

Characterization and antimicrobial properties of Matcha green tea

Milad Edraki^{a,b}, Issa Mousazadeh Moghaddampour^b, Mohammad Banimahd Keivani^c, Milad Sheydaei^{d,e,*}

^aPolymer Department, Technical Faculty, South Tehran Branch, Islamic Azad University, Tehran, Iran

^bDepartment of Chemistry, Technical and Vocational University (TVU), Lahijan, Iran

^cDepartment of Chemistry, Payame Noor University (PNU), P.O. Box 19395-4697, Tehran, Iran

^dFaculty of Polymer Engineering, Sahand University of Technology, P.O. Box 51335-1996, Tabriz, Iran

^eInstitute of Polymeric Materials, Sahand University of Technology, P.O. Box 51335-1996, Tabriz, Iran

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ABSTRACT

Matcha, made from the finely ground powder of green tea leaves, is used as a nutritious food ingredient because of its unique properties. In this study, Matcha was characterized using Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction analysis (XRD), energy-dispersive X-ray spectroscopy (EDX). Also, the antimicrobial properties of Matcha against 8 types of bacteria, 1 type of fungus, and 1 type of yeast were investigated. The results showed that Matcha has a completely amorphous structure and has a high content of carbon and oxygen. The results of antibacterial tests showed that Matcha has the ability to inhibit gram-positive and gram-negative bacteria as well as yeast, but has no effect on the fungus. Also, Matcha has a greater effect on gram-positive bacteria, which is due to the simple and reasonably porous cell wall of these bacteria. According to the results, the maximum and minimum inhibition zones created by Matcha belonged to *Pseudomonas aeruginosa* and *Escherichia coli*, respectively.

1. Introduction

Over the time drug resistance of many gram-positive, gram-negative bacteria and fungi has increased and has become one of the global public health challenges [1-6]. Every year, more than one billion people worldwide get fungal infections one of the most common of which is *Aspergillus* [7-9]. Gram-negative bacteria are much more resistant to drugs than gram-positive bacteria, and research has shown that two-thirds of bacterial deaths in Europe are due to gram-negative infections [10-15]. Environments with high population densities such as hospitals, subway and bus stations are major candidates for the cyclical transmission of bacteria and fungi [16-22]. Over the years there have been extensive efforts to obtain antimicrobial materials that among these which plants have a special place because due to their non-toxicity, they can be used in various fields such as medical equipment and food packaging [23-35]. Tea is one of the most popular and widely-consumed beverages, which in many societies second to water, mostly because distinctive flavour and aroma [36]. Matcha (*Camellia sinensis*) is a type of tea that originates from Japan and has amazing properties [37]. Matcha has potentially antioxidant, anti-viral and

anti-inflammatory function properties due to its high content of polyphenols, amino acids and caffeine [38,39]. Matcha can reduce of blood glucose and total cholesterol levels and also play a role in reducing stress [40]. In addition to using Matcha as a beverage, it has other uses as well. Research has shown that the use of Matcha in cooking rice noodles reduces cooking loss, thereby inncreasing chewability and stretchability. It also gives rice noodles a unique color and flavor and higher antioxidant capacity [41]. Moreover, Matcha phenolic compounds can stabilize the quality of rice cakes during long-term storage [42]. In this research, Matcha were investigated using FTIR, XRD, and EDX techniques. Also, the antimicrobial properties of Matcha against 4 types of gram-positive bacteria, 4 types of gram-negative bacteria, one type of yeast, and one type of fungus were investigated.

2. Results and Discussion

Figure 1 showed the FT-IR spectra of Matcha. Peaks at around 486, 1020, 1270, 1631, 2905-2954, and 3311 cm^{-1} are corresponding to the COOH, C-O-C vibrations, -C-OH bending, -OH bending and stretching vibrations, C-H stretching vibration, and -OH stretching vibration,

* Corresponding author. Tel.: +989211868265; e-mail: mi_sheydaei@sut.ac.ir, m.sheydaei@yahoo.com

respectively [43-52]. The peaks at 1143 and 1375-1523 cm^{-1} are related to the C=O vibrations [52].

flavonoid, and theanine [36,43]. All Matcha compounds are high in oxygen and carbon [43].

