Antioxidant, Antibacterial and Antiqourum Sensing Properties of Selected Wild Tea Leaves of Mountain Province

Lichelyn Moling Nasungan

Department of Science Education, Mountain Province State Polytechnic College, Bontoc, Philippines.

Abstract

This study investigated the potential biological activities by determining the antioxidant (DPPH Assay), antibacterial (Agar-well Diffusion Method), and antiquorum sensing (Crystal Violet based Microtitre Plate Biofilm Assay) of the selected wild tea leaves of Mt. Province, namely: Cinnamomum mercadoi S. Vidal, Gaultheria leucocarpa var. cumingiana (S.Vidal), Clausena sanki (Perr.) J.F. Molino var. mollis (Merr.) J.F Molino, Descasporum fruticosum (J.R. Forst and G. Forst) and Glycomis pentaphylla. It was shown that all of he wild tea leaves have antioxidant properties. G. leucocarpa var. cumingiana (S.Vidal) however, exhibited the highest radical scavenging activity of 88.67%, which indicates that it is the best antioxidant among the wild tea samples. Results also proved that the selected wild tea plants have antibacterial property against E. coli, P. aeruginosa, S. aureus, and B. subtilis. Moreover, the result of the Microtitre Plate Biofilm Assay showed that all selected wild tea had moderate antiquorum sensing activity against the bacterial species tested. It was concluded that the ethanolic leaves’ extracts of the selected wild tea samples had antioxidant, antibacterial, and antiquorum sensing property. Therefore, this study hopes to promote the consumption of wild tea on a commercial scale due to its additional health benefits. It may be worthy to consider natural products and alternative medicines as potential prevention and treatments for diseases.

Keywords: Antioxidant, Antibacterial, Antiquorum Sensing Property, Wild Tea Leaves
INTRODUCTION

Tea is the second most extensively consumed beverage in the world.1 Globally, the production and consumption of tea are driven by China that produces approximately 30.35% of the wild tea production. Tea consumption in the Philippines was popularized by the Chinese who migrated before the colonization of the Spaniards. Accordingly, 32.6% of the Filipinos consume functional beverages including tea several times a week.2

Tea beverages are claimed to contain many health-promoting abilities such as protection from cardiovascular diseases, control of obesity and diabetes, anticarcinogenic, antiaging, antihistaminic, antiarthritic, anti-inflammatory, antibacterial, antifungal, and antiviral effects.3 The substances present in tea plants, primary and secondary metabolites, can also be of great importance in other applications such as textile and drug production, nutraceuticals and cosmetics.4

At present, tea is continuously and scientifically investigated in terms of its potential to promote health. Antioxidants play an important role in inhibiting and scavenging free radicals, thus, providing protection to humans against infection and degenerative diseases. Tea has potent antioxidant activity5 because it is one of the richest sources of antioxidants and the three major forms of antioxidant tea are green tea, oolong tea, and black tea. The green teas are claimed to be the most powerful due to the presence of a large amount of flavan-3-ols known as catechins.6

Traditional herbal teas which may contain an extensive diversity of compounds, often with indefinite biological effects7 are widely used for the treatment of infections and inflammatory processes in the urinary tract, muscular pains and respiratory diseases.8

In Mountain Province, there are five plants consumed as tea which are generally called wild tea. These wild teas are present in the different municipalities, namely; Cinnamomum mercadoi S. Vidal locally named “kumayo” and Gaultheria leucocarpa var. cumingiana (S.Vidal), C. sanki (Perr.) J.F. Molino var. mollis (Merr.) J.F Molino, D. fruticosum (J.R. Forst and G. Forst) and G. pentaphylla.

MATERIALS AND METHODS

Plant Collection

The fresh mature leaves of C. mercadoi, G. leucocarpa var. cumingiana, C. sanki, D. fruticosum and G. pentaphylla were gathered and collected in the municipalities of Bauko, Barlig, Bontoc and Sagada of Mountain Province from November 2019 to March 2020 after acquiring the Wildlife Gratuitous Permit number DENR-CAR-010-2019.

