

BIOACTIVE COMPOUNDS OF TEA (CAMELLIA SINENSIS) FLOWERS

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ABSTRACT

Tea is an economically important non-alcoholic beverage-yielding plant for North East India and some parts of South India. Flower morphology is important to plant breeding as they provide important information on flower nature. Different bioactive compounds are present in Tea flowers which have multiple beneficial effects in tea drinkers. Genetically flowers are also different and major genes responsible for total catechin content in tea flowers such as chalcone synthase (CH2) and flavonol synthase (FLS) were found to be highly expressed during early flowering stage, while genes such as phenylalanine ammonia lyase (PAL1) and flavonoid 3'-hydroxylase (F3'H1) were expressed in the late flowering stage. Pserphids are main pollinators of tea. Though identification of morphological and genetically nature breeding becomes easy and higher success rate. The presence of health-beneficial bioactive molecules in tea flowers has been globally acknowledged.

KEYWORDS: Beverage, Bioactivity, Genetics, Tea Flowers

INTRODUCTION

The beverage "tea", made from the bud and first two leaves of the plant *Camellia sinensis*, has cemented its place as the world's second most loved and consumed non-alcoholic beverage, just after the "elixir of life", i. e., water. For years China had claimed to be the country of origin of this aromatic beverage. However, existence of its Indian counterpart (*Camellia assamica*) had also been eventually established (Mondalet *al*, 2019; Rawalet *al*, 2020). Tea is preferred mainly due to its strong aroma and stimulating effects. It is saturated with bioactive components which not only energize a drinker, but also contribute to innumerable health benefits (Chen *et al* 2012; Matsuda *et al* 2012; Wang *et al*, 2012; Wang *et al* 2017).

Bioactive Compounds

Tea flowers contain various bioactive chemicals which include

Catechins and Caffeine

The major health-promoting polyphenols in tea are catechins, theaflavins and thearubigins. As theaflavins and thearubigins are formed during black tea processing, as such are unavailable in flowers. Even though catechin content in tea flowers is lower as compared to tea leaves, however, it is significant enough to consider them as a second source. The major catechins found in tea flowers are +catechins (+C), epicatechin (EC), galocatechin (GC), epigallocatechin (EGC), catechins gallate (CG), epicatechingallate (ECG), galocatechingallate (GCG) and epigallocatechin gallate (EGCG) (Lin *et al*, 2003; Yang *et*

al, 2007, Yang *et al*, 2009; Morikawa *et al*, 2013b). Among all, EGC, ECG and EGCG have found to be the most prominent catechins in tea flowers. Caffeine, on the other hand, is present only at 0.3-1.1 % of total tea flower dry weight (Lin *et al*, 2003; Morikawa *et al*, 2013b) in comparison to 2-3 % of total tea leaf dry weight (Nagata and Sakai, 1984; Ashihara, 2006).

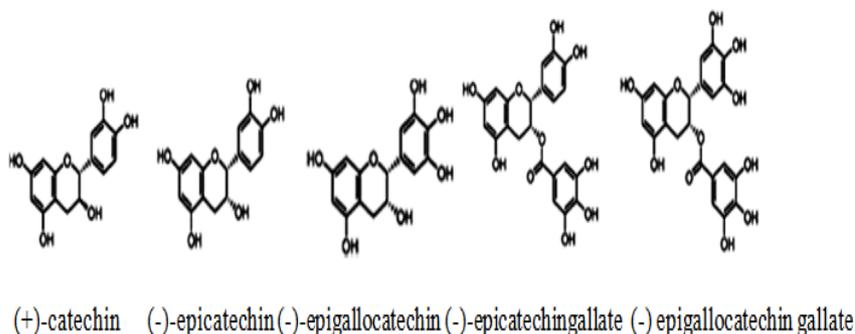


Figure 1: Structure of Major Catechins Found in Tea Flowers.

Volatile Compounds

Aromatic compounds in tea leaves are synthesized via the terpenoid or shikimic acid pathway, or are results of fatty acid and carotenoid degradation (Yang *et al*, 2013). In spite of a similar constitution of aromatic compounds in both tea leaves and flowers, the volatiles acetophenone and 1-phenylethanol are highly expressed in tea flowers (Dong *et al*, 2012, 2016; Zhou *et al* 2014, 2015). The composition of volatile aromatic compounds in tea flowers differ depending on the cultivar, geographical location and extraction procedure (Joshi *et al*, 2011a; 2011b; Wang *et al*, 2015). Supercritical Fluid Extraction (SFE) has been found to be superior to distillation extraction methods (SDE) as the heat-labile volatiles are retained during SFE resulting in better floral fragrance and aroma. Terpenoids, both free and glycosidically bound, form a major part of the aroma components in tea flowers. The major terpenoids found in tea flowers include linalool, linalool oxides, geraniol, nerolidol and α -terpineol, found at various stages of flowering. Additionally, other components such as benzaldehyde, glutaraldehyde, hexanoic acid, 2, 4-di-tert-butylphenol, methyl palmitate, methyl linoleate, methyl salicylate and β -ionone also add to the volatile components in tea flowers (Joshi *et al*, 2011a).

