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The science of matcha: Bioactive compounds, analytical techniques and biological properties

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Abstract

Background: The interest in the plant-derived healthy foods, nutraceuticals, functional foods and food supplements is increasing in recent times as potential agents in maintenance of health and the prevention and treatment of diseases. Matcha tea powder is obtained from the leaves of tea plant (*Camellia sinensis* (L.) Kuntze) grown under specific condition using about 90% shade. As compared to green tea, a hot water extract of tea leaves, matcha is consumed as a whole powder of leaves. Matcha powderis reported to have higher content of some bioactive components such as catechins, theanine and caffeine. In recent years, there is an increased market demand and consumption of matcha as a drink and as a component in various beverages, snacks and other food products.

Scope and approach: In this review, the available scientific information of the chemical constituents and their analysis and biological activities are critically analyzed. These results may help to understand current status of research on matcha and the gaps which help to guide future research related to evidence based product formulations.

Key findings and conclusions: Various studies have reported the difference in bioactive compounds in matcha as compared to green tea and other tea formulations. The content and composition were mostly affected by the cultivation and processing techniques. Analysis of marketed samples in various countries have shown the variable content of the bioactive compounds. Thus, there is a need for proper standardization for maintaining the quality. Matcha as a whole, its extract and compounds have shown promising biological activities in in vitro and animal studies. However, comparatively only a few clinical studies are performed, which need future attention. There should also be detailed study regarding matcha-containing foods' formulation.



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The Science of Matcha: Bioactive Compounds, Analytical Techniques and Biological Properties

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32 Abstract

33 *Background:* The interest in the plant-derived healthy foods, nutraceuticals, functional 34 foods and food supplements is increasing in recent times as potential agents in 35 maintenance of health and the prevention and treatment of diseases. Matcha tea powder 36 is obtained from the leaves of tea plant (Camellia sinensis (L.) Kuntze) grown under 37 specific condition using about 90% shade. As compared to green tea, a hot water extract 38 of tea leaves, matcha is consumed as a whole powder of leaves. Matcha powder is reported to have higher content of some bioactive components such as catechins, 39 40 theanine and caffeine. In recent years, there is an increased market demand and 41 consumption of matcha as a drink and as a component in various beverages, snacks and 42 other food products.

43 Scope and approach: In this review, the available scientific information of the chemical 44 constituents and their analysis and biological activities are critically analyzed. These 45 results may help to understand current status of research on matcha and the gaps which 46 help to guide future research related to evidence based product formulations.

47 Key findings and conclusions: Various studies have reported the difference in bioactive compounds in matcha as compared to green tea and other tea formulations. The content 48 49 and composition were mostly affected by the cultivation and processing techniques. 50 Analysis of marketed samples in various countries have shown the variable content of the bioactive compounds. Thus, there is a need for proper standardization for 51 52 maintaining the quality. Matcha as a whole, its extract and compounds have shown 53 promising biological activities in in vitro and animal studies. However, comparatively 54 only a few clinical studies are performed, which need future attention. There should also 55 be detailed study regarding matcha-containing foods' formulation.

56

57 Keywords: Matcha; green tea; *Camellia sinensis*; functional foods; market

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- 59

60 **1. Introduction**

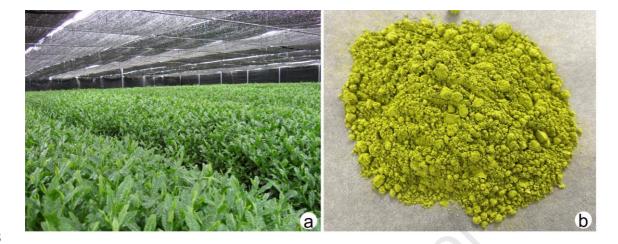
The interest in the plant-derived healthy foods, nutraceuticals, functional foods and food supplements is increasing in recent times as potential agents in maintenance of health and the prevention and treatment of diseases. Various phytochemicals including polyphenols present in tea, coffee, vegetables, legumes are gaining the importance (Ganesan & Xu, 2017; Petti & Scully, 2009; Pietta, 2000).

From ancient times, the young leaves of tea plant (Camellia sinensis (L.) Kuntze, Syn.: 66 67 Thea sinensis L., Theaceae) are used to prepare various tea formulations (Carloni et al., 68 2013; Y. Kim, Goodner, Park, Choi, & Talcott, 2011). Different reports suggest that the tea is second most consumed drink in the world after water (Hodgson & Croft, 2010; 69 70 Huang et al., 2018; Y. Kim et al., 2011; Weiss & Anderton, 2003) and its worldwide 71 market is growing day by day. Tea formulations are not only an important part of daily 72 cuisine but are also consumed for their health beneficial/functional effects of their high 73 content of polyphenols (e.g. catechins and phenolic acids), amino acids (eg. theanine, 74 gamma amino butyric acid) and caffeine (Devkota et al., 2021; Hodgson & Croft, 2010). 75