Preparation of Materials

The collected mature plant leaves were cleaned and washed with water and air-dried at room temperature for three weeks, after which these were ground into coarse powder using a high-capacity grinding machine. The bacterial isolates, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis used for the study were acquired from the Laboratory of Department of Biology -Benguet State University.

Dried leaves of each sample measuring 50 g were soaked in 300 mL of 95% ethanol for 2 days. The extracts were filtered with No.1 Whatman.
filter paper. The filtrates were then evaporated to 65°C under reduced pressure using a rotary evaporator to evaporate the alcohol.

**DPPH Radical Scavenging Assay**

The DPPH radical scavenging properties of the different wild tea leaves were evaluated using the method of Clarke et al. with few modifications. The DPPH radical scavenging activity was calculated using the formula:

\[
\% \text{ Radical Scavenging Activity} = \left( \frac{A_{\text{control}} - A_{\text{extract}}}{A_{\text{control}}} \right) \times 100\%
\]

**Agar-well Diffusion Method**

The antibacterial properties of the different wild tea were evaluated according to the procedures of Nimmakayala et al. with few modifications. The different leaf extracts were assessed on several strains of bacteria through the Agar-well diffusion method. The strains of bacteria that were used to test the antibacterial property of the different wild tea leaf extracts were *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis*.

The bacterial cultures were pipetted out into the well-plates. The plates were covered with aluminum foil and were incubated for 48 h. After 48 h of incubation, the supernatant of the bacterial cultures was discarded. The plates were washed with distilled water three times to eliminate the loosely associated cells. The plates were dried and after which, the wells were stained with 100 μL of 2% crystal violet for 15 min at room temperature. The plates were washed three times with distilled water to remove unabsorbed stains and then destained with ethanol.

**Crystal Violet based Microtitre Plate Biofilm Assay**

The bacterial cultures were pipetted out into the well-plates, covered with aluminum foil, and incubated for 48 h. After 48 h of incubation, the supernatant of the bacterial cultures was discarded. The plates were washed with distilled water three times to eliminate the loosely associated cells. The plates were dried and after which, the wells were stained with 100 μL of 2% crystal violet for 15 min at room temperature. The plates were washed three times with distilled water to remove unabsorbed stains and then destained with ethanol.

**Table 1. Interpretation of biofilm production**

| Average OD Value | Biofilm Production |
|------------------|--------------------|
| OD is < 0.120    | None/Weak (N/W)    |
| OD is 0.121 – 0.240 | Moderate (M)      |
| OD is > 0.240    | High (H)           |

**RESULTS**

**Potential of Selected Wild Tea Leaves Extracts as Antioxidants**

Radical scavenging activity is indicative of the antioxidant property of wild tea plants. Table 2 presents the radical scavenging activity of the selected wild tea leaves’ extracts. Specifically, *G. leucocarpa* var. *cumingiana* had the highest computed radical scavenging activity among the wild tea leaves’ extracts with a value of 88.67% and *G. pentaphylla* had the lowest radical scavenging activity with a computed value of 64.56%.

A substance, such as an antioxidant, helps in the protection of cells from the free radicals that cause damage to the cells. Free radicals are unstable molecules that are made during the normal metabolism of the cells. In this study, ascorbic acid has strong antioxidant property. The result implies that among the selected wild tea extracts, *G. leucocarpa* var. *cumingiana* and *C. mercadoi* have higher radical scavenging activity compared to the control ascorbic acid. The *C. sanki* and *G. pentaphylla* may have lower radical scavenging activity values but they are still capable of radical scavenging activity. It may also be possible that the biological compounds present in the different wild tea may affect the radical...
scavenging activity of each of the selected wild teas.

