Contributions to the quality control of two crops of economic importance : hops and yerba mate

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chapter 7

Yerba Mate: Review of Its Chemical Composition, Biological Activities and Uses

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Introduction

The use of Yerba mate can be traced back to the pre-colonnial times in S. America, when the aborigins who inhabited what is now NE. Argentina, Paraguay and SE. Brazil consumed it for its stimulant properties. Throughout the Spanish colonisation of the region, the use of this herbal drug made from *I. paraguariensis* leaves spread from a relatively restricted territory to all of what is today Argentina and Uruguay. A French botanist, Amadeo Bonpland dedicated a great part of his life to the study of its botanical characteristics, domestication and cultivation practices (1897) among others in the second half of the 19th century, but the investigation of its chemical composition only began during the first half of the 20th century. In the last 20 years, however, there has been an exponential growth in the number of papers published on mate, especially referring to its pharmacological activities. As occurs with a relatively young history of research in a certain plant material, some of the findings that were published originally were inexact, largely due to limitations of the analytical tools employed at the time. This is being corrected as more powerful and sensitive analytical methods are applied, but there is still a lot of work to be done to clarify some controversial issues.

When reviewing the chemical composition of mate, it is important to distinguish between the research done on the fresh or (conservatively) dried leaves of *I. paraguariensis*, and yerba mate that has undergone an industrial process involving drying and "roasting" that is quite drastic and produces significant changes in the original chemical composition. There is also a lot of work done on the infusions of yerba mate, where the amount of herbal drug, temperature, type of extraction and solvent: drug ratio is not always clearly stated and though very valid as a means of evaluating what is consumed by a regular user, does not necessarily reflect the qualitative and quantitative composition of yerba mate.

In the case of the investigation of pharmacological activities, the composition of the extract used for experimentation is often insufficiently defined, as it is based on the presence of certain groups of compounds that probably account for less than a 1 % of the total composition in organic compounds and are anyway ubiquitous in Nature. This is not a characteristic of yerba mate research, but rather a *modus operandi* of a lot of the pharmacological research on medicinal plants.

The application of techniques that can provide a complete profile of both primary and secondary metabolites allows the implementation of a holistic approach to the study of mechanisms behind possible pharmacological activities resulting most probably in more consistent and exact results.

Below is a review of the chemical composition of *I. paraguariensis* and diverse preparations made from its leaves. A brief description of the composition of a few cogeneric *Ilex* species used as adulterants of *I. paraguariensis* is also described.

Biological activities of mate have been reviewed as well as studies done to evaluate the safety of its use.

7. 2 Chemical composition of *Ilex paraguariensis* leaves

The main secondary metabolites of *I. paraguariensis* belong to three phytochemical groups: purine-derived alkaloids (methylxanthines), saponins and phenylpropanoids.

7.2.1 Xanthines

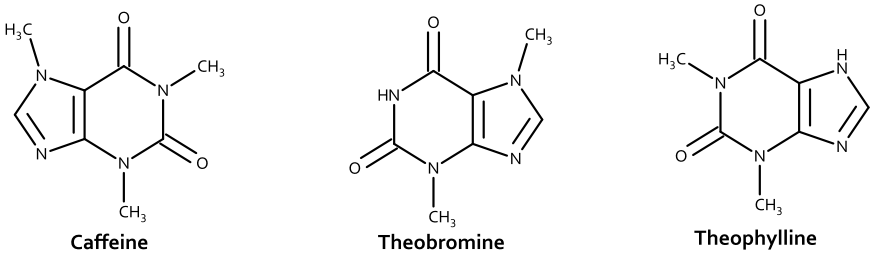


Fig. 7-1. Methylxanthines: of these only caffeine and theobromine have been detected consistently in I.paraguariensis.

These are by far, the most distinctive and significant secondary metabolites that have been detected in *I. paraguariensis*. Of the three most common purine derived-alkaloids - caffeine, theobromine and theophylline (Fig. 7-1) caffeine and theobromine are unequivocally present in greatest amounts in *I. paraguariensis* leaves and yerba mate.

Caffeine has been detected consistently in all aerial parts of *I. paraguariensis*, although stems have little or no caffeine as compared to the leaves (lamina). Caffeine is present in leaves usually bound to diverse acids such as tannic-, oxalic-, and acetic acid. In old publications and even in brochures published by the major mate manufacturers,

caffeine was referred to as matein, and claimed to be a more "beneficial" type of caffeine, with stimulant properties but less harmful side effects, such as its gastrointestinal irritant effects. This might possibly be due to differences in other components or the matrix of yerba mate as compared to coffee for example, since its caffeine content is high especially when drunk in the typical gourd-like manner.

There is an enormous discrepancy on the amount of caffeine present in *I. paraguariensis* that very likely reflects its variation according to the vegetative state of the plants in which the leaves were harvested, the season of the year, etc**.** As can be observed in Table 7-1, the content of caffeine in green leaves varies between 2.22 and 0,29 % (dry weight), but the average content of roasted *I. paraguariensis* leaves varies between 1 and 1.5 % of caffeine. Cardozo *et al.* (2007) obtained this data from the analysis of different progenies of *I. paraguariensis* plants collected from different locations of the Paraná state in SE. Brazil. Mazzafera (2004) studied caffeine in various parts of the plant - leaves, fruit and bark- and also in different vegetative states and grades of exposition to light, finding remarkable differences both in caffeine and theobromine content (*see Ref 11).* Coelho (2001) analysed the methylxanthine content in different seasons, finding that the highest was in February (that is summer in the S. hemisphere). Despite this, mate is harvested in winter due to agricultural practices. It is also interesting that *I. paraguariensis var vestita* has much less caffeine than the more popular *paraguariensis* variety, but its content decreases in summer (February).