76 Commonly available tea formulations are divided based on their processing such as 77 non-fermented teas (e.g. white tea, green tea), partially fermented oolong tea and fully 78 fermented black tea (Zhao et al., 2011). For obtaining above mentioned teas, tea plants are cultivated in open field without using any artificial shade and for the preparation of 79 80 normal green tea, dried tea leaves are extracted with hot water. However, one more variety of tea which is gaining worldwide interest is the matcha tea powder. For 81 82 preparing matcha tea, the tea plants are grown under shade (about 90% shade) by 83 covering the cultivation areas/fields with different materials (Yuki Kurauchi et al., 84 2019) (Figure 1). Thus cultivated tea leaves are picked, washed, dried and then ground using stone mills to make powder known as matcha tea powder (Fujioka et al., 2016) 85

(Figure 1). The use of stone mills to make tea leaves powder is reported to have started 86 in the 11th century in China and later started in Japan in the 14th century, which supports 87 the long history of use of tea powders (Fujioka et al., 2016). For consuming matcha 88 89 powder, the whole powder is mixed with hot water so that the whole content of leaves are consumed not only the extract (Yuki Kurauchi et al., 2019; Weiss & Anderton, 90 91 2003). The chemical composition of the matcha powder is reported to be different of 92 that of green tea due to various factors such as cultivation conditions, processing and also the consumption of whole powder not the extract and most of these studies report 93 94 that the content of catechins, caffeine and theanine is higher in tea leaves cultivated for preparing matcha as compared to those for normal tea (Dietz, Dekker, & 95 Piqueras-Fiszman, 2017; Komes, Horžić, Belščak, Ganić, & Vulić, 2010; Yuki 96 97 Kurauchi et al., 2019; Weiss & Anderton, 2003). In recent years, there is constant 98 growth in the market and consumption of matcha not only as a drink but as a functional 99 component in other beverages, snacks, chocolates, ice creams and many others both in Japan and overseas (Kochman, Jakubczyk, Antoniewicz, Mruk, & Janda, 2020; 100 101 Kurauchi et al., 2019). Although used only in tradition ceremony in past years, it has 102 received great attention in recent years as an ingredient in drinks, beverages, snacks, 103 and many other products. In 2016, the global matcha market size was valued at USD 104 2.62 billion and it is expected to grow at a CAGR of 7.6% from 2017 to 2025. The 105 modern use of matcha in different products such as snacks started from Japan in now 106 spreading to many countries in Asia to Australia, Europe and America. 107 (https://www.grandviewresearch.com/industry-analysis/matcha-market).

With increasing market demand, it is important to understand the current scientific information related to chemical constituents, biological activities and advancement in formulation techniques of matcha powder. Thus, the main aims of this review are to compile and analyze the bioactive chemical constituents, their analytic techniques and biological activities.



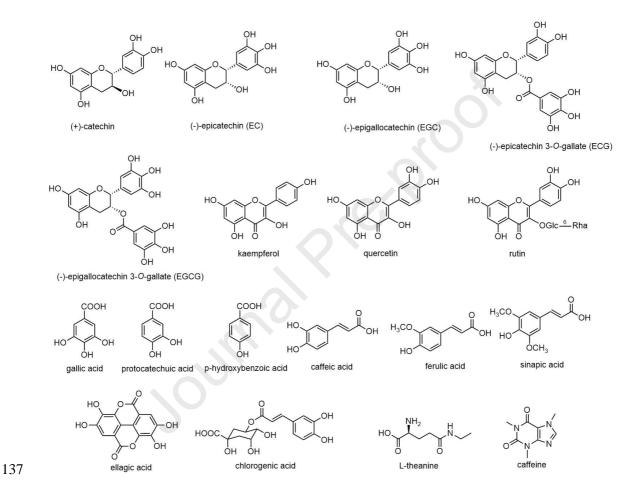
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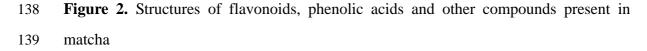
Figure 1. Photographs of Cultivation of tea plants for matcha using sun shades (a) and matcha powder (Photograph (a) is a courtesy of Aoiseicha Co., Ltd., Aichi, Japan and the matcha powder in photograph (b) was provided by AIYA Co. Ltd., Aichi, Japan)

117 **2. Bioactive chemical constituents and analytical techniques**

Since the first report of isolation and characterization of catechin from tea leaves by 118 119 Tsujimura in 1930 ((Tsujimura, 1930), there have been hundreds of studies related to 120 the chemical constituents an health beneficial activities of tea catechins (Devkota et al., 2021; Khan et al., 2019; Yeung et al., 2018). Flavonoids specially flavans, also known 121 122 as tea catechins, such as catechin, epicatechin (EC), epigallocatechin (ECG), 123 epicatechin 3-O-gallate (ECG) and epigallocatechin 3-O-gallate (EGCG) (Figure 2) 124 along with phenolic acids such as gallic acids are well known as bioactive compounds 125 of tea leaves (Carloni et al., 2013; Devkota et al., 2021). Apart from them, there are many other biologically important molecules such as flavonols (e.g. kaempferol, 126 quercetin, rutin), phenolic acids (protocatechuic acid, gallic acid, caffeic acid, sinapic 127 128 acid, ellagic acid, etc., amino acids (e.g. L-theanine, gamma amino butyric acid (GABA)), methyl xanthines (caffeine, theophylline, theobromine), chlorophyll and 129 130 volatile compounds (e.g. pentanal, heptanal, 2-butanone) (Ananingsih, Sharma, & Zhou, 2013; Carloni et al., 2013; Jakubczyk, Kochman, Kwiatkowska, Kałduńska, et al., 2020; 131 Y. Kim et al., 2011; Kochman, Jakubczyk, Antoniewicz, Mruk, & Janda, 2020; 132

Kurauchi et al., 2019; Zhao et al., 2011) (Figure 2). The composition of the bioactive compounds in tea leaves such as polyphenols, amino acids, caffeine are reported to be depending upon the cultivation condition, time of collection, processing and final formulations (Carloni et al., 2013; Y. Zhao et al., 2011).





