Antibacterial Activity Study of Liubao Chongcha Extract

Juxian Wu
School of Industrial Technology, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia, Guangdong Vocational College of Science and Trade, No. 36 Zhongsu Road, Qingyuan, Guangdong Province, China.

Joo Shun Tan*
School of Industrial Technology, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia.
Email: jooshun@usm.my

Ruyi Wang
Guangdong Food and Drug Vocational College, China

Huiqing Liu
Guangdong Vocational College of Science and Trade, No. 36 Zhongsu Road, Qingyuan, Guangdong Province, China

Jieqiong Yan
Guangdong Medical University, China

Jiale Tan
Guangdong Medical University, China

Chongchai is a popular beverage in China, enjoyed by people all over the country. Recent studies indicate that Chongcha has a refreshing impact on the body and offers numerous advantages for one's well-being. This study sought to conduct a preliminary investigation into the antibacterial properties of Liubao Chongcha and Liubao tea aqueous extract. The goal was to assess their effectiveness against common pathogenic bacteria by determining the minimum bactericidal concentration and the minimum inhibitory concentration**.** The test microorganisms utilised in the study included Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa. The Kirby-Bauer disc diffusion method was used to confirm the inhibitory effect of water extracts from Chongcha and Liubao black tea on the studied strains. The Mueller-Hinton broth dilution method was used to determine the minimum inhibitory concentration and minimum bactericidal concentration of the Liubao Chongcha and Liubao tea aqueous extracts against S. aureus. The aqueous extracts of Liubao Chongcha and Liubao tea demonstrated significant inhibitory effects against S. aureus, moderate inhibitory effects against E. coli and P. aeruginosa, and minimal inhibitory effects against B. subtilis. The Chongcha aqueous extract exhibited a minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of 0.625 mg/ml against S. aureus. Similarly, Liubao black tea water extract had an MBC and MIC of 1.25 mg/ml. The water extracts from Chongcha and Liubao black tea exhibited potent antibacterial activity against Gram-positive bacteria, while showing limited or no inhibitory effects on Gram-negative bacteria.

Keywords: Antibacterial Activity, Chongcha, Extract, Liubao, Minimum Bactericidal Concentration, Minimum Inhibitory Concentration.

Introduction

Contagious diseases pose a significant threat to both human and animal health, leading to illness and death. The discovery of antibiotics is considered a powerful tool in supporting medical treatment for bacterial infections and their associated risks and complications. Unfortunately, the extensive misuse of antibiotics contributes to the dissemination of antibiotic-resistant bacteria among the population. Therefore, it has a significant impact on the global community (Zhang et al., 2020).

Antibiotics have been shown to effectively treat bacterial diseases when accurately diagnosed by medical practitioners. Previous studies have examined the management of infectious diseases. Nevertheless, the use of antibiotics frequently leads to the emergence of resistant strains, which poses a threat to their effectiveness. Antimicrobial resistance occurs when bacteria or fungi develop the ability to reduce the effectiveness of drugs designed to kill them. The treatment of resistant infections is often challenging and can sometimes become impossible. The literature emphasises the need for alternative strategies to combat antibiotic resistance, as self-medication practices have contributed to its increasing rate. Scholars are actively searching for alternative approaches to address this issue.

The endangerment of human, environmental, and animal health makes it a global concern. The Food & Agriculture Organisation of the United Nations (FAO) states that antimicrobial resistance is a significant one health concern, resulting from the inappropriate use of antimicrobials in both healthcare and animal production. Exploring fractions with potential antibacterial activity from insects may serve as an alternative to overcome antibacterial resistance (Xu et al., 2013).

Tea is a widely consumed beverage known for its physiological effects on obesity, hypoglycemia, atherosclerosis, carcinogenicity, and antibacterial and antioxidant activities. Globally, more than 300 distinct commercial teas are consumed due to their widespread popularity. Teas are classified into different categories based on their manufacturing process, including non-fermented, semi-fermented, fully-fermented, and post-fermented. Catechins are recognised as the primary active components responsible for the biological activities of tea. The presence of various forms and derivatives in the drink enables it to exhibit diverse biological activities. Tea leaves undergo either steaming or panning processes, which prevent the oxidation of catechin. This preservation of polyphenols, such as epicatechol and epigallocatechin, occurs especially in green tea. In contrast, black tea undergoes grinding, wilting, and full-level fermentation. Enzymatic oxidation allows for the transformation of catechin derivatives into theaflavins and thearubigins.