Theobromine has been detected consistently in *I. paraguariensis* as can be appreciated in Table 7-1, varying between 0,029 and 0,068 in green leaves. Its content is also very variable, which is quite predictable considering that it is widely accepted to be mostly a precursor of caffeine.

The presence of **theophylline** on the other hand, is still a matter of controversy (Schubert, 2006) since many investigations performed with modern analytical methods both in older and more recent publications have not been able to detect it (Wilson *et al.*, 1981; Clifford *et al.*, 1990; Ashihara, 1993; Regginatto *et al.*, 1999; Filip *et al.*, 1998; Athayde *et al.* 2000; Choi *et al.*, 2005, 2010;Strassman *et al.*,2008; Sugimoto *et al.*,2009). In the case of Reginatto *et al.* (1999), the presence of other purine alkaloids in minor amounts is reported, but none of these were identified as theophylline. Similarly, Clifford and Ramirez-Martinez (1990) reported the finding of several minor alkaloid-type compounds that could be methylxanthines, but none of these were identified as theophylline nor 3-methylxanthine, 7-methylxanthine, 1,7-dimethyl-xanthine

(paraxanthine), uric acid, 1-methyluric acid, 1,3-dimethyluric acid and 1,7-dimethyluric acid. Other researchers, such as Vasquez and Moyna (0.02 mg/kg) (1986), Ashihara *et al.* (2004), Mazzafera (1994) and Ito *et al.* (1997) have, however, detected theophylline, using HPLC with UV detection; in the older papers, HPLC/UV detectors did not allow the acquisition of UV spectra, so that identification relied solely on the coincidence of retention times. Stronger evidence of its presence could be provided by reports of its isolation, albeit in low amounts from fresh leaves and from yerba mate using supercritical fluid extraction (SFE) (Saldaña, 1999; Mazzafera, 2004). Another paper reported its detection using high performance capillary electrophoresis (HPCE-UV detection), followed by its isolation and MS identification (Pomilio, 2002). Surprisingly, the authors did not quantify it, reportedly due to its low concentration.

A possible explanation for the inconsistent and rare presence of theophylline reported in a few cases could be the existence of a catabolic route of caffeine investigated in coffee and tea plants and in lesser degree in mate leaves (Clifford *et al.*, 1991; Ito *et al.*, 1997; Ashihara *et al.*, 1996; Mazzafera, 2004). In this route, theophylline appears as an intermediate when caffeine is demethylated in a first reaction rate limiting step, catalysed by N7-demethylase: caffeine → theophylline→ 3-methylxanthine → xanthine→ uric acid → allantoin → allantoic acid → urea → CO_2 and NH_3 pathway. Catabolism of caffeine has been studied using ^{14}C-labelled caffeine (Ashihara *et al.* 1996, 1997; Mazzafera 2004; Suzuki and Waller 1984). On the other hand, both in tea and mate, large amounts of [8-^{14}C] theophylline are also converted to theobromine and caffeine theophylline → 3-methylxanthine → theobromine→ caffeine salvage or *de novo* pathway- (Ito *et al.* 1997). Theobromine, in contrast to theophylline, is a precursor, as opposed to a catabolite, of caffeine and even though other minor catabolic pathways have been described (Koyama *et al.*, 2003; Zheng *et al.*, 2004) the major route leads to caffeine production. A very comprehensive review of this has been published by Ashihara *et al.* (2011), but it is important to note that most of the research of these metabolic and catabolic pathways has been done on coffee and tea plants and has not been confirmed to occur even in all organs of all *Coffea* species studied, for example. Therefore, more research should be done on *I. paraguariensis* to confirm the mechanisms in this case. In any case, though theophylline were present, the fact that there are only traces of the compound at the best, make the discussion rather irrelevant, since it would have no pharmacological activity whatsoever at these doses.

Xanthines in yerba mate and yerba mate beverages

According to the Código Alimentario Argentino (which is harmonised with other Mercosur countries' respective Codex), "yerba mate consists of the dried, slightly roasted, broken fragments of leaves of *Ilex paraguariensis* St. Hil, exclusively, mixed or not with young dry stalks, petioles or floral pedunculae". There are basically two types of yerba mate that can be marketed, one that contains up to 35% of stems (yerba mate elaborada con palo) and one that can only contain 10[1]% stems. It must contain a minimum of 0.6% caffeine.

There are a few reports of methylxanthine content of yerba mate and a lot more of the beverages prepared with it. These are very variable, depending heavily on the type of extraction (decoction or infusion) that is prepared, temperature of the water, amount of herb used, etc. and type of yerba mate. It is of course very important from a dietary viewpoint, since mate is consumed in very large amounts by a great number of people.

