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Comparative Composition Analysis of the Dried Leaves of *Ilex guayusa* (Loes.)

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Abstract Guayusa (*Ilex guayusa* Loes.) is a traditional herbal tea of western Amazon regions and an international commodity of increasing importance. Its consumption is rapidly growing in the USA and Canada, and the authorization of guayusa extract as a novel food in Europe signals further international market growth of this antioxidant and stimulant tea. However, little is known about the chemical composition of guayusa, despite much research on related *Ilex* species. There is an urgent need for a deeper understanding of the chemical composition of guayusa, to support assessments of its safety and its claimed nutritional value. This study follows the novel food assessment framework of the European Union Food Safety Authority, characterizing the proximate composition of guayusa and elucidating caffeine, amino acid and elemental components. It also evaluates potential microbial, mycotoxin and pesticide residue contaminants. The chemical composition of guayusa is analyzed in context with the related and well-characterized foods, yerba mate (*Ilex paraguariensis* A. St.-Hil.) and tea (*Camellia sinensis* L.). Guayusa's moisture content, caffeine concentration, amino acid compliment and elemental profile, including heavy metals, present no greater risk to human health than the consumption of tea or yerba mate. It is also established that there is a low risk of mycotoxin, bacterial or pesticide residue contamination of guayusa. The chemical composition of guayusa presents no barrier to the authorization of guayusa as a novel food in accordance with European Union novel food guidelines.

Keywords: *guayusa, yerba mate, traditional food, novel food, antioxidant, stimulant, herbal tea*

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1. Introduction

Guayusa (*Ilex guayusa* Loes.) is a flowering plant in the family Aquifoliaceae, and one of over 800 holly (*Ilex*) species [1]. Guayusa is a native tree found in the western Amazon, typically located on ancestral land of indigenous peoples used for agroforestry [2,3]. Its putative infertility and close association with human habitation have led to the conclusion that guayusa may be a domesticated species resulting from a long period of cultivation [4]. For centuries, guayusa leaves have been grown and consumed as a traditional food by indigenous peoples in the western Amazon regions of Ecuador, Peru, Colombia and Bolivia [3,4,5]. This study treats guayusa as a regularly consumed beverage, which is distinct from its use in different formulations as a traditional medicine [6,7,8]. Importantly the traditional consumption, and now international consumption of guayusa as a novel food currently occurs in the absence of any scientific evaluation of is proximate or elemental composition. Nor have scientific evaluations of possible microbial, mycotoxin or pesticide residue contaminants been published. This paucity of knowledge impedes the safe international growth of guayusa consumption.

A large consumer market for guayusa exists in Ecuador, being most evident in Ecuador's Amazon Napo province [4,9]. Furthermore, a recent increase in global guayusa consumption has resulted from strong industry development of guayusa as an Ecuadorian export commodity. In 2017 the volume of dried guayusa leaves exported from Ecuador exceeded 100 tons [10]. The modern international guayusa consumer market is currently most well established in the USA, where international commercialization and marketing first began in 2010 [2]. Consumer markets in other countries are currently positioned to expand rapidly with the novel food status of guayusa products having been considered now in Europe [11], Australia and New Zealand [12]. Against this backdrop of rapid guayusa internationalization as a novel food, emerges a strong need for the comprehensive characterization of its chemical composition.

Many foods that are commonly consumed have never been systematically assessed to determine their risk to human health [13]. Long histories of use support the public perception of those foods as being safe even in the

absence of chemical composition studies. However, the potential public health risk associated with large-scale consumer adoption of novel foods demands explicit investigation of their chemical compositions. European Union Food Safety Authority Panel guidance on notifications for authorization of traditional foods from third countries under Regulation (EU) 2015/2283 advises that analysis of botanical foods like guayusa should include proximate composition analysis in addition to consideration of nutritionally relevant constituents and substances of potential concern to human health, including potential microbial, heavy metal and pesticide contaminants [14]. Furthermore, principles for premarket assessment of novel plant foods include comparison with relevant or closely related taxa [15]. For guayusa, such a comparison with yerba mate (*Ilex paraguariensis* A. St.-Hil.) is advantageous since that *Ilex* species is already well studied, having significant commercial production and broad consumption in South America [16,17,18]. The comprehensive EU regulatory framework for authorization of traditional foods from third countries as novel foods in Europe, highlights that a deep understanding of chemical composition is critical for the safe adoption of novel foods.

