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Review

Identification of Epigallocatechin-3-Gallate (EGCG) from Green Tea Using Mass Spectrometry

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Abstract: In an era where humanity is reinstating its lost hope and expectation on natural products, green tea occupies quite a position for what it has proven to be, in its endeavors for human welfare and health. Epigallocatechin-3-gallate (EGCG) is the key to the vast biological activities of green tea. Green tea is no longer in the backdrop; it has emerged as the most viral, trending bioactive molecule when it comes to health benefits for human beings. This review focuses on the use of various analytical techniques for the analysis of EGCG. That which has been achieved so far, in terms of in vitro, pure component analysis, as well as those spikes in biological fluids and those in vivo in animal and human samples, was surveyed and presented. The use of MS-based techniques for the analysis of EGCG is elaborately reviewed and the need for improvising the applications is explained. The review emphasizes that there is plenty of room to explore matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) applications in this subject area.

Keywords: catechins; EGCG; mass spectrometry; detection; green tea; LC-MS; MALDI-TOF MS

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1. Introduction

Tea is an age-old beverage steeped in the memoirs of history. Almost three billion kilograms of tea is produced each year. *Camellia sinensis* is predominantly consumed in China and Japan [1]. Green tea can be produced in various ways, which becomes the fundamental basis for the differentiation of various green tea types. Yet, the most fundamental method is via the steaming of fresh *Camellia sinensis* leaves for 1 min, followed by drying. The main components of green tea responsible for its health benefits are the active biocomponents, the catechins [2]. Green tea is the richest source of catechins, that account for nearly 30% of the leaf's dry weight [1]. The other natural sources are red wine, broad beans, black grapes, apricots, and strawberries [3]. There are four predominant catechins in green tea, (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin-3-O-gallate (ECG) and (-)-epigallocatechin-3-O-gallate (EGCG). Amongst these, EGCG is the most abundant and most significant bioactive component [4,5]. Figure 1 shows the chemical structures of the predominant green tea catechins.

Green and black teas are widely consumed worldwide and host a wide range of potential health benefits. Catechin flavonoids, which are around 60 mg/g of the dry leaf weight [6], possess antioxidant properties and other biological activities that are key players in the claimed therapeutic activity of green tea [7]. Green tea is a broad terminology which can be further divided and subdivided based on various factors: the processing

style, the region grown, the time of harvest, the harvest season, the plant parts involved, and brewing method and the like. The three major popular Chinese green teas include: Biluochun, which is named based on its leaf shape which is curled like snails [8]; Chun Mee, which has a plum-like flavor [9]; Gunpowder tea, which is tumble-dried to look like a small pellet that resembles gunpowder [10]; Huangshan Maofeng, whose harvest includes two similar-sized leaves and buds together [10]; Longjing, which is pan-fired Chinese green tea [8]; Lu'an Melon Seed, which consists of a grassier flavor and harvested as two leaves separately from each branch, with no bud and stems [10]; Taiping Houkui, which is processed from unusually large leaves that are taken through a production process that flattens the tea leaves [10]; and Xinyang Maojian, which is a type of maojian tea grown in Xinyang, Henan province [11]. Maojian teas are harvested by plucking a bud and one leaf together [10]. Japanese green teas include: Bancha, which is a lower-grade tea with a bolder flavor, plucked after sencha production [12]; Genmaicha, which is a combination of sencha tea leaves with toasted puffs of rice; and Gyokuro, which is grown in shade for three weeks prior to plucking and is the most exclusive variety of Japanese tea [13,14]. Hōjicha, Kabusecha, Kukicha, Matcha [15], Sencha and Shincha are highly prized and expensive Japanese teas. Korean green teas are ideally classified based on the flush, or the time of the year when the leaves are plucked (also by leaf size). Korean green teas consist of Ujeon, Sejak, Jungjak [15], and Daejak [15] types. Table 1 gives the catechin composition of green tea.