Humans utilise insects and their products for various purposes. Insect-related products are utilised in the diets of humans, farm animals, and occasionally other insects. These products are also used in various forms to treat disease development, particularly in traditional medicine. China has accumulated extensive knowledge on tea cultivation, manufacturing, and consumption over a long period of time. China has developed a distinct tea culture.

The culture of the Tang Dynasty quickly evolved and subsequently deeply influenced Chinese society. The origin of tea is incredibly varied, influenced by a wide range of cultural, ecological, and ethnic factors. It is worth noting that there is an intriguing variation in ancient literature when it comes to tea. In the "Odes," tea is described as a sweet-tasting plant, whereas in the Eyra, it is referred to as bitter-tasting. When making tea beverages in different Chinese regions, a variety of herbs are taken into consideration. These herbs include vine, hawk, and bitter tea, among others. These types of herbs, also known as non-camellia varieties, are a unique sub-culture within the world of tea. One popular tea variety in Chinese culture is insect tea, known for its natural contribution to the vibrant tea tradition.

Insect tea is a traditional drink enjoyed by the ethnic minority communities in the southwest region of China. This product has a rich history and is well-known in China. According to Li Shizhen, this particular tea is made from the waste of insects that feed on plants. Surprisingly, it has been found to have medicinal properties and can be used to treat various ailments, such as ear infections. From a scholarly standpoint, insect tea is not your typical beverage due to its origin from insect waste. It stands out due to its consumption method and its vibrant colour. Steeping a small amount of tea leaves can yield a cup of tea that is free from any residue. The resulting yellow-red mixture boasts superior and more charming flavours compared to other types of teas. The term "boutique tea" is often used as another name for it. Researchers conducted a study, collecting data from individuals who have consumed insect tea, and found that this beverage provides various health benefits. The benefits of these include thermoregulation, humidity reduction, detoxification, gastrointestinal protection, and digestive support. QI, a specialised terminology in traditional Chinese medicine, highlights the significance of vital energy in alleviating surface-level symptoms. This approach offers advantages in the field. Studies have demonstrated the benefits of this tea for individuals working in hot environments, especially in tropical regions. Insect tea, a traditional export of China, is renowned for its exceptional qualities. The substance possesses a minimal dosage, notable transparency, and a potent fragrance. Insect tea is highly esteemed in Southeast Asia, particularly in countries such as Nansha, Singapore, and Malaysia, where it is referred to as a tea treasure.

Chongcha, a traditional Chinese medicinal tea, was initially recorded by Li Shizhen in his "Compendium of Materia Medica"(Elman, 2015). The tea was reported to be infested with insects, and the excrement of these insects was used medicinally. The excrement and remains were utilised for the treatment of ear discharge (Xu et al., 2013). Chongcha contains a variety of active constituents, including amino acids, polyphenols, and flavonoids. The production of this product is concentrated in the border regions of Hunan, Guangxi, Guizhou, Guangdong, and certain areas along the upper Yangtze River in Sichuan (Ya-Feng & Li-Zhang, 2019). The traditional beverage is popular among ethnic minorities such as the Miao, Yi, Tujia, and Gelao peoples. Chongcha is often associated with various effects in folk tradition, including heat clearance, detoxification, anti-inflammatory properties, enhancement of spleen and stomach function, digestion aid, diuresis, and reduction of phlegm (Wang et al., 2023). Underserved areas with limited access to medical facilities use Chongcha as a traditional medicine. People commonly use it to treat a variety of ailments, including wounds, abscesses, and paediatric onvulsions (Yuan et al., 2021) The local gazetteer "Chengbu Culture and History" (1989) suggests that consuming Chongcha offers a refreshing taste and various medicinal benefits. These include thirst-quenching, mental refreshment, blood pressure reduction, digestion promotion, treatment of stomach diseases, diuresis, qi regulation, phlegm reduction, detoxification, and swelling reduction (Gao et al., 2018).