Despite these human health, regulatory and industry development needs, chemical composition analyses of guayusa are fragmented. In 2013 Jara et al. [19] published a brief study of total phenolic and total carotenoid content of guayusa. That work was followed in 2016 and 2017 by a more detailed study by Garcia-Ruiz et al. [20,21], which also analyzed phenolic and terpenoid compounds. They quantified total phenolic and total carotenoid content as well as identifying 14 hydroxycinnamic acids and flavinols (principally chlorogenic acid and quercetin-3-O-hexose respectively), and five carotenoids. A similar analysis of those same compounds in guayusa leaves at different stages of ripening was published in 2017 by Villacís-Chiriboga et al. [22]. Also in 2017 Pardau et al. [23] identified 22 phenolics, major constituents being mono- and dicaffeoylquinic acid derivatives. Moldoveanu and Scott [24] analyzed terpenoid compounds in several bioactive botanicals, including guayusa, identifying four pentacyclic triterpenoid acids. Moldoveanu et al. [25,26] followed that study with analyses of the carbohydrate and amino acid profiles in guayusa and other bioactive botanicals. The caffeine and theobromine contents of dried, blanched or cold soaked guayusa leaves were characterized by Lewis et al. in 1987 [27] and 1991 [28]. Radice and Vidari also quantified caffeine content in guayusa leaves in a study of 2000 [29]. More recently, quantification of the caffeine content of guayusa has become a regular focus of Ecuadorian undergraduate student studies, for examples see Barriga Coronel [30], Cobos Morales [31], or Melo Gallegos [32]. The caffeine composition of a sample of ancient guayusa leaves has also been characterized by Holmstedt and Lindgren [33].

Despite the elucidation of its stimulant and antioxidant properties, a comprehensive chemical composition analysis of guayusa, as required for novel food risk assessment, is lacking. In order to reach an understanding of the chemical composition of guayusa, this study analyzes the proximate, caffeine, elemental and amino acid compositions of a broad selection of guayusa leaves, in line with the EU framework for safety assessment of novel foods. Furthermore we consider potential microbial, mycotoxin and pesticide contamination of guayusa samples from the Napo province of the Ecuadorian Amazon. We discuss these results in context with the known chemical compositions of two relevant or related taxa commonly used as tea or herbal tea infusions.

While this study has importance for safe guayusa consumption, it also has key agricultural significance. Because guayusa is a product of ancestral agroforestry systems, its development as an international commodity could stimulate positive impact for the economic resilience of Amazon indigenous communities. This in turn would support those agricultural systems, which have important environmental benefits in the sensitive Amazon basin [34,35,36].

2. Materials and Methods

2.1. Sample Collection and Preparation

This is a study of *Ilex guayusa* (Loes.), of the Aquifoliaceae family. Guayusa leaf samples were collected from traditional agroforestry operations across the Napo province of Ecuador as a part of the normal manual harvest process of commercial guayusa growers, using methods described by Krause and Ness [2]. Samples were then oven dried within one day of harvest. Dried leaves were mechanically chopped into a coarse residue then packaged and sealed to prevent contamination prior to transport for laboratory preparation and analysis.