The caffeine content in yerba mate is relevant since it varies a lot during the processing of *I. paraguariensis* leaves, not because of its chemical decomposition but basically because it sublimes and is thus lost during the heating and drying processes. Commercial samples of several commercial types of Brazilian yerba mate, including those used for the preparation of hot mate and "tereré" (cold or iced mate) contained between 0.66 and 1.20 % of caffeine respectively and 0.166 to 0.212% of theobromine as determined by capillary electrophoresis (UV detection at 206 nm). These authors also monitored the amount of methylxanthines extracted during a 30-extraction "round" with one same sample of yerba mate and a maximum of 17% of the caffeine content with hot water and 75% with cold water (tereré) (Meinhard *et al.,* 2010). The two surprising results in this paper are firstly the high theobromine content measured and secondly the fact that so little caffeine was extracted with hot water, especially considering the large percentage extracted with cold water. These results are quite different to those obtained by Wilson *et al.* (1982), Vasquez and Moyna (1986) and later Bastos *et al.* (2005) for whom the total ingestion of caffeine varies between 135 and 192 mg according to the brand and amount of yerba mate used to prepare the beverage, for hot mate and 85 mg for *tereré* (prepared with cold water (Bastos *et al.,* 2005). Regarding the theobromine content reported in Meinhard *et al.* (2010), another study done with capillary electrophoresis reported these uncommonly high contents of theobromine in commercial samples (both tea-bags and loose leaf samples), since they detected between 0.11 and 0.66 % using HPCE-UV (Pomilio *et al.,* 2002).

Table 7-1. Content of caffeine and theobromine in Ilex paraguariensis

Species	Type of extract	Part of plant	Caffeine (%)	Theobromine (%)	Ref
I. paraguariensis	Acid extraction	Roasted leaves	1,13	-	21
	Aqueous (as drunk)/ $CHCl_3$:isoprOH	Roasted leaves	0,89	0,12	21
I. paraguariensis var. vestita	Acid extraction	Leaves	0.003	-	1
I. paraguariensis		Leaf epicuticular waxes	0.016 - 12.76	0 to 0.95	2
I. paraguariensis	As drunk popularly	Roasted leaves	244.63 μg/mL	148.07 μg/mL	3
I. paraguariensis		Leaves	0.490–0.611	0.132–0.068	5
I. paraguariensis		Leaves	0.56	0.03%	6
I. paraguariensis		Leaves	0.78-1.25	0.34-0.43	7
I. paraguariensis		Leaves	1.92	0.484	8
I. paraguariensis		Leaves	0.88	0.08	9
I. paraguariensis		Leaves	0.89-1.73		10
I. paraguariensis	Also detects theophylline in low amounts (approx.0.02%)	Young leaves	0,8375	0.0768	11
		Old leaves	0,1626	0.0221	
		Young leaves nfb	0.9147	0.1565	
		Inmature fruit	0.0378	0.0014	
		Mature fruit	0.0132	--	
		Bark	0.1484	0.0695	
		Old leaves shade	0.8288	0.4320	

Species	Type of extract	Part of plant	Caffeine (%)	Theobromine (%)	Ref
I. paraguariensis	Acid extraction / $CHCl_3$: isoprOH	Leaves	Oct: 0,49-0,29 Feb:1,48-0,62	0,029-0,085 0,47-0,042	19
I. paraguariensis var *vestita*	Acid extraction/ $CHCl_3$:isoprOH	Leaves	Oct:0,022-0,81 Feb:0,009-1,23	0,001-0,4 0,003-0,81	19
I. paraguariensis	As drunk popularly	Leaves	1,67-2,22	-	20

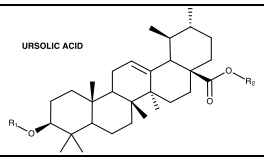
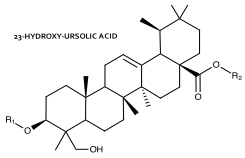
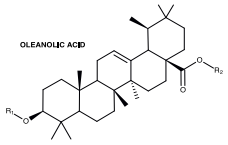
Ref:1: Reginatto et al.(1999);2:Athayde et al. (2000); 3:Strassman et al. (2008);5: Cardozo Jr et al. (2007); 6: Vazquez and Moyna (1986); 7: Baltassat et al. (1984); 8: Filip et al.(1998); 9: Nagata et al. (1985); 10: Clifford et al. (1990); 11: Mazzafera (2004); 19: Coelho et al. (2001), 20:Borille (2005); 21: Wilson et al. (1981). Abbr.: nfb=non-fruit bearing; isoprOH= 2-propanol

7.2.2 Saponins

The leaves of *I. paraguariensis* are rich in saponins, containing between 5 and 10% of crude saponins (Schenkel *et al.*, 1997). They are all glycosides either of oleanolic or ursolic acid. Among the latter the most abundant are matesaponin 1 (Gossman *et al.,* 1989), matesaponins 2, 3, 4 (Gossman *et al.*, 1997) and matesaponin 5 (Kraemer *et al.*, 1996). Later Martinet *et al.* (2002) isolated two oleanolic acid derivatives, guaiacin and nudicaucin C. Sugimoto *et al.* (2009) isolated another four saponins, two of which, mateglycoside A and D, are oleanolic acid derivatives and mateglycosides B and C which are 23-hydroxyursolic acid glycosides (Table 7- 2).

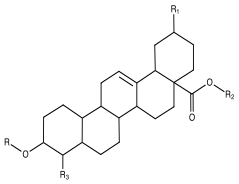
Saponins are believed to be responsible for the bitter taste of mate, but also produce the foam that is perceived as a quality attribute. *Ilex paraguariensis* and *I. dumosa* are saponin- rich species as reported by Pires *et al.* (6-10%), in opposition to other *Ilex* species that showed a lower saponin content (2-3%). However, there are remarkable differences in saponin bitterness and it is a known fact that adulterated yerba mate is often detectable due to its higher bitterness. This could be supported by a test described by Pires *et al.* (1997), which showed that when tested with the filter paper method, similar concentrations of the crude saponin fraction of *I. dumosa* were twice as bitter as that of *I. paraguariensis*. A recent paper reported the quantitation of matesaponins 1, 2 and 3 in leaves of *I. paraguariensis* as totalling between 0.3 and 1 % (dried weight) (Coelho, 2010).