Mass spectrometry (MS) is a powerful, multi-faceted technique used in food safety, environmental, pharmaceutical, biological, and forensic investigations [16–30]. This technique has brought about the detection of proteins, peptides, and lipids, and recently it has been extended to detect, quantitate, and localize metabolites and their counterparts. The unique ionization methods and the specificity and sensitivity of a mass analyzer make MS an attractive analytical option. A plethora of gas and liquid separation techniques are often used in conjunction with MS in order to increase sensitivity and ease interpretation; these include liquid chromatography (LC), gas chromatography (GC), capillary electrophoresis (CE), and gel electrophoresis (GE). The combination of methods is usually common in tandem with ICP-MS and DART-MS. Mass spectrometric imaging is an emerging powerful analytical methodology used for analyzing multiple molecules in complex samples without labeling, conferring a clear edge over preexisting methods for label-free and simultaneous drug and metabolite detection. There are a host of methods and ionization variants in MS. Gas phase methods include electron ionization (EI), chemical ionization (CI), direct analysis in real time (DART), and inductively coupled plasma (ICP); desorption methods consist of matrix-assisted laser desorption ionization (MALDI), fast atom bombardment (FAB), thermal ionization sources, plasma ionization sources, and liquid metal ion sources (LMIS); and spray methods include ultra-high electrospray ionization (ESI) and desorption electrospray ionization (DESI) [31–37]. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry profiling (MALDI-TOF MS profiling) is a promising approach for the rapid and high-throughput screening of biological samples, intact cells, and crude extracts [38]. This technique has been applied to clinically relevant medical applications, including cancer-disease-related biomarker identification and pathogenic microorganism diagnostics. It has now become a versatile tool for many applications. Clinical bacteria, mycobacteria, entomopathogenic soil fungi, yeasts, and viruses have been identified and clustered by MALDI-TOF MS profiling for quality control. MALDI-MS has also been successfully applied to quantitative studies of plant metabolites, plant alkaloids, anthocyanins, flavonoids, acetogenins, spirolides, curcuminoids, and rotenoids [39–54].

The following review aims to catalogue the green tea catechins that are well known for their diverse social and health benefits, with special emphasis on EGCG, which is notorious for its bioactivity. The various analytical methods that are employed to assess and assay green tea catechins are briefly presented. The application of mass spectrometry for the analysis of EGCG has been elaborately discussed and the challenges in MS applications for this expertise and their future perspectives are presented.