2.2. Analytical Methods

All chemical composition analyses were carried out by a certified European analytical laboratory, specifically accredited for all methodologies used in this study in accordance with ISO/IEC 17025:2005 2nd Edition. Moisture content was analyzed by air oven drying at 100-105°C using method 925.19 published by AOAC International [37]. Ash value was assessed through ignition in an ashing furnish using the Commission Regulation (EC) 152/2009 method of analysis for determination of ash. Crude lipid was analyzed by pulsed NMR spectroscopy. The dried samples were stabilized at 50°C in a heating block prior to being subjected to a pulsed low-resolution NMR field in a Bruker mq20 Series PC 120 20MHz NMR analyser, using the direct method published as the AOCS Official Method Cd 16b-93 [38]. The resulting fat content was determined by comparing the resonance of the sample with a two-point calibration curve determined using a certified olive oil standard. The Dumas method was used to determine total nitrogen content using an automated Dumas Leco Nitrogen Analyzer. Crude protein was calculated from total Nitrogen using a conversion factor of 6.25 as recommended for tea by AOAC international [37]. Total carbohydrate was calculated by difference whereby % carbohydrate = 100 - (% moisture + % fat + % protein + % ash). Crude fiber was determined by enzymatic digestion and denatured alcohol precipitation using method 985.29 published by AOAC International [37].

Caffeine was extracted from samples by boiling for one hour in deionized water. After cooling, extracts were

filtered through a 0.45 µm nitrocellulose filter. Diluted aliquots (20:1) were analyzed by HPLC using a Grace C18 reverse-phase column, 100 mm x 4.6 mm with a 4 µm particle size and eluted with citrate phosphate buffer at pH 7, having 6.5 ml 0.1 M citric acid and 43.6 ml 0.2 M dibasic sodium phosphate to 100 ml of water, and methanol (90:10), at a flow rate of 1 ml/min. UV detection at 272 nm was used.

Total and free amino acid composition was analyzed by HPLC using the method that is published in EC Regulation 152/2009 for the determination of amino acids (except tryptophane). Samples were heated under acidic conditions at 115°C to hydrolyze the protein chains. For the analysis of cystine and methionine, the samples were oxidized with performic acid prior to acid hydrolysis. The resulting hydrolysates were diluted, filtered and pH adjusted. The extracted amino acids were then derivatized prior to determination by gradient HPLC with fluorescence detection.

Determination of elements was carried out by atomic absorption spectrophotometry with inductively coupled plasma and stoichiometric calculation of concentration from measured values, using a method published in ISO 11885:2007 for atomic absorption elemental analysis. Samples were homogenized and mineralized by acids and hydrogen peroxide prior to analysis.

Aerobic colony count was enumerated using a horizontal method for the enumeration of microorganisms by means of the pour plate technique at 30°C after 72 hours, as published in ISO 4833-1:2013. Enterobacteriaceae (presumptive coliforms) were enumerated by means of the most probable number colony count technique published in ISO 21528-2:2017. A horizontal method for the enumeration and confirmation of coagulase-positive staphylococci with Baird-Parker agar medium was used as published in ISO 6888-1:1999. A horizontal method for the enumeration of presumptive *Bacillus cereus* by means of the colony-count technique at 30°C was used as published in ISO 7932 (2004). A horizontal method for the enumeration of viable osmophilic yeasts and xerophilic molds was used by means of the colony count technique at 25°C as published in ISO 21527-2:2008. A horizontal method for the detection, enumeration and serotyping of *Salmonella spp*. serovars was used as published in ISO 6579-1: 2017. A horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* was used by way of the colony-count technique at 44°C with membrane filtration and using 5-bromo-4-chloro-3-indolyl β-D-glucuronide, as published in ISO 16649-1:2001.

Fusarium-associated trichothecene mycotoxins were extracted from samples using an acetonitrile/water mixture. The extract was initially cleaned using alumina and charcoal column chromatography followed by a clean up by solid phase extraction using a Waters Oasis PRiME HLB column. The trichothecenes in the resulting extract were analyzed by UPLC-MS/MS using a Waters quadrupole time-of-flight MS system.