Various studies have reported the chemical constituents of matcha collected from
different places and their differences in chemical constituents and biological activities
(Goto, Horie, Ozeki, Masuda, & Warashina, 1994; Kenjiro, Hirotsugu, & Toyomasa,
1984; Koláčková, Kolofiková, et al., 2020). The contents of these bioactive compounds
in match tea powders vary greatly among the commercial samples available in market.

These contents also vary accordingly to the extraction procedures for these bioactive compounds. Use of hydroalcoholic solvents or water for extraction and the temperature of extraction condition and the duration of extraction time greatly affects the contents of these bioactive compounds (Koláčková et al., 2020; Komes et al., 2010). The detailed list of the bioactive compounds such as flavonoids (including catechins), methylxanthines and phenolic acids are provided in Table 1, 2 and 3, respectively.

151 The qualities of matcha available in Japanese market in relation to their prices have 152 been studied. Ikegaya, Takayanagi, & Anan (1984) reported that the contents of total 153 nitrogen, caffeine, free amino acids and L-theanine decreased along with decreasing prices. The contents of caffeine and catechins were reported to be 3.23-3.85% and 154 155 8.85-9.75%, respectively. Goto, Horie, Ozeki, Masuda, & Warashina (1994) compared 156 the qualities of various tea products available in Japanese markets and regarding the 157 matcha samples the contents of total amino acids, tannins and caffeine were reported to 158 3.50-5.50%, 7.83-10.57% and 2.93-3.29%, respectively. Ikeda (2002) also compared 159 the qualities and the marketing prices of total 25 commercial matcha powers available 160 in Japanese market and reported that the content of total tannins varied from 6.99% to 161 9.83%. Recently, Unno et al. (2018) analyzed the contents of theanine, amino acids, 162 GABA, catechins, caffeine and catechins in 76 matcha samples sold in Japanese market 163 and 67 matcha sample sold in market outside of Japan. The contents of each component 164 largely varied from one sample to another in samples from both markets. For example, 165 the content of L-theanine was reported to be in the range from 7.25 to 40.62 mg/g in samples from Japanese market and in the range from 0.32 to 27.09 mg/g in samples 166 167 from the markets outside of Japan. The contents of amino acids and GABA also varied 168 in similar pattern. The details of the contents of catechins and caffeine in these samples are given in Table 1 and 2, respectively. 169

Koláčková, Kolofiková, et al. (2020) analyzed the nutritional composition, phenolic
compounds and antioxidant activities of the water extract and 80% MeOH extract of 12

172 different matcha products available in Czech republic market using high performance 173 liquid chromatography (HPLC) and other methods. There was variation in the contents 174 of flavonoids (Table 1), caffeine (Table 2) and phenolic acids (Table 3). Caffeine 175 content was found to be 14.4-34.1 mg/g. Total phenolic content and flavonoid contents 176 were 169-273 mg gallic acid equivalents/g and 99-139 mg rutin equivalents/g, 177 respectively which were higher as compared to water extracts. Their antioxidative 178 activities also varied accordingly. Another recent study reported that matcha contains 179 44.8 mg/L of vitamin C, 1968.8 mg/L of flavonoids, and 1765.1 mg/L of polyphenols in an infusion made with 1.75 g of plant material in 100 mL of water (Jakubczyk, 180 181 Kochman, Kwiatkowska, Kałdunska, et al., 2020).

Table 1. Contents of catechins and other flavonoids in various matcha tea powdersamples

Bioactive	Analysis	Samples	Contents	Reference
compound	method			
classes/individual				
compounds				
Catechin	Micellar	Commercially	0.83 mg/g of dry	(Weiss &
	electrokinetic	available	leaf (MeOH	Anderton,
	chromatography	sample in US	extract)	2003)
		market	Not detected (water	
			extract)	
	HPLC	Commercially	6.62 mg/L of water	(Komes et al.,
		available	extract (80°C)	2010)
		sample in		
		Croatia market		
Epicatechin	Micellar	Commercially	4.0 mg/g of dry	(Weiss &
	electrokinetic	available	leaf (MeOH	Anderton,
	chromatography	sample in US	extract)	2003)
		market	2.4 mg/g of dry	

