Recent Advances in *Garcinia cambogia* Nutraceuticals in Relation to Its Hydroxy Citric Acid Level. A Comprehensive Review of Its Bioactive Production, Formulation, and Analysis with Future Perspectives

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ABSTRACT: *Garcinia cambogia* (Gaertn.) Desr. (known as Malabar tamarind) is a popular traditional herbal medicine and is one of the well-known folk medicines reported for the treatment of obesity and incorporated in several nutraceuticals worldwide. These effects are mediated by a myriad of bioactive compounds with most effects attributed to its hydroxy citric acid (HCA) content. This review aims to present a holistic overview on novel trends in the production of *G. cambogia* bioactive components and how extraction optimization is important to ensure best product quality with its reported nanoformulations with particular emphasis on HCA content. Further, an overview of the different analytical approaches used for quality control assessment of *G. cambogia* plant and its nutraceuticals is presented highlighting both advantages and limitations. Moreover, analytical approaches for detecting *G. cambogia* metabolites in biological fluids with emphasis on HCA level to determine its pharmacokinetics and proof of efficacy are presented for the first time.
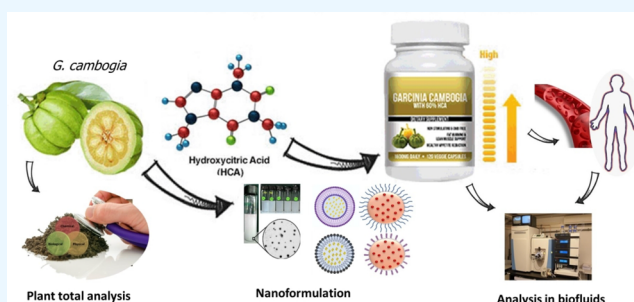
INTRODUCTION

Garcinia genus, a member of family Clusiaceae, comprises more than 300 species. They are native to Asia and Africa and are commonly called sap trees, kokum, *Garcinias* or mangosteens, and monkey fruits. *Garcinia* plants, besides being ornamental, find industrial pharmaceutical, and culinary applications.[1] *Garcinia cambogia* (Gaertn.) Desr., (syn. *Garcinia gummi-gutta* (L.) Roxb., known as Malabar tamarind, has been used for centuries in Southeastern Asia as an appetite suppressant to make meals more filling and satisfying.[2] The sour rind of the fruit is utilized as a condiment, and it is used in folk medicine to manage several ailments, *e.g.*, piles, constipation, edema, rheumatism, intestinal parasites, and irregular menstruation.[3] *G. cambogia* rind is considered a rich source of organic acids, amino acids, benzophenones, xanthones, and flavonoids[4] as depicted in Figure 1. Polyisoprenylated benzophenones precursors of xanthones were detected in Ceylon fruit rind to include garcinol and guttiferones K, I, J, M, and N. Moreover, xanthones, *viz.*, oxy-guttiferone K,[5] and oxy-guttiferones K2, I, and M were also reported from Ceylon fruits.[6] With regard to primary metabolites, fruit is enriched in amino acids including arginine, glutamine, proline, and γ-aminobutyric acid.[7] In addition to amino acids, organic acids represented by (−)-hydroxycitric acid (HCA) and its lactone are responsible for its sour taste,[2]
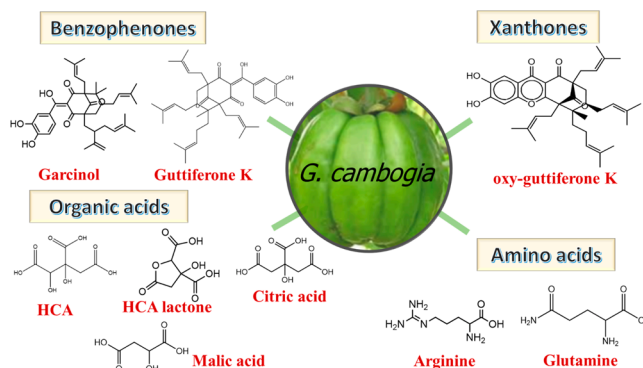

Figure 1. Representative examples of *G. cambogia* major chemical constituents. HCA, hydroxy citric acid.

followed by citric and malic acids.[8] It contains *ca.* 10−30% acid calculated as citric acid,[9] with HCA as the major one.[10]

Although herbal preparations are gaining more popularity worldwide, they often encounter contamination and adulteration affecting their safety and or efficacy.[11] The increasing interest in phytomedicine efficacy along with the increase in legal requirements for safety and reliable contents of active ingredients drives the development of analytical tools for proper quality control of such a complex, multicomponent mixture.[12] Targeted and untargeted analyses play a crucial role in the quality control of nutraceuticals. Whereas targeted methods are more selective, sensitive, and often quantitative, the untargeted approach embraces the complexity of natural samples and is more suited for analysis of nutraceuticals.[13] Modern techniques comprise hyphenated chromatography with spectroscopy, e.g., gas chromatography—mass spectrometry (GC—MS), high-performance liquid chromatography—diode array detection (HPLC-DAD), HPLC—MS, HPLC—nuclear magnetic resonance (NMR), and capillary electrophoresis-diode array detection (CE-DAD) have been widely used for qualitative and quantitative analysis. Recently, advanced analytical tools such as profiling and fingerprinting are often supplemented by multivariate data analysis, i.e., chemometrics to help analyze and dissipate such rich chemical info data set.[14]

The present review highlights the current analytical approaches employed for the quality control purposes of G. cambogia extracts and in nutraceuticals. Analysis of G. cambogia bioactivity in biofluids is presented to track its pharmacokinetics and efficacy. Furthermore, it represents an up-to-date comprehensive overview on extraction and optimization approaches together with the nano formulation of G. cambogia bioactives to improve efficacy, with particular emphasis on HCA content.