Samples were analyzed for the residues of 389 pesticides using GC/MS in accordance with the method published in BS EN 15662:2008, or using LC-MS/MS in accordance with the method published in BS EN 15637:2008. Both methods complied with the Codex Alimentarius CAC/GL 90-2017 guidelines of the Food and Agriculture Organization of the United Nations, on performance criteria for methods of analysis for the determination of pesticide residues in food and feed [39].

2.3. Statistical Analysis

Results for the analyses of guayusa samples are the mean of five determinations, plus or minus the standard deviation. Where possible, results for guayusa are compared with previously published results for *Camellia sinensis* using a Student's t-test with a confidence interval of ($p \leq 0.05$).

3. Results and Discussion

The proximate composition analysis of dried guayusa leaves is presented in Table 1 with units and significance values as recommended by the Food and Agriculture Organization of the UN [40].

Table 1. Proximate Composition of Dried Guayusa Leaves[a]

	Guayusa	Black Tea [41]	Yerba Mate [42]
Moisture content	5.4 ± 0.7	*6.8 ± 0.8	3.6 ± 0.4
Ash value	7.7 ± 0.9	6.3 ± 2.1	5.1 ± 0.2
Crude lipid	8.0 ± 0.2	*10.2 ± 0.5	4.3 ± 0.3
Total protein	14.8 ± 0.3	*8.9 ± 2.4	8.0 ± 0.2
Total carbohydrate	64.1 ± 1.5	*67.8 ± 2.0	79.0 ± 0.6
Crude fiber	37.0 ± 1.7	*15.8 ± 2.4	53.0 ±0.6

[a]Values are expressed as mean ± SD (n = 5). Units are g/100g. *Indicates a statistical difference to guayusa values ($p \leq 0.05$).

All values are expressed relative to wet weight, although all listed species have low moisture content, reflective of their nature as dried botanical products. Guayusa's moisture content of 5.4 ± 0.7 g/100g lies well below the maximum 10% moisture content that is considered safe in *Camellia sinensis,* with regards to the risk of microbial contamination [43]. Between guayusa and black tea there are statistically significant differences for all proximate values except ash, however all species are principally composed of carbohydrate and fiber. Significantly, the crude fiber content in guayusa is nearly twice as high as the value reported for black tea. The relatively low crude fiber value of black tea may reflect an inverse association between crude fiber and perceived tea quality [44,45]. Intense industry development activity that has generated *Camellia sinensis* cultivars and harvesting methods to maximize tea quality [46] may have have reduced fiber content in black tea.

The caffeine composition of guayusa reported in this study is 19.08 ± 0.31 mg/g, which is consistent with the caffeine composition reported in a previous study of Lewis (17.3 - 75.7 mg/g) [28]. Interestingly, these values are similar to the caffeine composition (18 mg/g) of ancient guayusa leaves sourced from a shaman's tomb in Bolivia dating back more then 1000 years [33]. Other studies published online show a much greater variation (8.13 – 75.8 mg/g) in reported caffeine composition [29,30,31,32]. These results collectively reinforce the commonly held view of guayusa as a stimulant beverage [28,47]. They also characterize guayusa leaves as a naturally caffeinated stimulant that probably has no

greater concentration of caffeine than yerba mate at 1% to 2% of dry weight (see Heck and Mejida [48] for a review) or green tea at 3.6 g/100g [49]. For this reason, and due to its minimal theobromine content [28], guayusa should present no greater risk to consumers as a stimulant than other commonly consumed tea infusions. The variations between some reported values for caffeine content in dried guayusa leaves may warrant further investigation to understand how caffeine accumulates in guayusa leaves during their growth period, and when to harvest leaves to achieve consistent caffeine levels across different batches.

