL) as the standard, and the TAC was expressed in terms of Ascorbic Acid Equivalent (AAE).

Determination of free radical scavenging activity using DPPH

A series of sample dilutions were prepared (2.5 – 182.5 µg/mL) using distilled water. 0.5mL of each concentration was mixed with 1mL of prepared 0.1mM DPPH solution in methanol, and were left to stand in the dark for 30 minutes (Lalhminghlui and Jagetia, 2018). The absorbance of each concentration was measured at 523 nm, and the scavenging activity was determined using the following equation:

Scavenging (%) = (A control - A sample)/A control × 100,

Where a sample is the absorbance of the test sample and a control is the absorbance of the control.

Then a dose-response curve was constructed for each sample, from which the half–maximal inhibition Concentration (IC50) was determined.

Antimicrobial susceptibility test

The antibacterial activity of the extracts was assessed using the modified version of well diffusion method (Manandhar, Luitel and Dahal, 2019), with gentamycin used as the positive control, and distilled water as the negative control. Bacterial suspensions of E.coli and S.aureus were adjusted to the 0.5 McFarland standard (9.95 mL of 1% BaCl2 and 0.05 mL of 1% H2SO4). Under aseptic conditions, the suspensions were then spread across prepared Muller Hinton agar plates using sterile cotton swabs. For well diffusion, wells of 6mm in diameter were bored in the agar using sterile pipette tips, where 50 µL of the extracts were pipetted into. The plates were then incubated for 24 hours at 37°C in an incubator. Afterwards, the zones of inhibition were measured.

Statistical analysis

The entire study was carried out in triplicates for each sample. The following analytics were performed using the IBM SPSS software. The student T test and analysis of variance (ANOVA) were carried for the determination of the significance of the values. In addition, Pearson's correlation coefficient was used to identify any correlation between the assays.

RESULTS

Determination of Total Phenolic Content (TPC)

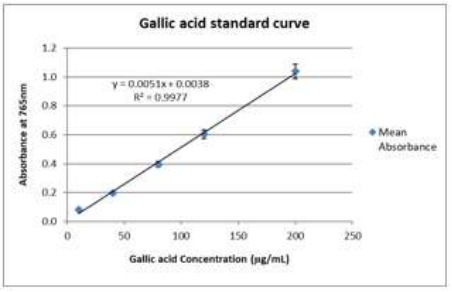

Figure 3: Gallic acid standard curve expressing absorbances values as mean ± SD (n=3)

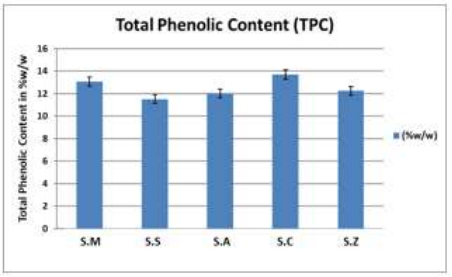

Figure 4: TPC of Syzygium extracts expressed in %w/w as mean ± SD (n =3)

Figure 4 shows that S. cumini possessed the highest phenolic content, with a value of 13.7% ± 0.57 (w/w), while S. samarangense had the lowest value of 11.5% ± 0.86 (w/w). S. mrytifolium produced a value of 13.1% ± 0.39 (w/w), while S. aromaticum and S. zeylanicum had values of 12.0% ± 0.54 and 12.2% ± 0.15 (w/w) respectively.

Determination of Total Flavonoid Content (TFC)

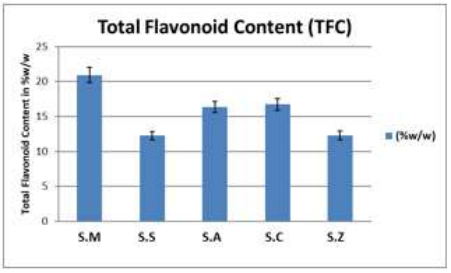

Figure 5: TFC of Syzygium extracts expressed in %w/w as mean ± SD (n =3)

Figure 5 shows that S. mrytifolium produced the highest value of 20.9% ± 3.14 (w/w), while S. samarangense and S. zeylanicum produced the lowest values of 12.3% ± 2.65 (w/w). S. aromaticum had the value of 16.4% ± 1.04 (w/w), while S. cumini produced the value of 16.7% ± 0.77 (w/w).

Determination of Total Antioxidant Capacity (TAC)

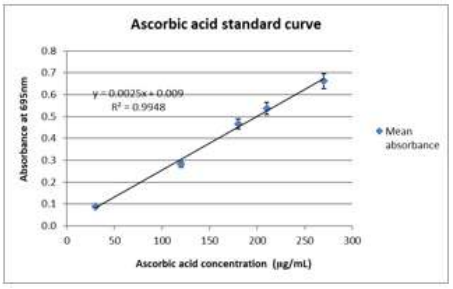

Figure 6: Ascorbic acid standard curve expressing absorbance values as mean ± SD (n =3)