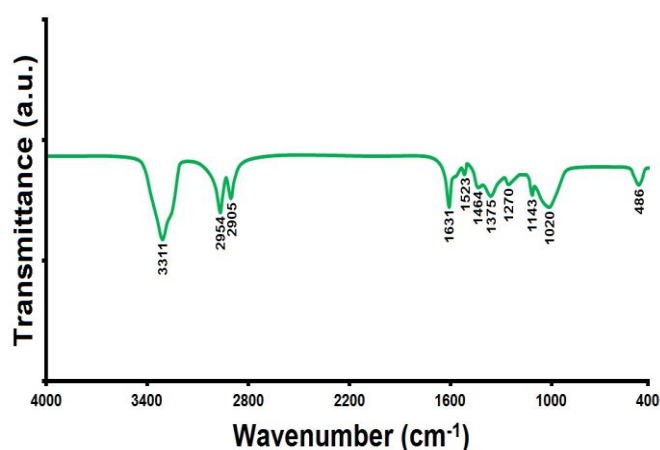


Figure 1. FT-IR pattern of Matcha

XRD pattern of Matcha are shown in Figure 2. As can be seen, Matcha has a completely amorphous structure. Research has shown that Matcha in combination with starch can reduce its crystallinity [41]. Matcha polyphenolic compounds can form hydrogen bonds with starch chains and prevent the formation of double helices of amylose and amylopectin [42].

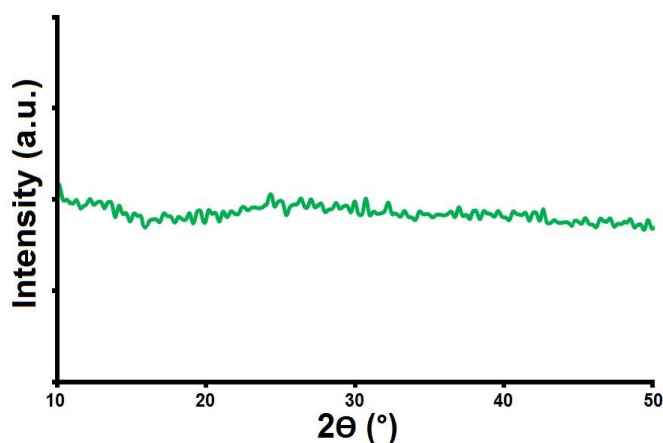


Figure 2. XRD pattern of Matcha

Figure 3 showed EDX analysis of Matcha. As can be seen, more than 80 wt.% of Matcha contains carbon and oxygen. Matcha has many chemical compounds, but a wide range of compounds consists of four main catechins (epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate) that originate from a type of phenolic compound [36]. Other chemical compounds in Matcha include caffeine, phenolic acids, rutin, quercetin, vitamin C, chlorophyll,

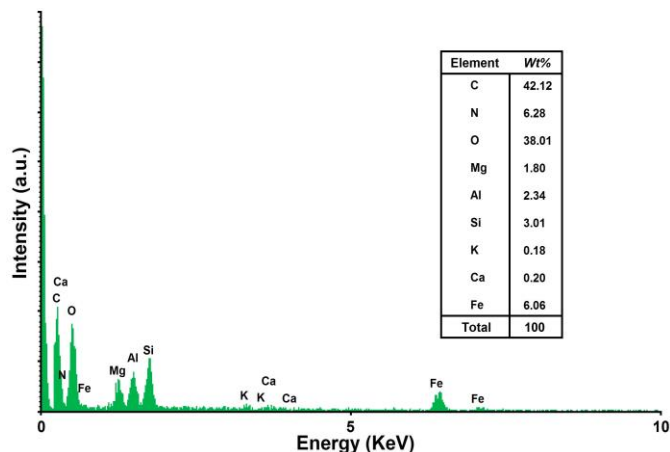


Figure 3. Elemental analysis of Matcha

In recent years, the high resistance of many bacteria has become relatively worrying, therefore having great costs on human societies and has become one of the biggest health challenges [53-59]. Bacteria have the ability to form biofilm structures, which exacerbates the infection [60-61]. In fact, biofilm is a community of microbial cells that are surrounded by an extracellular matrix consisting of exopolysaccharide, protein, and DNA [60-63]. This matrix has a protective role against various factors that prevent the penetration of antimicrobial compounds and proper function [60,61,64]. One of the best candidates for controlling antibiotic-resistant bacteria are medicinal plants, the benefits of which include reduced production costs, low side effects and no environmental problems [65,66]. Many medical systems use herbal remedies to treat various infections caused by bacterial strains [67]. Plant-derived antimicrobials may have weak performance compared to antibiotics, but drug resistance, side effects, limit the effectiveness of antibiotics [67,68]. In addition, herbal remedies can have inhibitory effects, even at very low doses [67]. Much research has been done on the antibacterial properties of plants. Ilić et al. [69] investigated the antimicrobial properties of methanol extracts goji berries were investigated against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica*, *Pseudomonas aeruginosa*, and *Candida albicans*. The results showed that the yellow goji berry extract showed the highest level of flavonoids and the best antimicrobial properties. Bendjedid et al. [70] investigated the antimicrobial of leaves extracts and fractions of Aloe vera against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. The results has shown that yields of methanol, chloroform, ethyl acetate, n-butanol, aqueous,