Table 7-2 Saponins detected in leaves of I. paraguariensis

Name	Substituents		Ref
			
	R1	**R2**	
Matesaponin 1	-D-glc-(1-->3)- -L-ara]	-D-glc	13
Matesaponin 2	-D-glc-(1-->3)-[-L-rha-(1-->2)]]- -L-ara]-(28-->1)	-D-glc	18
Matesaponin 3	-D-glc-(1-->3)- -L-ara]-(28-- >1)	-D-glc-(1-->6)- -D-glc	18
Matesaponin 4	-D-glc-(1-->3)-[-L- rha-(1-->2)- -L-ara]-(28-->1)	-D- glc-(1-->6)- -D-glc	18
Matesaponin 5	-D-glc-(1->3)-[-L-rha-(1->2)]- -L- ara}-(28->1)-	-D-glc-(1->4)- -D-glc-(1 ->6)- -D-glc] ester.	16
Jla/b	α-L-rha-(1-->2)-α-L-ara	H	15
J2 a/b	β-D-glc-(1-->3)-α-L-ara	H	15
J3a/b	α-L-rha-(1-->2)-α-L-ara	β-D-glc	15
-	β -D-glc(1-->3)-α - L-2-O-acetyl-ara	β-D-glc	19,20
			
Mateglycoside B	β-D-glc(1-->3)-α-L-ara	β-D-glc	20
Mateglycoside C	α-L-ara	β-D-glc	20
			
Guaiacin B	-D-glc-(1-->3)- -L-ara	28-->1)- -D-glc	14
Nudicaucin C	-D-glc-(1-->3)-(-L-rha (1-->2))- -L-ara)	28-->1)- -D-glc	17,14
Mateglycoside A	α-L-rha(1-->2)[β -D-glc(1-->3)]-α -L-ara	-β -D-glc(1-->6)-β -D- glc	20
Mateglycoside D	α-L-rha-(1-->2)-α-L-ara	β-D-glc	20

Ref: 13: Gossman et al. (1989); 14: Martinet et al. (2005); 15: Gnoatto et al. (2005); 16: Kraemer et al. (1996); 17: Nishimura et al.(1999); 18: Gosmann et al. (1997); 19: Pezzuto et al.(2002); 20: Sugimoto et al. (2009). Abbreviations: Glc: glucose; Ara: arabinose; Gal: galactose; Rha: rhamnose.

Table 7-3 Saponin content of Ilex cogeneric species (Adapted *from Heck and Mejía, 2007)*

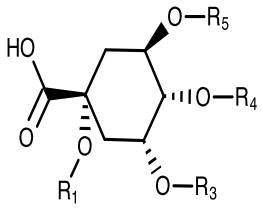


Species	Compound name	Sapogenin	Substituents				Ref
			R (C3)	R1 (C20)	R2 (C28)	R3 (C23)	
I. argentina	Pedunculoside	Rotundic acid*	H	H	β-D-glc	CH_2OH	I
	N/A	Rotundioc acid** (20-S-isomer)	H	H	β-D-glc	COOH	
	IL-A	Hydroxyursolic acid	α-L-ara		CH_2OH	-β-D-glc	4
I. brevicuspis	Brevicuspisaponin I	Hydroxyursolic acid	ara	H	H	CH_3	2
	Breviscuspisaponin II	Dihydroxyursolic acid	ara	H	H	CH_2OH	
	BrevicuspisaponinIII	Hydroxyursolic acid	α-L- ara	$-CH_3$	$-CH_3$	-β-D-glc	3
	Brevicuspisaponin IV	Hydroxyursolic acid	H	COO^-Na	CH_2OH	-β-D-glc	
	IL-A	Hydroxyursolic acid	α-L-ara	H	CH_2OH	-β-D-glc	
I. dumosa	ChikusetsusaponinIVa	Oleanolic acid	Glc	H	β-D-Glc	H	5
	Chikusetsusaponin IVa methyl ester	Oleanolic acid	GlcAOMe	H	β-D-Glc	H	
	Dumosasaponin5	Mesembryanthemoi-digenic acid	glc(1-->2)gal	OH	β-D-Glc	H	
	Dumosasaponin 6	Oleanolic acid	ara(1-->2)ara	H	β-D-glc	H	
	Dumosasaponin7	Oleanolic acid	β-D-galac	H	β-D-glc	H	
	E1	Oleanolic acid	β-D-gal	H	H	H	6
	E3	Oleanolic acid	α-L-ara-(1-->2) β-D-gal	H	H		
	E6	Oleanolic acid	β-D-glc(1-->2) β-D-gal	H	H		
	E7	Oleanolic acid	α-L- ara(1-->2) - β-D-gal	H	H		
	E8	Oleanolic acid	β-D-glc(1-->2)- β-D-gal	H	β-D-Glc		
I. theezans	Pedunculoside 1	Rotundic acid	H	H	β-D-glc	CH_2OH	7
		20-S-Rotundioc acid	H	H	β-D-glc	COOH	
I. integerrima	N/A	19α,24-dihydroxy-ursolic acid	H	H	ß-D-glc	COOH	9
I. pseudobuxus	N/A	Rotungenic acid	H	H	α-L-rha(1-->2)p-D-Glc		8
	N/A	Pomolic acid	H		α-L-rha(1-->2)p-D-Glc		