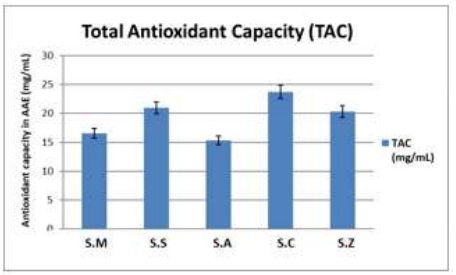

Figure 7: TAC of Syzygium expressed in AAE (Ascorbic Acid Equivalent) as mean ± SD (n =3)

Figure 7 shows that S. cumini possessed the highest total antioxidant capacity, with a value of 22.8 ± 0.78 mg/mL AAE, while S. aromaticum showed the lowest of 14.7 ± 0.17 mg/mL AAE. S. mrytifolium had the value of 16.0 ± 0.25 mg/mL, while S. samarangense had 20.2 ± 0.99 mg/mL, and S. zeylanicum had 19.5 ± 0.24 mg/mL AAE.

Determination of DPPH radical scavenging activity

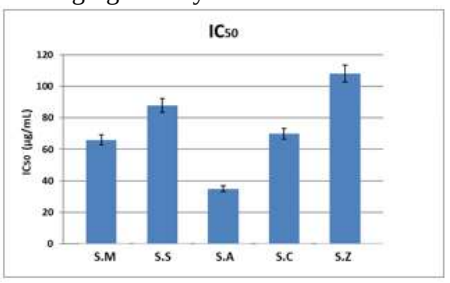

Figure 8: IC50 of Syzygium aqueous extracts expressed in µg/mL as mean ± SD (n = 3)

Figure 8 shows S. aromaticum producing the lowest IC50 value of 35µg/mL, being the strongest free radical scavenger out of all the tested samples, while S. zeylanicum had the highest value of 108µg/mL. S. mrytifolium produced an IC50 of 66µg/mL, while S. samarangense and S. cumini gave 88µg/mL and 77µg/mL respectively. All five extracts demonstrated strong free radical scavenging activity against DPPH free radical.

Antimicrobial susceptibility testing

Table 1: Antibacterial activity for each extract against E.coli and S.aureus after 24hr of incubation at 20mg/mL

Sample	Diameter of zone of inhibition (mm)	
	E.coli	*S.aureus*
S.M	None	4
S.S	None	None
S.A	None	12
S.C	None	8
S.Z	None	None

Table 1 shows that inhibition zones were observed for S.aureus with diameters of 4, 12 and 8mm by S.mrytifolium, S.aromaticum and S.cumini respectively. However, no zones of inhibition were observed for E.coli by all extracts at the end of 24hr at 20mg/mL.

Statistical analysis

Pearson correlation coefficient results showed a weak negative and positive correlation between the TAC with TFC and TPC respectively (figure 9), however it is not significant ($p > 0.05$). The analysis also showed that there is a negative correlation between the phytochemical content and free radical scavenging activity, and positive correlations between TAC and scavenging activity (figure 10), and phytochemical content with antimicrobial activity (figure 11). However, these were not significant ($p > 0.05$).

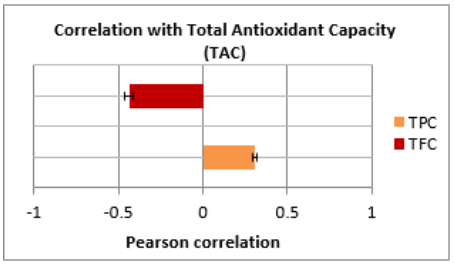

Figure 9: Pearson correlation coefficients of TPC and TFC with TAC ($p > 0.05$)

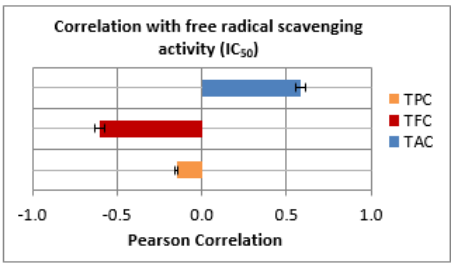

Figure 10: Pearson correlation coefficients of TPC, TFC and TAC with free radical scavenging activity ($p > 0.05$)

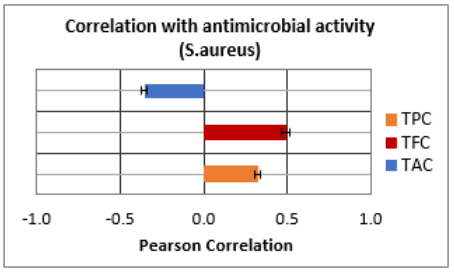

Figure 11: Pearson correlation coefficients of TPC, TFC and TAC with antimicrobial activity (S.aureus) ($p > 0.05$)

Student T test reveals that there is a significant difference between the highest and lowest values for TPC, TAC, TFC and IC50 ($p < 0.05$). One way ANOVA showed that the phytochemical content and free radical scavenging activity is significantly different ($p < 0.05$).