acetone extracts are respectively $20.56\% \pm 0.38$, $3.4\% \pm 0.11$, $1.58\% \pm 0.12$, $14.16\% \pm 0.11$, $13.56\% \pm 0.78$, $0.68\% \pm 0.50$. Moreover, the n-butanol fraction on acetone and methanol extracts has positive effect against all the bacteria. Subramani et al.[71] investigated the antibacterial and textural properties of cotton fabrics using herbal nanoparticles from *Azadirachta indica* (neem) against *Staphylococcus aureus* and *Escherichia coli*. The results showed that the agar loaded with nanoparticles had the maximum zone of inhibition against *Escherichia coli* and *Staphylococcus aureus* bacteria at a concentration of 100 mg ml^{-1} . Here, the antimicrobial properties of Matcha against 8 types of bacteria, 1 type of fungus and 1 type of yeast were investigated (see Table 1) and determination of tiny growth inhibitory concentration (MIC) and determination of minimum bacterial lethal concentration (MBC) has been done and the results are shown in Tables 2 and Figure 4. Also, Macha antimicrobial results was compared with the effectiveness antibiotics rifampin, gentamicin, and nystatin (see Table 3).

Table 1. Microorganisms used to evaluate the antimicrobial activity of Matcha

Yeasts	Fungus	Gram-negative bacteria	Gram-positive bacteria
<i>Candida albicans</i> (ATCC 10231)	<i>Aspergillus niger</i> (ATCC 16404)	<i>Escherichia coli</i> (ATCC 10536)	<i>Bacillus subtilis</i> (ATCC 6633)
		<i>Pseudomonas aeruginosa</i> (ATCC 27853)	<i>Staphylococcus aureus</i> (ATCC 29737)
		<i>Salmonella paratyphi-A serotype</i> (ATCC 5702)	<i>Staphylococcus epidermidis</i> (CIP 81.55)
		<i>Shigella dysenteriae</i> (PTCC 1188)	<i>Streptococcus pyogenes</i> (ATCC 19615)

Table 2. Antimicrobial activity of Matcha

Type of microorganism	Matcha		
	DD	MIC	MBC
ATCC 6633	7	500	1000
ATCC 29737	12	1000	>1000
CIP 81.55	14	500	>1000
ATCC 19615	11	125	125
ATCC 10536	6	250	500
ATCC 27853	18	250	1000
ATCC 5702	8	500	1000
PTCC 1188	10	500	1000
ATCC 10231	2	250	250
ATCC 16404	—	—	—

Note:

* DD: Disk diffusion method, inhibition zones in diameter (mm) around the impregnated disk.