Garcinia Health Benefits. G. cambogia potential use in body weight loss has been validated through several in vivo animal[15−18] and clinical studies[17,19] mainly due to (−)-hydroxycitric acid (HCA). Nevertheless, few studies failed to show the same outcome; a randomized clinical trial of 66 subjects to administer 1500 mg HCA/day for 12 weeks, while they were on a high-fiber and low-energy diet, did not reduce body weight or fat mass[20] contrary to other studies with positive results using a simple diet rich in carbohydrates.[21] Discrepancy may be attributed to the low HCA administered dose or the high-fiber diet that may have inhibited its gastrointestinal absorption.[10] Furthermore, treatment with 3 g per day for 3 days did not influence energy expenditure or respiratory quotient, suggesting that HCA does not affect the fasting rate of fat oxidation in the short term.[22] In contrast, longer administration of G. cambogia supplementation (45 mg/kg for 6 months) did not affect cats' body weight.[23] Thus, further controlled, long-term clinical studies ought to be conducted on a large scale to assess the efficacy of G. cambogia or HCA with monitoring of its bioavailability. G. cambogia supplementation was found to reduce high-fat-diet-induced obesity by suppressing epididymal and inguinal subcutaneous white adipose tissue mass and adipocyte size.[24] Morover, G. cambogia administration resulted in significant blood glucose-lowering effects.[25] HCA has attracted attention in Garcinia among its many other bioactives owing to its antiobesity effect and various action mechanisms in obesity management. Chiefly, HCA is a competitive inhibitor of the ATP-citrate lyase enzyme that converts mitochondria-derived citrate into acetyl CoA, a precursor for both fatty acid and mevalonate synthesis pathways. Reduction of acetyl-CoA units leads to less fatty acid

biosynthesis and lipogenesis from a high carbohydrate diet. On the other hand, it stimulates liver gluconeogenesis and glycogen storage, which sequentially impacts the liver glucoreceptors bringing about satiety through the vagus nerve. Gluconeogenesis also could contribute to the ATP wasting and calorie consumption.[9] HCA-SX, a Ca/K-HCA rich extract, enhanced the release and availability of serotonin and inhibited its uptake in the rat brain cortex like SRRIs, and consequently could control appetite and manage several serotonin-deficient conditions, e.g., insomnia, depression, headaches, and migraine.[26] These effects are suggestive for multiple mechanisms through which Garcinia fruit aids in weight loss. Additionally, it upregulated serotonin receptor genes involved in satiety.[2] The concurrent use of G. cambogia extract with probiotics further ameliorated weight gain and adiposity, through modifying gut microbiota composition, especially suppressing Clostridium aminophilum, the bacteria highly correlated with obese phenotypes.[27] Such an effect is suggestive of a synergistic action of Garcinia and probiotics in weight reduction. Moreover, the effects of HCA extracts from G. cambogia on metabolic, atherogenic, and inflammatory biomarkers were examined in obese women with nonalcoholic fatty liver disease. Results revealed that weight, body mass index, waist circumference, fasting blood sugar, hip circumference, triglyceride, and low-density lipoprotein cholesterol showed a decrease, concurrent with an increase in high-density lipoprotein cholesterol.[28] Interestingly, fruit rinds of G. cambogia, G. indica, and G. atroviridis contain (2S,3S)-HCA with a potential to inhibit ATP-citrate lyase,[10] while the calyx of Hibiscus subdariffa and H. rosa-sinensis produce (2S,3R)-HCA which suppresses intestinal α-glucosidase and pancreatic α-amylase, reducing carbohydrate metabolism. Likewise, (2S,3R)-HCA is produced by Streptomyces sp. U121 and Bacillus megaterium G45C at trace levels.[29]

Pharmacological and clinical studies confirm the safety of HCA intake at levels up to 2800 mg/day.[9] Nevertheless, several side effects were reported including ocular complications[30] and hepatic toxicity[31] either upon consumption of G. cambogia with multiple ingredients[32−34] or due to its concurrent use with other drugs[35] or its administration beyond the recommended doses.[30]

G. cambogia and HCA derivatives, e.g., calcium, potassium, and sodium salts, are widely available in pharmaceutical preparations used for weight loss, lipid abnormalities, improving exercise endurance, and cardio protection.[10] Super CitriMax, a Ca/K salt of 60% HCA extract from G. cambogia, is more soluble and bioavailable than a Ca-based one proven safe and effective in weight control.[36] Other health benefits revealed for Garcinia include, e.g., anti-ulcerogenic, antiseptic,[37] anti-inflammatory, hepatoprotective, antioxidant, erythropoietic, and cytotoxic effect, though most are based on animal studies and less explored in humans to be conclusive compared to the slimming effect reported before.[35] These effects are likely attributed to garcinol, the polyisoprenylated benzophenone that exhibits anti-ulcer, anti-inflammatory, antimicrobial, and anticarcinogenic activities,[5] while the hypolipidemic effect of its flavonoid fraction (10 mg/kg BW/day) in rats exceeded that of Myristica fragrance, Cocos nucifera, and Saraka asoka without exerting any toxic effects.[38]

Analysis and QC Approaches of G. cambogia and in Nutraceuticals. G. cambogia fruit is a valued weight-loss supplement owing to its richness in organic acids, mainly hydroxycitric acid (HCA) and HCA lactone, benzophenones,