			leaf (water extract)	
	HPLC	Commercially	120.7 mg/L of	(Komes et al.,
		available	water extract (80°	2010)
		sample in	C)	
		Croatia market		
	HPLC	Commercial	0.87-10.75 mg/g	(Unno et al.,
		samples from		2018)
		Japanese		
		market	Å	
	HPLC	Commercial	0.18-15.40 mg/g	(Unno et al.,
		samples from		2018)
		markets	0	
		outside of		
		Japan		
Epigallocatechin	Micellar	Commercially	12.6 mg/g of dry	(Weiss &
	electrokinetic	available	leaf (MeOH	Anderton,
	chromatography	sample in US	extract)	2003)
		market	0.75 mg/g of dry	
			leaf (water extract)	
	HPLC	Commercially	324.88 mg/L of	(Komes et al.,
		available	water extract (80°	2010)
		sample in	C)	
		Croatia market		
	HPLC	Commercial	4.09-44.43 mg/g	(Unno et al.,
		samples from		2018)
		Japanese		
		market		
	HPLC	Commercial	1.86-55.22 mg/g	(Unno et al.,
		samples from		2018)
		market outside		
		of Japan		
Epicatechin	Micellar	Commercially	12.8 mg/g of dry	(Weiss &

3-O-gallate	electrokinetic	available	leaf (MeOH	Anderton,
	chromatography	sample in US	extract)	2003)
		market	0.25 mg/g of dry	ŕ
			leaf (water extract)	
	HPLC	Commercially	102.67 mg/L of	(Komes et al.,
		available	water extract (80°	2010)
		sample in	C)	
		Croatia market	6	
	HPLC	Commercial	8.58-16.37 mg/g	(Unno et al.,
		samples from		2018)
		Japanese		
		market	\mathbf{O}	
	HPLC	Commercial	0.63-27.96 mg/g	(Unno et al.,
		samples from		2018)
		market outside		
		of Japan		
Epigallocatechin	Micellar	Commercially	57.4 mg/g of dry	(Weiss &
3-O-gallate	electrokinetic	available	leaf (MeOH	Anderton,
	chromatography	sample in US	extract)	2003)
	0	market	0.32 mg/g of dry	
			leaf (water extract)	
	HPLC	Commercially	345.55 mg/L of	(Komes et al.,
		available	water extract (80°	2010)
		sample in	C)	
		Croatia market		
	HPLC	Commercial	13.15-86.76 mg/g	(Unno et al.,
		samples from		2018)
		Japanese		
		market		
	HPLC	Commercial	3.02-88.23 mg/g	(Unno et al.,
		samples from		2018)

		market outside		
		of Japan		
Gallocatechin	HPLC	Commercially	70.49 mg/L of	(Komes et al.,
		available	water extract (80°	2010)
		sample in	C)	
		Croatia market		
Gallocatechin	HPLC	Commercially	6.62 mg/L of water	(Komes et al.,
3-O-gallate		available	extract (80°C)	2010)
		sample in		
		Croatia market		
Kaempferol	HPLC	Commercially	4.2-20.4 µg/g mg/g	(Koláčková,
		available	of weight (80%	Kolofiková, et
		samples in	MeOH extract)	al. 2020)
		Czech	1.7-16.2 μg/g of	
		Republic	weight (water	
		market	extract)	
Quercetin	HPLC	Commercially	8.4-17.2 μg/g mg/g	(Koláčková,
		available	of weight (80%	Kolofiková, et
		samples in	MeOH extract)	al. 2020)
	\sim	Czech	10.5-84.9 µg/g of	
		Republic	weight (water	
		market	extract)	
Rutin	HPLC	Commercially	570-2870 μg/g of	(Koláčková,
		available	weight (80%	Kolofiková, et
		samples in	MeOH extract)	al. 2020)
		Czech	361-1590 μg/g of	
		Republic	weight (water	
		market	extract)	

Table 2. Contents of methylxanthines including caffeine in various matcha tea powdersamples

Bioactive	Analysis method	Samples	Contents	Reference
compound				
classes/individual				
compounds				
Total methylxanthines	HPLC	Commercially available sample in Croatia market	322.30 mg/L of water extract (80°C)	(Komes et al., 2010)
Caffeine	Micellar electrokinetic chromatography	Commercially available sample in US market	6.4 mg/g of dry leaf (MeOH extract)	(Weiss & Anderton, 2003)
		R	23.9 mg/g of dry leaf (water extract)	
	HPLC	Commercially available sample in Croatia market	300.00 mg/L of water extract (80°C)	(Komes et al., 2010)
	HPLC	Commercial samples from Japanese market	5.95-50.16 mg/g	(Unno et al., 2018)
	HPLC	Commercial samples from market outside of Japan	0.85-40.96 mg/g	(Unno et al., 2018)
	HPLC	Commercially available samples in Czech Republic	14.4-34.1 mg/g	(Koláčková, Kolofiková, et al. 2020)

		market		
Theobromine	HPLC	Commercially	12.18 mg/L of	(Komes et al.,
		available sample	water extract	2010)
		in Croatia	(80°C)	
		market		
Theophyline	HPLC	Commercially	10.12 mg/L of	(Komes et al.,
		available sample	water extract	2010)
		in Croatia	(80°C)	
		market		

187

- 188 **Table 3.** Contents of phenolic acids ($\mu g/g$ of dry powder) of various matcha tea powder
- 189 samples commercially available samples in Czech Republic market as analyzed by
- 190 HPLC (Koláčková, Kolofiková, et al. 2020)

Phenolic acid	80% MeOH extract	Water extract
Gallic acid	45.4-423.0	39.4-184.0
Protocatechuic acid	54.4-299.0	46.1-291.0
p-Hydroxybenzoic	9.7-243.0	13.5-167.0
acid		
Chlorogenic acid	3040-4800	2640-3920
Caffeic acid	13.3-223.0	9.3-78.3
Ferulic acid	45.5-289.0	5.6-171.0
Sinapic acid	104.0-1400	89.0-592.0
Ellagic acid	38.1-371.0	79.4-246.0

From above data, it is relevant that is very difficult to generalize the contents of bioactive compounds in marketed samples of matcha. There should be more proper guidelines and standardization methods to evaluate the chemical composition and their respective biological activities.