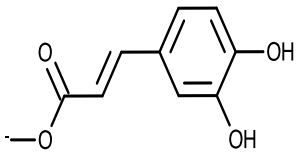
*Abbr: Glc: glucose; Ara: arabinose; Gal: galactose. *Rotundic acid: 3β,19α,23-trihydroxyurs-12-en-28-oic acid; **Rotundioc acid: 3β,19α,2,3-trihydroxyurs-1,2-en-24,28-oic acid; mesembryan-themoidigenic acid: 29-OH-oleanolic acid; pomolic acid: 3β,19-Dihydroxy-5α-urs-1,2-en-28-oic acid . Refs: 1:Athayde et al. (2001); 2: Taketa et al.(2000); 3: Taketa et al.(2002); 4: Schenkel et al.(1995); 5: Pires et al. (1997) 6: Heinzmann et al.(1995); 7: Athayde et (1999); 8: Taketa (1994); 9: Constantin (1995).*

7.2.3 Polyphenols

The other main group of secondary metabolites present in *I. paraguariensis* is polyphenols, mainly phenylpropanoids known generically as chlorogenic acids (CGAs), caffeoylshikimates (CSAs) and in lesser amount, flavonol glycosides and their aglycones. The CGAs are a family of mono- and di-acyl quinic acids (Clifford, 1985a,b). Quinic acid is 1,3,4,5-tetrahydroxycyclohexanecarboxylic acid (IUPAC, 2011). Common acylating residues are caffeic acid (3,4-dihydroxycinnamic acid), ferulic acid (3-methoxy-4-hydroxycinnamic acid) and p-coumaric acid (4-hydroxycinnamic acid), thus producing caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA), feruloylquinic acids (FQA), p-coumaroylquinic acids (p-CoQA) and caffeoylferuloylquinic acids (CFQA) (Clifford *et al.*, 1989a).



Quinic acid



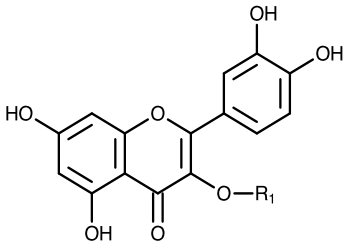
Caffeoyl-

Monocaffeoylquinic acid derivatives:

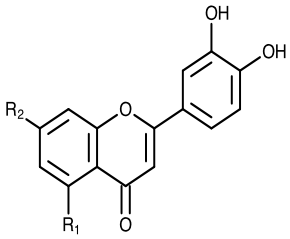
R_1=R_4=R_5=H; R_3=-Caffeoyl: Chlorogenic acid
R_1=R_3=R_4=H; R_2=-Caffeoyl: Neochlorogenic acid
R_1=R_2=R_4=H; R_3=-Caffeoyl: Cryptochlorogenic acid

Dicaffeoylquinic acid derivatives:
(Isochlorogenic acid)

R_1=R_4=H; R_3= $R_{5=}$-Caffeoyl
R_1=R_5=H; R_3= $R_{4=}$-Caffeoyl
R_1=R_3=H; R_4= $R_{5=}$-Caffeoyl



R_1=H= Quercetin
$R_{1=}$ Glu-O-rha= Rutin



Luteolin diglycoside:
$R_{1=}$H; R_2= Glu-O-Rha or
R_1=Glu-O-Rha; R_2= H

Fig. 7-2 Main polyphenolic derivatives found in mate

Caffeic acid was first isolated from *I. paraguariensis* by Woodward and Cowland (1935) as a product of the hydrolysis of a substance that they named caffetannin, pseudotannin while the same year, Hauschild (1935) reported the isolation of a substance that he thought was related to chlorogenic acid. This was confirmed years later by Deulofeu *et al.* (1943, 1945), a prominent researcher in Argentina, who additionally reported that its hydrolysis yielded caffeic acid. Later on, Descartes isolated chlorogenic acid, identifying it as 3-caffeoylquinic acid (1953,1956) and Roberts (1956) reported the finding of two caffeoylquinic isomers, chlorogenic acid (3-caffeoylquinic acid) and neochlorogenic acid (5-caffeoylquinic acid), and isochlorogenic acid which is actually a mixture of three dicaffeoylquinic acid isomers: 4,5-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid and 3,5-dicaffeoylquinic acid. (Alkaridis,1987). In 1990, Clifford and Ramirez-Martinez reported the identification of the above-mentioned CGAs, the three isomers of isochlorogenic acid, 5-feruoylquinic acid plus 10 compounds they described as chlorogenic acid-like compounds. They also identified rutin (quercetin-3-rutinoside).

A comparative study on of the content of CQAs and flavonoids in different *Ilex* species (*I. dumosa*, *I. argentina*, *I. brevicuspis*) that grow in the same region as *I. paraguariensis* was done using HPLC/UV analysis. Results showed that *I. paraguariensis* contained the highest amount of chlorogenic acid (2.8%), caffeic acid (0.023%) and the three isomers of isochlorogenic acid (3,4 DCQ: 3,5DCQ and 4,5 DCQ)(Filip, 2001). In another paper, Filip *et al.*(2001) also determined the total chlorogenic acid content of these species, using a spectrophotometric method. They determined that *I. paraguariensis* contained 10,71% (DW, referred to chlorogenic acid) while the other *Ilex* species varied between 0,96 and 4,26%.