Total and free amino acid composition of dried guayusa leaves is shown in columns two and three of Table 2. These data are compared with previously reported free amino acid values for guayusa and green tea. We determined values for all 17 assayed total amino acids and seven free amino acids in guayusa, while the other 10 assayed free amino acids, if present, were below limits of detection. The values of all seven free amino acids were all markedly lower than those reported for green tea. With the exception of aspartic acid, serine and glutamic acid, all free amino acid values were lower than those reported previously for guayusa. This may reflect a methodological difference between the gradient HPLC method with fluorescence detection used in this study and the non-derivatized detection method that was trialed in the pre-existing study. It may also be a consequence of sampling difference since the pre-existing study used guayusa samples of unknown geographical and agricultural provenance, while we have used mixed samples that represent a mean value for guayusa grown across the Napo province of Ecuador. While these results indicate that the amino acid profile of guayusa is generally lower than that of green tea, any nutritional significance of these differential values is untested and remains a potential focus for future study.

Table 2. Total and Free Amino Acid Composition of Dried Guayusa Leaves[a]

	Total Amino Acids	Free Amino Acids	Guayusa Free Amino Acids [26]	Green Tea Free Amino Acids [26]
Asp	1.09 ± 0.01	83.8 ± 8.3	53.3	517.9
Ser	0.54 ± 0.01	31.4 ± 20.4	10.7	101.7
Glu	1.21 ± 0.03	105.3 ± 46.7	50.1	600.1
Gly	0.53 ± 0.02	<LOD[b]	10	0.9
His	0.22 ± 0.01	6.2 ± 1.2	12.9	22.8
Arg	0.56 ± 0.02	<LOD	42.9	203.1
Thr	0.51 ± 0.02	5.5 ± 0.5	13.6	60.9
Ala	0.60 ± 0.02	20.8 ± 1.5	106.9	128.6
Pro	0.53 ± 0.01	10.2 ± 2.1	25.3	56.1
Cys	0.10 ± 0.01	<LOD	-	-
Tyr	0.38 ± 0.01	<LOD	12.9	88.6
Val	0.60 ± 0.02	<LOD	17.4	66.5
Met	0.21 ± 0.01	<LOD	5.2	<0.0181
Lys	0.64 ± 0.01	<LOD	9.2	42.4
Ile	0.48 ± 0.02	<LOD	13.2	45.9
Leu	0.86 ± 0.03	<LOD	12.5	41.5
Phe	0.55 ± 0.02	<LOD	11.0	80.1

[a]Column two and three values were determined in this study and are expressed as mean ± SD (n = 5). These values are compared with previously published values for guayusa and green tea. The units of total amino acids are mg/g. Units for free amino acids are µg/g. [b]<LOD = below limit of detection.

Elemental analyses of teas are most comprehensive for *Camellia sinensis*. That species contains elements such as Ca, Na, K, Mg, F, Al and Mn at milligram per gram levels and Cr, Fe, Co, Ni, Zn, Cd, Pb, As and Hg at microgram per gram levels, although values vary with agricultural conditions, harvest methods and post-harvest processing, see Vázquez and Vélez [50] for a recent review. As shown in Table 3, the dried guayusa leaves assayed in this study also possessed the essential macroelements K, P and Mg at milligram per gram levels while a much lower level for Na was determined. This macroelemental profile is mirrored in yerba mate and black tea [51,52,53]. Essential micronutrients of guayusa include Mn, Fe, Ni, Zn, with Mn appearing at markedly high concentrations than the others. Again this micronutrient profile is generally reflected in yerba mate and black tea [50,51,52].

Al and F are non-essential trace elements that appear with the highest concentrations in infusions of black tea [50]. While F has not been analyzed in guayusa or yerba mate, we identified an elevated concentration of Al in guayusa, as has been reported previously for yerba mate [51,52,54]. *Camellia sinensis* is known to be an Al accumulator, high concentrations of Al (300-1500 µg/g) have been reported in its leaves [55]. However, Al accumulation has not been studied in guayusa. Its identification at an elevated level in guayusa might warrant further investigation of its speciation, possible health implications and any biogeochemical reasons for its strong presence. Based on the current results, the total concentration of Al in guayusa leaves is of lower concern than the relatively higher concentration of total Al found in *Camellia sinensis* leaves.