195 There have also been attempts to develop new analytic methods for the determination of 196 bioactive components in green tea samples including matcha. Weiss and Anderton 197 analyzed the contents of catechins and caffeine in methanol (MeOH) and water extracts 198 of commercially available match in US market by using micellar electrokinetic 199 chromatography (Weiss & Anderton, 2003). Content of caffeine was very high in water 200 extract as compared to MeOH extract (Table 2) but the content of catechins was very 201 low in water extract (Table 1). Similarly, the content of EGCG while drinking matcha 202 would be around at least 3 times higher as compared to other green tea samples. Guo et 203 al. (2021) et al. reported near infrared (NIR) based methods for the quantification of 204 various constituents such as polyphenols, aminoacids, etc. in matcha powder. Similarly, 205 Ouyang, Wang, Park, Kang, & Chen (2021) also reported visible-near infrared 206 hyperspectral imaging technology for the analysis of components in matcha. Baba, Amano, Wada, & Kumazawa (2017) developed gas chromatography-olfactometric 207 208 techniques for the determination of volatile components and odorants responsible for 209 the characteristic aroma of matcha.

Traditionally, stone mills are used to prepare matcha powder from tea leaves. Recently, Fujioka et al. (2016) et al. reported that the use of ceramic mills to make powders can enhance the catechin extraction from the powder and also the extract obtained from such powder had stronger antioxidant activity.

214 **3. Biological activities of matcha**

Bioactive components present in tea leaves such as catechins, phenolic acids, amino acids, vitamins and caffeine are reported to be beneficial for human health having various biological activities such as chemopreventive, antioxidant and mood enhancing activities (de Souza, Gambeta, Stern, & Zanoveli, 2018; Higashiyama, Htay, Ozeki, Juneja, & Kapoor, 2011; Kurauchi et al., 2019; Pervin et al., 2018; Schröder et al., 2019; Smith, 2002; Unno et al., 2019). On the other hand, as matcha powder contains all components of the tea leaves, it is suggested to have improved biological activities as 222 compared to green tea (water extract) (Bonuccelli, Sotgia, & Lisanti, 2018; Dietz et al., 223 2017; Koláčková et al., 2020; Yuki Kurauchi et al., 2019; Pervin et al., 2018; Weiss & 224 Anderton, 2003). Matcha has been first identified and employed to treat diverse human 225 ailments in Japan, in the form of matcha green tea. Biological activities of matcha are 226 closely associated with its bioactive phytochemicals such as caffeine, tannins and other polyphenols. Due to the presence of bioactive polyphenols and vitamin C, matcha could 227 have crucial biological effects. In the following sections, the reported biological 228 activities of matcha based on recently reported scientific evidences are discussed in 229 230 detail.

231

232 **3.1. Antioxidant activities**

Matcha infusion possess strong antioxidant properties because catechins and 233 234 polyphenols compounds in the matcha are previously reported to have strong antioxidant effects (Koláčková et al., 2020; Zhao, Li, Liu, & Yang, 2014). Matcha tea 235 236 infusion showed potent free radical scavenging activity as evidenced by increased 237 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity and ferric ion reducing antioxidant power. The antioxidant effects of matcha was influenced by the 238 temperature as matcha infusion at higher temperature showed better antioxidant 239 properties than lower temperature infusion (Jakubczyk, Kochman, Kwiatkowska, 240 Kałdunska, et al., 2020). Therefore, consumption of matcha in the form of matcha tea 241 242 (green tea) could results increased antioxidant efficacy. Another study also confirms the antioxidant of DPPH 243 potential matcha employing and 244 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging assays (Kim et al., 2020). This study also reports that storing matcha in higher 245 temperature for long period leads to the reduced free radical scavenging activity. 246 247 catechins, crucial bioactive compound in matcha, could also leads to the profound 248 antioxidant activity of matcha (Henning et al., 2003).

249

250 **3.2. Anxiolytic and stress-reducing activities**

251 Drinking tea is a mood freshener suggesting that matcha can counter anxiety. In fact, a 252 recent experimental study revealed the anxiolytic properties of matcha (Kurauchi et al., 253 2019). Matcha tea powder significantly increased the time-duration and distance 254 travelled in open arm maze indicating that matcha can improve open field behavior of 255 mice. These open-field behaviors of anxious-mice were also improved by different organic solvent extract fractions of matcha. The possible mechanism of action for the 256 anxiolytic activity of matcha was associated with the activation of dopaminergic and 257 258 serotonergic neurotransmission system, because the anxiolytic activity of matcha was abolished when these signaling were blocked by using specific inhibitors. Matcha 259 260 extract also increased overall locomotor actions in mice. Another study also reported 261 that continued ingestion of high-quality matcha reduced the anxiety-like behavior in 262 psychologically and physiologically stressed mice (Monobe et al., 2019). It was notable 263 that sencha, a common Japanese green tea, did not demonstrate anxiolytic effect in this 264 model mice. The anxiolytic effects was expected by the higher molar ratio of (caffeine + 265 EGCG)/(theanine + arginine) in the Matcha.