DISCUSSION

The pharmacological properties of a plant are dependent on their phytochemical content. These properties are mostly antioxidant effects, anti-cancer or antimicrobial activity. Accessing such important constituents from plants would open up potential sources of medicinal drugs, which are safer and more acceptable in the society. This study screened for the phytochemical contents of five selected species from the Syzygium genus, which have not been extensively

studied in Sri Lanka for their phytochemical content, particularly in their leaves. However, previous researches overseas demonstrated their potential as medicinal plants and use in therapeutics (Uddin et al., 2022). Bioactive compounds found in Syzygium plants such as gallic acid, carotenoids, polyphenols and myricetin contribute to numerous pharmacological properties that benefit overall health (Chhikara et al., 2018). Two flavonoids isolated from the leaves of S. campanulatum, named 2S)-7-Hydroxy-5-methoxy-6,8-dimethyl flavanone and (S)-5,7-dihydroxy-6,8-dimethyl-flavanone, were shown to have anti-proliferative activity against HCT 116 (colon cancer) cell line (Memon et al., 2015). Another study demonstrated the antimicrobial activity of the aqueous seed extract of S.aromaticum, which contained bioactive components such as eugenol acetate, β-carophyllene and eugenol (Ajiboye et al., 2016). These compounds are able to contribute antioxidant and anti-inflammatory activities as well (Uddin et al., 2022).

The extraction procedure used in this study was maceration, which is an easy and convenient method suitable for exposing the plant material to the solvent long term (Abubakar and Haque, 2020). It is suitable for extracting phytochemicals from thermolabile plants as it does not require any heat. Thus, preventing any thermal degradation of bioactive compounds and effecting their concentration (Abubakar and Haque, 2020). The leaves of the species in this study were extracted using distilled water as the solvent, as a previous study showed that solvents with a high polarity index yielded a greater phytochemical content when used for extraction (Wakeel et al., 2019). It is also cheap and nontoxic, therefore, it was deemed suitable for this study. For determining the TPC, the Folin-Ciocalteu method was used, which involves the formation of a reduced form of Folin C reagent due to oxidation by the hydroxyl groups of phenolic compounds present in the plant extract (Eddy et al., 2022). The reduced complex produces a blue colour whose intensity reflects on the amount of phenolic compounds present, which can then be measured using the spectrophotometer. All selected Syzygium species showed high phenolic content, with S.cumini expressing the highest value of 13.7% ± 0.57 (w/w) (figure 4). The value varies with a previous study conducted by Ahmed et al (2019), possibly due to different extraction procedures of the extract made, as well as climate factors. In general, S.cumini has shown to possess relatively a higher phenolic content when studied with other species of the same genus (Sheela and Cheenickal, 2017). Therefore, it is viewed as a valuable herbal plant with the highest potential as a phytochemical. S.samarangense produced the lowest TPC value of 11.5% ± 0.86 (w/w). The independent T test revealed that mean difference between the highest and lowest TPC values is significant ($p < 0.05$).

The TFC was determined using the Aluminium chloride colourimetric assay. This involves any present flavonoids in the plant extract binding to the metal ion Al (III), which acts as a complexing agent forming metal chelates that can be measured spectrophotometrically (Shraim et al., 2021). S.mrytifolium showed the highest TFC with a value of 20.9% ± 3.14 (w/w) (figure 5). This value comes in close with the value (25%) obtained by Ahmad et al (2022), who carried out a similar technique on the aqueous extract of the same species. S. samarangense and S.zeylanicum produced the lowest value of 12.3% ± 2.65 (w/w). The difference between the highest and lowest values was shown to be significant ($p < 0.05$). A study by Sheela and Cheenickal (2017) showed that the leaves of S.samarangense contained the highest TFC amongst other tested Syzygium species including S.cumini. There are many factors, including the climate and age of plant,

which may have contributed to the difference in outcomes. Research conducted by Idris et al (2023), reveals that the age of leaves of a species of the Syzygium genus acts as an important factor in effecting the phytochemical content present, with younger leaves containing more constituents than older ones. This explains the difference in the phytochemical content the species used in this study, with regional origins playing a role as well.

For the determination of TAC, the phosphomolybadate method was applied, which is based on the reduction of the reagent molybdenum VI to molybdenum V, creating a blue-green phosphomolybdeum complex in the presence of an antioxidant (Bibi Sadeer et al., 2020). The intensity of the colour reflects on the antioxidant capacity of the extract, which can be measured using a spectrophotometer. S. cumini showed the highest TAC value of 22.8 ± 0.78 mg/mL AAE (figure 7). Ahmed et al (2020) reported a similar value of 21.0 ± 0.85 mg/mL AAE. Interestingly, the same study also reported that the TAC of the same plant obtained from different regions in Bangladesh, varied drastically from one another, indicating that regional factors play a major contribution in the antioxidant capacity of a plant species. Therefore, any variation in the results may possibly be due to a difference in their locations. The lowest TAC was exhibited by S.aromaticum (figure 7), which varies drastically with many available literature indicating it to be the strongest antioxidant (Chang et al., 2020). The difference in TAC may have been strongly affected by location or climate factors in this study. The free radical scavenging activity was assessed using DPPH as the free radical, as it is stable, and commercially available. The radical provides a purple colour to the solution, which turns yellow or colourless in the presence of an antioxidant that reduces the radical to DPPH-H (Baliyan et al., 2022). Therefore, the plant extract with more free radical scavenging activity produces a clearer solution, which is measured spectrophotometrically. Using the calculated scavenging activity from various extract concentrations, a dose-response curve was constructed from which the concentration at which 50% of the free radicals are scavenged is determined. This is the IC50 of the sample, which is the most effective indicative of a potential drug's efficacy, and it provides a measure of potency that can be used in the development of the drug (Aykul and Martinez-Hackert, 2016). S.aromaticum had the lowest IC50 value of 35 ± 2.31 µg/mL (figure 8), having the strongest scavenging activity amongst the tested samples. Though this value varies with previous studies such as Afanyibo et al., (2019), it agrees with current literature indicating S.aromaticum having the strongest scavenging activity amongst most selected species (Chang et al., 2020).