Table 1. Different Analytical Methodologies Employed for the Analyses of *G. cambogia* Fruit Bioactive Metabolities

| Sample	Composition	Methodology	Advantages	Results	Ref
Dried and smoked *Garcinia cambogia* fruit rinds	(−)-HCA	UV Spectrophotometry	Reliable, sensitive and specific method for HCA determination	828 μg/mL of HCA was resulted from 100 g *G. cambogia* and the absorbance was measured at 467 nm after addition of sodium meta vanadate solution	44
G. cambogia fruit rinds	Xanthochymol and isoxanthochymol	LC/ESI−MS/MS	Sensitive, short analysis time and selective	The limits of detection for xanthochymol and isoxanthochymol were 1.0 ng/mL and 0.5 ng/mL, and xanthochymol was quantified (4.93 ng/mL)	54
Dried leaves of *G. cambogia, G. indica, G. xanthochymus,* and *G. morella*	(−)-HCA, lactone, and citric acid	HPLC	Rapid and sensitive methods in isolation of HCA and lactones	The amount of (−)-HCA, lactone, and citric acid in *G. cambogia* were estimated at 7.95% w/w, 3.25%w/w, and 0.13%w/w, respectively	45
The fruit rinds of *G. cambogia*	Isoxanthochymol and camboginol	(LC/ESI-MS/MS)	A validated, sensitive and selective, rapid method	The limits of detection for isoxanthochymol and camboginol were 2.0 and 5.0 ng/mL and quantified 11.3 and 57.7 ng/mL, respectively	55
G. cambogia fruit rinds	HCA	HPLC-UV	Selective method for HCA analysis, validated for levels ranging from 2−10 mg/mL with acceptable reproducibility and accuracy	The method showed good reproducibility and accuracy at concentrations ranging from 2 to 10 μg of HCA. The mean recovery of the HCA from *G. cambogia* extracts ranged from 98.4−100.5%	8
Fruit rinds of *G. cambogia*	Isoxanthochymol and camboginol	HPLC-LC-ESI-MS	Validated in terms of repeatability and precision	LOD and LOQ were 5 and 10 g/mL for isoxanthochymol and 50 and 100 g/mL for camboginol, respectively. The intra- and interday precisions were 2.34% and 3.41% for isoxanthochymol and 3.35% and 3.66% for camboginol. Quantification of isoxanthochymol and camboginol in *Garcinia* extracts were 16.6 and 88.2 mg/g	56
Fruit rinds of 8 different *Garcinia* species	α-mangostin, β-mangostin, γ-mangostin, xanthochymol, and isoxanthochymol	UHPLC-PDA	The method is accurate, precise, linear, and reproducible	xanthochymol, and isoxanthochymol were detected in different *Garcinia* species extracts and in *G. cambogia* was 21.42 and 0.89%w/w, respectively	57
Ten herbal products that included either *G. cambogia* or *G. indica*	(−)-hydroxycitric acid and (−)-hydroxycitric acid lactone	^{1}H NMR	Rapid, no need for additional cleanup of extracts or derivatization, possible to detect of all kind of organic molecules in the same sample, highly reproducible with little instrument-instrument variation, noninvasive, and nondestructive method	(−)-hydroxycitric acid and (−)-hydroxycitric acid lactone content in *G. cambogia* and *G. indica* ranged from 1.7% to 16.3%, and 3.5% to 20.7% respectively.	53
Garcinia commercial formulation, 20 mg/kg in rat plasma	HCA	UPLC−MS/MS	Highly sensitive, accurate, precise and linear method for determination of HCA quality control in rat plasma	the concentration range of precision was 10.5−5000 ng/mL, LLOQ, 31.25 ng/mL	59
Human plasma	HCA	UPLC-MS/MS	Rapid, selective and sensitive UPLC-MS/MS method	5.02−12.01% (CV%). 0.29 to 9.20% (RE%). Linearity range 0.05−10 μg/mL	58
Human plasma	HCA	GC-MS	The derivatizing reagent used was N,O-bis(trimethylsilyl)fluoroacetamide and 10% trimethylchlorosilane	HCA plasma level ranged from 0.8 to 8.4 mg/mL 30 min and 2 h after ingestion of 2 g HCA, respectively	50
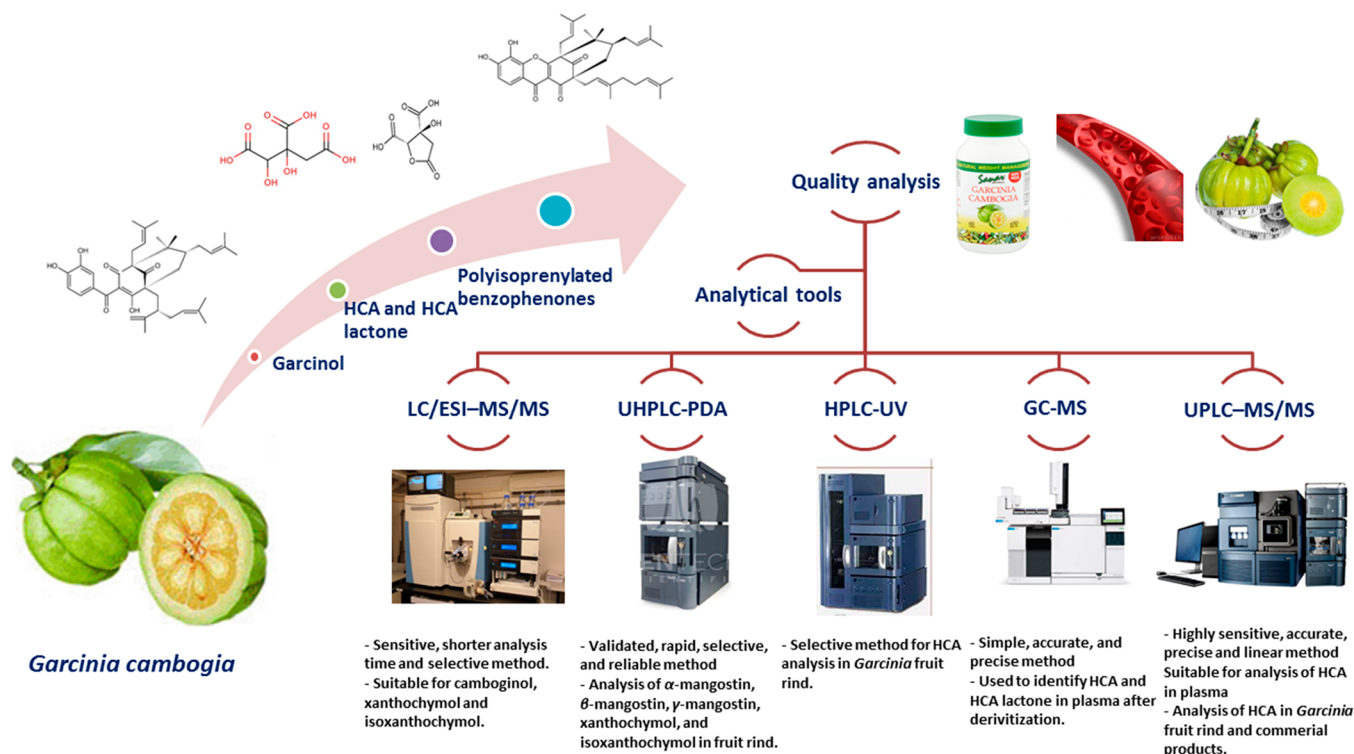
Figure 2. Different analytical methods applied for the analysis and quality control of *G. cambogia*.