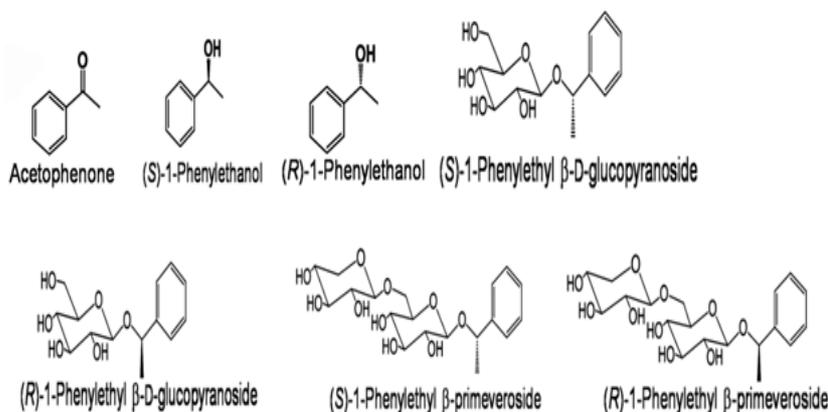


Figure 2: Major Volatiles in Tea Flower.

L-Theanine

L-theanine is a non-proteinogenic amino acid found most commonly in tea (genus Theaceae). It is the most common free amino acid found in tea and accounts to 1-2 % of total tea leaf dry weight (Wan, 2003; Wan and Xia, 2015), up to 6 % of total tea root dry weight (Li *et al*, 2019) and around 0.8 % of total tea flower dry weight (Wang *et al*, 2010a).

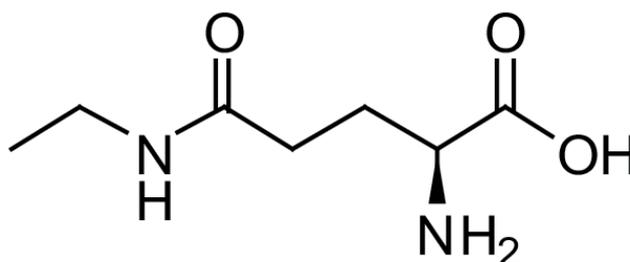


Figure 3: Structure of L-Theanine Found In Tea Flowers.

Polysaccharides

Polysaccharides have two major roles in plants- as structural components and/or as reserved components. Consequently, the composition of polysaccharides in different parts/organs of plants differs. The polysaccharide content in tea flowers (5.24 %) have been found to be higher as compared to tea leaves (3.64 %) (Wang *et al*, 2010b). As for the composition, tea flower polysaccharide (TFPS) composition differ depending on the method of extraction used. Contrary to microwave or ultrasonic-assisted extraction, traditional water extraction was found to be optimal for the extraction of TFPS (Wei *et al*, 2010). Furthermore, water extract was found to contain glucose: xylose: rhamnose: galactose in the ratio 1.0:1.2:0.81:0.98, whereas ethanol extract comprised of glucose: xylose: rhamnose: arabinose in the ratio 1.0:0.76:2.3:2.3 (Han *et al*, 2011b).

Saponins

Saponin concentration in different parts of a plant varies, with its highest concentration found in seeds (Wan and Xia, 2015). Tea flowers have been found to contain higher saponin content (0.47-4.23 % dry weight) (Morikawa *et al*, 2012) than tea leaves (0.04-0.07 % dry weight) (Zhen, 2002). Till date, 26 saponins have been identified in tea flowers which include Floratheasaponin A-K, Chakasaponin I-VI, Floraassamsaponin I-VIII and Assamsaponin E (Sugimoto *et al* 2009; Matsuda *et al*, 2016; Ohta *et al*, 2015, 2017). Composition of saponins, however, differ depending on the geographical region. The saponin composition in tea flowers of India were found to be similar to those in Anhui Province in China but were different from the tea flowers in Japan (Yoshikawa *et al*, 2008).