Statistical analysis shows that there is a weak negative correlation between TPC/TFC and IC50 value (figure 10), indicating that the phytochemical content affects scavenging activity. However, it was not significant ($p > 0.05$), so it cannot be assumed that the phytochemical content of the leaf extracts in this study directly contributes to its antioxidant activity. IC50 and TAC also showed a positive correlation which was insignificant. Reasons may include equipment and pipetting errors resulting in inaccuracy. Nevertheless, results open up the potential of the selected species to be used in the treatment of diseases caused by oxidative stress, mainly cancer and cardiovascular diseases. For this study, the agar well diffusion method was used to assess the antibacterial properties of the aqueous extracts against E.coli and S.aureus, with gentamycin used as the positive control. Gentamycin is a strong aminoglycoside antibiotic which inhibits bacterial protein synthesis, and has been shown to effectively prevent the growth of many Gram-positive and Gram-negative

bacteria (Buechler and Daveluy, 2022). The method involves the creation of wells in the agar medium, where the antimicrobial agent is placed. The agent then diffuses in the medium and inhibits the growth of microorganisms on it, creating zones of inhibition (Balouiri, Sadiki and Ibnsouda, 2016). Antimicrobial activity was observed against the bacteria, S.aureus, with S.aromaticum showing the largest zone of inhibition of 12mm in diameter after 24hr of incubation. This confirms that Syzygium species do possess antibacterial activity against Gram-positive bacteria, and can be used to develop new antibiotics. No zones were observed for E.coli, possibly due to the aqueous extracts being ineffective against Gram-negative bacteria at the end of 24hr. However previous research has shown that the Syzygium species are able to exhibit antibacterial activity against E.coli after 24hr (Ahmad et al., 2021). The study by Famuyide et al (2019) showed leaf extracts of Syzygium plants exhibited antimicrobial activity against both Gram-positive and Gram-negative bacteria, with the capacity to reduce biofilm formations. Phytochemicals present in most Syzygium plants, such as phenols and terpenoids, contribute to effective antimicrobial activity against bacteria like E.coli and S.aurues (Uddin et al., 2022). A reason as to why no activity was shown in this study may be due to differences in methodology with regard to extraction procedure and solvents used for extraction, seasonal factors or storage conditions of the extract, which may have affected the phytochemical content of the extracts or their properties. Figure 11 shows a weak positive correlation with the phytochemical content of this study with antimicrobial activity of the extracts. However, it is not significant ($p > 0.05$).

The findings of this study adds to current evidences of Syzygium plants possessing high phytochemical content that contribute to numerous pharmacological properties, which can be used as an plant based approach in treating diseases mainly caused by the elevated levels of free radicals in the body, including different forms of malignancies and cardiovascular diseases, as well as infections led by S.aureus. Further studies involving in vitro tests and animal studies must be conducted on these species to evaluate their cytotoxicity, potency, and efficacy. Other antimicrobial susceptibility tests, such as agar disc diffusion and broth dilution, must also be conducted using a wider range of test microorganisms for a broader perspective on the plants' antimicrobial activity. These will pave the way to clinical trials, leading up to their use in the pharmaceutical and nutraceutical industry.

In conclusion, the study unfolds that all five selected Syzygium species has high phenolic and flavonoid content, and antioxidant capacity, with S.cumini having the highest TPC and TAC. In addition, all the tested species demonstrated strong free radical scavenging activity (<200 µg/mL), and further exhibited antibacterial activity against S.aureus. These results provide proof that Syzygium species have the potential to act as sources of free radical scavengers against cancer and cardiovascular diseases and also, as antibacterial agents to be used in healthcare treatments.

Acknowledgements

I thank the School of Science at BMS Campus, Sri Lanka which is affiliated to Northumbria University (UK), for providing the laboratory facilities, equipment and necessary reagents, and the lab staff for aiding when necessary. I then express my thanks to my assigned supervisors, Ms. S.A.M.A. Gayani Madara Senanayake and Mr. A.I.A. Imthikab for overlooking the entirety of the project and giving their continuous support.

REFERENCES

Abubakar, A. and Haque, M. (2020) 'Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes', Journal of Pharmacy And Bioallied Sciences, 12(1), pp.1–10. [Online] Available at: https://pubmed.ncbi.nlm.nih.gov/32801594/ (Accessed: 16 May 2024).