and polyisoprenylated benzophenones, *viz.*, garcinol (camboginol or guttiferone E) and isogarcinol (cambogin), guttiferone I, guttiferone N, guttiferone J, guttiferone K, and guttiferone M.[39] Xanthones were also reported in *G. cambogia* such as garbogiol, rheediaxanthone A, oxy-guttiferone I, oxy-guttiferone K, oxy-guttiferone K2, and oxy-guttiferone M[39] (Figure 1). Xanthones, benzophenones, and HCA as the major constituents in *G. cambogia* were reported for their biological activities such as antiobesity,[40] hypolipidemic,[41] and anticancer activity.[42]

Several analytical methodologies were reported in the literature for the determination and quantification of *G. cambogia* fruit bioactive metabolites and its commercial products (Table 1, Figure 2). Moreover, the bioavailability and pharmacokinetics of these metabolites in the human body have been monitored to prove drug efficacy and safety inside the body. The next sections shall cover different approaches employed for the analysis of *Garcinia* major bioactive classes, highlighting both advantages and limitations for each technique.

Hydroxycitric Acid (HCA) and Lactones. HCA is the major organic acid identified in *G. cambogia* fruit and the main chemical used in weight loss programs due to its antiobesity action and appetite suppressant effect.[39,43] HCA content in *G. cambogia* fruits was analyzed spectrophotometrically by forming a unique, specific, and stable colored complex with sodium meta vanadate and measuring its absorbance at 467 nm.[44] HCA amounted for *ca.* 3−5% of *G. cambogia* fruits. The average concentration versus absorbance plot showed positive correlation at a concentration range of HCA (5−50 μg/mL).[44]

In another study, HCA and other organic acid levels in *G. cambogia* were estimated compared to other species in that genus to include *G. indica*, *G. xanthochymus*, and *G. morella* using HPLC-UV. Results revealed that *G. cambogia* encompassed (−)-HCA, HCA lactone, and citric acid at 7.9, 3.2, and

0.13% w/w, respectively. Additionally, *G. cambogia* and *G. indica* were found to contain the highest (−)-HCA and its lactone levels,[45] and suggestive that *G. indica* can be used instead of *G. cambogia* especially if targeting its HCA. Likewise, (−)hydroxycitric acid was analyzed in *G. cambogia* commercial samples using HPLC/UV.[46,47] The mean recovery of HCA from *G. cambogia* extracts ranged 98.4−100.5% with coefficients of variation of 0.25−0.63% and accuracy range of 2−10 μg, reflecting that the method is validated for the routine analysis of HCA in commercial *G. cambogia* extracts.[46] The method also offered selectivity compared to traditional acid base titration methods which quantify for total acids, not only targeting HCA.[46] HCA quantification in two *G. cambogia* marketed extracts containing 50% of HCA was performed using HPLC-DAD.[48] HCA quantification in samples ranged 36.1−41.5%, and in agreement with results specified by the distributors. Another LC-based method employing capillary electrophoresis coupled to UV detection at 200 nm was reported for the qualitative determination of HCA in the presence of other organic acids, *viz.*, citric, malic, and tartaric in *G. cambogia* fruits.[49] Lactone and nonlactone from HCA can also be identified and quantified in the fruit rind of *G. cambogia* using gas chromatography coupled with mass spectrometry (GC/MS) post derivatization.[29,50] The derivatization step in GC/MS though complicates the procedure and can affect HCA recovery from its matrix and should be accounted for during absolute quantification.[51]

Compared to chromatographic techniques extensively reported for HCA determination, direct spectroscopic techniques such as NMR were reported for the quantification of (−)-HCA and its lactone in raw herbal drugs and *Garcinia* food supplements. Compared to hyphenated techniques, NMR is more robust and can quantify for targeted metabolites with no need for standard curve preparation being normalized to internal standard spiked in sample such as hexamethyldisilox-

ane (HMDS). Nevertheless, NMR suffers from low sensitivity and signal overlap hampering quantification in certain signals.[52] The NMR method was used with DNA barcoding for the authentication of *Garcinia* fruit rinds and food supplements,[53] with ^{1}H NMR spectroscopy found effective for distinguishing between both diasteriomers (2*S*,3*S*) and (2*S*,3*R*) HCA.[29]