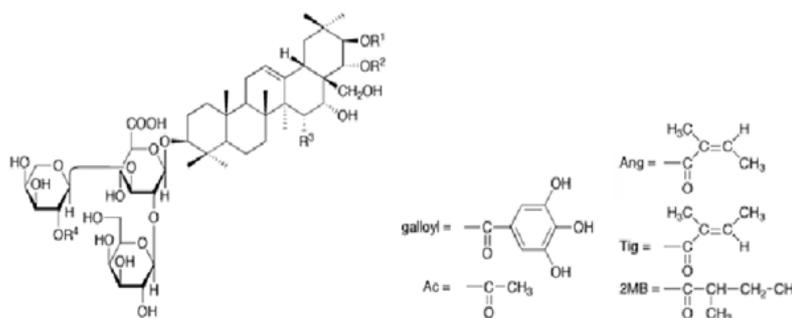


Figure 4: Basic Backbone Structure of Saponin In Tea Flower.

Proteins

The total protein content in tea flowers is 30-50 % of dry weight as compared to 20-30 % of dry weight in tea leaves (Weng, 2004). One of the most important commercially used proteins is the protease enzymes which convert complex proteins to amino acids for quicker absorption. A protease enzyme extracted from tea flowers have been found to increase the amino acid content of tea infusion up to 177 %, a competence far greater than any commercially available protease (Chen *et al*, 2016).

Sperm Dine Derivatives

The composition of spermidine derivatives in tea leaves and flowers are considerably distinct. A principal component analysis of the different metabolites in tea leaves and flowers using ultra-performance liquid chromatography/time-of-flight mass spectrometry revealed differences in metabolite profiles between the two types of samples. Four spermidine derivatives namely N¹, N⁵, N¹⁰-tri-coumaroyl spermidine, coumaroyl di-feruloyl spermidine, feruloyl di-coumaroyl spermidine, and tri-feruloyl spermidine were detected and isolated from tea flowers which were absent in tea leaves. Furthermore, a reduction in the content of spermidine conjugates was observed during flower development and was found to concentrate primarily in the anthers (Yang *et al*, 2012). The role of spermidine derivatives in plants range from floral induction and flower formation to sexual differentiation, tuberization, cell division, cytomorphogenesis and even defense against insects, pathogens and wounding (Facchini *et al*, 2002).

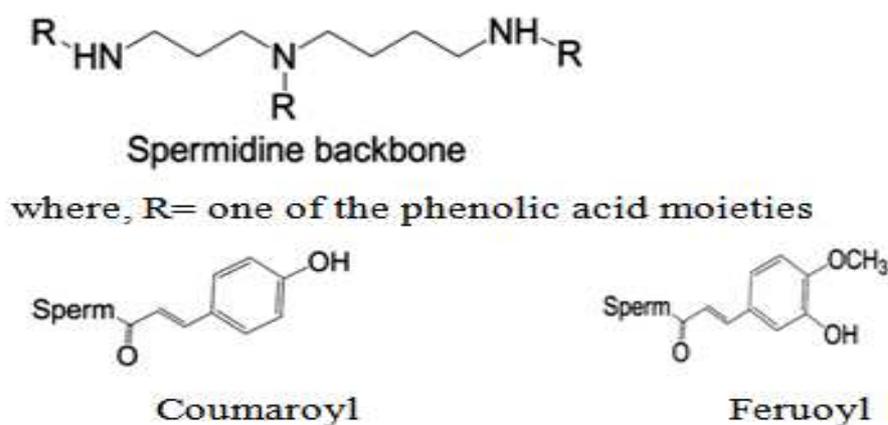


Figure 5: Structure of Spermidine Found in Tea Flowers.

Gene Expression with Bioactivity of Tea Flowers

The process of flower induction and development involves an intricate physiological process comprising of numerous endogenous as well as extraneous factors (Liu *et al*, 2017). During the past decade, molecular and genetic mechanisms involved in tea flower induction, differentiation and development have been inferred. Floral induction has been found to be influenced by the expression of genes such as gibberellic acid intensive dwarf 1B (GID1B) and GID1C, gibberelin 3-oxidase 1 (GA3ox1), GIGANTEA (GI), pseudo-response regulator (PRR7) and flowering locus T (FT), whereas expression of genes such as leafy (LFY), pound-floolish (PNF) and pennywise (PNY) were correlated with floral bud formation (Liu *et al*, 2020). 207 unigenes and transcription factors such as WRKY, ERF, MYB, bHLH and MADS-box have been particularly identified with flowering-associated roles in tea (Liu *et al*, 2017).

Tea flower differentiation and development includes various processes. The tubulin-encoding *Tua1* and pollen coat protein (*Pcp*) genes have been observed to promote pollen tube growth (Fang et al, 2006) and anther development (Ye et al, 2008) in tea. Pollen tube elongation in tea flowers is also regulated via the nitric oxide (NO) pathway under low temperature stress by the CAMTA TFs, COBRA-like genes and phosphatidylinositol-4-kinase (PI4K) (Pan et al, 2016).

