Chinese Herbal Medicines 15 (2023) 33–36

Contents lists available at ScienceDirect

Chinese Herbal Medicines

journal homepage: www.elsevier.com/locate/chmed

Review

Dark tea: A popular beverage with possible medicinal application

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ARTICLE INFO

Article history:
Received 15 May 2022
Revised 15 July 2022
Accepted 24 August 2022
Available online 24 November 2022

Keywords:
biological activities
dark tea
metabolites
medicinal development perspectives

ABSTRACT

Tea is a famous beverage that is produced from leaves of *Camellia sinensis*. Amongst the six major tea categories in China, dark tea is the only one that involves microbial fermentation in the manufacturing process, which contributes unique flavors and functions for the tea. In the recent decade, the reports about the biofunctions of dark teas have increased rapidly. Therefore it may be the proper time to consider dark tea as one potential homology of medicine and food. In this viewpoint, our current understanding of the chemical constituents, biological activities and possible health beneficial effects of dark teas were introduced. Some future directions and challenges to the development perspectives of dark teas were also discussed.

Contents

1. Introduction

Tea is a popular beverage made from the leaves of the plant *Camellia sinensis* (Fig. 1), which was first mentioned in a legend of Shen-Nong tasting herbs. *The Classic of Tea* records, "As a beverage, tea originated from the Chinese Shen-Nong Era, and became popular in the period of the Duke of Lu and Zhou." Although there is no definitive evidence for Shen-Nong's discovery of tea, this legend does reflect the fact that early humans realized the medical value of tea. From then on, the usage of tea leaves had been developing through the succession of dynasties in China, from medicine to edible food, and currently as a healthy beverage.

Amongst the six major tea categories in China, dark tea is the only one that involves microbial fermentation in the manufacturing process. It is worth noting that the fermentation of dark tea is different from that of black tea (full-fermented tea) and oolong tea (semi-fermented tea). The so-called fermentation in black tea and oolong tea refers to the catalysis by endogenous enzymes, such as polyphenol oxidases and peroxidases, when the tea leaves are crashed. However, the activity of these tea endogenous oxidases is passivated by heat before microbial fermentation of dark tea. It is the microbial enzymes and metabolites that plays essential role in the formation of unique flavors and functions in dark tea.

The widely accepted origin of dark teas is the Ancient Tea Horse Road, a long and moist journey for transportation of tea products, which provided a favorable condition for microbial fermentation to make dark teas. The popular dark teas include ripen Pu-erh tea, Liubao (or Liupao, Liupu) tea, Fuzhuan brick tea, Qingzhuan brick tea and Kangzhuan brick tea, which are respectively from Yunnan,

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https://doi.org/10.1016/j.chmed.2022.08.005
1674-6384/

Fig. 1. (A) Dark tea is a popular beverage. (B) An ancient tea tree in Yunnan Province (Picture is from https://app.zznews.gov.cn/print.php?contentid=228219). (C) A local cultivar variety of *Camellia sinensis* in Hunan Province, Yuntai Daye, which is commonly used for production of dark tea (Picture is from https://www.ytscljt.com/h-nd-156.html).

Guangxi, Hunan or Shaanxi, Hubei, and Sichuan in China (Fig. 2) (Zhu et al., 2020). Pu-erh tea and Liubao tea were named according to their producing or commercial places. Fuzhuan brick tea was named because it was traditionally processed in the hottest days of summer -- "San Fu Tian", that's the origin of the name "Fu". While the name of Heizhuan brick tea means a piece of black brick tea, Qingzhuan brick tea means a piece of cyan brick tea, and Qianliang tea means a strapped column of tea leaves weighing 36.25 kg.

In recent decades, reports about the biological activities of dark teas have been increasing (Liu, Liu, Zhang, Ren, & Xu, 2017). This article reviews our current understanding of the chemical constituents, biological activities, and possible health beneficial effects of dark teas. Some future directions and challenges in dark tea research are also discussed.