Table 3. Elemental Composition of Dried Guayusa Leaves[a]

	Dried Guayusa Leaves	Yerba Mate [51]	Yerba Mate [52]
Al	252.4 ± 91.7	413 ± 23	361 ± 108
Sb	<LOD[b]	-	<LOD
As	<LOD	-	0.052 ± 0.251
Ba	82.6 ± 3.3	-	61.5 ± 20.8
Be	<LOD	-	0.023 ± 0.009
Bi	<LOD	-	<LOD
B	28.4 ± 13.1	-	-
Cd	0.99 ± 0.08	-	0.41 ± 0.18
Cr	<LOD	-	0.528 ± 0.24
Co	<LOD	-	0.169 ± 0.956
Cu	8.68 ± 0.72	14 ± 2	11.9 ± 2.06
Fe	31.2 ± 0.9	254 ± 27	205 ± 89.1
Pb	<LOD	-	0.314 ± 0.181
Li	<LOD	-	0.085 ± 0.079
Mg	6030 ± 124	1315 ± 113	4591 ± 842
Mn	689 ± 319	5025 ± 186	1078 ± 377
Mo	2.33 ± 0.65	-	0.066 ± 0.325
Ni	2.01 ± 0.07	-	2.74 ± 0.945
P	4240 ± 1981	1404 ± 73	3409 ± 548
K	17740 ± 3100	15599 ± 422	12317 ± 2094
Se	<LOD	-	<LOD
Ag	<LOD	-	<LOD
Na	27.1 ± 3.8	-	-
Sr	117 ± 17	-	34.5 ± 5.51
Tl	<LOD	-	-
Sn	<LOD	-	-
Ti	0.32 ± 0.05	-	9.87 ± 5.25
V	<LOD	30 ± 3	0.377 ± 0.247
Zn	85.2 ± 19.4	72 ± 5	63.6 ± 25

[a]Values are expressed as mean ± SD (n = 5). Values determined in this study are compared with two previously reported studies. All units are µg/g. [b]<LOD = below limit of detection.

The toxicity of Ni at levels that exceed its function as an essential micronutrient is unlikely to be a concern for guayusa since Ni appears at an extremely low level, as it does in yerba mate [52] and black tea [56]. Other toxic trace elements such as As, Cd, Cr and Pb have been reported in low concentrations in black tea infusions [56]. While all of these toxic elements have also been reported in yerba mate [52], only Cd appeared in guayusa, and at a very low level, the others being below this study's limits of detection using atomic absorption spectrophotometry with inductively coupled plasma detection. Legislation to control for the presence of toxic elements in tea infusions exists in many countries and where it does not, WHO guidelines for maximum permissible levels in drinking water can be used [50]. Toxic elements identified in this study of dried guayusa leaves appeared at very low levels, lower than those reported for yerba mate [52]. Even if they should appear more elevated in other analyses of dried guayusa leaves, As, Cd, Cr, and Pb are poorly extractable in aqueous solution [57], consequently they present negligible concern for human health when consumed as a tea infusion. Further elemental toxicity studies specifically for infusions of guayusa would be helpful to permit direct comparison with maximum allowed concentrations in foods.