Moreover, major genes responsible for total catechin content in tea flowers such as chalcone synthase (CH2) and flavonol synthase (FLS) were found to be highly expressed during early flowering stage, while genes such as phenylalanine ammonia lyase (PAL1) and flavonoid 3'-hydroxylase (F3'H1) were expressed in the late flowering stage and negatively correlated with the total catechins content in the flowers (Sun et al, 2019). As is evident, aroma compound formation during tea flowering increases. This occurs due to the increasing activity of hydrolytic enzymes such as glycosidases (Watanabe et al, 1993; Hayashi et al, 2004). Consequently, flavor precursors formed during anthesis are converted to volatile compounds leading to the gradual development of unique odors (Watanabe et al, 1993).

CONCLUSION

Tea flowers have been used as deodorizer, skin care, flavoring agent, etc in China and Japan for centuries. However, in the last two decades, the presence of health-beneficial bioactive molecules in tea flowers has been globally acknowledged. Tea flowers, earlier regarded as waste, are now being increasingly used as an additive in health drinks and various other beverages. In an initial study, tea flower extracts have also been found to be non-mutagenic and non-toxic. Nevertheless, comprehensive research on tea flowers needs to be carried out to decipher the restrictive factors, if any, in their commercial use. Ideal plucking procedures need to be devised as flowers often undergo browning easily due to the high concentration of moisture and polyphenol oxidases. Moreover, optimal extraction procedures of the individual biomolecules from tea flowers also need to be developed targeting their specific uses. Tea flowers, thus, provide an avenue of applied research with extensive potential in pharmaceutical, cosmetic and food industry.

REFERENCES

1. Ariyaratna HACK, Gunasekare MTK, Kottawa-Arachichige JD, Paskarathavan R, Ranaweera KK, Ratnayake M, Kumara JDBAP (2011) Morpho-physiological and phenological attributes of reproductive biology of tea (*Camellia sinensis* (L.) O. Kuntze) in Sri Lanka. *Euphytica*, 181: 203-215.
2. Ashihara H (2006) Metabolism of alkaloids in coffee plants. *Braz. J. Plant Physiol.*, 18: 1–8. <https://doi.org/10.1590/S1677-04202006000100001>
3. Balentine DA, Wiseman SA, and Bouwens LCM(1997) The chemistry of tea flavonoids. *Crit. Rev. Food Sci. Nutr.*, 37: 693-704. <https://doi.org/10.1080/10408399709527797>.
4. Cai X, Wang YF, Mao FF, Lan YU, Liu CX, Zhang H and Wei XL (2011) Hypoglycemic and hyperglycemia-prevention effects of crude tea flower polysaccharide. *Modern Food Science & Technology*, 27(3), 262-266 (in Chinese). <https://doi.org/10.13982/j.mfst.1673-9078.2011.03.002>.
5. Chen BT, Ki WX, He RR, Li YF, Tsoi B, Zhai YJ and Kurihara H (2012) Anti-inflammatory effects of a polyphenols-rich extract from tea (*Camellia sinensis*) flowers in acute and chronic mice models. *Oxid. Med. Cell Longev.*, Vol. 2012, Article ID 537923. <https://doi.org/10.1155/2012/537923>