Due to its origins in ethnic minority regions in China, there has been limited international research conducted on Chongcha. Chongcha in China has a rich history that can be traced back to 992 AD. Prior to Li Shizhen's time, there were historical accounts of utilising Chongcha to address specific ailments (Elman, 2015). The usage of Chongcha has been documented for more than a thousand years. Research on the medicinal value and food safety of Chongcha has been on the rise in China since the 1990s (Fu et al., 2018b). Liubao tea is a type of dark tea made from the fresh leaves of specific tea tree varieties and cultivars in Cangwu County, Guangxi (Feng et al., 2023). It undergoes specific techniques to attain its distinct quality characteristics (Zhu et al., 2020).

Various types of tea, such as green, black, yellow, oolong, dark, and white, are available to individuals for further discussion. Liubao tea is a traditional example of dark tea, similar to popular teas like Pureh and Fuzhuan. Liubao tea, a renowned cultural product of China, is produced in China. In 2021, the production of Liubao tea in Wuzhou reached 25,000 tonnes, generating an output value of 11 billion RMB. The Liubao tea produced in Wuzhou has been recognised as one of the most prominent brands in China in 2021. The tea is made from Camellia sinensis and brewed with a water content of 15 to 18% at temperatures ranging from 40 to 55 °C for a period of 20 to 30 days. The distinct flavour of tea is formed through fermentation, which occurs when moisture and heat interact in the presence of microorganisms like mould and staph. The fermentative process decreases the levels of polymeric polyphenols, total phenolic compounds, and flavonoids, while increasing the levels of oxidised polyphenols and saccharides. The alteration of a component in Liubao tea results in the development of a unique and distinctive colour, along with a subtle flavour that also imparts a wood-like taste. The outer wall of tea undergoes degradation by microorganisms during fermentation.

Liubao tea is primarily made using Wuzhou tea leaves from China. The tea production process involves natural fermentation, pile fermentation, parching, sterilising, and maturing. Thus, Wang et al. (2023) identified a specific black tea that undergoes post-fermentation. The tea is named after its geographical location, Liubao, in Wuzhou city, Guangxi province, China. Tea has historically been used for preventive care. Pu'er and Fuzhuan tea, as well as

Hunan black tea, have been the primary focus of most studies. However, there is a lack of research specifically related to Liubao tea. Recent literature suggests that liubao tea has been found to have potential benefits in reducing cholesterol levels, regulating glucose and fat metabolism, promoting antioxidant activity, modulating immunity, and influencing the microbiota. The health benefits of liubao tea are attributed to its content of polyphenols, soluble carbohydrates, caffeine, flavonoids, and non-proteinogenic amino acids.

The Liubao insect tea, also known as ChongCha tea, is produced in Guangxi, China and is marketed as a medicinal or health tea. This tea is made from tea leaves that have been consumed and excreted by insects. The excreted droppings originate from various insect species, including Orthopygia glaucinalis, Hydrillodes, Simplicia niphona Butler, Tea Tabby, and Leech. The insects feed on Red Clover and other plants, which is important for tea production. The collected products were processed to produce insect tea. Insect tea is a mineral-rich beverage that contains 17 minerals and 10 trace elements, including potassium, calcium, sodium, iron, zinc, and magnesium. These minerals and trace elements are essential for the human body. Some argue that insect tea contains higher levels of iron, zinc, calcium, and magnesium compared to other types of tea. Insect tea contains protein, fibre, fat content, polyphenols, vitamins, and amino acids. The tea, known as a traditional Chinese beverage, has been documented to have detoxifying properties and the ability to flush toxins from the body in clinical therapy. Therefore, enhancing the digestion process to strengthen the stomach. Favourable outcomes have been observed when using this tea to treat diarrhoea, nosebleeds, rectal bleeding, and gum bleeding.