As to concentrations, 500 µg/mL had the highest antioxidant property with a computed radical scavenging activity of 88.46% while the 62.50 µg/mL concentration had the least antioxidant property of 53.35%. Statistical analysis showed that the different concentrations of the wild tea leaves’ extracts were significantly different. In this study, it was shown that the higher concentrations of the wild tea samples, the higher radical scavenging activity and consequently higher antioxidant property. This finding also indicates that higher concentrations of these selected wild tea samples are best recommended, as shown in the result that 500mg/mL concentration has higher radical scavenging activity. This result corroborates with the study of Jadid et al.12, where they reported that the highest concentration of the plant extracts demonstrated higher radical scavenging activity.

Potential of Selected Wild Tea Leaves’ Extracts as Antibacterial in Terms of Zone of Inhibition

Table 3 presents the antibacterial potential of the selected wild tea plants against the different strains of bacteria. Based on the analysis, all the wild tea leaves had a similar effect on the growth of E. coli as shown by the values for inhibition zones which did not differ significantly. The results may not be comparable to the positive control, azithromycin, however, the result of the present study shows that the various wild tea leaves’ extracts significantly inhibited the growth of E. coli as compared to the negative control. The presence of the bioactive compounds may be linked to the antibacterial properties of the selected wild tea leaves against E. coli.13

On the other hand, it was observed that the wild tea leaves’ extracts have a potential antibacterial property against P. aeruginosa. Particularly, C. sanki significantly inhibited the bacterial growth with the highest mean zone of inhibition of 11.77mm. Based on the statistical analysis, significant differences were observed in the mean zone of inhibition of the different wild tea leaves’ extracts against P. aeruginosa. This statistical result shows that C. sanki and D. fruticosum is the most effective against P. aeruginosa based on the zone of inhibition. It may not be comparable to the positive control but results show that the wild tea plants have antibacterial potential against P. aeruginosa. G. leucocarpa var. cumingiana is the least effective among the different wild tea plant. This finding implies that the different wild tea leaves’ leaves’ extracts possess antibacterial properties against P. aeruginosa. The inhibition of the growth of the P. aeruginosa indicates that the different phytochemicals present in the different wild tea leaves have antibacterial properties against P. aeruginosa.
Furthermore, the zone of inhibition of the various wild tea leaves' extracts against *S. aureus* was also observed. *D. fruticosum* had the highest mean zone of inhibition with a value of 9.67. On the other hand, *G. pentaphylla* had the lowest zone of inhibition on *S. aureus* with a mean value of 6.85 mm.

Based on the analysis, the measured diameter of the different wild tea leaves' extracts was significantly lower than the positive control, however, the presence of an inhibition zone indicates the presence of the antibacterial substances from the different wild tea plants. This is shown by the significant differences with the negative control. This finding simply indicates that the various treatments used had different effects in terms of the zone of inhibition of *S. aureus*. The result shows that the selected wild tea leaves have antibacterial properties against *S. aureus*.

Lastly, Table 3 also presents the zone of inhibition of the various wild tea leaves' extracts along *B. subtilis*. *C. sanki* had the highest mean zone of inhibition with a value of 9.83 mm. On the other hand, it was revealed that *G. leucocarpa var. cumingiana* had the lowest zone of inhibition of 7.97 mm against *B. subtilis*. The result shows that the selected wild tea leaves have antibacterial properties against *S. aureus*.

| Treatment                                                | Mean (Mm) |
|----------------------------------------------------------|-----------|
| Negative Control (Distilled water)                       | 0.00      |
| *C. mercadoi* (Kumayo)                                   | 10.47     |
| *G. leucocarpa var. cumingiana* (Tayugtog)               | 9.95      |
| *C. sanki* (Gutmo)                                       | 11.47     |
| *D. fruticosum* (Mountain Tea)                           | 11.38     |
| *G. pentaphylla* (Itsa)                                  | 11.35     |
| Positive Control (Azithromycin)                          | 16.86     |

Means of the same letter are not significantly different using LSD at 5%.

Furthermore, the zone of inhibition of the various wild tea leaves' extracts against *S. aureus* was also observed. *D. fruticosum* had the highest mean zone of inhibition with a value of 9.67. On the other hand, *G. pentaphylla* had the lowest zone of inhibition on *S. aureus* with a mean value of 6.85 mm.