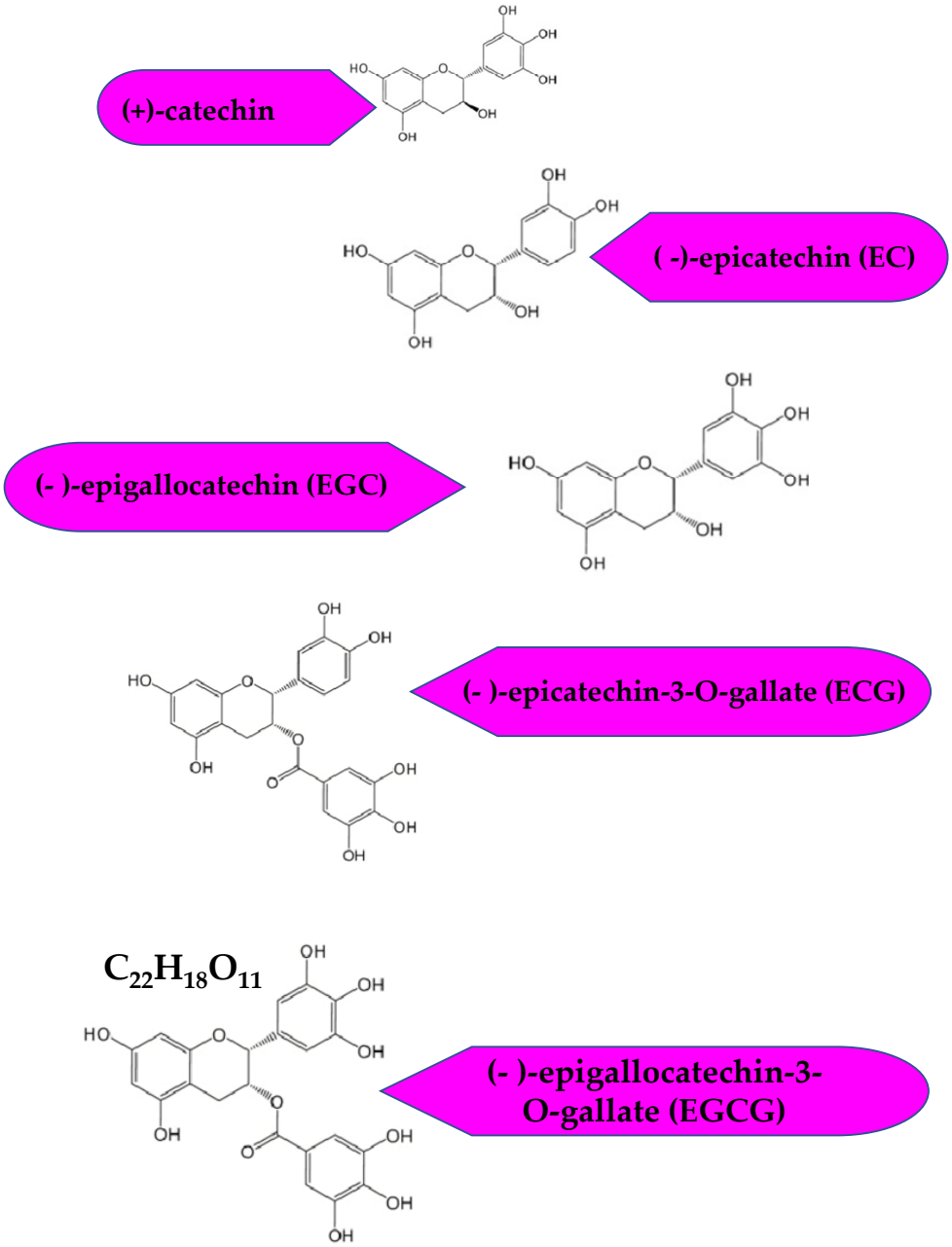

Figure 1. Chemical structures of the green tea catechins.

Table 1. Composition of Green tea.

Green Tea Components	Concentrations	References
Epigallocatechin gallate (EGCG)	38.7–64.5	[13–15]
Epicatechin gallate (ECG)	1.8–37.9	
Epigallocatechin (EGC)	2.2–38.8	
Gallocatechin gallate (GCG)	0.5–4.6	
Gallocatechin (GC)	0–9.8	
Gallic acid (GA)	0.3–1.7	
Catechin	0–3.8	
Epicatechin (EC)	3.9–9.8	

2. A Snapshot of EGCG Applications

Green tea catechins are well known for their biological and pharmacological impacts: the anticarcinogenic [55] and antioxidant activities [56]; lowering of plasma lipid [57] and glucose levels [58]; reduction in obesity [59,60]; prevention of cardiovascular diseases; and antiarthritic, antibacterial, antioxidative, anti-inflammatory, antiangiogenic, antiviral, neuroprotective, and cholesterol-lowering actions [7,61]. EGCG not only holds the position of being the major polyphenolic component in green tea, but it also contributes predominantly towards green tea's properties. In this review, we essentially focus on and highlight the biological functions of EGCG. It protects the system against free-radical-induced cellular damage, shields the cells from oxidative-stress-induced damage, and suppresses the pro-inflammatory activity of chemicals produced in the body. EGCG promotes heart health by reducing blood pressure, cholesterol, and plaque accumulation in blood vessels, which are triggers of cardiac diseases [62,63]. Catechins are also associated with the development of lung, gastric, and breast cancers [64–66]. A well-established connectivity between diabetes, improved immunity, and green tea is well-documented [67–72].

EGCG further inhibits inflammation and prevents chronic heart diseases, diabetes, and cancers. EGCG improves neurological function and prevents degenerative brain diseases and assists in the regeneration of neural cells in mice with spinal cord injuries [73,74]. In vitro studies validate the antibacterial property of EGCG, whereby they are able to suppress the growth of bacteria and lower the risk of infections [75–78]; there is a handful of evidence that green tea may reduce halitosis or bad breath [79,80]. Most importantly, EGCG has been associated with affecting age-related brain decline, as well as Alzheimer's and Parkinson's disease [81].

In the food industry, EGCG prevents lipid peroxidation of oily foods, scavenges free radicals, and inhibits the autooxidation of lipids as well as lipid degradation [82]. EGCG is almost 20 times more potent than vitamin E in preventing lipid peroxidation and four times higher than butylated hydroxyanisole (a food additive that preserves fat/oil) in this regard [83]. Furthermore, EGCG inhibits the formation of mutagens, which occur during the broiling or frying of meats and are known to increase the risk of cancers [84]. In the Asian food industry, EGCG has been used as functional food components, enhancing product shelf-life and adding health benefits for consumers [85]. Tea catechins have been used to fortify various food commodities and beverages [86]. In the pharmaceutical industry, applications such as mouthwashes, toothpastes, and breath fresheners to improve oral health are prevalent [86]. In addition, EGCG supplement tablets or drinks are commercialized to enhance consumer health. EGCG has been reported to be incorporated into air filters in "antiinfluenza" masks [86]. In the cosmetics industry, they have been added to shampoos, face masks, moisturizing creams, perfumes, and sunscreens, as they can relay soothing effects on the skin and protect it from free-radical damage [86].