The manufacturing process of insect tea is unique as it involves providing insect larvae with primary leaves, and then processing the larval droppings to create the final drink. Furthermore, the type of insect and the species of material leaves are important factors that influence the quality and function of the insect. Several types of teas exist, such as Kuding tea, toringo tea, and Liubao tea, which is also known as camellia sinensis. Several studies have shown that polyphenols found in insect tea have gastric injury prevention properties and also possess potential benefits in preventing cancer, reducing blood sugar levels and blood pressure, and decreasing body fat. Tea has the potential to regulate the microenvironment and calcium levels of the digestive system due to its anti-viral and anti-bacterial properties. Therefore, it effectively modulates the body's immune response while reducing inflammation. Tea, as an old-fashioned remedy, has a traditional role in managing inflammation and avoiding the need for surgery. The implementation of geographical indication protection for "Liubao Tea" was approved by the former General Administration of Quality Supervision, Inspection and Quarantine of China on March 16, 2011 (Hao et al., 2013). Although different types of Chongcha have been investigated within the country, there is currently no documented research on the antibacterial properties of Liubao Chongcha. The current study utilised in vitro antibacterial tests, specifically the Kirby-Bauer (K-B) disc diffusion method and the Mueller-Hinton broth dilution method, to examine the inhibitory effects of the substance on intestinal microorganisms, namely S. aureus, E. coli, P. aeruginosa, and B. subtilis.

Materials and Method

The current study assessed the inhibitory effect of Liubao Chongcha extract on specific microorganisms including S. aureus, E.coli, P.aeruginosa, and B. subtilis. The test strains were obtained from Guangdong Food and Drug Vocational College in Guangdong Province, China. Liubao Chongcha, a type of Liubao tea, was purchased from Wuzhou Chuanxiang Tea Co., Ltd.

K-B Disk Diffusion Method

The Liubao Chongcha extract was prepared by adding 100 g of Liubao Chongcha to 2 L of boiling water. The mixture was steeped for 12 hours at room temperature, with multiple extractions. The extract underwent filtration, and the resulting filtrate was collected and concentrated to a thick consistency using a rotary evaporator. The concentrated extract was dried using a water bath, freeze-dried, and dissolved in purified water to create solutions of varying concentrations. The sterile paper discs were immersed in these solutions for 1.5 hours and then dried at 55 °C under sterile conditions (Fu et al., 2018a).

A bacterial suspension was prepared by adjusting the concentration of a selected bacterial strain to approximately 108~109 CFU/ml. Approximately 100 µl of bacterial suspension was transferred onto the plate containing casein hydrolysate agar medium for bacteria and evenly distributed. The antibiotic discs for sample and control were placed on the slightly dried inoculated plate. The negative control in this experiment was a water disc, while the positive control was a gentamicin antibiotic disc used to target bacteria. The plates were incubated at 37°C and 28°C for 18 hours after a 15-minute period.The diameter of the inhibition zones was measured using a vernier calliper, specifically employing the cross-sectional method. The experiment was conducted in triplicate (Fu et al., 2018a).

Mueller-Hinton (MH) Broth Dilution Method

The Mueller-Hinton (MH) broth dilution method was used to evaluate the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Chongchawater (Lei & Lu, 2001)·The sample solution was prepared by weighing 1 g of dry Chongcha extract and adjusting its concentration to 100 mg/ml with the addition of water. A 1ml aliquot of the sample solution was diluted in casein hydrolysate broth to achieve a concentration of 10 mg/ml.

A 96-well bacterial cell culture plate was used to add 100 µl of MH broth to the second to the tenth well. Approximately 100 µl of the sample solution was added to the first and second wells. The solution was mixed in the second well, and then 100 µl was transferred to the third well. The process was iterated until the tenth well, with 100 µl from the tenth well being excluded. This resulted in sample concentrations of 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, 0.039, and 0.0195 mg/ml in each well. Control groups were established

for both broth without bacterial solution and test bacterial growth without antibacterial agent.

The S.aureus bacterial suspension was diluted with 0.9% saline to a concentration of 108~109 CFU/ml. One millilitre of the suspension was transferred into a 100 mL volumetric flask and then filled to volume with casein hydrolysate broth. Each well was filled with 100 µl of bacterial suspension, resulting in final sample concentrations ranging from 5 to 0.00975 mg/ml. The 96-well bacterial cell culture plate was incubated at 37 ℃ for 24 hours to cultivate S. aureus. The minimum inhibitory concentration (MIC) was determined as the lowest concentration at which bacterial growth was not observed in the wells. The incubation period was extended by 12 hours in order to determine the minimum bactericidal concentration (MBC). The experiment was conducted three times (Lei et al., 2001).