6. Chen YY, Fu XM, Mei X, Zhou Y, Du B, Tu YY and Yang ZY (2016) Characterization of functional proteases from flowers of tea (*Camellia sinensis*) plants. *J. Funct. Foods*, **25**: 149–159. <https://doi.org/10.1016/j.jff.2016.05.017>
7. Curr. (1962) *Tea Classification Revised*, Sci. July, 1962.
8. Chen YY, Zhou Y, Zeng LT, Dong F, Tu YY and Yang ZY (2018) Occurrence of functional molecules in the flowers of tea (*Camellia sinensis*) plants: Evidence for a second resource. *Molecules*, **23(4)**: 790. <https://doi.org/10.3390/molecules23040790>.
9. Dong F, Yang ZY, Baldermann S, Kajitani Y, Ota S, Kasuga H, Imazeki Y, Ohnishi T, Watanabe N (2012) Characterization of L-phenylalanine metabolism to acetophenone and 1-phenylethanol in the flowers of *Camellia sinensis* using stable isotope labeling. *J. Plant Physiol*, **169**: 217–225. <https://doi.org/10.1016/j.jplph.2011.12.003>
10. Dong F, Zhou Y, Zeng LT, Peng QY, Chen YY, Zhang L, Su XG, Watanabe N and Yang ZY (2016) Elucidation of differential accumulation of 1-phenylethanol in flowers and leaves of tea (*Camellia sinensis*) plants. *Molecules*, **21**: 1106. <https://doi.org/10.3390/molecules21091106>
11. Facchini PJ, Hagel J and Zulak KG (2002) Hydroxycinnamic acid amide metabolism: physiology and biochemistry. *Can. J. Bot.*, **80**: 577–589. <https://doi.org/10.1139/b02-065>
12. Fang WP, Jiang C, Yu M, Ye AH and Wang Z (2006). Differentially expression of *Tua1*, a tubulin-encoding gene, during flowering of tea plant *Camellia sinensis* (L.) O. Kuntze using cDNA amplified fragment length polymorphism technique. *Acta Biochimica et Biophysica Sinica*, **38(9)**: 653-662. <https://doi.org/10.1111/j.1745-7270.2006.00202.x>.
13. Hamao M, Matsuda H, Nakamura S, Nakashima S, Semura S, Maekubo S, Wakasugi S and Yoshikawa M (2011). Anti-obesity effects of the methanolic extract and chakasaponins from the flower buds of *Camellia sinensis* in mice. *Bioorg. Med. Chem.*, **19(20)**: 6033-6041. <https://doi.org/10.1016/j.bmc.2011.08.042>.
14. Han Q, Ling ZJ, He PM and Xiong CY (2010). Immunomodulatory and antitumor activity of polysaccharide isolated from tea plant flower. *Prog. Biochem. Biophys.*, **37(6)**: 646-653. <https://doi.org/10.3724/SP.J.1206.2009.00656>.
15. Han Q, Yu QY, Shi J, Xiong CY, Ling ZJ and He PM (2011a) Molecular characterization and hypoglycemic activity of a novel water-soluble polysaccharide from tea (*Camellia sinensis*) flower. *Carbohydr. Polym.*, **86(2)**: 797-805. <https://doi.org/10.1016/j.carbpol.2011.05.039>.
16. Han Q, Yu QY, Shi J, Xiong CY, Ling ZJ and He PM (2011b) Structural characterization and antioxidant activities of 2 water-soluble polysaccharide fractions purified from tea (*Camellia sinensis*) flower. *J. Food Sci.*, **76(3)**: C462-C471. <https://doi.org/10.1111/j.1750-3841.2011.02063.x>.
17. Heber D, Zhang Y, Yang J, Ma JE, Henning SM, Li Z (2014) Green tea, black tea, and oolong tea polyphenols reduce visceral fat and inflammation in mice fed high-fat, high-sucrose obesogenic diets. *J. Nutr.*, **144(9)**: 1385-93. <https://doi.org/10.3945/jn.114.191007>

18. Hayashi S, Yagi K, Ishikawa T, Kawasaki M, Asai T, Picone J, Turnbull C, Hiratake J, Sakata K, Takada M, Ogawa K and Watanabe, N. (2004). Emission of 2-phenylethanol from its β -D-glucopyranoside and the biogenesis of these compounds from [2H8] L-phenylalanine in rose flowers. *Tetrahedron*, **60(33)**: 7005-7013. <https://doi.org/10.1016/j.tet.2003.10.130>.
19. Iqbal MCM, Wijesekare KB (2002) Cells of the connective tissue differentiate and migrate into pollen sacs. *Nturwissenchaft* 89:39–42.
20. Joshi R, Poonam and Gulati, A (2011a) Biochemical attributes of tea flowers(*Camellia sinensis*) at different developmental stages in the Kangra region of India. *SciHortic-Amsterdam*, **130(1)**: 266-274. <https://doi.org/10.1016/j.scienta.2011.06.007>.
21. Joshi R, Poonam, Saini R, Guleria S, Babu GDK, Kumari M and Gulati A(2011b) Characterization of volatile components of tea flowers (*Camelliasinensis*) growing in Kangra by GC/MS. *Nat. Prod. Commun.*,**6(8)**:1155-1158. <https://doi.org/10.1177/1934578X1100600829>.
22. Li F, Dong C, Yang T, Ma J, Zhang S, Wei C, Wan X and Zhang Z (2019) Seasonal theanine accumulation and related gene expression in the roots and leaf buds of tea plants (*Camellia sinensis* L.). *Front Plant Sci.*, **10**:1397. <https://doi.org/10.3389/fpls.2019.01397> Lin YS, Wu SS and Lin JK (2003) Determination of tea polyphenols and caffeine in tea flowers (*Camellia sinensis*) and their hydroxyl radical scavenging and nitric oxide suppressing effects. *J. Agric. Food Chem.*, **51(4)**:975-980. <https://doi.org/10.1021/jf020870v>.
23. Liu F, Wang Y, Ding Z, Zhao L, Xiao J, Wang L and Ding S (2017) Transcriptomic analysis of flower development in tea (*Camellia sinensis*. (L.)). *Gene*, **631**: 39-51. <https://doi.org/10.1016/j.gene.2017.08.013>.
24. Liu Y, Hao X, Lu Q, Zhang W, Zhang H, Wang L, Yang Y, Xiao B and Wang X (2020) Genome-wide identification and expression analysis of flowering-related genes reveal putative floral induction and differentiation mechanisms in tea plant (*Camellia sinensis*). *Genomics*, **112(3)**: 2318-2326. <https://doi.org/10.1016/j.ygeno.2020.01.003>
25. Matsuda H, Hamao M, Nakamura S, Kon'I H, Murata M and Yoshikawa M (2012) Medicinal flowers. XXXIII. Anti-hyperlipidemic and anti-hyperglycemic effects of chakasaponins I-III and structure of chakasaponin IV from flower buds of Chinese tea plant (*Camellia sinensis*). *Chem. Pharm. Bull.*, **60**:674–680. <https://doi.org/10.1248/cpb.60.674>
26. Matsuda H, Nakamura S, Morikawa T, Muraoka O and Yoshikawa M (2016) New biofunctional effects of the flower buds of *Camellia sinensis* and its bioactive acylatedoleanane-type triterpene oligoglycosides. *J. Nat. Med.*, **70(4)**: 689-701. <https://doi.org/10.1007/s11418-016-1021-1>.
27. Mondal TK, Rawal HC, Bera B, Kumar PM, Choubey M, Sahab G, Das B, Bandyopadhyay T, Ilango RVJ, Sharma TR, Barua A, Radhakrishnan B and Singh NK (2019) Draft genome of a popular Indian tea genotype TV-1 [*Camellia assamica* L.(O). Kuntze]. <http://dx.doi.org/10.1101/762161>