The flip side of EGCG is also being carefully investigated; it is important to note that EGCG has been reported not to be 100% safe or risk-free. EGCG supplements have been associated with serious side effects such as [87]: liver and kidney failure, dizziness, low blood sugar, and anemia. Taking supplemental EGCG is not recommended for pregnant women, as it is confirmed to interfere with the metabolism of folate, which is a B vitamin needed for fetal growth and development, the deficiency of which results in birth defects such as spina bifida [88]. It is still ambiguous as to the safety of EGCG supplements for breastfeeding women [89]. EGCG interferes with the absorption of certain types of cholesterol-lowering and antipsychotic drugs [90]. The pro-oxidation action of EGCG is a crucial mechanism key to its protective functions, one of which includes the induction of adaptive responses and detoxification [91]. Intriguingly, it is also reported that EGCG at higher doses can induce hepatotoxicity in both animals and human beings. It is further postulated that EGCG may undergo auto-oxidation, generating reactive oxygen species (ROS), inducing toxicity [92]. These are a few of the health concerns that have been raised; there could be a host of other unknown aspects as well which, if disclosed, could guide us towards the better utilization of EGCG, knowing its pros and cons. Thus, it becomes

essentially crucial to weigh their benefits against toxicity aspects to carefully work on discovering the safe physiological dosages of EGCG. Because of the high rates of tea consumption in the global population, even minor hazards could have seriously larger implications on public health. It is in this direction that the disposition of catechins within biological systems, involving their absorption, distribution, metabolism, and excretion, have been investigated in mice, rats, and humans [93]. The metabolism of catechins in animals and humans has been elaborately reviewed [94]. These pharmacokinetic profiles of catechins in rats and humans yield a clearer perspective on the movement and absorption of EGCG within a system; although, this is far from being fully elucidated.

3. Separation and Identification of Green Tea Catechins/EGCG

Green tea catechins consist of four major polyphenols, that include: epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), epigallocatechin (EGC), and epicatechin (EC) [2]. EGCG is the chieftain among the catechins; it constitutes about 50–80% of the total catechin content [95–97]. Catechins are not only soluble in water, but also in ethanol, methanol, and acetone. Pure catechins from tea are obtained following a separation protocol. Separation and isolation procedures for tea catechins involve extraction processes such as (i) treatment of the starting material, (ii) the extraction of tea constituents from tea leaves into infusions, (iii) the concentration of the extracted constituents, (iv) the separation and isolation of catechins from other impure components; and (v) drying of the catechins to obtain an extract powder for industrial use [98].

The specialized methods that have been applied for the analysis of tea catechins [99,100] in plasma samples include liquid chromatography (LC) connected to an ultraviolet detector (UVD), a chemiluminescence detector (CLD), a fluorescence detector (FLD), and an electrochemical detector (ECD) [101]. When it comes to the estimation of EGCG with marked specificity, spectrophotometry [102], HPLC [103,104], reverse-phase (RP) HPLC [100,101], ultra-performance liquid chromatography (UPLC) [103,104], ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) [105], electrospray ionization -mass spectrometry (ESI-MS), ultra-performance liquid chromatography-diode array detector-mass spectroscopy (UPLC-DAD-MS) [106,107], and ultra-performance liquid chromatography—time-of-flight—mass spectrometry (UPLC-TOF-MS) have been successfully applied [108].

3.1. MS-Coupled Chromatographic Techniques for Detection and Identification of Catechins/EGCG

The absorbance wavelengths of catechins lie in the range of 210 and 269–280 nm [109,110], and so, UV-spectrophotometry and diode array detectors have become easy and simple analytical methods for the determination of catechins. Various analytical methodologies have been used for identifying and quantifying tea catechins [111]. These methods determine the yield, concentration, and purity of catechins in the separated products. The identification and quantification of catechins are predominantly steered by chromatographic techniques, such as HPLC and CE associated with various UV, electrochemical, and MS detectors that enable the detection of individual catechins [112]. Near-infrared reflectance spectroscopy, high-speed countercurrent chromatography, TLC, and GC are the other alternate options [113], of which HPLC with ultraviolet (UV) detection is a rather simple, highly reproducible technique with low limits of detection (more than 500 ng/mL) [114]. HPLC with chemiluminescence detection overcame this detection limit, but its sample preparation and column preparation procedures are difficult [111]. Lee et al. reported the electrochemical detection (ECD) of catechins with HPLC [111]. This was a more sensitive approach, capable of detecting EGCG at concentrations as low as 0.5 ng/mL; the assay took almost 35 min. HPLC is the most common, as it leads to good separation and can be combined with many detectors [112]. A reverse-phase C18 column is mostly used for HPLC [112]. Ramakrishna et al. [113] used standard EGCG for EGCG optimization experiments using ultra-high-performance liquid chromatography (UHPLC). A preliminary trial run yielded good peak shapes and acceptable system suitability, leading to validation. The method was

specific and had an acceptable recovery rate in the range of 99.1% to 100.4% with a relative standard deviation (less than 2%). This method was identified to be robust and rugged and was validated as a routine compliance test in the laboratory.