Polyisoprenylated Benzophenones. Xanthochymol and isoxanthochymol were identified and quantified in *G. cambogia* fruit rind using the LC/ESI−MS/MS technique, found more sensitive and accurate than classical HPLC-UV methods.[54] Two polyisoprenylated benzophenones known as isoxanthochymol and camboginol were identified and quantified in *G. cambogia* fruit rinds using a sensitive LC/ESI-MS/MS method.[55,56] The method was validated in terms of linearity, accuracy, specificity, and precision, posing it to be used for screening of these benzophenones in different *G. cambogia* extracts including commercial products.[55,56] The LC-ESI/MS/MS method was shown to be more sensitive than the classical HPLC-UV method, with the lowest limit of quantification (LLOQ) of 2−4 ng/mL for isoxanthochymol and 5−10 ng/mL for camboginol, employed for the assay of these two compounds in other *Garcinia* species.[55,56] Isoxanthochymol and camboginol were detected in *G. cambogia* fruit rind at levels of 11.3 and 57.7 ng/mL, respectively, while in the study of Kumar et al. (2009) they were quantified at 16.6 and 88.2 mg/g, respectively.[55,56] Three xanthones and two polyisoprenylated benzophenones were identified and quantified in 8 Indian *Garcinia* fruit rinds using a validated ultra-HPLC (UHPLC)-photodiode array (PDA) method. Metabolites included α-mangostin, β-mangostin, γ-mangostin, xanthochymol, and isoxanthochymol.[57] Quantification of each metabolite was reported in extracts of different polarities, *viz.*, *n*-hexane, chloroform, ethyl acetate, and methanol. α-Mangostin was quantified in *G. mangostana* detected only in nonpolar extract, *i.e.*, *n*-hexane at (5.56%, w/w) and absent from polar ones, *i.e.*, chloroform, ethyl acetate, and methanol, whereas β-mangostin was detected at the highest level in chloroform (0.29%, w/w).[57] γ-Mangostin was detected in all tested *Garcinia* species excluding *G. pedunculata* with the highest level detected in *G. mangostana n*-hexane extract (0.76%, w/w).[57] Among the four examined *Garcinia* species, *G. xanthochymus n*-hexane extract showed the maximum level of xanthochymol at 30.38% w/w.[57] In addition, isoxanthochymol was quantified in all extracts, with the highest level detected in the chloroform extract of *G. indica* 3.07%, w/w.[57] Ultimately, results revealed that the *n*-hexane extract is most rich in xanthones and polyisoprenylated benzophenones posing *n*-hexane as the optimum solvent for higher recovery of these active metabolites from *Garcinia*.

Pharmacokinetics Determination of G. cambogia Metabolites in Biofluids. Analysis of *G. cambogia* active constituents and their metabolites in body fluids is performed typically using most sensitive hyphenated analytical techniques, i.e., MS detection to assess active metabolites bioavailability and pharmacokinetics. The first determination of HCA in plasma has been reported using ion chromatography with a linear gradient elution of NaOH ranging 0.5−38.25 mM in water.[43] Plasma HCA level was determined after the ingestion of a single bolus dose of HCA solution (4.4 g) over a 3.5 h period revealing maximal plasma level at 60−90 min of 0.12 mmol/L.[43] Later, Loe et al. (2001) developed a GC/MS method for the determination of HCA as a marker for *G. cambogia* administration in human plasma post derivatization, and

verified for the determination of HCA bioavailability after ingestion of 2 g HCA in 4 subjects, with plasma HCA level ranging from 0.8 to 8.4 mg/mL 30 min and 2 h after ingestion, respectively.[50] The method efficiency was aided by the high separation power of the GC column with improved resolution than typical LC for minimal sample preparation. The advances of high resolution GC and LC coupled to time-of-flight (TOF) mass spectrometers has yet to be reported for profiling of *G. cambogia* or determination of its biotransformed products inside the body.

The quantification of HCA in humans and rats was evaluated using a rapid, selective, and sensitive UPLC-MS/MS method.[58,59] HCA was analyzed in 16 healthy subjects after oral administration of *G. cambogia* extract revealing an excellent mean peak area ratio of 0.98 and coefficient of variation CV% of 7.17 from normal, lipemic, and hemolyzed plasma samples.[58] Excellent intra-assay and inter-assay precision were achieved, ranging from 5.02% to 12.01% (CV%) as well as intra- and interassay accuracy ranging from 0.29% to 9.20% relative errors (RE%). UPLC-MS/MS is highly efficient to be applied for HCA pharmacokinetic study on a large scale, as it can analyze more than 670 plasma samples.[58] Likewise, HCA was analyzed in rat plasma after i.v. administration of a dose of 1 mg/kg, where the half-life and the apparent volume of distribution of HCA reached 2.1 ± 0.4 h and 0.6 ± 0.15 L/kg, respectively, whereas with oral administration of 20 mg/kg, HCA showed maximum plasma concentration C_{max} at 14.4 μg/mL reached at 1 h. The method was reproducible, sensitive, accurate, precise, and linear in the concentration range of 10−5000 ng/mL.[59] Further, the method was successfully used to determine HCA pharmacokinetics (PKs) in rat plasma after the administration of pure HCA and a commercial *Garcinia* preparation. The absolute bioavailability of HCA after the administration of commercial preparation was at 61.3%, after correcting the dose for the actual content of HCA (i.e., 12 mg/kg). The method was successfully used to determine *in vivo* PKs of HCA revealing that it is rapidly absorbed with moderate apparent volume of distribution and a good bioavailability. The method reported can be further used routinely for the analysis of HCA and could be helpful for further toxicological evaluation of *Garcinia* products alone or in a mixture.[59] A validated analytical method for quantification of HCA in rat plasma and fetal homogenate using LC-MS/MS analysis was reported. Results revealed that at a range of 20−800 ng/mL the method was linear with limits of quantitation (LOQ) and detection (LOD) equal to 20.0 and 3.9 ng/mL plasma, respectively. The accuracy and precision using quality control standards were ±7.5% and 9.5%, respectively.[60]