Later on, more reliable and complete studies were performed by Carini *et al.* (1998) Bastos *et al.* (2007) and Jaiswal *et al. (*2010). Carini *et al.* (2010) identified 10 compounds using LC/MS in commercial green mate leaves, including the 3 naturally occurring isomers of caffeoylquinic acid (CQA), neo-chlorogenic acid, chlorogenic acid and crypto-chlorogenic acid, as well as 3 isomeric dicaffeoyl quinic acids, rutin (quercetin-3-rutinoside), a diglycosyl derivative of luteolin, and 2 isomeric caffeoyl-glucosides. Additionally, all chlorogenic acid isomers were quantified using DAD detector acquired data and the total content of a methanolic extract was found to be 17,7% (calculated as chlorogenic acid) of which 5.1% corresponded to 3-CQA (chlorogenic acid); 8.2% to 5-CQA (neochlorogenic acid) and 4.4% to 4-CQA (cryptochlorogenic acid); this corresponds, according to the reported sample preparation to approximately 1.17% chlorogenic acid, 1,85% of neochlorogenic acid CGA and 0.99% cryptochlorogenic acid. In these papers no distinction was made

between the stereoisomers of chlorogenic acid. In Nature, chlorogenic acids are usually found in the - configuration and conversion to *cis*- isomer occurs throught exposition to light. Thus, the presence of the *cis*-isomers may be another feature to consider as a possible quality parameter.

Using direct infusion electrospray insertion mass spectrometry (ESI-MS), Bastos *et al.* (2007) identified the main phenolic compounds from aqueous, ethanolic and ether extracts from green and roasted yerba mate. Compounds identified in water and ethanolic extracts from green *maté* were caffeic acid, quinic acid, caffeoyl glucose, caffeoylquinic acid, feruloylquinic acid, dicaffeoylquinic acid and rutin. The roasted yerba mate polar extracts also contained caffeoylshikimic acid and dicaffeoylshikimic acid. These compounds have also been isolated from prunes, produced during the drying process of plums (Fang *et al,* 2002).

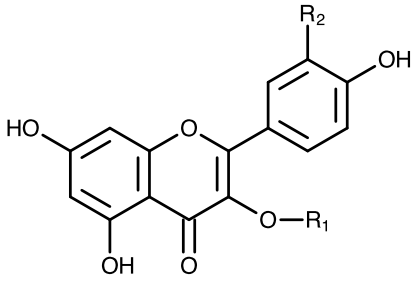
The most complete information yet on yerba mate caffeoylquinic, hydroxy-cynnamoylshikimate esters and feruoylquinic acids, was published by Jaiswal *et al. (*2010), who reported the detection and characterization of 42 caffeoylquinic acids by LC-MS^n, 24 of which had never been published. Additionally 9 CSAs were detected. The material used for this determination was identified as green dry yerba mate leaves (presumably commercial yerba mate) or roasted green dry yerba mate leaves bought in Germany (these products are not usually available in S. America). Samples were prepared by methanol extraction. Assignment to the level of individual regioisomers resulted in the identification of eight caffeoylquinic acids, five dicaffeoylquinic acids, one tricaffeoylquinic acid (3,4,5-tri-O-caffeoylquinic acid), six feruoylquinic acids, two diferuloyl quinic acids, five *p*-coumaroylquinic acids, four caffeoyl-*p*-coumaroyquinicc acids, seven caffeoyl-feruloylquinic acids, three caffeoyl-sinapoylquinic acids and one dicaffeoyl-feruloylquinic acid.

The shikimates that were detected were four caffeoylshikimates, three dicaffeoylshikimates, one tricaffeoylshikimate and one feruloylshikimate.

Figure 7-2 shows the chemical structure for neochlorogenic, cryptochlorogenic and chlorogenic acid, 4,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 3,4-dicaffeoylquinic acid which are the most abundant CQAs in yerba mate, particularly, neochlorogenic acid. Yerba mate does not contain a great amount of flavonoids, representing less than a 5 % of the total polyphenolic content of mate (Bravo, 2007). Rutin has been detected inequivocally in all *I. paraguariensis* samples and in yerba mate (Sugimito *et al.,* 2009; Heck *et al.,* 2008; Jaiswal *et al.*, 2007; Bravo *et al.*, 2007; Carini *et al.*, 1998; Clifford and Ramirez-Martínez, 1991; Filip *et al.,* 2000). Quercetin, its aglycon

has not always been detected and Carini *et al.* (1998) and Heck *et al. (*2008) reported only rutin and a diglycoside of luteolin.

There are still differences, however regarding the identity of flavonoids in mate since a recent study published by Rostagno *et al.* (2011) confirmed the presence of rutin, but detected kaempferol -3-O-glucoside and quercetin-3-O-glucopyranoside and did not detect luteolin-3-O-glucoside. This coincided with Sugimoto *et al.* (2009), who detected rutin and kaempferol-3-O-rutinoside only and no luteolin glycosides.



R1= H R2= H: Kaempferol
R1= H R2= OH= Quercetin
R1=3β-D-rutinoside; R2=OH=Rutin

The ^{1}HNMR metabolomic study of 11 *Ilex* species : *I. argentina, I. brasiliensis, I. brevicuspis, I. dumosa var. dumosa, I. dumosa var. guaranina, I. integerrima, I. microdonta, I. paraguariensis var. paraguariensis, I. pseudobuxus, I. taubertiana, and I. theezans* showed the phenylpropanoid content to be one of the major discriminating factors among species. Among the species analysed, chlorogenic acid as well as the three dicaffeoylquinic (DCQ) acids :3,4-O-DCQ , 3,5-O- DCQ and 4,5-O-DCQ acids were identified in I. *paraguariensis* and *I. theezans* extracts . In other species, instead of dicaffeoylquinic acids, 3-O- and 4-O-caffeoylquinic acids were detected as major phenylpropanoids as well as chlorogenic acid. (Choi *et al.* ,2004; Kim *et al.* 2010).