Saito et al. presented an HPLC analytical methodology development for the simultaneous determination and quantification of caffeine (CAF), catechin (C), epicatechin (EC), and epigallocatechin gallate (EGCG) in samples of *Camellia sinensis* (green tea) grown in Brazil and harvested in spring, summer, and autumn, in comparison to Brazilian black tea, to samples of Japanese and Chinese green tea, and to two standardized dry extracts of green tea [114]. Lambert et al. [115] detected intravenously administrated EGCG in the plasma of male CF-1 mice. The kinetics of EGCG in the liver, lung, intestine, and kidney of rats after intravenous administration of decaffeinated green tea was also reported [116]. The tissue levels of EGCG, EGC, and EC in rats, as well as the liver and lung levels in mice, after administration of green tea has been documented [116]. Wangkarn et al. [117] recently elaborately reviewed the HPLC-based detection of green tea catechins.

3.2. Direct MS Analysis of EGCG

MS-assisted methods for analysis of EGCG have also been reported; we present a brief overview of these reports in this section. EGCG gives a high intensity of the deprotonated molecular ion, $[M\text{-}H]^-$, at m/z 457 in negative ESI analysis. Diverse methods to measure the catechin concentration in biological fluids have been reported for pharmacokinetic and metabolic studies [118–122]. This enables the tracking of catechin inside the biological system, assisting in a better understanding of the process of absorption, distribution, metabolism, and excretion via analyzing the plasma and urine levels. Numerous high-performance liquid chromatographic (HPLC) methods have been published on the analysis of tea catechins in blood plasma. Most of these methods involve the extraction of tea catechins from plasma or tissue homogenates using solvents such as ethyl acetate [123–127], acetonitrile [128], or methanol [129], followed by an HPLC separation coupled with electrochemical or MS detection [130]. Liquid chromatography coupled with mass spectrometry (LC/MS/MS) is another emerging analytical technique for the quantitative determination of metabolites in different biomatrices, due to its sensitivity and selectivity through MS/MS experiments and the fact that it enables structural identification [121,131–133].

Recently, HPLC with electrospray ionization (ESI) mass spectrometry (MS) was reported by Masukawa et al. [121]; a minimum detectable concentration of less than 1 ng/mL for EGCG in human plasma was achieved, but the assay time was over 50 min. However, in a more time-efficient assay, Lin et al. [134] detected EGCG in rat plasma and brain tissue using LC/MS/MS within 10 min. Despite the highly sensitive EGCG detection, quantification (LLOQ) is still a challenge. Ultraperformance liquid chromatography (UPLC) is a recently developed method in LC; this led to a marked separation time reduction and solvent consumption with high resolution [135].

LC-MS, for quite a while, had not been used for the detection of EGCG from actual plasma after ingestion; although, it has been used for analyzing EGCG in model plasma with a few spiked catechins. Columns packed with sub-2 m particles working at elevated pressure (UHPLC strategy) could improve the chromatographic performance. Identification required a liquid-liquid extraction procedure before UHPLC-UV analysis to decrease the complexity of the sample. UHPLC coupled with ESI-MS/MS could successfully enhance sensitivity and selectivity [136].

In another study [137], LC-ESI MS was used for the separation of eight catechins using an Inertsil ODS-2 column equipped with a gradient elution system. Detection using MS was performed under negative ESI. The key difference between positive and negative ionization in mass spectrometry is that positive ionization is the process that forms positively charged ions, whereas negative ionization is the process that forms negatively charged ions. In the negative-ion mode operation, peaks corresponding to deprotonated analyte molecules are observed. The negative mode allows better sensitivity for small-molecule detection compared to the positive mode. Eight catechins in human plasma after oral ingestion

of a commercial green tea beverage were detected in human blood for the first time. Another rapid and valid method led to the simultaneous detection of catechin, epicatechin, and epicatechin gallate in rat plasmas. Three analytes were recovered; the lower limits of quantitation (LLOQ) in rat plasma for catechin, epicatechin, and epicatechin gallate were identified [124]. The results demonstrated that the present LC-MS/MS method was sensitive enough for pharmacokinetic study of catechins following oral administration of *C. songaricum* extract; since this method was successful in the detection of catechins that are less dominant than EGCG, this method can certainly be applied to EGCG as well.