Afanyibo, Y., Esseh, K., Idoh, K., Koudouvo, K., Agbonon, A. and Gbeassor, M. (2019) 'Toxicity and antioxidant activity of Syzygium aromaticum, Mondia whitei, Carissa spinarum and Caesalpinia bonduc', The Journal of Phytopharmacology, 8(3), pp.124–128. [Online] Available at: https://www.phytopharmajournal.com/Vol8_Issue3_07.pdf (Accessed: 04 Jul 2023).

Agidew, M.G. (2022) 'Phytochemical analysis of some selected traditional medicinal plants in Ethiopia', Bulletin of the National Research Centre, 46(1). [Online] Available at: https://bnrc.springeropen.com/articles/10.1186/s42269-022-00770-8 (Accessed: 3April 2023)

Ahmad, M.A., Lim, Y.H., Chan, Y.S., Hsu, C.-Y., Wu, T.-Y. and Sit, N.W. (2021) 'Chemical composition, antioxidant, antimicrobial and antiviral activities of the leaf extracts of Syzygium myrtifolium', Acta Pharmaceutica, 72(2), pp.317–328 [Online] Available at: https://sciendo.com/article/10.2478/acph-2022-0013 (Accessed: 04 July 2023).

Ahmad, M.A., Lim, Y.H., Chan, Y.S., Hsu, C.Y., Wu, T.Y. and Sit, N.W. (2022) 'Chemical composition, antioxidant, antimicrobial and antiviral activities of the leaf extracts of Syzygium myrtifolium', Acta Pharmaceutica, 72(2), pp.317–328. [Online] Available at: https://sciendo.com/article/10.2478/acph-2022-0013 (Accessed: 3 April 2023)

Ahmed, R., Tariq, M., Hussain, M., Andleeb, A., Masoud, M.S., Ali, I., Mraiche, F. and Hasan, A. (2019) 'Phenolic contents-based assessment of therapeutic potential of Syzygium cumini leaves extract', PLOS ONE, 14(8), p.e0221318. [Online] Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6715210/ (Accessed: 05 Jul 2023).

Ahmed, S., Ahmed, K.S., Hossain, M.S., Azam, M.S., Rahman, M. and Hoque, M. (2020) 'Proximate composition and antioxidant activity of Syzygium cumini fruit grown at different regions in Bangladesh', Food Research, 4(5), pp.1693–1699. [Online] Available at: https://www.myfoodresearch.com/uploads/8/4/8/5/84855864/_39__fr-2020-162_ahmed.pdf (Accessed: 10 Jul 2023).

Álvarez-Martínez, F.J., Barrajón-Catalán, E., Herranz-López, M. and Micol, V. (2021) 'Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action', Phytomedicine, 90, p.153626. [Online] Available at: https://www.sciencedirect.com/science/article/pii/S0944711321001690 (Accessed: 3 April 2023).

Ajiboye, T.O., Mohammed, A.O., Bello, S.A., Yusuf, I.I., Ibitoye, O.B., Muritala, H.F. and Onajobi, I.B. (2016). Antibacterial activity of Syzygium aromaticum seed: Studies on oxidative stress biomarkers and membrane permeability. Microbial Pathogenesis, 95, pp.208–215. [Online] Available at: https://www.sciencedirect.com/science/article/abs/pii/S0882401015302400?via%3Dihub (Accessed: 16 May 2024).

Amir Rawa, M.S., Mazlan, M.K.N., Ahmad, R., Nogawa, T. and Wahab, H.A. (2022) 'Roles of Syzygium in Anti-Cholinesterase, Anti-Diabetic, Anti-Inflammatory, and Antioxidant: From Alzheimer's Perspective', Plants, 11(11), p.1476. [Online] Available at: https://www.mdpi.com/2223-7747/11/11/1476 (Accessed: 3 April 2023).

Anoop, M. V. and Bindu, A. R. (2014) 'Pharmacognostic and physico-chemical studies on leaves of Syzygium zeylanicum (L.) DC', International Journal of Pharmacognostic and phytochemical research 6(4) pp.685-689. [Online] Available at: https://www.semanticscholar.org/paper/Pharmacognostic-and-Physico-Chemical-Studies-on-of-Anoop/f19f7b142c7b6662b0e88c4cb9789386d8c377e5 (Accessed: 04 Jul 2023).

Aykul, S. and Martinez H. E. (2016) 'Determination of half-maximal Inhibitory concentration using biosensor-based protein interaction analysis', Analytical Biochemistry, 508(1), pp.97–103. [Online] Available at: https://pubmed.ncbi.nlm.nih.gov/27365221/ (Assessed: 05 Jul 2023).

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R.P. and Chang, C.-M. (2022) 'Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa', Molecules, 27(4), p.1326. [Online] Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8878429/ (Assessed: 05 Jul 2023).

Balouiri, M., Sadiki, M. and Ibnsouda, S.K. (2016) 'Methods for in Vitro Evaluating Antimicrobial activity: a Review', Journal of Pharmaceutical Analysis, 6(2), pp.71–79. [Online] Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5762448/ (Accessed: 05 Jul 2023).