** DD: 900 μg per well.

*** Concentrations of MIC and MBC as $\mu\text{g}/\text{mL}$

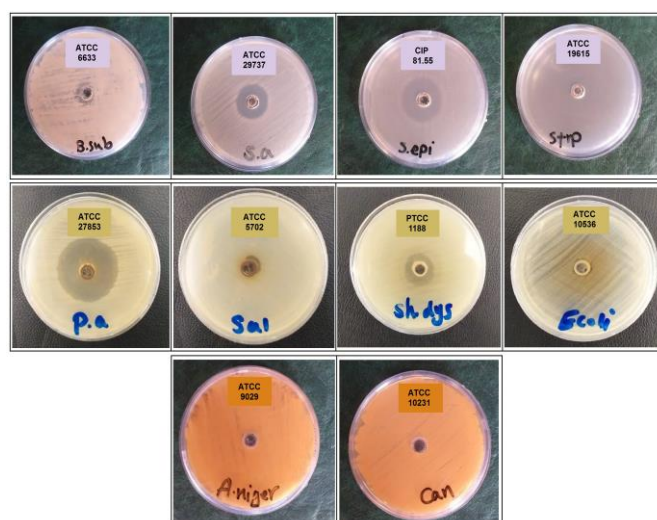


Figure 4. Observations on inhibition zone

Table 3. Results of antimicrobial activity of antibiotics on microorganisms

Type of microorganism	Rifampin		Gentamicin		Nystatin	
	MIC	DD	MIC	DD	MIC	DD
ATCC 6633	62.5	19	7.80	30	NA ^a	NA
ATCC 29737	31.25	21	3.90	27	NA	NA
CIP 81.55	3.90	27	3.90	45	NA	NA
ATCC 19615	7.80	15	3.90	30	NA	NA
ATCC 10536	31.25	10	62.50	23	NA	NA
ATCC 27853	62.50	-	15.60	20	NA	NA
ATCC 5702	31.25	8	7.80	18	NA	NA
PTCC 1188	31.25	9	7.80	18	NA	NA
ATCC 10231	NA	NA	NA	NA	125	33
ATCC 16404	NA	NA	NA	NA	31.2	30

Note:

* NA: No Active

As the results in Table 2 show, Matcha has the ability to inhibit the yeast and all bacteria but has no effects on the fungus. Several factors contribute to the antibacterial activity of Matcha. Polyphenols are present in all plants and an important role as a defensive shield against plant pathogens, animal herbivore aggression, rainfall and ultraviolet rays. Polyphenols have the ability to suppress by membrane perturbation, reduction of host ligands adhesion, and neutralizing bacterial toxins [72]. They inhibit *Pseudomonas aeruginosa* via damaging the cell membrane by releasing small cell molecules [73,74]. Epigallocatechin gallate (one of the four main catechins in Matcha) can bind directly to peptidoglycan (a major component of the bacteria cell wall) and break cross-linking bridges of the peptidoglycan layer [75]. Also, epigallocatechin gallate can perturb the function of cell walls and membranes by disrupting the signaling pathways of bacteria [76]. Research has shown that epigallocatechin gallate can prevent influenza virus infection by binding to the viral hemagglutinin [72]. Another chemical compound in Matcha is flavonoids, that due to their hydrophobicity, can penetrate cell phospholipid membranes and exert their antibacterial activity inside the cell [72,75]. Different mechanisms for the antibacterial activity of theanine in inhibiting bacterial growth have been reported, such as cell the permeabilization of the cell membrane, destabilization of the cytoplasmic membrane, and direct actions on microbial metabolism [72,76]. Research has shown that phenolic acids have antibacterial activity against *Staphylococcus aureus*, *Listeria monocytogenes*,

Escherichia coli, and *Pseudomonas aeruginosa* [72]. As the results show, Matcha is more effective against gram-positive bacteria. In fact, gram-positive bacteria have a simple cell wall that is reasonably porous and it is easier to penetrate them, but gram-negative bacteria have complex cell wall structures with a less porous outer layer [77-80]. It can be said that Matcha contains an army of antimicrobial agents. According to the results, it can be said that in addition to a beverage, Matcha can be considered as an antibiotic of plant origin and can be used in many sanitary such as toothbrushes or medical devices such as urinary catheters.

3. Experimental

3.1. General

Matcha Green Tea Powder (*Camellia sinensis*) was purchased from Arifoğlu Company (Turkey). Dimethyl sulfoxide was purchased from Merck Company. FT-IR spectra of the samples were recorded on an Equinox 55 spectrometer (Bruker, Germany). XRD measurements were carried out using an Xpert Pro MPD diffractometer (Panalytical, Netherlands). Also, EDX was carried out on a JSM 6360LV instrument.

3.2. Antimicrobial test

The antimicrobial properties of Matcha was investigated according to the procedure described in the literature [23,81,82]. Briefly, plates containing Mueller Hinton Agar culture medium were prepared, wells were created on the culture medium, and then bacterial suspensions in uniform conditions at the culture medium have been followed. Also, Matcha dissolved in dimethyl sulfoxide used to determine inhibition zones by disk diffusion method. Moreover, the MIC for microorganisms' sensitivity to Matcha was calculated by microdilution method.

4. Conclusion

To sum up, Matcha characterize and antimicrobial properties were investigated. The results showed that Matcha has inhibitory and kill properties against bacteria and yeast. These antibacterial properties are from compounds of flavonoids, theanine, phenolic acids and especially from catechins. The four main catechins, which include epicatechin, epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate are the most important antimicrobial compounds of Matcha, which originate from a phenolic compound. Based on the results, it can be said that Matcha is one of the most valuable antibiotics of plant origin, which unfortunately has not yet been introduced in many countries and is not used.

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