The simultaneous detection and quantification of green tea catechins using UPLC/ESI-MS have also been reported. In 3.5 min analytical run time, EC, ECG, EGC, and EGCG were detected in rat plasma [138]. The assay was successfully applied to a pharmacokinetic tracking of catechins following intravenous and intragastric administrations of green tea extract in rats. Plasma concentrations of four catechins were detected up to 5–24 h after administration. LC-ESI-MS/MS was used to identify green tea catechin metabolites in plasma and urine, following oral intake of a green tea extract [139]. LC-ESI-MS/MS was applied for the identification and structural assignments of the polyphenolic extracts of green tea (*Camellia sinensis*) [140]. UPLC-MS/MS was used to obtain the concentration of catechins in blood after one-time ingestion of green tea extract (630 mg) with digoxin and consumption of 630 mg green tea for 15 days [114]. Green, oolong, and black tea samples were analyzed. Catechin standards were obtained from Cerilliant® (Round Rock, TX, USA). Liquid chromatography and mass spectrometry analysis was used for acquisition and data analysis [140].

Juang et al. [141] described the use of surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS) for the detection of catechins. They incubated catechins with titanium dioxide (TiO_2) nanoparticles (NPs) and graphene flakes (GF) and subjected the mixture to microwave irradiation for enriching the analytes. TiO_2 nanoparticles enriched the catechins while GF enhanced the desorption/ionization efficiency. The dual nanomaterial matrix system yielded higher desorption/ionization efficiency, enhanced analyte enrichment, and reduced the run time to less than 10 min. Several tea samples were tested using the optimized method; good shot-to-shot, sample-to-sample, and quantitative linearity were obtained. (m/z 457 $[M\text{-}H]^-$). Table 2 lists the reports involving MS-based identification/detection of EGCG.

Table 2. MS-assisted identification of EGCG.

MS Technique	Source of EGCG	References
a. MS-coupled chromatographic methods		
LC-MS	Tea samples	[142]
LC-MS	Tea samples	[143]
LC-MS	Tea samples	[144]
LC-MS	Pure EGCG	[145]
LC-MS/MS	Rat plasma and liver tissue	[146]
LC-MS/MS	Tea samples	[147]
LC-Q-TOF-MS/MS	Tea samples	[148]
HR-LC-MS/MS	Biotransformed products of green tea extract	[149]
UPLC-Q-TOF/MS	Tea samples	[150]
UPLC-MS/MS	Tea samples	[151]
UPLC-MS-MS	Pure EGCG	[152]
UPLC-Q-TOF-MS	Crayfish liver and muscle	[153]
UPLC-QQQ-MS/MS	Tea samples	[154]
UFLC-MS/MS	Rat plasma	[155]
UPLC-Q-TOF/MS	Tea samples	[156]
HPLC-MS	Tea samples (purified EGCG)	[157]
HPLC-MS/MS	Human plasma	[158]
HPLC-ESI-MS/MS	Tea samples	[159]
HPLC-FTMS	Tea samples	[160]

Table 2. *Cont.*

MS Technique	Source of EGCG	References
a. MS-coupled chromatographic methods		
UHPLC-ESI-QTOF-MS	Tea samples	[161]
UHPLCESI-IT-MS	Human fecal materials	[162]
UHPLC-Q Exactive-MS	Tea samples	[163]
UHPLC-MS	Tea samples	[99]
b. Direct MS techniques		
ESI-MS	EGCG-sucrose solution	[164]
UHPLC-ESI-QTOF-MS	Tea samples	[165]
QTOF-MS	Pure EGCG Interaction between EGCG and sunitinib	[166]
ESI-QTOF-MS MALDI-TOF MS	Pure EGCG Interaction between EGCG and model peptides	[167]
MALDI-TOF MS	Interaction of EGCG with albumin	[168]
MALDI-TOF/TOF MS	Interaction between EGCG and calmodulin-dependent protein kinase II	[169]
MALDI-MSI	Mammalian tissues	[170,171]
SALDI-MS	Catechins	[142]

Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) is a trending offshoot of MALDI-TOF MS, which has been presently used for the physiological visualization of targets in organs. MALDI-MSI has been used as a visualization tool to view the intestinal absorption of polyphenols [170]. Nifedipine/phytic acid-aided MALDI-MSI was performed to visualize theaflavin-3′-O-gallate (TF3′G) and epicatechin-3-O-gallate (ECG) in the jejunum of rats. MALDI-MSI was also performed to determine the transport routes of the target metabolites. MALDI-MSI could provide critical spatial information on intestinal absorption of targets. Kim et al. [170] established a 1,5-diaminonaphthalene (1,5-DAN)-based MALDI-MSI technique for visualizing EGCG, within mammalian tissue microregions after oral dosing. Using a combination of label-free MALDI-MSI and a standard-independent metabolite identification method, the authors were able to achieve isotopic fine structure analysis using an ultra-high-resolution mass spectrometer. This led to the informative visualization of spatially resolved biotransformation based on the simultaneous mapping of EGCG and its phase II metabolites. The caliber of information derived hereby was unique. The only limitation of this technique was its detection sensitivity. An overview of the analytical analysis of EGCG, with the list of techniques, attempted so far, is shown in Figure 2.

