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RESEARCH ARTICLE OPEN ACCESS

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

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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

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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 activity[5] 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 effects[7] 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) locally named "tayugtog" in Barlig; the *Clausena sanki* (Perr.) J.F. Molino var. *mollis* (Merr.) J.F Molino as "gutmo" thriving in boundaries of Bauko; *Descaspermum fruticosum* (J.R. Forst and G. Forst) from Bontoc that locals call mountain tea; and *Glycomis pentaphylla* growing in Sagada with a local name of "itsa". Local folks gather these wild tea leaves and consume them as a beverage. The natives claimed that the use of the wild tea is to ease diarrhea or any stomach pain and prevent coughs and colds.

So far, there has been little discussion about the probable medicinal properties of the target plants used as tea in this study. Hence, this can be a ground for pursuing a study on the potential properties such as antioxidant and medicinal properties, consequently, promoting the consumption of naturally grown tea and relating results with their known ethnobotanical importance. Specifically, this study was conducted to determine the antioxidant and potential antibacterial and antiquorum sensing properties of ethanolic extracts of the wild tea leaves; *C. mercadoi* S. Vidal, *G. 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.[9] with few modifications. The DPPH radical scavenging activity was calculated using the formula:

$$\% \text{ Radical Scavenging Activity} = \frac{^{A}\text{control} - {}^{A}\text{extract}}{^{A}\text{control}} X100\%$$

Agar-well Diffusion Method

The antibacterial properties of the different wild tea were evaluated according to the procedures of Nimmakayala et al.[10] 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.

Quantitative assessment of biofilm formation. The amount of biofilm mass was measured by adding 100 µL of 95% ethanol to solubilize the absorbed stain. The solutions were transferred in cuvettes and 900 µL was added to make a 1 mL solution and were mixed by pipetting up and down. The optical density (OD) was obtained at a wavelength of 570 nm using a visible spectrophotometer. The criteria used in the interpretation of biofilm production shown in Table 1 were based on the criteria of Stepanovic et al.[11] which describes a classification of bacterial formation.

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

Table 2. The potential of the selected wild tea leaves' extracts plants as antioxidants

Treatments	Radical Scavenging Activity (%)	F_{VAL}	P_{VAL}
Factor A (Wild Tea Plants)			
Ascorbic Acid	73.45[c]	50159.810**	0.000
C. mercadoi (Kumayo)	87.39[b]		
G. leucocarpa var. *cumingiana* (Tayugtog)	88.67[a]		
C. sanki (Gutmo)	68.21[e]		
D. fruticosum (Mountain Tea)	73.41[d]		
G. pentaphylla (Itsa)	64.56[f]		
Factor B (Concentration)			
500 ug/mL	88.46[a]	159966.448**	0.000
250 ug/mL	85.23[b]		
125 ug/mL	73.74[c]		
62.50 ug/mL	53.35[d]		
Coefficient of Variation (%)			0.29%

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

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*.

Table 3. Mean zone of inhibition of the selected wild tea leaves' extracts against *E. coli, P. aeruginosa, S. aureus* and *B. subtilis*

Treatment	Mean (Mm)			
	E.coli	*P.aeruginosa*	*S.aureus*	*B.subtilis*
Negative Control (Distilled water)	0.00^{b}	0.00^{e}	0.00^{g}	0.00^{e}
C. mercadoi (Kumayo)	10.47^{b}	9.54^{c}	7.49^{e}	9.49^{c}
G. leucocarpa var. *cumingiana* (Tayugtog)	9.95^{b}	8.87^{d}	8.46^{d}	7.97^{d}
C. sanki (Gutmo)	11.47^{b}	11.77^{b}	9.24^{c}	9.83^{b}
D. fruticosum (Mountain Tea)	11.38^{b}	11.42^{b}	9.67^{b}	9.78^{b}
G. pentaphylla (Itsa)	11.35^{b}	9.78^{c}	6.85^{f}	9.68^{b}
Positive Control (Azithromycin)	16.86^{a}	16.88^{a}	16.59^{a}	16.62^{a}

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.

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*. Statistical analysis of the different wild tea leaves' extracts showed that the selected wild tea plants significantly affected the growth of *B. subtilis*. *C. sanki*, *D. fruticosum* and *G. pentaphylla* were not significantly different from each other. These were the most effective wild tea plants in terms of inhibiting the growth of *B. subtilis*. This finding also suggests that *C. mercadoi* and *G. leucocarpa* var. *cumingiana* had the least effect in terms of zone of inhibition on *B. subtilis*. This simply implies that all the wild tea leaves' extracts had a significant effect in inhibiting the *B. subtilis* in terms of zone of inhibition.

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. aeroginosa*. 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

Table 4. The antiquorum sensing property of the selected wild tea leaves' extracts in the prevention of *E. coli, P.aeruginosa, S.aureus, B.subtilis* to form a biofilm

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

different extracts. Furthermore, in terms of the antiquorum sensing property of the selected wild tea leaves' extracts against *S. aureus*. The analysis revealed that the antiquorum sensing property of the selected wild tea leaves' extracts against *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 *S. aureus*.

Lastly, as shown in Table 4, it was detected that *D. fruticosum* had a high quorum sensing inhibitory effect as indicated by the lowest mean OD value of 0.15 against *B. subtilis*. The statistical analysis revealed that *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 *B. subtilis*. The result, suggests that the selected wild tea leave's extracts possess moderate antiquorum sensing property that inhibits the biofilm formation of *B. subtilis*. This further shows that the different wild tea leaves extracts have different levels of antiquorum sensing property with regard to *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[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.[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.[16] The presence of phytochemicals indicates a main role in the antioxidant activity.[17]

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

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

In the antibacterial screening of *C. sanki*, the present result is supported by the study of Fakruddin et al.[25] wherein another species, *Clausena 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.[26] which also suggested that phenols, flavonoids and terpenoids from *G. leucocarpa* var. yunnanensis have antibacterial activity. Moreover, Murugan and Natarajan[27] 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*.[20] Also, Fakruddin et al.[25] observed in the antibacterial screening of *Clausena heptaphylla*, a moderate zone of inhibition (6.5-9.0 mm in diameter) against gram-positive *B. subtilis* ATCC 11774. Fuentes et al.[28] 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.[29] Additionally, an antibacterial study of another species of Gaultheria by Pandey et al.[30] 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.[31] 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.[32]

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.[33], 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-medicated bioluminescence, restrict with QS detection and influence.[34] Olawuwo et al.[35] observed a comparable observation in their determination of antibiofilm 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.

FUNDING

None.

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.

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