Statistical Analysis

The results were expressed as mean ± standard deviation (x±s). Data analysis was conducted using SPSS 19.0 statistical software. Variance analysis, t-tests, and the LSD method were employed to analyse the experimental results. The significance level was set at α=0.05, with a p-value<0.05 indicating statistical significance.

Results

The antibacterial spectrum of Chongcha was assessed against common pathogens using the K-B disc diffusion method. The Liubao Chongcha and Liubao black tea extracts (aqueous) exhibited varying degrees of inhibitory effects against the tested strains. The diameter of the inhibition zone caused by Liubao Chongcha and Liubao black tea water extracts against S. aureus were 19.07 mm and 16.06 mm, respectively. Liubao Chongcha exhibited superior antibacterial activity compared to Liubo black tea, particularly against the Gram-positive bacterium S. aureus. Both teas showed weaker inhibition against Gram-negative bacteria, including P. aeruginosa and E. coli. No inhibition zone was observed against B. subtilis, suggesting that neither tea extract was sensitive to this bacterium. The antibacterial spectrum of Liubao Chong includes S. aureus, E. coli, and P. aeruginosa (Table 1&2).

Table 1: Antibacterial Activity of Liubao Chongchawater Extract.

Extract type	Chongcha	Gentamicin	Water
Concentration (g/100 ml)	5.00	10.00	15.00
	Inhibition zone diameter (mm)		
S.aureus	11.07±0.46	14.34±0.49	15.62±0.65
P.aeruginosa	6±0	7.11±0.04	7.83±0.56
E.coli	6±0	7±0.22	7.88±0.22
B.subtilis	6±0	6±0	6±0

Table 2: Antibacterial Activity of Liubao Black Tea Water Extract.

Extract type	Liubao black tea	Gentamicin	Water
Concentration (g/100 ml)	5.00	10.00	15.00
	Inhibition zone diameter (mm)		
S.aureus	9.77±0.96	12.5±0.39	14.22±0.68
P.aeruginosa	6±0	6±0	7.27±0.32
E.coli	7.74±0.06	7.78±0.45	8.1±0.14
B.subtilis	6±0	6±0	6±0

Table 3: Antibacterial Activity of Liubao Chongcha and Liubao Black Tea Against S.Aureus (MIC& MBC).

	Minimum inhibitory concentration									
Concentration (mg/ml)	**5**	**2.5**	**1.25**	**0.625**	**0.313**	**0.156**	**0.078**	**0.039**	**0.02**	**0.01**
Liubao Chongcha	-	-	-	-	+	+	+	+	+	+
Liubao black tea	-	-	-	+	+	+	+	+	+	+
	Minimum bactericidal concentration									
Liubao Chongcha	-	-	-	+	+	+	+	+	+	
Liubao black tea	-	-	+	+	+	+	+	+	+	

"-" indicates no turbidity, suggesting antibacterial effect; "+" indicates turbidity, suggesting no antibacterial effect.

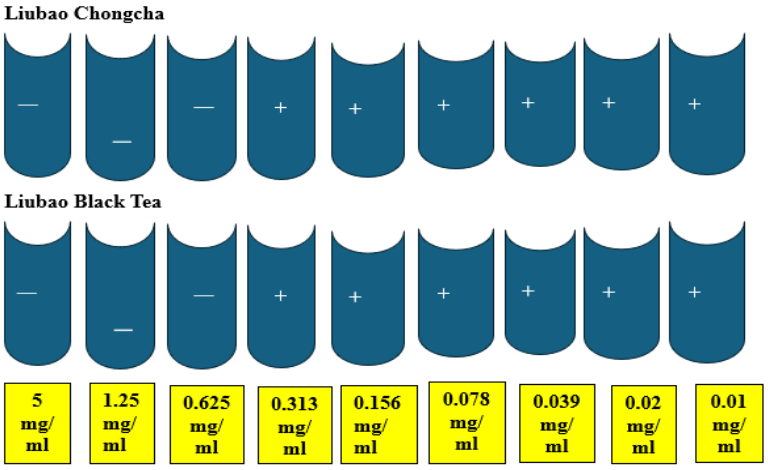

Figure 1: Antibacterial Activity of Liubao Chongcha and Liubao Black Tea Against S.Aureus.