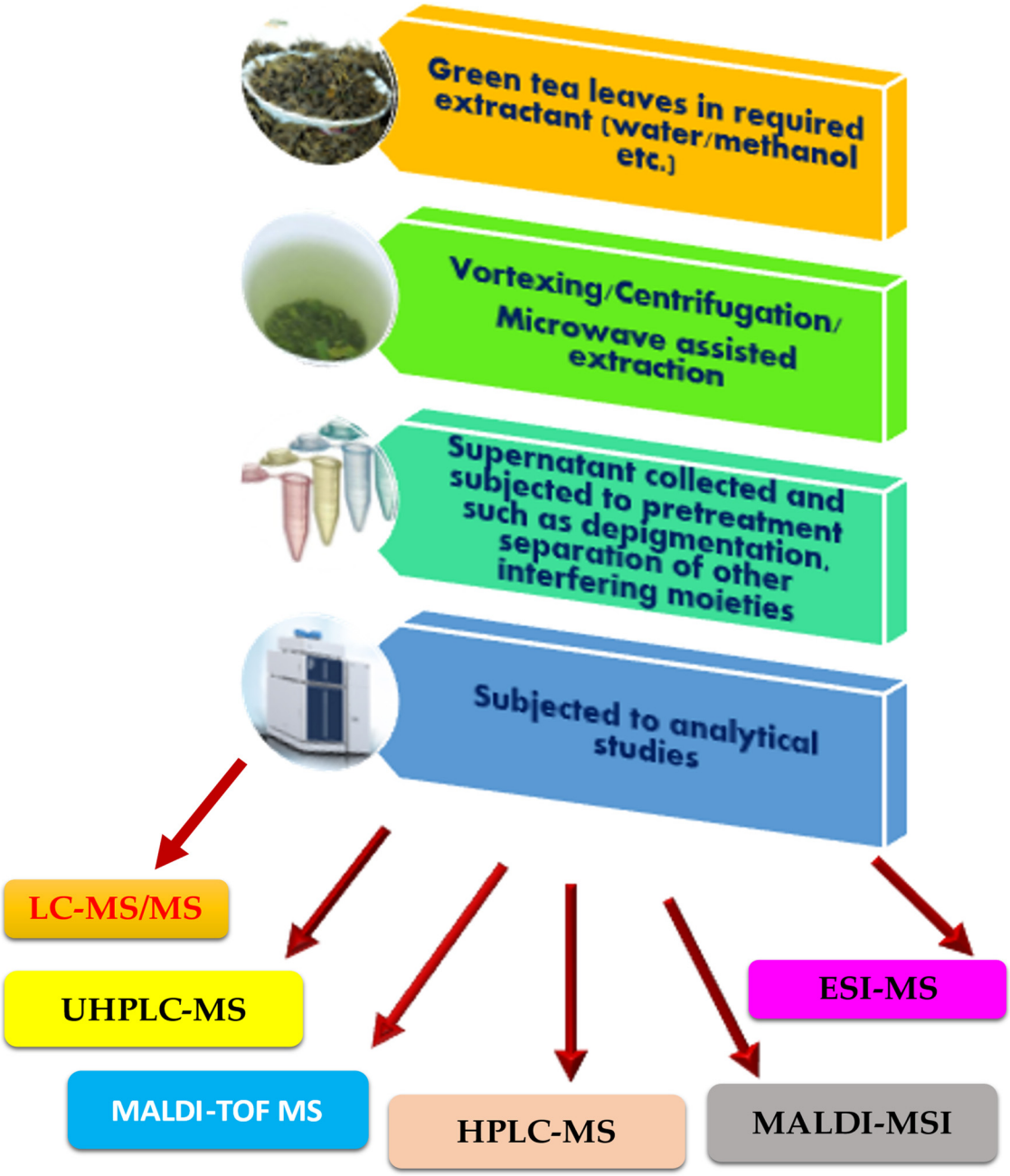

Figure 2. Workflow of EGCG MS-based analysis.