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Results of the antibacterial tests revealed that all of the selected wild tea plants have potential antibacterial properties against the test organisms. It is shown from the results that there is no significant difference in the inhibition zones of the different wild tea extracts against *E. coli*. However, for the *P. aeruginosa*, *S. aureus* and *B. subtilis*, the inhibition zones significantly differed among the different wild tea leaves' extracts.

### Potential of the Selected Wild Tea Leaves’ Extracts in the Prevention of Quorum Sensing Activity

The capability of the selected wild tea leaves’ extracts with antiquorum sensing property against the bacterial strains was investigated through Crystal Violet-based Microtitre Plate Assay. The OD values indicate the measure of biofilm formation of microorganisms. Table 4 presents the antiquorum sensing property of selected wild tea leaves’ extracts against different bacterial strains. Specifically, statistical analysis revealed that there were no significant differences in the computed OD values of the different wild tea leaves’ extracts against *E. coli*. The computed OD values are equivalent to moderate biofilm formation of *E. coli*.

Table 4 also presents the antiquorum sensing property of selected wild tea leaves’ extracts against *P. aeruginosa*. The finding suggests that the selected wild tea plants with significantly different OD values have varied effects on the biofilm formation of *P. aeruginosa*. However, *P. aeruginosa* formed a moderate biofilm formation regardless of the OD values of the
different extracts. Furthermore, in terms of the antiquorum sensing property of the selected wild tea leaves’ extracts against \textit{S. aureus}. The analysis revealed that the antiquorum sensing property of the selected wild tea leaves’ extracts against \textit{S. aureus} is significantly different from each other. The OD values may significantly differ, however, all selected wild tea samples moderately inhibited biofilm formation of \textit{S. aureus}.

Lastly, as shown in Table 4, it was detected that \textit{D. fruticosum} had a high quorum sensing inhibitory effect as indicated by the lowest mean OD value of 0.15 against \textit{B. subtilis}. The statistical analysis revealed that \textit{D. fruticosum} had significantly higher OD value as compared to the other wild tea plants. The wild tea leaves’ extracts may have OD values which are significantly different from each other but all of the wild tea plants showed a moderate effect on the biofilm formation of \textit{B. subtilis}. The result, suggests that the selected wild tea leave’s extracts possess moderate antiquorum sensing property that inhibits the biofilm formation of \textit{B. subtilis}. This further shows that the different wild tea leaves extracts have different levels of antiquorum sensing property with regard to \textit{B. subtilis}.

### DISCUSSION

The DPPH activity of the different wild tea leaves’ extracts may be attributed to the phenolics and flavonoids that were detected and measured during the study. The findings are in line with previous findings of Kumari & Kumar\textsuperscript{14} which showed the antioxidant activities of herbal teas. Several studies have been performed to determine the property of plant extracts to scavenge the free radicals.\textsuperscript{15} Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide and thus inhibit the oxidative mechanisms that lead to degenerative diseases.\textsuperscript{16} The presence of phytochemicals indicates a main role in the antioxidant activity.\textsuperscript{17}

The antibacterial property of the different wild tea leaves’ extracts against \textit{E. coli} coincides with the finding of Tarnam\textsuperscript{18} wherein \textit{Clausena} sp. showed a maximum zone of inhibition against \textit{E. coli}. \textit{G. pentaphylla} leaves and stems portrayed the suppression of the growth of \textit{E. coli} which is one of the microbes causing the plaque formation. Bin et al.\textsuperscript{19} observed antibacterial activity and minimum inhibitory concentration of \textit{Cinnamomum} spp. stick extract exhibited significant antibacterial property against \textit{E. coli}.\textsuperscript{20} In addition, \textit{G. leucocarpa} sp. also revealed inhibitory effects against \textit{E. coli} using different extracting solutions.\textsuperscript{21}

The result of the present study on the potential antibacterial property of \textit{C. sanki} against \textit{P. aeruginosa} is supported by the study of Agyepong et al.\textsuperscript{22} whereby they concluded that \textit{C. sanki} demonstrated antibacterial property against the bacteria, \textit{P. aeruginosa} through agar-well diffusion method and microdilution methods. On the other hand, the result of the \textit{D. fruticosum} (Myrtaceae) having an antibacterial property corroborates numerous studies on the antibacterial activity of some species under the family Myrtaceae (Auricchio et al.),\textsuperscript{23} against \textit{P. aeruginosa}. Howlader et al.\textsuperscript{24} also supports the result of the present study whereby an inhibition of the growth of \textit{P. aeruginosa} was observed which indicates the presence of antibacterial agents in \textit{G. pentaphylla}.