Effect of administration route on blood *G. cambogia*/HCA level and antioxidative activity was examined in a randomized study on healthy women compared between fasted and fed conditions post-ingestion of 1500/750 mg of *G. cambogia*/HCA under 8 h of fasting.[61] In the fed period a high calorie breakfast (∼600 cal) was given after dosing revealing reduced HCA plasma concentrations in the fed state with a higher volume of distribution and clearance and large interindividual variations observed.[61] HCA was found to be affected by the fed state indicating a possible adsorption between HCA and food, so administration is preferable on fasted stomach or at least 2 h before a meal, avoiding against food/HCA interaction.[61]

In another study, GC/MS was utilized to analyze serum metabolite changes in broiler chickens after the administration
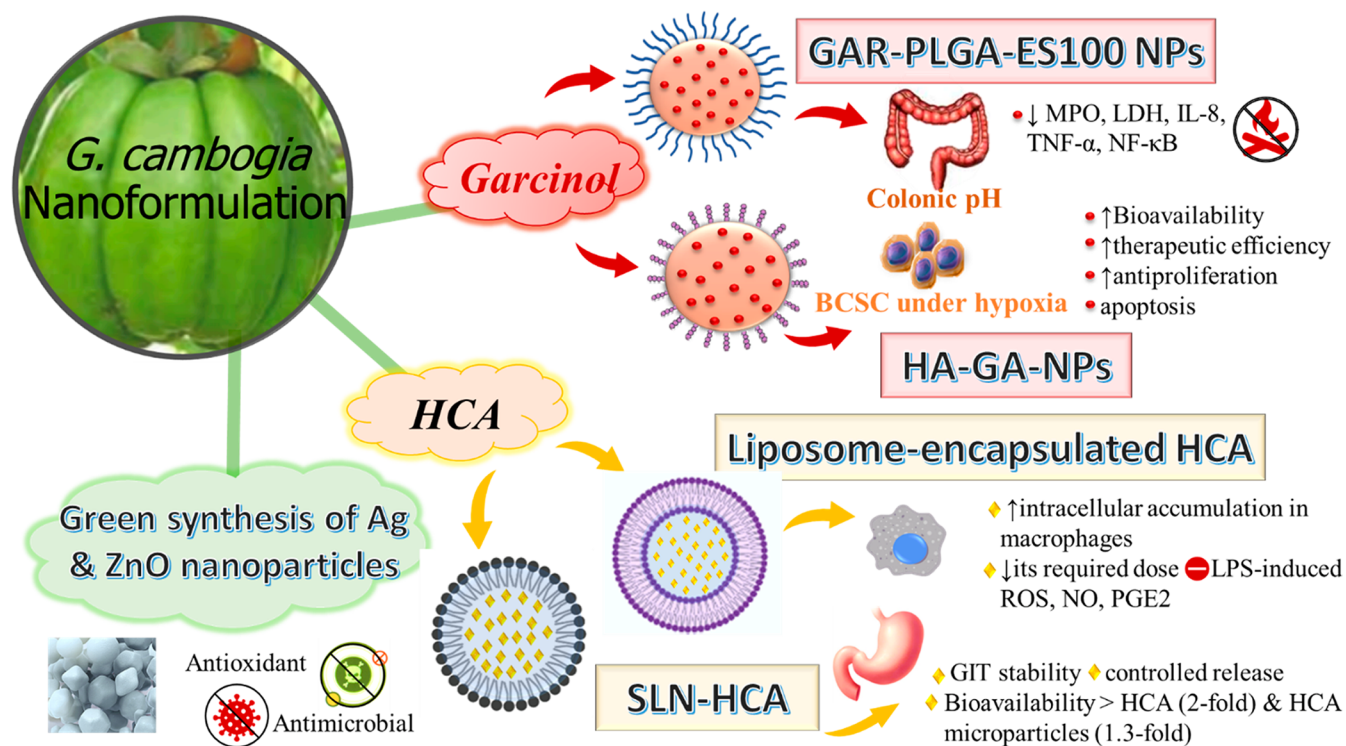
Figure 3. Nanoformulations prepared from *G. cambogia* to improve bioavailability of selective metabolites and efficacy. BCSC, breast cancer stem cells; GAR-PLGA-ES100 NPs, garcinol-poly(lactic-*co*-glycolic acid)-Eudragit S100; HA-GA-NPs, garcinol-hyaluronic acid-poly(lactic-*co*-glycolic acid); IL-8, interleukin-8; LDH, lactate dehydrogenase; MPO, Myeloperoxidase; NF-κB, nuclear factor kappa light chain enhancer of activated B-cells; NO, nitric oxide; PGE2, Prostaglandin E2; ROS, Reactive oxygen species; SLN-HCA, hydroxy citric acid loaded nanoparticles; TNF-α, tumor necrosis factor-α.

of (−)-HCA at 0, 1000, 2000, and 3000 mg/kg diets for 28 days. Results showed that 20 metabolites in the 1000 mg/kg (−)-HCA treatment group and 16 metabolites in the 3000 mg/kg (−)-HCA treatment group were significantly altered. The data indicated that (−)-HCA promoted protein biosynthesis by regulating the metabolic directions of amino acids, to depend on the dose of administered (−)-HCA. Likewise, (−)-HCA decreased body weight gain by reducing the abdominal fat deposition in broiler chickens and inhibited fatty acid synthesis by promoting the citric acid cycle.[62] Reduction in cytosolic acetyl-CoA level is associated with decreased lipids biosynthesis and is likely to account for the *Garcinia* slimming effect.