28. Morikawa T, Lee IJ, Okugawa S, Miyake S, Miki Y, Ninomiya K, Kitagawa N, Yoshikawa M and Muraoka O (2013a) Quantitative analysis of catechin, flavonoid, and saponin constituents in "tea flower", the flower buds of *Camelliasinensis*, from different regions in Taiwan. *Nat. Prod. Commun.*, **8(11)**: 1553-1557. <https://doi.org/10.1177/1934578x1300801114>.
29. Morikawa T, Miyake S, Miki Y, Ninomiya K, Yoshikawa M and Muraoka O (2012) Quantitative analysis of acylatedoleanane-type triterpene saponins, chakasaponins I-III and floratheasaponins A-F, in the flower buds of *Camelliasinensis* from different regional origins. *J. Nat. Prod.*, **66(4)**: 608-613. <https://doi.org/10.1007/s11418-012-0627-1>.
30. Morikawa T, Ninomiya K, Miyake S, Miki Y, Okamoto M, Yoshikawa M and Muraoka O (2013b) Flavonol glycosides with lipid accumulation inhibitory activity and simultaneous quantitative analysis of 15 polyphenols and caffeine in the flower buds of *Camellia sinensis* from different regions by LCMS. *Food Chem.*, **140(1-2)**: 353-360. <https://doi.org/10.1016/j.foodchem.2013.02.079>.
31. Nagata T and Sakai S (1984) Differences in caffeine, flavanols and amino acids contents in leaves of cultivated species of *Camellia*. *Jpn. J. Breed.*, **34**: 459-467. <https://doi.org/10.1270/jsbbs1951.34.459>
32. Ohta T, Nakamura S, Nakashima S, Matsumoto T, Ogawa K, Fujimoto K, Fukaya M, Yoshikawa M and Matsuda H (2015) Acylatedoleanane-type triterpene oligoglycosides from the flower buds of *Camellia sinensis* var. *assamica*. *Tetrahedron*, **71(5)**: 846-851. <https://doi.org/10.1016/j.tet.2014.12.049>.
33. Ohta T, Nakamura S, Matsumoto T, Nakashima S, Ogawa K, Matsumoto T, Fukaya M, Yoshikawa M and Matsuda H (2017) Chemical structure of an acylatedoleanane-type triterpene oligoglycoside and anti-inflammatory constituents from the flower buds of *Camellia sinensis*. *Nat. Prod. Commun.*, **12(8)**: 1193-1196. <https://doi.org/10.1177/1934578X1701200811>.
34. Pan J, Wang W, Li D, Shu Z, Ye X, Chang P and Wang Y (2016) Gene expression profile indicates involvement of NO in *Camellia sinensis* pollen tube growth at low temperature. *BMC Genomics*, **17(1)**: 809. <https://doi.org/10.1186/s12864-016-3158-4>.
35. Rains TM, Agarwal S and Maki KC (2011) Antiobesity effects of green tea catechins: a mechanistic review. *J. Nutr. Biochem.*, **22(1)**: 1-7.
36. <https://doi.org/10.1016/j.jnutbio.2010.06.006>
37. Ramasubramanian, B. *Studies on variability, genetic divergence and crop improvement in tea (Camellia assamica (masters) wight)*, Thesis. Department of Botany, University of Calicut, Kerala, 2005.
38. Rawal HC, Mazumder A, Borchetia S, Bera B, Soundararajan S, Ilango RVJ, Barooah AK, Singh NK, Mondal TK (2020) Comparative analysis of chloroplast genomes indicated different origin for Indian Tea (*Camellia assamica*) cv TV-1 as compared to Chinese tea. <http://dx.doi.org/10.1101/762161>
39. Shi L, Gu Y, Wu D, Wu X, Grierson D, Tu Y and Wu Y (2018) Hot air drying of tea flowers: effect of experimental temperatures on drying kinetics, bioactive compounds and quality attributes. *Int J Food Sci Technol*, **54(2)**: 526-535. <https://doi.org/10.1111/ijfs.13967>