The MIC and MBC of the aqueous extracts of Liubao Chongcha and Liubao black tea were determined using the MH broth dilution method. S. aureus was selected as the representative bacterium for this measurement. The experimental results demonstrated significant antibacterial activity against S. aureus using both Liubao Chongcha (MIC and MBC = 0.625 mg/ml) and Liubao black tea (MIC and MBC = 1.25 mg/ml). The results indicate that the aqueous extract of Liubao Chongcha exhibits a more potent antibacterial effect against S. aureus compared to Liubao black tea (Table 3& Figure 1).

Discussion

Chongcha, a Chinese medicinal tea, is highly valued for its health-promoting properties and its association with longevity among mountain residents. Chongchain China exhibits a diverse range of traditional types that are distinguished by their elevated amino acid content, extensive variety, and well-balanced proportions. Additionally, they contain a wide range of minerals and trace elements that are advantageous for human health (Jiang, 2000; Liang et al., 2023).

Pathogenic microorganisms, including S. aureus, P. aeruginosa, and E. coli, are widely distributed and can have adverse effects on human health. Liubao Chongcha exhibited significant inhibitory effects against S. aureus, as well as inhibitory effects against E. coli and P. aeruginosa. Tan et al. demonstrated comparable outcomes by showing strong antibacterial inhibition against a range of microorganisms, including S. aureus and B. subtilis (Tan et al., 2002). Hence, it can be argued that the consumption of Liubao Chongcha may have potential benefits for specific intestinal diseases caused by S.aureus, suggesting its potential role in modulating intestinal homeostasis.

The antibacterial effect of aqueous extracts in vitro is directly proportional to the concentration of the extract. Nevertheless, the use of a high concentration of Liubao Chongcha extract in this experiment resulted in increased viscosity and the presence of both antibacterial and non-antibacterial components. This could have contributed to the observed variability in the experimental results. Pan et al. conducted a study on fermented samples of Liubao Chongcha, using omics analysis to identify critical microorganisms, biomarker chemicals, and a stable microbial community structure (Pan et al., 2024).

This study utilised the disc diffusion method to investigate the antibacterial effects of aqueous extracts from Liubao Chongcha and Liubao black tea on various Gram-positive and Gram-negative microorganisms. Liubo black tea showed weaker antibacterial activity compared to the former, especially against Gram-positive S. aureus. Both teas exhibited reduced inhibition against Gram-negative bacteria, such as E. coli and P. aeruginosa. Additionally, neither of the tea extracts showed sensitivity to B. subtilis, as indicated by the lack of an inhibitory zone. The results of this study support the findings of another study that showed the potential of Liubao Chongcha polyphenols in preventing gastric injury in mice (Zhang et al., 2020).

The MIC and MBC of the tea extracts against the representative microorganism, S. aureus, were determined using the M-H broth dilution method. The antibacterial activity of both tea extracts against S. aureus was found to be promising. The aqueous extract of Liubao Chongcha exhibited a stronger antibacterial effect compared to Liubao black tea. Liubao tea extract has been reported to have potential in inhibiting harmful bacteria such as Streptococcus and Delftia acidovorans, while promoting the growth of Firmicutes and Actinobacteria and reducing the abundance of Proteobacteria, thus helping to maintain a balanced oral microecology (Liu et al., 2024; Zhou et al., 2023).

The literature documents additional benefits of Liubao Chongcha and Liubao black tea. Guo et al. (2023) observed significant reductions in the production of proinflammatory cytokines and inflammatory cells associated with allergic asthma. The tea contains various compounds such as alkaloids, peptides, free amino acids, polyphenols, polysaccharides, and volatile compounds. These compounds have been linked to numerous health benefits, including antioxidant, anti-obesity, anti-diabetic, anti-cancer, gastrointestinal and cardiovascular protection, hepatoprotection, neuroprotection, and photoprotection (Cao et al., 2020; Lin et al., 2021).The bioactive components of Chongcha are known to possess remarkable antioxidant properties (Yi et al., 2020; Zhu et al., 2019).