Extraction Optimization and Nanoformulation of *G. cambogia* Bioactives. *Extraction Optimization.* Several methods have been described for the extraction and isolation of HCA from *G. cambogia*. First methods reported on (−) HCA isolation in the form of its lactone on a large scale from dried rinds, used either acetone followed by aqueous extraction of the acid with a yield of *ca.* 15%[63] or hot water for extraction, followed by alcohol precipitation of pectin; then KOH is added to obtain *ca.* 20% of HCA potassium salt after purification.[64] A more recent method used an anion exchange column for the adsorption of (−)-HCA from a salt-free water extract of *Garcinia* rinds, eluted with sodium/potassium hydroxide, and then loaded on a cation exchange column to yield concentrate containing 23−54% free HCA and 6−20% HCA lactone.[65]

Although fewer techniques were reported for *G. cambogia* extraction, several methods including novel extraction techniques have been described for the better utilization and recovery of other *Garcinia* species, of potential to be applied in

the future to *G. cambogia* considering its close chemical composition. Microwave-assisted extraction followed by micro-encapsulation was applied to *G. cowa* to reduce the lactonization of HCA, and to produce higher HCA yield with an overall improved antioxidant activity compared to that extracted using autoclave.[66] Moreover, response surface methodology was adopted for *G. mangostana* rind to optimize for the extraction of antioxidant pectic-polysaccharide (*ca.* 12.0−12.4%) with higher uronic acid content (*ca.* 20.2−21.1 mg/g) and antioxidant activity 225−252% DPPHsc/g,[67] total phenolics,[68] and xanthone-rich extract.[69] Applying response surface methodology for *G. mangostana* revealed that using 0.05 solid to solvent ratio and methanol (69.77%) for 2 h extraction yielded the maximum total phenolic content (140.66 mg gallic acid equivalent (GAE)/g). While optimization of microwave-assisted extraction revealed that using 25 mL/g (solvent to solid ratio), ethanol (71%) and irradiation time of 2.24 min were found optimum for obtaining antioxidant-rich xanthone extract from *G. mangostana*. Likewise, and from another *Garcinia* sp., response surface methodology was employed to optimize for the aqueous two-phase system extraction of *G. indica* fruit rind bioactives, *viz.* anthocyanins, garcinol, isogarcinol, and hydroxycitric acid. To clarify, an aqueous two-phase system made up of 1-propanol (15.202% w/w) and $(NH_4)_2SO_4$ (10.242% w/w) with 2.5 g crude load achieved the maximum yield of garcinol (97.39%) and isogarcinol (92.38%) in the top phase, anthocyanins (99.19%) and HCA (99.83%) in the bottom one with a purity higher than 99% by applying secondary aqueous two-phase system.[70] Furthermore, high-intensity ultrasound-assisted extraction for *G. madruno* increased the

yield of bioflavonoids by *ca.* 3-fold (370 mg/100 mL of the total BF),[71] while cellulase-assisted extraction was applied for the direct and enhanced recovery of mangostins (potential xanthone compounds) from *G. mangostana* pericarps[72] which possess promising biological activities.[73] Additionally, α-mangostin was extracted from *G. mangostana* using natural deep eutectic solvents designed as green solvents leaving no solvent residue in the final product.[74] Such success in improving the extraction output in other *Garcinia* species should encourage its application for extraction optimization in *G. cambogia* especially for inclusion in nutraceuticals.

Nanotechnology and Nanoformulations. The use of natural products for the treatment of metabolic diseases is limited by their low bioavailability. Formulation of these phytochemicals such as polyphenols and flavonoids by nanotechnology has the potential to improve their hydrophilicity, stability, bioavailability, and safe and controlled delivery to target sites. Nanoformulations such as nanoemulsion, nanoliposomes, solid lipid nanoparticles, nanostructured lipid carriers, and polymeric nanoparticles are the most common reported in the literature.[75] Nanotechnology has been rapidly developing in the past decade in nutraceuticals.[76] Nanotechnology is increasingly utilized to design novel formulations of nutraceuticals for the following objectives: to protect degradation of compounds during storage or processing, mask undesirable tastes, enhance their solubility and dispersion, control their release, and improve their oral bioavailability.[77] Nanocarriers, *e.g.*, nanodispersion, nanoemulsion, liposomes, and polymer micelles, enclose and deliver bioactive compounds to increase their gastrointestinal tract uptake through active endocytosis or to enhance their bioavailability.[78] *G. cambogia* has been employed for the green synthesis of silver nanoparticles with strong antimicrobial potential compared to the original plant extract.[79] Likewise, it was used in the synthesis of multifunctional ZnO nanoparticles with antioxidant properties (DPPH radical scavenging activity with IC$_{50}$ 93.16 μg/mL), cytotoxic activity (IC$_{50}$ 27.5 μg/mL and 68.8 μg/mL for MCF 7 and HeLA cell lines, respectively using MTT assay), and antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* with 19, 13, and 14 mm zones of inhibition at a concentration of 500 μg/mL (Figure 3).[80]