#### Table 4. The antiquorum sensing property of the selected wild tea leaves’ extracts in the prevention of \textit{E. coli}, \textit{P. aeruginosa}, \textit{S. aureus}, \textit{B. subtilis} to form a biofilm

| Treatment                        | \textit{E. coli} | \textit{P. aeruginosa} | \textit{S. aureus} | \textit{B. subtilis} | Biofilm Formation |
|----------------------------------|------------------|------------------------|-------------------|---------------------|------------------|
| \textit{C. mercadoi} (Kumayo)    | 0.13\textsuperscript{a} | 0.16\textsuperscript{ab} | 0.15\textsuperscript{h} | 0.18\textsuperscript{h} | M                |
| \textit{G. leucocarpa} var. cumingiana (Tayugtog) | 0.14\textsuperscript{a} | 0.16\textsuperscript{ab} | 0.17\textsuperscript{a} | 0.16\textsuperscript{b} | M                |
| \textit{C. sanki} (Gutmo)       | 0.15\textsuperscript{a} | 0.17\textsuperscript{a}  | 0.16\textsuperscript{c} | 0.16\textsuperscript{b} | M                |
| \textit{D. fruticosum} (Mountain Tea) | 0.14\textsuperscript{a} | 0.15\textsuperscript{a}  | 0.11\textsuperscript{a} | 0.15\textsuperscript{a} | M                |
| \textit{G. pentaphylla} (Itsa)  | 0.15\textsuperscript{a} | 0.15\textsuperscript{a}  | 0.16\textsuperscript{c} | 0.18\textsuperscript{h} | M                |

Means of the same letter are not significantly different using DMRT at 5%.
Legend: H= high biofilm formation; M= moderate biofilm formation; N/W= none/weak biofilm formation
In the antibacterial screening of *C. sanki*, the present result is supported by the study of Fakruddin et al., wherein another species, *Claussenia heptaphylla*, demonstrated a moderate inhibition zone (6.5-9.00mm) against *S. aureus*. In addition, the 95% ethanol extract of the stems or roots of *G. leucocarpa* significantly inhibited *S. aureus* in the report of Liu et al., which also suggested that phenols, flavonoids and terpenoids from *G. leucocarpa* var. yunnanensis have antibacterial activity. Moreover, Murugan and Natarajan also investigated the antibacterial activity of *G. pentaphylla* and their study suggests that *G. pentaphylla* plant extract has antibacterial activity against *S. aureus*.

It was also studied that plants consumed by primates, one of them was *D. fruticosum* had a potent bactericidal property against *B. subtilis*. Also, Fakruddin et al. observed in the antibacterial screening of *Claussenia heptaphylla*, a moderate zone of inhibition (6.5-9.0 mm in diameter) against gram-positive *B. subtilis* ATCC 11774. Fuentes et al. also concluded that the bark extract of *C. mercadoi* Vidal had the ability to inhibit the bacterial activity of *B. subtilis*. Furthermore, *G. pentaphylla* also provided significant antibacterial property, producing a varied range of inhibition zone where the average diameter is 8-22mm. Additionally, an antibacterial study of another species of Gaultheria by Pandey et al. reported that this plant is a rich source of phenolic and flavonoid compounds and showed good antibacterial activity against *B. subtilis*. Overall, the various wild tea plants have potential antibacterial against Gram-positive and Gram-negative bacteria, due to the presence of the bioactive compounds that are responsible for their inhibitory effect. Green tea extracts showed the highest antibacterial activity against the test microorganisms with the lowest MIC values, followed by oolong tea extracts, Fuzhuan tea extracts, and black tea extracts. The green tea catechins also manifest wide-range of antibacterial activity through different mechanisms. Specifically, this antibacterial property is due to the presence of phenolic compounds and flavonoids which are hydroxylated phenolic substances known to be produced by plants as a protective mechanism against microbial infection, and they have been determined to have antimicrobial substances against a broad array of microorganisms in vitro.