Conclusion

Health should not be neglected in today's fast-paced society, and proper dietary therapy is essential for maintaining optimal well-being. Consuming tea is a prevalent practice among individuals, thus highlighting the significance of creating tea beverages that promote gastrointestinal health. The study found that the extract of Liubao Chongcha has a significant antibacterial effect on Gram-positive bacteria, specifically S. aureus. This suggests that it may have potential in promoting gastrointestinal health. Chongcha polyphenols have significant antioxidant and anti-aging properties.

Moreover, the reliance on labour and expertise, coupled with our inadequate understanding of the intricate interconnections and underlying mechanisms associated with processing, bioactivities, and organoleptic quality, still hinder the advancement of Liubao Chongcha and Liubao black tea. Therefore, further research is necessary to address these gaps.

Acknowledgement

This work was supported by the 2022 Guangdong Provincial Administration of Traditional Chinese Medicine Research Project [Grant number: 20222153]

References

Cao, S.-Y., Li, B.-Y., Gan, R.-Y., Mao, Q.-Q., Wang, Y.-F., Shang, A., et al. (2020). The in vivo antioxidant and hepatoprotective actions of selected Chinese teas. *Foods, 9*(3), 262. doi: https://doi.org/10.3390/foods9030262

Elman, B. A. (2015). *Science in China, 1600?1900*. World Scientific / World Century Publishing Corporation. doi: https://doi.org/10.1142/9466

Feng, X., Chen, M., Song, H., Ma, S., Ou, C., Li, Z., et al.

(2023). A systemic review on Liubao tea: A time-honored dark tea with distinctive raw materials, process techniques, chemical profiles, and biological activities. *Comprehensive reviews in food science and food safety, 22*(6), 5063-5085. doi: https://doi.org/10.1111/1541-4337.13254

Fu, J., Zhu, F., & Wen, L. (2018a). Influence of tri-leaf insect tea extract on certain intestinal bacteria. *Central China Entomological Research, 5*, 156–159.

Fu, Y., Yang, J.-c., Cunningham, A. B., Towns, A. M., Zhang, Y., Yang, H.-y., et al. (2018b). A billion cups: The diversity, traditional uses, safety issues and potential of Chinese herbal teas. *Journal of ethnopharmacology, 222*, 217-228. doi: https://doi.org/10.1016/j.jep.2018.04.026

Gao, Y., Wang, D., Xu, M.-L., Shi, S.-S., & Xiong, J.-F. (2018). Toxicological characteristics of edible insects in China: A historical review. *Food and Chemical Toxicology, 119*, 237-251. doi: https://doi.org/10.1016/j.fct.2018.04.016

Guo, S., Shi, Y., Xu, A., Wang, Y., & Xu, P. (2023). Liubao tea extract ameliorates ovalbumin-induced allergic asthma by regulating gut microbiota in mice. *Food & Function, 14*(23), 10605-10616. doi: https://doi.org/10.1039/D3FO03470D

Hao, Z. B., Wu, Q., Jiang, Z. J., Zhang, J. W., & Luo, Y. F. (2013). Biological properties of Guangxi Liu Pao black tea. *J Northeast Agri Univ, 44*, 68–72.

Jiang, S. (2000). Resources and Health Benefits of Chinese Insect Tea. *Special Economic Animals and Plants, 5*, 36.