Bibi Sadeer, N., Montesano, D., Albrizio, S., Zengin, G. and Mahomoodally, M.F. (2020). 'The Versatility of Antioxidant Assays in Food Science and Safety—Chemistry, Applications, Strengths, and Limitations', Antioxidants, 9(8), p.709. [Online] Available at: https://www.mdpi.com/2076-3921/9/8/709 (Accessed: 05 Jul 2023).

Buechler, C.R. and Daveluy, S. (2022) 'Topical Therapeutics', Elsevier eBooks, pp.145–154. [Online] Available at: https://www.sciencedirect.com/science/article/abs/pii/B9780323777247000152 (Assessed: 05 Jul 2023).

Chang, H.-J., Kim, Y., Yun Chan Kang, Choi, M. and Lee, J.-H. (2020) 'Antioxidant and antibacterial effects of medicinal plants and their stick-type medicinal concentrated beverages,' 29(10), pp.1413–1423. [Online] Available at: https://pubmed.ncbi.nlm.nih.gov/32999749/ (Accessed: 14 May 2024)

Chassagne, F., Samarakoon, T., Porras, G., Lyles, J.T., Dettweiler, M., Marquez, L., Salam, A.M., Shabih, S., Farrokhi, D.R. and Quave, C.L. (2021) 'A Systematic Review of Plants With Antibacterial Activities: A Taxonomic and Phylogenetic Perspective', Frontiers in Pharmacology, 11. [Online] Available at: https://www.frontiersin.org/articles/10.3389/fphar.2020.586548/full (Accessed: 3April 2023).

Chhikara, N., Kaur, R., Jaglan, S., Sharma, P., Gat, Y. and Panghal, A. (2018) 'Bioactive compounds and pharmacological and food applications ofSyzygium cumini– a review', Food & Function, 9(12), pp.6096–6115. [Online] Available at: https://pubmed.ncbi.nlm.nih.gov/30379170/ (Accessed: 16 May 2024).

Cock, I.E. and Cheesman, M. (2019). 'The Potential of Plants of the Genus Syzygium (Myrtaceae) for the Prevention and Treatment of Arthritic and Autoimmune Diseases', Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases, pp.401–424. [Online] Available at: https://www.sciencedirect.com/science/article/abs/pii/B9780128138205000234 (Accessed: 3 April 2023).

Eddy, D.R., Nursyamsiah, D., Permana, M.D., Solihudin, Noviyanti, A.R. and Rahayu, I. (2022). Green Production of Zero-Valent Iron (ZVI) Using Tea-Leaf Extracts for Fenton Degradation of Mixed Rhodamine B

and Methyl Orange Dyes. Materials, 15(1), p.332. [Online] Available at: https://www.mdpi.com/1996-1944/15/1/332 (Accessed: 05 Jul 2023).

Famuyide, I.M., Aro, A.O., Fasina, F.O., Eloff, J.N. and McGaw, L.J. (2019) 'Antibacterial and antibiofilm activity of acetone leaf extracts of nine under-investigated south African Eugenia and Syzygium (Myrtaceae) species and their selectivity indices', BMC Complementary and Alternative Medicine, 19(1). [Online] Available at: https://bmccomplementmedtherapies.biomedcentral.com/articles/10.1186/s12906-019-2547-z (Accessed: 3April 2023).

Ghosh, D., Maiti, S., Ali, K., Jana, K., Chatterjee, K. and De, D. (2013) 'Ameliorating effect of mother tincture of Syzygium jambolanum on carbohydrate and lipid metabolic disorders in streptozotocin-induced diabetic rat: Homeopathic remedy', Journal of Natural Science, Biology and Medicine, 4(1), p.68. [Online] Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3633306/ (Accessed: 3 April 2023).

Gonelimali, F.D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M. and Hatab, S.R. (2018) 'Antimicrobial Properties and Mechanism of Action of Some Plant Extracts Against Food Pathogens and Spoilage Microorganisms', Frontiers in Microbiology, 9. [Online] Available at: https://www.frontiersin.org/articles/10.3389/fmicb.2018.01639/full (Accessed: 3 April 2023).

Gunawardana, S.L.A. and Jayasuriya, W.J.A.B.N. (2019) 'Medicinally Important Herbal Flowers in Sri Lanka', Evidence-Based Complementary and Alternative Medicine, 2019, pp.1–18. [Online] Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6556806/ (Accessed: 3 April 2023).

Huang, Y., Xiao, D., Burton-Freeman, B.M. and Edirisinghe, I. (2016) 'Chemical Changes of Bioactive Phytochemicals during Thermal Processing', Reference Module in Food Science. [Online] Available at: https://www.semanticscholar.org/paper/Chemical-Changes-of-Bioactive-Phytochemicals-during-Huang-Xiao/f293f8f20b3ad26fcd1f1ae40db2de070ff79949 (Accessed: 3 April 2023).