2. Diverse metabolites in dark teas

Fresh tea leaves contain a diverse group of constituents. In general, the tea polyphenols, represented as flavan-3-ols and flavonol glycosides, as well as theanine, caffeine are the most important compounds for both biological activities and flavors. The remarkably enriched metabolite diversity caused by microbial fermentation forms the mellow, sweet, and smooth taste of dark teas (Zhu et al., 2020). During fermentation, the microbes not only produce metabolites through *de novo* biosynthesis, but also transform the pre-existing compounds in tea leaves (Fig. 3). The microbial metabolites in dark teas are more likely to contribute to the unique biological activities and therefore worthy investigating. However, most of the microbial metabolites are difficult to be isolated and identified by classic approaches of natural product chemistry, due to their low concentration and high diversity in chemical structures.

A recent investigation on the metabolites of both diverse dark teas and fungi using Feature-Based Molecular Networking (FBMN), has unveiled not only many shared metabolites between microbes and dark teas, but also the relative quantities of these metabolites among dark teas (Kong et al., 2022). In this study, more than three thousand metabolites were presented simultaneously. The dark tea metabolites contributed from fungi include prenylated cyclic dipeptides, B-vitamins, anthraquinones, flavan-3-ol B-ring fission analogues, fatty acids, triterpenoids, guanidine derivatives, cholesterol, lipopeptides, acarbose, asperphenamate, prenylated benzaldehydes, and many other natural products. This work greatly expanded our knowledge of the natural products in dark teas and helped us understand the biological activities and flavors of dark teas.

3. Beneficial biological activities of dark teas

It has been reported that dark teas and their constituents can display many biological activities that may be beneficial to human health, including antioxidation, anti-bacteria, anti-inflammation, anti-obesity, ant-diabetes, anti-diarrhea, anti-cancer, gastrointestinal regulation, cardiovascular protection, immune-regulation, hepatoprotection, neuroprotection, and photoprotection (Lin et al., 2021).

The antioxidant activity is one of the most important biological properties of dark teas (Lin et al., 2021). Therein, tea polyphenols

Fig. 2. Some commercial dark tea products (Zhu et al., 2020).

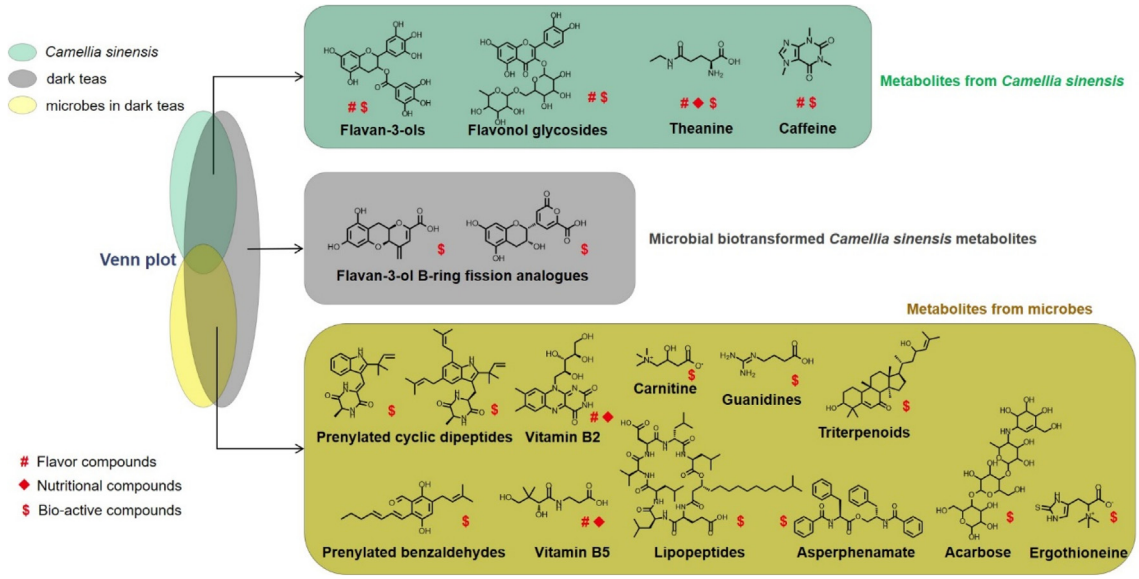

Fig. 3. Some typical metabolites in dark teas originated from those in *Camellia sinensis* and fermentation microbes.