Lei, M., & Lu, X. (2001). China's worm tea resources and research status. *Food Science, 22*(11), 100-102. Retrieved from https://www.spkx.net.cn/CN/Y2001/V22/I11/100

Liang, Y.-N., Li, H., Huang, X.-Y., Bin, Y.-J., Zhen, Y.-M., & Qin, X.-M. (2023). The complete chloroplast genome and phylogenomic analysis of Camellia sinensis var. sinensis cultivar 'Liupao', a landrace from Guangxi, China. *Mitochondrial DNA Part B, 8*(8), 921-926. doi: https://doi.org/10.1080/23802359.2023.2250072

Lin, F.-J., Wei, X.-L., Liu, H.-Y., Li, H., Xia, Y., Wu, D.-T., et al. (2021). State-of-the-art review of dark tea: From chemistry to health benefits. *Trends in Food Science & Technology, 109*, 126-138. doi: https://doi.org/10.1016/j.tifs.2021.01.030

Liu, H., Huang, Y., Liu, Z., Pang, Y., Yang, C., Li, M., et al. (2024). Determination of the variations in the metabolic profiles and bacterial communities during traditional craftsmanship Liupao tea processing. *Food Chemistry: X*, 101516. doi: https://doi.org/10.1016/j.fochx.2024.101516

Pan, J., Wang, J., Teng, J., Huang, L., Wei, B., Xia, N., et al. (2024). Deciphering the underlying core microorganisms and the marker compounds of Liupao tea during the pile-fermentation process. *Journal of the Science of Food and Agriculture, 104*(5), 2862-2875. doi: https://doi.org/10.1002/jsfa.13177

Tan, Z. J., Wen, L. Z., & Xiao, Q. M. (2002). Study on antibacterial property of several extracts made from some insect excrements of different Chinese Chongcha species. *Food Science, 23*(6), 104–107.

Wang, K., Wu, K., & Li, N. (2023). Insect tea originated from ethnic minority regions in Southwest China: A review on the types, traditional uses, nutrients, chemistry and pharmacological activities. *Journal of Ethnopharmacology, 309*, 116340. doi: https://doi.org/10.1016/j.jep.2023.116340

Xu, L., Pan, H., Lei, Q., Xiao, W., Peng, Y., & Xiao, P. (2013). Insect tea, a wonderful work in the Chinese tea culture. *Food research international, 53*(2), 629-635. doi: https://doi.org/10.1016/j.foodres.2013.01.005

Ya-Feng, W., & Li-Zhang, W. (2019). A survey of the historical origins, medicinal value and food safety of Chinese insect tea. *Chinese Journal of Applied Entomology, 56*(5), 957. doi: https://doi.org/10.7679/j.issn.2095-1353.2019.106

Yi, R., Chen, X., Li, W., Mu, J., Tan, F., & Zhao, X. (2020). Preventive effect of insect tea primary leaf (Malus sieboldii (Regal) Rehd.) extract on D-galactose-induced oxidative damage in mice. *Food Science & Nutrition, 8*(9), 5160-5171. doi: https://doi.org/10.1002/fsn3.1821

Yuan, D.-Y., Chen, J.-X., Zhang, F., Huang, S.-H., Peng, J.-R., & Wen, L.-X. (2021). The current situation and prospect of insect tea. *Tianjin Food Research Institute Co. Ltd, 42*(9), 198-204. doi: https://doi.org/10.12161/j.issn.1005-6521.2021.09.029

Zhang, J., Lu, D.-Y., Yuan, Y., Chen, J., Yi, S., Chen, B., et al. (2020). Liubao Insect tea polyphenols prevent HCl/ethanol induced gastric damage through its antioxidant ability in mice. *Rsc Advances, 10*(9), 4984-4995. doi: https://doi.org/10.1039/C9RA09641H

Zhou, S., Bao, Z., Ma, S., Ou, C., Hu, H., Yang, Y., et al. (2023). A local dark tea–Liubao tea–extract exhibits remarkable performance in oral tissue regeneration, inflammation relief and oral microbiota reconstruction. *Food & Function, 14*(16), 7400-7412. doi: https://doi.org/10.1039/D3FO02277C

Zhu, K., Zeng, X., Tan, F., Li, W., Li, C., Song, Y., et al. (2019). Effect of insect tea on D-galactose-induced oxidation in mice and its mechanisms. *Food Science & Nutrition, 7*(12), 4105-4115. doi: https://doi.org/10.1002/fsn3.1278

Zhu, M.-z., Li, N., Zhou, F., Ouyang, J., Lu, D.-m., Xu, W., et al. (2020). Microbial bioconversion of the chemical components in dark tea. *Food Chemistry, 312*, 126043. doi: https://doi.org/10.1016/j.foodchem.2019.126043