40. Sugimoto S, Yoshikawa M, Nakamura S and Matsuda H (2009) Medicinal flowers. XXV. Structures of floratheasaponin J and chakanoside II from Japanese tea flower, flower buds of *Camellia sinensis*. *Heterocycles*, **78(4)**: 1023-1029.
41. Sun LT, Wang Y, Ding ZT and Liu F (2019) The dynamic changes of catechins and related genes in tea (*Camellia sinensis*) flowers. *Acta Physiol. Plant*, **41(2)**: 30. <https://doi.org/10.1007/s11738-019-2822-0>.
42. Tsou CH (1997) Embryology of the Theaceae-anther and ovule development of *Camellia*, *Franklinia* and *Schima*. *Am J Bot* **84**:369–381.
43. Wang H, Cui XX, Zhao XG, Gao S, Zhao JA and Yuan ZL (2015) Differences of biochemical constituents and contents of eight cultivars flowers of *Camellia sinensis*. *J. Essent. Oil-Bear. Plants*, **18(2)**: 320-328. <https://doi.org/10.1080/0972060x.2014.961036>.
44. Wang L, Xu RJ, Hu B, Li W, Sun Y, Tu YY and Zeng XX (2010a) Analysis of free amino acids in Chinese teas and flower of tea plant by high performance liquid chromatography combined with solid-phase extraction. *Food Chem.*, **123(4)**: 1259-1266. <https://doi.org/10.1016/j.foodchem.2010.05.063>.
45. Wang YF, Yang ZW and Wei XL (2010b) Sugar compositions, alpha-glucosidase inhibitory and amylase inhibitory activities of polysaccharides from leaves and flowers of *Camellia sinensis* obtained by different extraction methods. *Int. J. Biol. Macromol.*, **47(4)**: 534-539. <https://doi.org/10.1016/j.ijbiomac.2010.07.007>.
46. Wang YF, Yu L and Wei XL (2012) Monosaccharide composition and bioactivity of tea flower polysaccharides obtained by ethanol fractional precipitation and stepwise precipitation. *CyTA-J. Food*, **10(1)**: 1-4. <https://doi.org/10.1080/19476337.2010.523901>.
47. Wang YM, Ren N, Rankin, GO, Li B, Rojanasakul Y, Tu Y and Chen YC (2017) Anti-proliferative effect and cell cycle arrest induced by saponins extracted from tea (*Camellia sinensis*) flower in human ovarian cancer cells. *J. Funct. Foods*, **37**: 310-321. <https://doi.org/10.1016/j.jff.2017.08.001>.
48. Wan XC (2003) *Tea Biochemistry*, 3rd ed.; China Agriculture Press: Beijing, China, pp. 8–67.
49. Wan XC and Xia T (2015) *Secondary Metabolism of Tea Plant*, 1st ed.; Science Press: Beijing, China, pp. 39–64. (In Chinese)
50. Watanabe N, Watanabe S, Nakajima R, Moon JH, Shimokihara K, Inagaki J, Etoh H, Asai T, Sakata K and Ina K (1993) Formation of flower fragrance compounds from their precursors by enzymic action during flower opening. *Biosci. Biotechnol. Biochem.*, **57(7)**: 1101-1106. <https://doi.org/10.1271/bbb.57.1101>.
51. Way TD, Lin HY, Hua KT, Lee JC, Li WH, Lee MR, Shuang CH and Lin JK (2009) Beneficial effects of different tea flowers against human breast cancer MCF-7 cells. *Food Chem.*, **114(4)**: 1231-1236.
52. Wei XL, Chen MA, Xiao JB, Liu Y, Yu L, Zhang H and Wang YF (2010) Composition and bioactivity of tea flower polysaccharides obtained by different methods. *Carbohydr. Polym.*, **79**: 418-422.
53. Weng W (2004) Study on the main bioactive compounds of tea (*Camellia sinensis*) flower and its application perspectives. Master's thesis, Zhejiang University, Hangzhou, China.