With regard to nanoformulation employment as a drug delivery system imparting higher clinical efficacy, several assembled drug nanoparticles have been developed, *e.g.*, liposomes, polymeric nanoparticles, niosomes, dendrimers, metallic nanoparticles, and nanostructured lipid carriers.[81] A nanodelivery system encompassing garcinol, hyaluronic acid, and poly(lactic-*co*-glycolic acid) (HA-GA-NPs) has been developed as a nanocarrier for garcinol targeting breast cancer stem cells under hypoxia (Figure 3). The HA-GA-NPs nanodelivery system improved the bioavailability, and consequently the therapeutic efficiency of garcinol, the aqueous insoluble polyisoprenylated benzophenone derivative. HA-GA-NPs exhibited higher antiproliferative activity using WST-1 cell proliferation assay (IC$_{50}$ 5.54, 4.03, and 0.81 μg/mL at 24, 48, 72 h, respectively) compared to that of pure garcinol (IC$_{50}$ 21.16, 17.06, 10.22 μg/mL, respectively). It also showed enhanced cellular uptake via receptor-mediated endocytosis, and apoptosis potential confirmed by caspase-3/7 activation.[82] Moreover, pH-sensitive biodegradable nanoparticles has been formulated using garcinol, poly(lactic-*co*-glycolic acid), and Eudragit S100 (GAR-PLGA-ES100 NPs) as illustrated in

Figure 3. It specifically targeted the colonic pH where it releases the drug to exert its anti-inflammatory effect of potential for application in ulcerative colitis. GAR-PLGA-ES100 NPs decreased myeloperoxidase (MPO) and lactate dehydrogenase (LDH) activity, downregulated interleukin (IL)-8, tumor necrosis factor (TNF-α), and nuclear factor kappa light chain enhancer of activated B-cells (NF-κB) expression suggestive that it could be beneficial in inflammatory bowel disease.[83]

On the other hand, HCA loaded nanoparticles (SLN-HCA) demonstrated better HCA gastrointestinal stability (*ca.* 88%) with controlled release (Figure 3). They showed superior bioavailability compared to unencapsulated HCA and microparticles (SLM-HCA) by 2- and 1.3-fold, respectively.[84] Furthermore, liposome-encapsulated HCA improved HCA intracellular accumulation in macrophages (*ca.* 4 times) and decreased the required dose to combat LPS-induced ROS, NO, and PGE2 (*ca.* 10 times), suggestive of its possible use in inflammatory conditions.[85]

Although the *G. cambogia* whole extract has not yet been nanoformulated to the best of our knowledge, an interesting formula has been prepared from *G. mangostana* extract. The extract as well as α-mangostin were individually encapsulated into the mucoadhesive, relatively acid stable nanocarriers ethylcellulose and methylcellulose (ECMC). The *G. mangostana* loaded naonocarrier showed sustained release of the extract at pH 2.0 and 7.4. The encapsulated extracts exhibited a strong *in vitro* activity against *Helicobacter pylori* nearly similar to the free ones, where α-mangostin showed nearly half MIC values (31.3−62.5 μg/mL) compared to *G. mangostana* extract against 5 *H. pylori* strains (62.5−125 μg/mL). Nevertheless, α-mangostin exhibited nearly the same activity as metronidazole; however it was weaker than both clarithromycin (higher MIC by 130−4170-fold) and amoxicillin (higher MIC by 100−7800-fold). The encapsulated forms not only retained the *in vitro* anti-*Helicobacter pylori* effect but also exhibited anti-adhesion potential even stronger than that of free extracts. *In vivo* study revealed the ability of orally administered encapsulated forms to tackle *H. pylori* in mice, contrary to the unencapsulated forms which may be attributed to the mucoadhesive properties of ECMC nanospheres and the sustained release,[86] and to be considered for inclusion in future clinical trials.

■ CONCLUSION

The current review capitalizes on the increasing application of *G. cambogia* fruit in nutraceuticals mainly for its antiobesity effects. Nevertheless, its effectiveness as an antiobesity agent remains contradictory. On the other hand, it exerts other promising biological activities, *viz.*, anti-ulcerogenic, antiseptic, anti-inflammatory, hepatoprotective, antioxidant, erythropoietic, and cytotoxic effects which are clinically less well explored. Henceforth, further controlled, large-scale clinical trials are recommended to unravel its effectiveness related to its bioavailability. Moreover, the pharmacological effects of its individual compounds should be further studied and their synergistic effects should be assessed. In addition, its interaction with gut microbiota should be further explored and related to its antiobesity effect. Several analytical procedures employed for the quality control as well as pharmacokinetics evaluation of *G. cambogia* phytochemicals were reviewed. Analytical techniques targeted either qualitative and quantitative analyses of *G. cambogia* bioactive metabolites

in fruit rind and further commercially available products. UPLC coupled to high-resolution MS appeared to be the most suitable tool offering high sensitivity and accuracy for the analysis of HCA in fruit rind, commercial products, and human plasma, while GC-MS analysis post derivatization enabled the characterization of HCA and its metabolites in plasma to be considered for detection of HCA metabolic fate post administration. The application of NMR analysis and other metabolome tools is still limited, and coupling with multivariate data analysis has yet to be considered for the analysis and quality control of *G. cambogia* metabolites in comparison to other *Garcinia* species. Based on the published data, it is evident that traditional methods of extraction are still widely applied for *G. cambogia*. On the other hand, recent strategies for extraction optimization are less explored in *G. cambogia*, while the success of using optimization methodologies for other *Garcinia* species should be considered as a sign to be examined for *G. cambogia*. Few nanoformulations have been prepared from *G. cambogia* bioactive constituents, *i.e.*, garcinol and HCA, and successfully induced their targeted activities. Future research should focus now on the development of nanoformulations for different bioactive compounds of *G. cambogia*, as well as its whole extract to benefit from their synergistic effects. Also, such nanoformulations should be biologically assessed compared to the original extracts, and their safety should be addressed prior to their clinical and safety assessments being investigated.

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Notes

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