4. Challenges and Future Perspective

The applications of EGCG have been briefly reviewed and the analytical instruments that have been used for the detection, identification, and analysis of green tea catechins and EGCG have been presented. Going with the theme of this review, more emphasis has been given to reviewing the MS-based analysis of EGCG. Through the course of the review, it has become exceedingly evident that green tea catechins and EGCG have huge potential and hold voluminous promises for human welfare. In spite of this reputation, the deployment of analytical techniques towards the analysis of EGCG in vitro and in vivo is represented by merely a handful of studies. In particular, MS-based applications for EGCG detection and analysis are less numerous. LC-MS is a somewhat more accomplished instrumentation when it comes to catechin detection, but this does not necessarily confirm the monopoly of this technique. The lack of availability of reports on other parallel techniques is the reason why such a comparison could not be raised.

MALDI-TOF MS is well known as a rapid and sophisticated technique which, except for a couple of studies in terms of EGCG detection, has not been well reported. MALDI-TOF MS is well established for its role in the detection of plant secondary metabolites. Rush et al. [171] reported the use of MALDI-TOF MS for the analysis of small molecules such as procyanidins, which constitute a unique class of polymeric plant secondary metabolites with a variety of biological properties including potent antioxidant activity. Its structural determination has been challenging, and the structures of many complex procyanidins remain uncertain. Negative-ion MALDI-TOF/TOF has expediated the characterization of procyanidins. The interpretation of the tandem mass spectra enabled the sequencing of A-type, B-type, mixed-type, linear, and branched procyanidins.

The identification of catechin oligomers from apple has been successfully demonstrated using MALDI-TOF MS and fast-atom-bombardment mass spectrometry. MALDI-TOF MS provided evidence for the pentadecamer (*trans*-3-indoleacrylic acid has been used as the matrix in the presence of silver ions). Given these facts, it is rather intriguing that the utility of MALDI-TOF MS has been proven for the identification, elucidation, and analysis of various bioactive small molecules, yet nearly nothing has been attempted to apply it in EGCG analysis [172]. This review stresses that there is much that MALDI-TOF MS can offer, especially in terms of reducing the elaborate sample preparation and instrumentation protocols that rise from LC-MS [147]. MALDI-TOF MS, as an option for EGCG analysis, should be strongly considered and explored; we, however, do not rule out the utility of standard chromatographic techniques that enable separation and the other MS-assisted chromatographic methods that enable detection. What we emphasize here is that MALDI-TOF MS is also able to contribute to EGCG detection, and more attention in the direction of checking what it has to offer is encouraged.

As evident from the previous section, LC-MS is unable to perform with respect to EGCG analysis as a standalone technique. LC/ESI MS and MS/MS; UPLC-MS/MS; LC/ESI-MS; and UPLC-DAD-MS are the various combinations of LC-MS and MS that have made progress in EGCG research. MALDI-TOF MS has the potential to function as a standalone technique, which will prove highly advantageous since this will simplify the preparative procedures as well as save time and resources and curtail involving multiple instrumentations [172].

Speculating why MALDI-TOF MS has not been used for the analysis of EGCG, it is understandable that chromatography methods enable the separation, leading to effective detection. Conventional MALDI-TOF MS did suffer from the interferences of the other compounds in an analyte, but with the introduction of the nanoparticle-based preconcentration techniques, as well as nanoparticle-based affinity-probe-assisted MALDI-TOF MS; other state-of-the-art techniques such as SALDI-MS, NALDI-MS, MALDI-TOF MSI; and other sophisticated LDI-MS platforms [173–186], MALDI-TOF MS has overcome its own limitations and is progressing rapidly in small-molecule analysis. This review urges the readers to utilize MALDI-TOF MS for the detection of EGCG and other catechins. MALDI-TOF MSI can be explored and applied, since it is a versatile and robust technique that can become very resourceful, especially when tracking the pharmacokinetics of EGCG in vivo. There can be more that MALDI-TOF MS and its variants have to offer, in terms of specificity, linearity, accuracy, system suitability, method precision, robustness, and ruggedness, which need to be investigated as soon as possible.

5. Conclusions

The benefits of green tea catechins, especially EGCG, were reviewed and the current status quo of the analytical techniques that have been used to analyze EGCG in vitro and in vivo were comprehensively presented and discussed. The use of MS-coupled chromatographic methods and direct MS methods for the analysis of EGCG were comprehensively presented. MS-based methods have proved useful when it comes to the analysis of EGCG; however, there are few MS techniques that are unattempted. In particular, MALDI-TOF MS has not been used much, in spite of its proven usefulness in small-molecule analysis.

This is poised as a huge gap, since MALDI-TOF MS is a standalone technique and there are other, more evolved variants of MALDI-TOF MS that are in the field. Yet, as this review identified, this is an area which lacks research attention. This review prompts the need for exploring the available MALDI-TOF MS options for EGCG analysis.

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