54. Westerterp-Plantenga MS (2010) Green tea catechins, caffeine and body-weight regulation. *Physiol.Behav.*,**100(1)**:42-6.
55. Xia C, Tu Y, Yang Z, Jin Y and Xia H (2018) Antioxidant activity of essential oil of flowers of tea (*Camellia sinensis* L.) plants extracted by supercritical carbon dioxide, *J. Biomed. Sci.*, **7(3:10)**. <https://doi.org/10.4172/2254-609X.100089>
56. Xu RJ, Ye H, Sun Y, Tu YY and Zeng XX (2012) Preparation, preliminary characterization, antioxidant, hepatoprotective and antitumor activities of polysaccharides from the flower of tea plant (*Camellia sinensis*). *Food Chem. Toxicol.*, **50(7)**: 2473-2480. <https://doi.org/10.1016/j.fct.2011.10.047>.
57. Yang Z, Xu Y, Jie G, He P and Tu Y (2007) Study on the antioxidant activity of tea flowers (*Camellia sinensis*). *Asia Pac. J.Clin. Nutr.*,**16 (suppl 1)**: 148-152.
58. Yang ZY, Baldermann S and Watanabe N (2013) Recent studies of the volatile compounds in tea. *Food Res. Int.*, **53**: 585-599. <https://doi.org/10.1016/j.foodres.2013.02.011>
59. Yang ZY, Dong F, Baldermann S, Murata A, Tu YY, Asai T and Watanabe N (2012) Isolation and identification of spermidine derivatives in tea (*Camellia sinensis*) flowers and their distribution in floral organs. *J. Sci. Food Agric.*, **92(10)**: 2128-2132. <https://doi.org/10.1002/jsfa.5596>.
60. Yang ZY, Tu YY, Baldermann S, Dong F, Xu Y and Watanabe N (2009) Isolation and identification of compounds from the ethanolic extract of flowers of the tea (*Camellia sinensis*) plant and their contribution to the antioxidant capacity. *LWT Food Sci. Technol.*, **42**: 1439–1443. <https://doi.org/10.1016/J.LWT.2009.03.017>
61. Yang ZY, Xu Y, Jie GL, He PM and Tu YY (2007) Study on the antioxidant activity of tea flowers (*Camellia sinensis*). *Asia Pac. J. Clin. Nutr.*,**16**: 148–152. <https://doi.org/10.6133/APJCN.2007.16.S1.28>
62. Ye AH, Yu M, Zhu L, Jiang CJ, Wang ZX, Wei CL and Li YY (2008) Transcriptional profiling by cDNA-AFLP and its modified cDNA-AFLP technique reveals gene expression of tea (*Camellia sinensis*) during flower bud development stage. *Acta Laser Biology Sinica*, **17(6)**: 733-738.
63. Yoshikawa M, Wang T, Sugimoto S, Nakamura S, Nagatomo A, Matsuda H and Harima S (2008) Functional saponins in tea flower (Flower buds of *Camellia sinensis*): Gastroprotective and hypoglycemic effects of floratheasaponins and qualitative and quantitative analysis using HPLC. *Yakugaku Zasshi- J. Pharm. Soc. Jpn.*, **128(1)**: 141-151. <https://doi.org/10.1248/yakushi.128.141>.
64. Yu XL and He Y (2018) Optimization of tea-leaf saponins water extraction and relationships between their contents and tea (*Camellia sinensis*) tree varieties. *Food Sci.Nutr.*, **6**: 1734-1740. <https://doi.org/10.1002/fsn3.724>.
65. Zhang L, Ho CT, Zhou J, Santos JS, Armstrong L and Granato D (2019) Chemistry and biological activities of processed *Camellia sinensis* teas: A comprehensive review. *Compr. Rev. Food Sci. Food Saf.*,**18**: 1474-1495. <https://doi.org/10.1111/1541-4337.12479>.

66. Zhen Y (2002) *Tea: Bioactivity and Therapeutic Potential*; CRC Press, Taylor and Francis: New York, NY, USA, p. 58.
67. Zhou Y, Dong F, Kunimasa A, Zhang Y, Cheng S, Lu J, Zhang L, Murata A, Mayer F, Fleischmann P, Watanabe N and Yang Z (2014) Occurrence of glycosidically conjugated 1-phenylethanol and its hydrolase β -primeverosidase in tea (*Camellia sinensis*) flowers. *J. Agric. Food Chem.*, **62**: 8042–8050. <https://doi.org/10.1021/jf5022658>
68. Zhou Y, Zhang L, Gui JD, Dong F, Cheng S, Mei X, Zhang LY, Li YQ, Su XG, Baldermann S, Watanabe N and Yang Z (2015) Molecular cloning and characterization of a short chain dehydrogenase showing activity with volatile compounds isolated from *Camellia sinensis*. *Plant Mol. Biol. Rep.*, **33**: 253–263.