7.2.4 Volatiles

Taste and aroma are undoubtedly considered to be important quality attributes of mate and have been attributed to the caffeine content, but also to saponins present in great quantities. Mate is considered to be an acquired taste since its rather bitter and "smoked" taste confers a rather peculiar flavour that is not instantly accepted by new consumers. It is perhaps the way in which it is consumed, with the gourd and straw and the ritual associated to this manner of consumption that makes it more attractive.

Above 250 compounds were detected in the volatile fraction of Yerba mate (Kawakami and Kobayashi 1991). Using GC/MS the researchers compared the volatile

profile of yerba mate and green tea finding that of 196 identified compounds, 144 were present in both products. Some compounds were particularly characteristic of mate, for example, 2-butoxy-ethanol, present in high concentrations and 3,3,5-trimethylcyclohexanone-related compounds.

Bastos (2006) published an interesting comparison between the volatiles of green and roasted leaves. Not surprisingly, the roasting and drying process produced considerable quali- and quantitative differences. Compounds that could be associated to the floral aroma of green leaves such as limonene decreased from 18 to 4.5% and linalool was oxidized to linalool oxides during the roasting process. Other compounds, such as methylfurfural and furfural, which might contribute to the smoked flavor and aroma of mate tea infusions, were detected after the roasting process. The major compounds identified in the green mate essential oil were limonene, linalool and geranylacetone while the major compounds identified in the mate tea essential oil were geranylacetone, limonene and β-E-ionone.

Lozano *et al.* (2007) also studied the aroma of commercial yerba mate using three different types of Argentine yerba mate selected according to their levels of polyphenolic compounds, agronomic factors and flavour strength. Using three different methods - dynamic headspace analysis (DHA), solvent-assisted flavour evaporation-solvent extraction (SAFESE) and column adsorption extraction - volatiles were isolated from hot infusions prepared with the dried leaves. Aroma-active components were identified by gas chromatography olfactometry (GCO) and GC-MS. Interestingly each method allowed the identification of compounds which had not been detected with other methods, showing the importance of using several different methods to obtain the best information (Heck and Mejía, 2010). SAFE-SE analysis allowed the identification of most compounds followed by aroma extract dilution analysis (ACE-AEDA) and dynamic headspace dilution analysis (DHDA). The predominant aroma components of Mate tea included geraniol, β-damascenone, 2-methoxyphenol, linalool, β-ionone, eugenol, 2-acetyl-1-pyrroline, (*E,Z*) 2,6-nonadienal, and geranial.

Volatile and semi-volatile components of yerba mate were analysed by headspace solid-phase micro-extraction (HS-SPME) coupled to gas chromatography and mass spectrometry. Seventy compounds were identified in the sample headspace, including propanal, (*E*)-2-pentenal, hexanal, (*E*)-2-hexenal, 6-methyl-5-hepten-2-one, (*E,Z*)-2,4-heptadienal, (*E,E*)-2,4-heptadienal, (*E,Z*)-3,5-octadien-2-one, β-cyclocitral, 3-ethyl,4-methyl-(1*H*)-pyrrole-2,5-dione, α-ionone, geranylacetone, β-E-ionone, dihydroactinidiolide and caffeine (Araujo et al., 2007).

7.2.5 Other components

Several other compounds that are considered to be bioactive have been isolated. Among them, a monoterpene oligoglycoside: (R)-linalyl 6-O-α-L-arabinopyranosyl-β -D-glucopyranoside (Sugimoto *et al.*, 2009).

Apart from these, two acetylated megastigmane glycosides: matenosides I and II, (Fig 7-4) which exhibited HNE (human neutrophil elastase) inhibitory activity were isolated from methanolic extracts of yerba mate. HNE is associated to the appearance of wrinkles with age (Xu *et al.*, 2010).

The same group had isolated a new pyrrole alkaloid, pyrrolezanthine-6-methyl ether, along with seventeen known compounds, including caffeine, theobromine, diverse CQAs, and the flavonoids, quercetin, rutin and kaempferol-3-O-rutinoside (Xu, 2009).

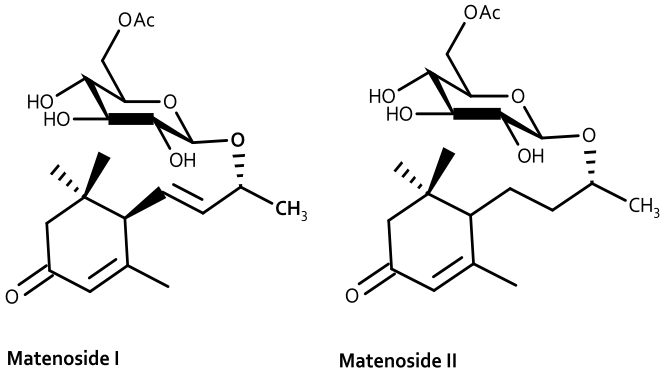


Fig. 7 -4: Matenosides I and II isolated from I. paraguariensis leaves (Xu, 2010)

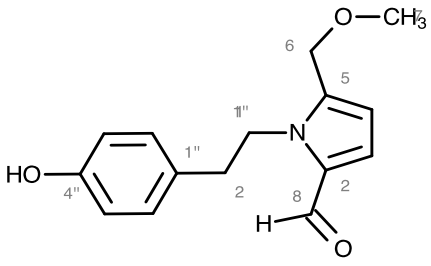


Fig. 7-5: Pyrrolezanthine-6-methyl ether, a new pyrrolinic alkaloid isolated from I. paraguariensis leaves (Xu, 2009)

There is only one report of the detection of melanoidins in roasted leaves. Melanoidins are dark pigments of high molecular mass that are formed by oxidation and polymerisation of polyphenols. Sava *et al.,* (2001) have described the formation of these compounds during the production of black tea. In this case Souza obtained a fraction of melanoidins approximately equivalent to 1% of dry weight of roasted yerba mate leaves that varied according to the commercial brand (Souza, 2009).