In this present study, all of the wild tea plants have the potential antiquorum sensing property through the prevention of the formation of biofilm of the different bacterial species. Natural compounds present in the plant extracts may be responsible for the various antiquorum sensing and antibiofilm activities. In another study by Alam et al., it was reported that the secondary metabolites demonstrated significant antibiofilm activity of plant derived extracts. Numerous secondary metabolites from medicinal plants have anti-QS effects have been isolated such as cinnamaldehyde, flavanones, and flavonoids have been studied and demonstrated promising anti-QS activities specifically, inhibition of HAI- and AI-2-mediated bioluminescence, restrict with QS detection and influence. Olawuwo et al. observed a comparable observation in their determination of antibiotic formation activity of the medicinal leaf extracts against bacterial biofilms.

**CONCLUSION**

All selected wild tea plants have potential antioxidant property but *G. leucocarpa* var. *cumingiana* had the highest radical scavenging activity percentage, hence, the best antioxidant among the wild tea plant samples. The selected wild tea plant extracts showed antibacterial potential against Gram-positive (*E. coli* and *P. aeruginosa*) and Gram-negative bacteria (*S. aureus* and *B. subtilis*). The ethanolic extracts of the selected wild tea plants have potential antiquorum sensing property by inhibiting the biofilm formation of *E. coli* and *P. aeruginosa* and Gram-negative bacteria (*S. aureus* and *B. subtilis*). The result of the study is of help to the scientific community that would provide knowledge and a basis for more intensive research about the selected wild tea leaves.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

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DATA AVAILABILITY
All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT
This article does not contain any studies on human participants or animals performed by any of the authors.