266

Supporting the anxiolytic effect of matcha another independent study revealed the stress 267 268 relieving ability of matcha green tea on animals as well as in clinical trials. Consumption of matcha tea lowered adrenal hypertrophy, which is an important stress 269 270 marker. In addition, consumption of matcha enriched cookies significantly inhibited the 271 stress marker salivary α -amylase activity in comparison to the placebo group. These 272 findings clearly revealed that consumption of matcha either in the form of drink or 273 beverages or cookies can reduce the impact of stress (Unno et al., 2019). Presence of 274 threonine was reported to be responsible for the stress reducing effect of matcha. The 275 anxiolytic or stress reducing effects of matcha was influenced by the presence of 276 caffeine and epigallocatechin gallate (EGCG) in the matcha. Higher amount of threonine and the lesser ration of caffeine, EGCG, and arginine against theanine isnoticed to have high beneficial effect of matcha (Unno et al., 2018).

279

280 **3.3. Matcha as memory and cognitive function booster**

281 Biological roles of matcha in central nervous system is also associated with cognition and memory. Besides its stress reducing ability, matcha was also evaluated for the 282 improvement of cognitive function. Presence of active ingredient such as 283 epigallocatechin gallate, theanine, and caffeine was responsible for the cognition 284 285 improvement functions of matcha (Dietz et al., 2017). A recent study reported that consumption of matcha tea partially improved the cognitive performance of matcha tea 286 287 in human participants, however, matcha tea consumption significantly improved the 288 memory and task measuring dimension of attention. In addition, participants consuming 289 matcha showed a better episodic secondary memory and speed of attention (Dietz et al., 290 2017) indicating that matcha can boost cognition and memory in humans. Therefore, 291 matcha could be an appealing therapeutic strategy in dementia and Alzheimer's disease, 292 where improving memory can lead to the significant social benefits in patients.

293

294 **3.4. Neuroprotective activities**

295 Matcha could have possible neuroprotective effects because of its antioxidant, 296 anxiolytic, stress reducing, and memory boosting properties. Indeed, a few previous 297 studies reported the neuroprotective effects of matcha and its bioactive phytochemicals. 298 Theanine and catechins showed neuroprotective effects against neuronal cell death. 299 Theanine inhibited the glutamic acid-induced cortical neuronal death in rats. Theanine 300 administration through ventricular route also protected the hippocampal CA1 pyramidal 301 neurons and CA3 neurons against transient forebrain ischemia-induced neuronal death 302 in gerbils. Alteration of the glutamate receptor functioning and glutamate transporter 303 by theanine was mechanistically associated with the neuroprotective effect of theanine. 304 In addition, theanine mediated prevention of oxidative alteration of LDL together with

305 its antioxidant effects is responsible to lower the prevalence and complication of 306 atherosclerosis which is a reason behind the protection against ischemic cerebrovascular 307 diseases (Kakuda, 2002). Beneficial effect of green tea against Alzheimer's disease 308 (AD) and Parkinson's disease (PD) pathogenesis has also been reported previously. 309 Catechins from matcha was reported to have supportive neuroprotective effects through lowering inflammation, oxidative stress, apoptosis, and fibrous protein (AB and 310 311 a-synuclein) accumulation. Catechins, more specifically EGCG, prevented fibrous 312 protein accumulation suggesting its possible role in prevention as well as management 313 of the AD and PD complications (Pervin et al., 2018). Neuroinflammation is a key 314 mechanism responsible for the neuronal toxicity and neurodegeneration in most of the 315 neurological complications (Adhikari-Devkota et al., 2019; Collins, Toulouse, Connor, 316 & Nolan, 2012; Cui et al., 2010; Williams & Spencer, 2012). Treatment of EGCG was 317 reported to inhibit the neuroinflammation by lowering the level of inflammatory mediators and their responsible pathways. EGCG inhibited the production of 318 319 proinflammatory cytokines like TNF- α , IL-6, and IL-1 β in palmitic acid- activated 320 microglia together with inhibition of lipid accumulation. All these anti-inflammatory functions of EGCG was possible through the inhibition of the JAK2/STAT3 signaling 321 322 pathway in microglia following EGCG treatment (Mao et al., 2019; Ohishi, Goto, 323 Monira, Isemura, & Nakamura, 2016).