such as epigallocatechin-3-gallate (EGCG), other flavonoids, theabrownins, as well as polysaccharides in dark teas were found to exhibit *in vitro* or *in vivo* antioxidant activities. These include scavenging free radicals, promoting synthesis of other antioxidants, and increasing antioxidant enzyme activities. Interestingly, some dark teas showed much stronger *in vivo* antioxidant activities than green teas, even though the contents of antioxidant tea polyphenols in dark teas are much lower than those in green teas (Lin et al., 2021).

The beneficial effects of dark tea in the gastrointestinal system have also been studied in animal models. It was reported that dark tea polysaccharides could modulate the ratio between beneficial and detrimental microorganisms, and consequently alter the activities and metabolites of gut microbiota (Bond & Derbyshire, 2019). In that way, the extract of dark tea significantly ameliorates some chronic diseases, such as colitis. Meanwhile, the regulation of the gut microbiome also contributes to the anti-obesity property of dark teas. For example, *Eurotium cristatum*, a dominant fungus from Fuzhuan brick tea, could increase acetate- and butyrate-producing bacteria in mouse gut, and subsequently alleviate obesity (Lin et al., 2021). Theabrownins, extracted from Pu-erh tea, could increase the levels of ileal conjugated bile acids (BAs) which, in turn, inhibit the intestinal FXR-FGF15 signaling pathway, resulting in increased hepatic production and fecal excretion of BAs, reduced hepatic cholesterol, and decreased lipogenesis (Huang et al., 2019). Besides, extracts of dark tea could decrease the lipid droplet size, reduce the mass of subcutaneous adipose tissue, up-regulate the genes related to both uptake and beta oxidation of fatty acids, as well as down-regulate the genes for fatty acid synthesis. Moreover, several studies reported a beneficial effect of dark teas in ameliorating obesity-related inflammation in the adipose, liver, intestine, skeletal muscle and other tissues of mice (Tang et al., 2019).

The cardiovascular protective effect of dark teas could be due to the following types of activities. First, dark tea flavanols, acyl glycosides of flavones, and their derivatives, could play a preventive role against hyperlipidemia (Chen et al., 2021). Second, dark tea theabrownins and caffeine could prevent hypertension. In addition, dark teas show a strong anti-diabetic effect through ameliorating insulin sensitivity, inhibiting the expression of signal proteins, and possibly other mechanisms (Lin et al., 2021). Furthermore, dark tea could effectively attenuate the formation of foam cell leading to atherosclerosis which is mainly caused by hyperlipidemia and hypertension (Lin et al., 2021).

4. Future directions and challenges

Although there have been numerous studies showing that dark teas have many biological activities that may be beneficial to human health, such effects and the mechanisms involved remain to be further investigated.

Some interesting activities of dark tea have been reported recently and deserve further investigation (Lin et al., 2021). For instance, some metabolites from dark teas such as L-theanine, 3,3′-azanediylbis (4-hydroxybenzoic acid) and 8-C *N*-ethyl-2-pyrrolidinone substituted flavan-3-ols could relieve the age-related neurodegenerative disorders; Fuzhuan brick tea and Anhua dark tea-derived 2*S*,3*R*-6-methoxycarbonyl gallocatechin could protect skin from ultraviolet B (UVB) induced damages. Some dark tea constituents with unique biological activities may be explored in the future for medicinal uses and the yield of certain metabolites may be increased by selecting the microbial species used for the manufacturing of the dark tea.