REFERENCES

1. Mukhopadyay M, Bantawa P, Das A, et al. Changes of growth, photosynthesis and alteration of leaf antioxidative defense system of tea (Camellia sinensis (L.) O. Kuntze) seedling under aluminum stress. BioMetals. 2012;25:1141-1154. doi: 10.1007/s10534-012-9576-0
2. Statista Research Department. Frequency of Drinking Functional Beverages Philippines 2019, by age group. 2019. www.statista.com.
3. Patel SH. Camellia sinensis: Historical Perspectives and Future Prospects. J Agromedicine 2005;10(2):57-64. doi: 10.1300/J096v10n02_08
4. Guerriero G, Berni R, Munoz-Sanchez JA, et al. Production of Plant Secondary Metabolites: Examples, Tips and Suggestions for Biotechnologists. Genes (Basel). 2018;9(6):309. doi: 10.3390/genes9060309
5. Ozkan G, Sagdic O, Ozcan M, Ozcelik H, Unver A. Antioxidant and Antibacterial Activities of Turkish Endemic Sideritis Extracts. Grasas Aceites. 2005;56(1):16-20. doi: 10.3989/gya.2005.v56.i.129
6. Chen D, Daniel K, Kuhn D, Kazi A, Bhuiyan M, Li L, Dou Q. 2004. Green tea and tea polyphenols in cancer prevention. Frontiers in Bioscience. 9(1-3): 2618-2631.
7. Konan NA, Bacchi EM, Lincopan N, Varela SD, Varanda EA. Acute, Sub-Acute Toxicity and Genotoxic Effect of Hydroethanolic Extract of the Cashew (Anacardium occidentale L.). J Ethnopharmacol. 2007;110(1):30-38. doi: 10.1016/j.jep.2006.08.033
8. Jovanova B, Kulevanova S, Panovska TK. Cytotoxic Potential of Selected Commercial Herbal Teas According to the Brine Shrimp Lethality Assay. International Bioscience Conference. 2016;T4-P-BBS51. 248-249. https://www.researchgate.net/publication/308938635
9. Clarke G, Ting KN, Wiart C, Fry J. High Correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging, Ferric Reducing Activity Potential and Total Phenolic Content Indicates Redundancy in Use of All Assays to Screen for Antioxidant Activity of Extracts of Plants from Malaysian Rainforest. Antioxidants. 2013;2(1):1-10. doi: 10.3390/antiox2010001
10. Nimmakayala S, Duggirala SL, Puchakayala G. Antimicrobial Activity of Ethanolic Extracts of Justicia neesi. Bangladesh J Pharmacol. 2011;4(4):624-627. doi: 10.3329/bjp.v4i4.20571
11. Stepanovic S, Vukovic D, Dakic I, Savic B, Svbatic-Vlahovic M. A Modified Microtitter Plate Test for Quantification of Staphylococcal Biofilm Formation. J Microbiol Methods. 2000;40(2):175-179. doi: 10.1016/S0167-7012(00)00122-6
12. Jadid N, Hidayati D, Hartanti SR, Arranny BA, Rachman RY, Wikanta W. Antioxidant Activities of Different Solvent Extracts of Piper retrofractum Vahl. using DPPH Assay. Am Int Proceedings. 2017;1854:020019. doi: 10.1063/1.4985410
13. Wintola OA, Afolayan AJ. The Antibacterial, Phytochemicals and Antioxidants Evaluation of the Root Extracts of Hydrona Africana Thum. Used as Antidysenteric in Eastern Cape Province, South Africa. BMC Complement Altern Med. 2015;15:307. doi: 10.1186/s12906-015-0835-9
14. Kumar A, Kumar D. Evaluation of Antioxidant and Cytotoxic Activity of Herbal Teas From Western Himalayan Region: A Comparison with Green Tea (Camellia sinensis) and Black Tea. Chemical and Biological Technologies in Agriculture. 2022;9:33. doi: 10.1186/s40538-022-00294-3
15. Pavithra K, Vadivukkarasi S. Evaluation of Free Radical Scavenging Activity of Various Extracts of Leaves from Redrostris foetidissima (Jacq.) Cogn. Food Sci Hum Wellness. 2015;4(1):42-46. doi: 10.1016/j.fshw.2015.02.001
16. Wu YY, Li W, Xu Y, Jin EH, Tu YY. Evaluation of the Antioxidant Effects of Four Main Theaflavin Derivatives Through Chemiluminescence and DNA Damage Analyses. J Zhejiang Univ Sci B. 2011;12(9):744-751. doi: 10.1631/jzus.B1100041
17. Maslov O, Kolinsky A, Komisarenko M, Golik M. Study of Total Antioxidant Activity Green tea Leaves (Camellia sinensis L.). Herba Polonica. 2002;68(1):1-9. doi: 10.2478/hepo-2002-0003