324

325 **3.5. Anti-angiogenic and anti-inflammatory activities**

326 Catechins from matcha such as EGCG were reported to inhibit or alter the neutrophil 327 function by lowering the amount of neutrophil elastase. Neutrophil though is involved 328 in hosting immune response; it also plays a critical inflammatory role in several acute 329 and chronic inflammatory conditions. Inhibition of neutrophil elastase by Matcha green 330 tea and EGCG might be responsible for the anti-inflammatory effect of matcha. A recent 331 report suggested that even the 3 μ M concentration of EGCG lowered the apoptosis and 332 the subsequent cell death mediated by activated neutrophils (Donà et al., 2003). Green

tea catechins EGCG also pronouncedly reduced neutrophil chemotaxis that has been 333 334 induced by chemokines or inflammatory mediators. Additionally, EGCG as well as the 335 green tea extract significantly inhibited angiogenesis in inflammatory angiogenesis 336 model, especially neutrophil induced angiogenesis in in vivo conditions. Interestingly, 337 inhibition of angiogenesis, fibrosis and inflammation in the pulmonary system is reported following EGCG treatment. This study revealed the strong anti-inflammatory, 338 339 anti-fibrosis, or antiangiogenic potential of green tea itself as well as its active 340 catechins.

341

342 **3.6. Regulating blood glucose and microbiota by matcha**

343 Phytochemicals presents in matcha green tea were reported to be involved in the 344 digestion or metabolism of food products. EGG present in green tea inhibited the 345 digestion of the starch suggesting their possible role in altering the functions of gut 346 microbiota and to prevent the sudden increase of glucose level in patients (Forester, Gu, 347 & Lambert, 2012). Another study supported this result by reporting that consumption of 348 matcha in a supplement was useful for controlling the gut microbiota and blood glucose 349 level. Altogether, consumption of green tea can be considered as a beneficial functional 350 food for hyperglycemic/diabetic patients (Zhang et al., 2020)

351

352 **3.7.** Activities related to anti-obesity and other metabolic diseases

353 The effect of matcha on different physiological parameters of diabetic patients, including oxidative stress, metabolic conditions, and anthropometric conditions were 354 355 reported in a recent study, which revealed that drinking matcha tea 4 times a day 356 significantly lowered the systolic blood pressure, body weight, body mass index, and waist circumference. Altogether, consumption of green tea can help in maintaining 357 358 blood pressure, body weight, and body mass index (Mousavi, Vafa, Neyestani, 359 Khamseh, & Hoseini, 2013). Presence of antioxidant and biologically active catechins 360 make matcha tea this an important functional food. Matcha green tea powder (MGTP)

enriched bread biscuit was developed to study the consumer acceptability and health
benefits. However, biscuits with low MGTP did not significantly affect the postprandial
effect in comparison to the non-enriched one was well its well acceptability in the
consumers. The reason could be because of very less amount of phytochemicals in low
MGTP biscuits (Phongnarisorn, Orfila, Holmes, & Marshall, 2018).

366

Catechins from matcha green tea are reported to reduce total cholesterols as well as its 367 absorption which were associated with less cardiac abnormalities in experimental rats 368 369 consuming high cholesterol food. Rats were treated with EGCG in different 370 concentration to evaluate their effects on attenuating cholesterol, lipids and glucose. 371 Plasma level of low-density lipoproteins and total amount of cholesterol were 372 dramatically attenuated upon EGCG administration. Additionally, animals administered 373 oral dose of EGCG showed less cholesterol absorption (Raederstorff, Schlachter, Elste, 374 & Weber, 2003). Another study also supported these findings as feeding of matcha in 375 high-fat diet administered mice significantly lowered the serum level of triglycerides 376 and cholesterol. Additionally, matcha also decreased the level of low-density lipoprotein 377 and increased high density lipoproteins. In addition, matcha can downregulate the lipid 378 and glucose level in mice (Xu, Ying, Hong, & Wang, 2016). Therefore, consumption of 379 matcha can lead to the attenuation of bad cholesterol, which indicate that matcha could 380 have potential health benefit to counter cardiovascular diseases associated with 381 increased cholesterol and lipids.

382

Exercise-induced fat oxidation was reported to be increased by the consumption of caffeine, EGG and catechin which are the key ingredients of Matcha. Consumption of 1 g of Matcha in different drinks by female participants caused the increased fat oxidation, which was associated with balancing metabolic effects (Willems, Şahin, & Cook, 2018). Another study also reported that green tea consumption can boost the fat oxidation in resting as well as in post exercise (Gahreman, Wang, Boutcher, & Boutcher, 2015). In

addition, similar results were observed by other research groups, in which the expression of fat metabolism genes and adipogenesis genes were altered upon matcha consumption. Matcha increased fat metabolism genes in skeletal muscle and decreased adipogenesis genes in the liver, which was possibly mediated through PPAR- γ coactivator 1- α and PPARs activation was responsible for the increase fat oxidation in rest as well as exercise induced state (Gahreman et al., 2015).