The context for the analysis of microbial, mycotoxin and pesticide residue contamination of guayusa differs from the characterization of its endogenous chemical composition. Such analysis places the composition of dried guayusa leaves within an agricultural and manufacturing context, consideration of which is necessary for risk assessment of novel foods. We report negative LC-MS/MS and GC/MS multi-residue screens for 389 pesticides. This is reflective of both the ethno-agricultural and regulatory environment for guayusa's cultivation. Guayusa is a low impact, environmentally sensitive agricultural product, growing and cropping rapidly in the western Amazon, where it is an endemic species [2]. As such, within traditional agroforestry systems such as those from which our samples were collected, guayusa is grown without the use of pesticides or fertilizers. Furthermore, each of the collectives from which guayusa was sourced, possess USDA Organic certification, administratively reinforcing a pesticide-free growing environment. Our negative pesticide residue screen confirms the veracity of this information regarding current growing conditions, but we highlight the need for future consideration of pesticide residues in guayusa leaves if grown using higher intensity non-organic agricultural systems. We also highlight the need to study pesticide residues in herbal tea infusions made from guayusa leaves, since pesticides have been shown to infuse with high efficiency during a normal tea brewing procedure [58].

No internationally ratified parameters for microbial contaminants are established specifically for *Camellia sinensis* or other dried leaf herbal teas. This is likely due to their long history of safe use, low moisture content and inherent antimicrobial factors [43]. As a dried leaf herbal tea, guayusa is likely to possess similar inherent protection from microbial contamination. Table 4 presents the results of a bacteriological analysis of dried guayusa leaves. The negative coliform test compares favorably with a study of black teas using the same most probable number technique, which returned colony counts between 14 ± 0.41 CFU/g and 93 ± 1.22 CFU/g [59]. All other determined values are orders of magnitude lower than the safe limits established or proposed for *Camellia sinensis* or other herbal products.

Table 4. Microbiological Analysis of Dried Guayusa Leaves by Culture Media[a]

	Value	Safe Limit
Presumptive coliforms	<10	10^{b}
Coagulase + staphylococci	<20	10^{3c}[60]
Bacillus cereus	70 ± 87	10^{2c}[60]
Aerobic colony count	354 ± 266	$10^{7d,e}$[61]
ß-glucuronidase + *E. coli*	<10	10^{2e}[61]
Ealmonella	not detected	absent in 125g[e][61]
Yeasts	<100	10^{4e}[61]
Molds	<100	10^{5e}[61]

[a]All numerical values are expressed in CFU/g. Safety limits are in accordance with [b]ISO 4831:2006; [c]the New Zealand safety limit for herbs; and [e]Tea and Herbal Infusions Europe. [d]The safety limit is for total plate count.

A screen of nine *Fusarium*-associated trichothecene mycotoxins determined that if present in dried leaves, all mycotoxin concentrations were below the limit of detection (<10 µg/kg). Assayed mycotoxins were Deoxynivalenol; Diacetoxyscirpenol; 3-Acetyldeoxynivalenol; 15-Acetyldeoxynivalenol; Fusarenone X; Nivalenol; T2 Toxin; HT2 Toxin; and T2-triol. For context, EU limits for the presence of *Fusarium* toxins such as Deoxynivalenol, range from 50-200 µg/kg [62].

4. Conclusions

This study reports broad similarities between the chemical composition of guayusa, the closely related herb yerba mate, and *Camellia sinensis*. While the total and free amino acid profiles of guayusa are lower than that of green tea, we demonstrated that the elemental composition of guayusa (including heavy metals) presents no greater risk to human health than that of *Camellia sinensis* and yerba mate. Furthermore our negative mycotoxin, pesticide and microbiological assays constitute evidence that the agricultural and manufacturing setting for the production of guayusa in the Napo province of Ecuador, presents no risk to human health related to contaminants from the soil, agricultural chemicals or postharvest processing. The caffeine composition findings of this study further support the acceptance of guayusa as a stimulant herbal tea. We conclude that the chemical composition of guayusa as guided by the novel food assessment framework of the European Union Food Safety Authority, presents no barrier to its use as an herbal tea infusion for human consumption. These chemical composition findings contribute to a positive risk assessment of guayusa as a novel food that has been traditionally consumed in western Amazon regions.

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Statement of Competing Interests

The authors have no competing interests.

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