18. Tarnam A. Antibacterial activity of silver nanoparticles synthesized from Clausena anisate (Willd.) Hook F. Ex Benth (Rutaceae). Innovare J Ayurvedic Sci. 2016;4(3). https://innovareacademics.in/journals/index.php/ijas/article/view/12802
19. Bin S, C Yi-Zhong, JD Brooks, Corke H. Antibacterial Properties and Major Bioactive Components of Cinnamon Stick (Cinnamomum burmanni): Activity Against Foodborne Pathogenic Bacteria. J Agric Food Chem. 2007;55(14):5484-5490. doi: 10.1021/jf070424d
20. Abdolah R, Milanda T, Sugijanto M, et al. Antibacterial Properties of Selected Plants Consumed by Primates Against Escherichia coli and Bacillus subtilis. Asian J Trop Med Public Health. 2017;48(1):109-116. PMID: 29644827
21. Ma XJ, Zhao L, Du CF, Gong YJ, Zheng JH, Chen XZ. Screening of Antibacterial Activity of Extracts of Gautheria leucocarpa var. yunnanensis. Chin Med J. 2001;26(4):223-226. PMID: 12525043
22. Agyepong N, Agyare C, Adarkwa-Yiadom M, Gbedema S. Phytochemical Investigation and Anti-Microbial Activity of Clausena anisata (wild) Hook. Afr J Tradit Complement Altern Med. 2014;11(3):200-209. doi: 10.4314/atcam.v11i3.28
23. Auricchio MT, Bugno A, Barros SB, Bacchi EM. Antimicrobial and Antioxidant Activities and Toxicity of Eusenia uniflora. Lat Am J Pharm. 2007;26(1):76-81.
24. Howlader MD, Rizwan AF, Sultana S, et al. Antimicrobial, Antioxidant and Cytotoxic Effects of Methanolic Extracts of Leaves and Stems of Glycosmis pentaphylla (Retz.). J Appl Pharm Sci. 2011;1(8):137-140.
25. Fakruddin M, Mannan KSB, Mazumdar RM, Afroz H. Antibacterial, Antifungal and Antioxidant Activities of the Ethanolic Extract of the Stem Bark of Clausena heptaphylla. BMC Complement Altern Med. 2012;12:232. doi: 10.1186/1472-6882-12-232
26. Liu, S, Zhang Q, Li H, Qiu Z, Yu Y. Comparative Assessment of the Antibacterial Efficacies and Mechanisms of Different Tea Extracts. Foods. 2022;11(4):620. doi: 10.3390/foods11040620
27. Murugan N, Natarajan D. Phytochemical, Antioxidant and Antibacterial Activities of Glycosmis pentaphylla (Rutaceae) Leaf Extracts Against Selected Multi-drug Resistant Bacteria. J Chem Pharm Res. 2016;8(1):737-744.
28. Fuentes R, Díly F, Tan I, Balanquít BJ. Antioxidant and Antibacterial Properties of Crude Methanolic Extracts of Cinnamomum mercadoi Vidal. Philipp J Sci. 2010;15:9-15.
29. Bulbul IJ, Jahan N. Study on Antioxidant and Antimicrobial Activities of Methanolic Leaf Extract of Glycosmis pentaphylla against Various Microbial Strains. Journal of Pharmacy and Biochemistry. 2016;5(4):53-57.
30. Pandey BP, Thapa A, Upreti A. Chemical composition, antioxidant and antibacterial activities of essential oil and methanol extract of Artemisia vulgaris and Gaultheria fragrantissima collected from Nepal. Asian Pac J Trop Med. 2017;10(9):952-959. doi: 10.1016/j.aptm.2017.09.005
31. Siriphap A, Kiddee A, Duangjai A, et al. Antimicrobial Activity of the Green Tea Polyphenol (−)-Epigallocatechin-3-Gallate (EGCG) against Clinical Isolates of Multidrug-Resistant Vibrio cholerae. Antibiotics. 2022;11(4):518. doi: 10.3390/antibiotics11040518
32. Kumar AR, Rathinam KM, Prabakar G. Antibacterial Activity of Selected Plants of the Family Caesalpinioideae. J Ecolobiol. 2007;20(4):351-354.
33. Alam K, Al Farraj D, Mah-e-Fatima S, et al. Anti-biofilm Activity of Plant Derived Extracts Against Infectious Patogen- Pseudomonas aeruginosa PAO1. J Infect Public Health. 2022;13(11):1734-1741. doi: 10.1016/j.jiph.2020.07.007
34. Truchado P, Gimenez-Bastida JA, Larrosa M, et al. An Inhibition of Quorum Sensing (QS) in Yersinia enterocolitica by Orange Extract Rich in Glycosylated Flavanones. J Agric Food Chem. 2012;60(36):8885-8894. doi: 10.1021/jf301365a
35. Olawuwo OS, Famuyide IM, McGaw LJ. Antibacterial and Antifilm Activity of Selected Medicinal Plant Leaf Extracts Against Pathogens Implicated in Poultry Diseases. Front Vet Sci. 2022;9:20304. doi: 10.3389/fvets.2022.820304