395

In another study, Zhou et al. (2020) reported the anti-obesity activity of matcha in 396 397 experimental high-fat diet-induced obese mouse model. Authors reported that the supplementation of matcha in diet decreased the weight gain, fat accumulation and the 398 399 levels of sugar and fats in blood. The ethanolic extract of matcha also showed the potent 400 anti-inflammatory activity in palmitic acid-induced inflammation of microglial BV-2 401 cells through inhibition of the release of inflammatory cytokines and the expression and phosphorylation of JAK2 and STAT3. Matcha is also reported to prevent the 402 403 accumulation of visceral and hepatic lipid, increase in blood glucose level, abnormal 404 liver function and steatosis hepatitis in high fat diet fed male C57BL/6 mice (Zhou, Yu, 405 Ding, Xu, & Wang, 2021). The anti-obesity activity of green tea powder along with 406 three major components i.e. catechins, caffeine and L-theanine is also reported using 407 female ICR mice model (Zheng, Sayama, Okubo, Juneja, & Oguni, 2004)

408

409 **3.8. Hepatoprotective and renoprotective activities**

Potential effects of matcha on protecting liver and kidney was also reported. EGCG which is an active ingredient of matcha was reported to protect kidney damage that has been induced in the diabetic nephropathy in rats. Oral administration of different concentrations of matcha in diabetic rats reduced the progression of liver and kidney damage in rats with type-2 diabetes. Matcha lowered the formation of different toxic AGEs including N(6)-(carboxylethyl)lysine (CEL) and N(6)-(carboxylmethyl)lysine (CML), inhibition of the expression of AGEs receptor further validated the protective 417 effect of matcha from AGEs and their downstream pathways signaling that critically play a key role. Additionally, matcha treatment significantly lowered the level of total 418 419 cholesterol, triglyceride, and glucose in the serum and liver which not only enhanced 420 the liver protection but also helped to boost overall cardiovascular system. AGEs and 421 their reactive products accumulation are the key players in the renal toxicity following 422 Type 2 DM and inhibition of this important cascade undoubtedly can help renal system 423 to be protected from DM mediated toxicities. Altogether, administration of matcha might play a critical role in the protection of hepatic, renal, and cardiovascular system 424 425 against type 2 diabetes complications (Yamabe, Kang, Hur, & Yokozawa, 2009).

426

427 **3.9. Anti-arthritic activity**

428 Prevention of collagen-induced arthritis in mice by a polyphenolic fraction from green 429 tea has been reported recently (Haqqi et al., 1999). Polyphenolic fractions of green tea 430 (GTP) showed strong anti-arthritic effect against collagen-induced arthritis in animals. 431 Polyphenols from green tea was supplied to animals orally, which inhibited the incident 432 of arthritis almost 50 to 70% in comparison to the control group. GTP also lowered the 433 arthritis index and significantly inhibited inflammatory markers including TNF- α , 434 interferon gamma, and cyclooxygenase in comparison to the control group animals 435 indicating that its anti-inflammatory effects were associated with the reduction of 436 arthritis index. In addition, fewer inflammatory cells infiltration was observed upon 437 GTP-administration. Treatment of animal with GTP showed dramatic reduction in endopeptidase activity indicating matcha as a potential natural remedy for arthritis 438 439 (Haqqi et al., 1999).

440

441 **3.10. Other biological activities**

442 Catechins like EGCG showed a strong antibacterial effect. It also helps to reverse the 443 tetracycline resistance by certain bacteria. EGCG stopped the bacterial growth and 444 invasion indicating the potential antibacterial effect of matcha (Roccaro, Blanco,

Giuliano, Rusciano, & Enea, 2004). In addition, catechins present in green tea were
suggested to have strong antiviral potency because they inhibit the proteases which are
involved in the pathogenesis viral infection like influenza and human adeno virus.
EGCG significantly lowered the viral infection by blocking the viral replication. (Song,
Lee, & Seong, 2005). Oral care product containing matcha lowered the accumulation of
dental plaque. Prevalence of gingivitis and calculus was also reduced notably upon
matcha consumption (Lindinger, 2016)

452

453 **4. Conclusion and future prospects**

454 Matcha, powder of specially cultivated tea leaves, is gaining popularity in functional 455 food and other food markets all over the world. Various studies have reported the 456 difference in bioactive compounds in matcha as compared to green tea and other tea formulations. The content and composition were reported to be affected by the 457 cultivation and processing techniques. Analysis of marketed samples in various 458 459 countries have shown the variable content of the bioactive compounds and it is very 460 difficult to generalize the contents of bioactive compounds in marketed samples of matcha. There should be more proper guidelines and standardization methods to 461 462 evaluate the chemical composition and their respective biological activities. Matcha as a 463 whole, its extract and compounds have shown promising biological activities in in vitro and animal studies. However, comparatively only a few clinical studies are performed, 464 465 which need future attention. The pharmacokinetic studies related to the components in matcha, their synergistic or antagonistic activities with therapeutic agents should be 466 studied in detail. There should also be detailed research regarding formulation of 467 468 matcha-containing foods.

469

470

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475

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470	runung

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479

480 **Conflict of interest**

K.H. is an employee of Astellas Pharma Inc., Japan. The authors declare that they have
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484

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709 Figure legends

- Figure 1. Photographs of Cultivation of tea plants for matcha using sun shades (a) and
 matcha powder (Photograph (a) is a courtesy of Aoiseicha Co., Ltd., Aichi, Japan and
- the matcha powder in photograph (b) was provided by AIYA Co. Ltd., Aichi, Japan)

Figure 2. Structures of flavonoids, phenolic acids and other compounds present in
matcha

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Highlights

- \bullet Matcha tea powder is obtained from the leaves of tea plant grown under specific condition.
- The food market demand of matcha is increasing rapidly in recent years.
- Catechins, phenolic compounds, amino acids, and caffeine are major components
- Studies have shown its antioxidant, antianxiety and anti-stress activities among others

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