A recent study showed that the combination of Pu-erh tea and silibinin has a significant synergistic preventive effect on nonalcoholic steatohepatitis (Lin et al., 2021). The possible synergistic effects of dark tea with other food ingredients may be a promising area for future dark tea research.

The traditional manufacturing procedure of dark tea commonly lacks both standardized management and controlled targeted fermentation; the safety assessment of dark teas should not be ignored. There have been several reports concerning heavy metals, pesticide residues, and mycotoxins in some dark tea products (Tang et al., 2019). Even though these unhealthy ingredients were suggested to be below hazard levels, it is still important to improve the environment of tea plantations, control the use of pesticides, and find ways to eliminate potential safety problems, such as mycotoxin contaminations. The relationship between dark tea microbes and flavor compounds needs to be further studied. Based on the results, we may use specific single or mixed microorganisms to perform precisely monitored fermentation to make customized flavors and avoid the formation of mycotoxins.

More studies on dark tea in animal models and humans are needed. In relating biological activities to dark tea constituents,

both the systemic effects (by compounds that are bioavailable) and effects mediated by gut microbiota need to be considered. If dark tea is to be considered as a homology of medicine and food, it is important to provide consumers with the recommended, minimum, and maximum daily doses. In addition, the optimal duration of intake to achieve a significant preventive and ameliorating effects of diseases need to be studied.

In summary, the reported various biological activities and the long history of beverage utilization, suggest the potential medicine and food homology for dark teas. However, we are still facing challenges in this area of research. Further basic research and human studies are needed before dark tea can be evaluated as a medicine homologue.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study is supported by the Fund of Anhui Key Research and Development Program (No. 202104a06020009), and the Fund of Natural Science Research Project of Anhui Colleges and Universities (No. YJS20210245).

References

Bond, T., & Derbyshire, E. (2019). Tea compounds and the gut microbiome: Findings from trials and mechanistic studies. *Nutrients, 11*, 2364.

Chen, G. J., Peng, Y. J., Xie, M. H., Chen, C. X., Zeng, X. X., & Liu, Z. H. (2021). A critical review of Fuzhuan brick tea: Processing, chemical constituents, health benefits and potential risk. *Critical Reviews in Food Science and Nutrition, 518*, 1–18.

Huang, F. J., Zheng, X. J., Ma, X. H., Jiang, R. Q., Zhou, W. Y., Zhou, S. P., & Jia, W. (2019). Theabrownin from Pu-erh tea attenuates hypercholesterolemia via modulation of gut microbiota and bile acid metabolism. *Nature Communications, 10*, 1–17.

Kong, Y. S., Ren, H. Y., Liu, R., da Silva, R. R., Aksenov, A. A., Melnik, A. V., & Ling, T. J. (2022). Microbial and nonvolatile chemical diversities of Chinese dark teas are differed by latitude and pile fermentation. *Journal of Agricultural and Food Chemistry, 70*, 5701–5714.

Lin, F. J., Wei, X. L., Liu, H., Xia, Y., Wu, D. T., Zhang, P. Z., & Gan, R. Y. (2021). State-of-the-art review of dark tea: From chemistry to health benefits. *Trends in Food Science & Technology, 109*, 126–138.

Liu, J. Y., Liu, D., Zhang, H., Ren, X. H., & Xu, Y. N. (2017). Research progress on chemical constituents of Anhua dark tea and their activities. *Chinese Traditional & Herbal Drugs, 48*(7), 1449–1457.

Tang, G. Y., Meng, X., Gan, R. Y., Zhao, C. N., Liu, Q., Feng, Y. B., & Li, H. B. (2019). Health functions and related molecular mechanisms of tea components: An update review. *International Journal of Molecular Sciences, 20*, 6196.

Zhu, M. Z., Li, N., Zhou, F., Jian, O. Y., Lu, D. M., Xu, W., & Wu, J. L. (2020). Microbial bioconversion of the chemical components in dark tea. *Food Chemistry, 312*, 126043.