Idris, N.S., Khandaker, M.M., Rashid, Z.M., Majrashi, A., Alenazi, M.M., Nor, Z.M., Mohd Adnan, A.F. and Mat, N. (2023) 'Polyphenolic Compounds and Biological Activities of Leaves and Fruits of Syzygium samarangense cv. 'Giant Green' at Three Different Maturities', Horticulturae, 9(3), p.326. [Online] Available at: https://www.mdpi.com/2311-7524/9/3/326 (Accessed: 04 Jul 2023).

Kumla, J., Suwannarach, N., Tanruean, K. and Lumyong, S. (2021) 'Comparative Evaluation of Chemical Composition, Phenolic Compounds, and Antioxidant and Antimicrobial Activities of Tropical Black Bolete Mushroom Using Different Preservation Methods' Foods, 10(4), p.781. [Online] Available at: https://www.mdpi.com/2304-8158/10/4/781 (Accessed: 01 June 2023).

Kytidou, K., Artola, M., Overkleeft, H.S. and Aerts, J.M.F.G. (2020) 'Plant Glycosides and Glycosidases: A Treasure-Trove for Therapeutics', Frontiers in Plant Science, 11. [Online] Available at: https://www.frontiersin.org/articles/10.3389/fpls.2020.00357/full (Accessed: 21 Jul 2023).

Lalhminghlui, K. and Jagetia, G.C. (2018) 'Evaluation of the free-radical scavenging and antioxidant activities of Chilauni, Schima wallichii Korth in vitro', Future Science OA, 4(2), p.FSO272. [Online] Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5778377/ (Accessed: 01 June 2023).

Liu, J., Wu, M., Zhang, R. and Xu, Z.P. (2021) 'Oxygen-derived free radicals: Production, biological importance, bioimaging, and analytical detection with responsive luminescent nanoprobes', VIEW, 2(5), p.20200139. [Online] Available at: https://onlinelibrary.wiley.com/doi/full/10.1002/VIW.20200139 (Accessed: 3 April 2023).

Lobo V, Patil A, Phatak A, and Chandra N. (2010) 'Free radicals, antioxidants and Functional Foods: Impact on human health', Pharmacognosy Reviews. 4(8) pp.118-126 [Online] Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3249911/ (Accessed at: 3 April 2023).

Madaan, R., Bansal, G., Kumar, S. and Sharma, A. (2011) 'Estimation of Total Phenols and Flavonoids in Extracts of Actaea spicata Roots and Antioxidant Activity Studies', Indian Journal of Pharmaceutical Sciences, 73(6), pp.666–669 [Online] Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3480753/ (Accessed: 22 June 2023).

Manandhar, S., Luitel, S. and Dahal, R.K. (2019) 'In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria', Journal of Tropical Medicine, 2019, pp.1–5. [Online] Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6466868/ (Accessed: 12 June 2023).

Mfotie Njoya, E. (2021) 'Medicinal plants, antioxidant potential, and cancer', Cancer, pp.349–357 [Online] Available at: https://www.sciencedirect.com/science/article/pii/B978012819547500031 6 (Accessed: 3 April 2023).

Memon, A., Ismail, Z., Al-Suede, F., Aisha, A., Hamil, M., Saeed, M., Laghari, M. and Majid, A. (2015) 'Isolation, Characterization, Crystal Structure Elucidation of Two Flavanones and Simultaneous RP-HPLC Determination of Five Major Compounds from Syzygium campanulatum Korth', Molecules, 20(8), pp.14212–14233. [Online] Available at: https://www.mdpi.com/1420-3049/20/8/14212 (Accessed: 16 May 2024).

Michalak, M. (2022) 'Plant-Derived Antioxidants: Significance in Skin Health and the Ageing Process', International Journal of Molecular Sciences, 23(2), p.585 [Online] Available at: https://www.mdpi.com/1422-0067/23/2/585 (Accessed: 3 April 2023).

Najmi, A., Javed, S.A., Al Bratty, M. and Alhazmi, H.A. (2022) 'Modern Approaches in the Discovery and Development of Plant-Based Natural Products and Their Analogues as Potential Therapeutic Agents', Molecules, 27(2), p.349. [Online] Available at: https://www.mdpi.com/1420-3049/27/2/349 (Accessed: 3 April 2023)

Napagoda, M.T., Sundarapperuma, T., Fonseka, D., Amarasiri, S. and Gunaratna, P. (2019) 'Traditional Uses of Medicinal Plants in Polonnaruwa District in North Central Province of Sri Lanka', Scientifica, 2019, pp.1–11 [Online] Available at: https://www.hindawi.com/journals/scientifica/2019/9737302/ (Accessed: 20 April 2023).

Qamar, M., Akhtar, S., Ismail, T., Wahid, M., Abbas, M.W., Mubarak, M.S., Yuan, Y., Barnard, R.T., Ziora, Z.M. and Esatbeyoglu, T. (2022) 'Phytochemical Profile, Biological Properties, and Food Applications of the Medicinal Plant Syzygium cumini', Foods, 11(3), p.378. [Online] Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8834268/ (Accessed: 21 Jul 2023).

Rajkumari, J., Dyavaiah, M., Siddhardha, S. J., and Busi, S.,(2018) 'Evaluation of in vivo antioxidant potential of Syzygium jambos (L.) Alston and Terminalia citrina Roxb. towards oxidative stress response in Saccharomyces cerevisiae. J Food Sci Technol 55(11), pp.4432–4439.

[Online] Available at: https://link.springer.com/article/10.1007/s13197-018-3355-z (Accessed: 01 June 2023).

Russell-Smith, J., Karunaratne, N.S. and Mahindapala, R. (2006) 'Rapid inventory of wild medicinal plant populations in Sri Lanka', Biological Conservation, 132(1), pp.22–32. [Online] Available at: https://www.sciencedirect.com/science/article/abs/pii/S0006320706001121 (Accessed: 3 April 2023).

Sadef, Y., Javed, T., Javed, R., Mahmood, A., Alwahibi, M.S., Elshikh, M.S., AbdelGawwa, M.R., Alhaji, J.H. and Rasheed, R.A. (2022) 'Nutritional status, antioxidant activity and total phenolic content of different fruits and vegetables' peels' PLOS ONE, 17(5), p.e0265566. [Online] Available at: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0265566 (Accessed: 01 June 2023)

Selvakumar, P.M., Rajkumar S, R.J. and MSA, M.N. (2018) 'Phytochemicals as a potential source for anti-microbial, anti-oxidant and wound healing - a review', MOJ Bioorganic & Organic Chemistry, 2(2). [Online] Available at: https://medcraveonline.com/medcrave.org/index.php/MOJBOC/article/view/14440 (Accessed: 14 Jul 2023).

Sheela, D. and Cheenickal, M. (2017) 'Total Phenolics and Flavonoids among The Selected Species of Syzygium, Gaertn,' Research Journal of Pharmacognosy and Phytochemistry, 9(2), p.101. [Online] Available at: https://rjpponline.org/AbstractView.aspx?PID=2017-9-2-9 (Accessed: 14 May 2024).

Shekhar, S., Yadav, Y., Singh, A.P., Pradhan, R., Desai, G.R., Dey, A.B. and Dey, S. (2018) 'Neuroprotection by ethanolic extract of Syzygium aromaticum in Alzheimer's disease like pathology via maintaining oxidative balance through SIRT1 pathway', Experimental Gerontology, 110, pp.277–283. [Online] Available at: https://pubmed.ncbi.nlm.nih.gov/29959974/ (Accessed: 3 April 2023).

Shraim, A.M., Ahmed, T.A., Rahman, M.M. and Hijji, Y.M. (2021) 'Determination of total flavonoid content by aluminum chloride assay: A critical evaluation', LWT, 150, p.111932. [Online] Available at: https://www.sciencedirect.com/science/article/abs/pii/S0023643821010859 (Assessed: 05 Jul 2023).

Sindhia, V. and Bairwa, R. (2010) 'Plant review: Butea monosperma,' International Journal of Pharmaceutical and Clinical Research, 2(2) pp. 90–94. [Online] Available at: https://impactfactor.org/PDF/IJPCR/2/IJPCR,Vol2,Issue2,Article9.pdf (Accessed: 3 April 2023).

Uddin, A.B.M.N., Hossain, F., Reza, A.S.M.A., Nasrin, Mst.S. and Alam, A.H.M.K. (2022) 'Traditional uses, pharmacological activities, and phytochemical constituents of the genus Syzygium: A review', Food Science & Nutrition. [Online] Available at: https://onlinelibrary.wiley.com/doi/full/10.1002/fsn3.2797 (Accessed: 3 April 2023).

Ullah M.A, Sarkar B, Hossain S, Mohammad N.U.R and Islam M.S., (2019) 'Phytochemicals and Metabolites as Antimicrobial Agents from Medicinal Plants of Bangladesh: A Review', PharmaTutor; 7(6) pp. 1-11 [Online] Available at: https://www.semanticscholar.org/paper/Phytochemicals-and-Metabolites-as-Antimicrobial-of-Ullah-Hossain/7a4096e0b8164739b99443f606cf29f131fa7a39 (Accessed: 11 April 2023).

Waisundara, V.Y. and Watawana, M.I. (2014) 'The Classification of Sri Lankan Medicinal Herbs: An Extensive Comparison of the Antioxidant Activities', Journal of Traditional and Complementary Medicine, 4(3), pp.196–202. [Online] Available at: https://www.sciencedirect.com/science/article/pii/S2225411016302334 (Accessed: 3 April 2023).

Wakeel, A., Jan, S.A., Ullah, I., Shinwari, Z.K. and Xu, M. (2019) 'Solvent polarity mediates phytochemical yield and antioxidant capacity of Isatis tinctoria,'PeerJ, 7, p.e7857. [Online] Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6790100/ (Accessed: 21 Jul 2023).

Yoo, S., Kim, K., Nam, H. and Lee, D. (2018) 'Discovering Health Benefits of Phytochemicals with Integrated Analysis of the Molecular Network, Chemical Properties and Ethnopharmacological Evidence', Nutrients, 10(8). [Online] Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6115900/ (Accessed: 11 April 2023).