7.2.6 Minerals

Yerba mate leaves have a high content of minerals, concentrated largely in the lamina rather than in the stems. A study performed on the commercial product revealed the presence of potassium, phosphorous, calcium magnesium, sulphur, boron, copper, iron, manganese, nickel, aluminiumn, chromium, cobalt and sodium (analysed by atomic absorption) and nitrogen (analysed by Kjeldahl) out of which the K, Mg and Mn (a micronutient) proved to be the most abundant representing a 1,2; 0,4 and 0.06% over dry weight respectively. The Mn content detected is at least five times that detected in coffee or chocolate. Heavy metals such as Cd and Pb were not detected in contrast to previous reports, implying that the presence of these metals depends on the proximity to industrial zones or urban centers (Heinrichs *et al.*, 2001). In the infusion the most abundant metals are K and Mg, followed by other elements such as S and Mn. In all cases Pb and Cd were not detected. Aluminium was found in extremely low quantities. This confirmed results obtained by Sanz and Torija (1991) using atomic absorption after nitric/hydrochloric acid digestion that showed yerba mate was a good source of Mg, K and Mn. Another recent study confirmed these results. Using electrothermal atomic absorption spectrometry after nitric/perchloric acid digestion, commercial yerba mate was found to contain low amounts of Al 369 μg/g (equivalent to 0,037 %) and 2223 μg/g (0,2%) of Mn, 48.1% of which was available in the infusion of these leaves (Wrobel *et al.*, 2000*).*

7.3 Biological activities of yerba mate

Throughout the last 20 years, the number of papers published on diverse biological activities of yerba mate has been increasing. This is basically due to the also increasing interest in widening the market of this herb outside its traditional borders. This is quite natural considering that yerba mate has a great amount of compounds that are considered very attractive nowadays for diverse reasons: caffeine, caffeoylquinic acids (CQAs) and the presence of a substantial amount of saponins to increase the solubility of all these compounds in water aside from their biological activities. The resulting

beverage thus, could potentially have all the benefits conferred by this high caffeine content together with the purported beneficial effects of large amounts of CQAs with their alleged antioxidant properties.

Unfortunately, to my knowledge no serious epidemiologic study has been performed to determine the real effect of the high consumption of yerba mate on the population of heavy mate drinkers in S. America. For centuries, great numbers of people of all ages, sex, social status and health conditions have been consuming yerba mate most of all in the infusion-maceration type extraction (gourd+straw) which provides a highly concentrated beverage. Unlike what is observed in regions in which certain diets lead to low obesity or longevity such as the Mediterranean diet, people of this region are not characterized by their longevity nor is there a low incidence of cancer or obesity. Thus, although the interest in promoting its use as a functional food is reasonable, it would be very important to carry out well-designed clinical studies to assess its biological activities, both desirable and undesirable.

One aspect however should be thoroughly researched. In S. America, the mate-drinking habit consists in sharing the mate in rounds, in a way such that everyone uses the same straw to drink the mate. Wiping or cleaning the straw before drinking is considered to be impolite or even offensive, so that any orally spread diseases should be easily transmitted. This, however, has never happened and there have been neither severe hepatitis nor flu epidemics for example, in all this time. Considering that no special hygienic rules have been implemented to avoid this, it is reasonable to suspect that mate (prepared this way) might have some type of antiseptic or antimicrobial properties.

7.3.1 Antimicrobial activity

Some research has been done on this, though results and studies have not been as interesting as expected nor have conclusions been scientifically rigourous.

- Antiviral

Aqueous extracts of several herbs, including *I. paraguariensis* leaves (no details on treatment of the leaves prior to extraction, neither the concentration of aqueous extract nor temperature or time of extraction is included in the paper) were tested for their activity against HSV-1 KOS strains and rabies virus. *Ilex paraguariensis* showed a strong *in vitro* activity against against HSV-1 KOS (IS = 15.8) and 29-R (IS = 12.6) strains but no activity against rabies virus was observed (Müller *et al.*, 2007). No work was done on the detection of the compound or compounds responsible for this activity,

though authors relate the presence of CQAs and/or saponins to the activity through former references. However, it is important to note that CQAs are ubiquitious and present in most of the other species tested in this paper, for example, in which less or practically no activity was detected and that an aqueous extract of *I. paraguariensis* has a great variety of secondary metabolites due to the presence of abundant tensioactive saponins. Again, it would be interesting to give more thought to the issue, considering the possible correlation between a good response to *in vivo* tests and the solubility required for the bioavailability of the proposed compounds apart and their metabolic fate once ingested.

- Antibacterial

The antimicrobial activity of the essential oil of yerba mate leaf was tested against selected microorganisms (Kubo *et al*., 1993) but mainly against *Streptococcus mutans* owing to it importance in the formation of dental decay (Hamada and Slade, 1980). All the components of this volatile fraction showed activity, although the individual effect of components proved to be moderate to low.

Sari *et al.* (2007) tested the inhibitory activity of diverse types of yerba mate extracts against diverse bacteria. For this, several extracts were prepared using 50 % mixtures of water with ethanol, dimethylformamide (DMF), methanol and acetone at room temperature (23 C) and different extraction times. All yerba mate extracts proved to be active against all tested strains, *Staphylococcus aureus, Listeria monocytogenes*, *Escherichia coli* O+/1: H1, *Hafnia alvei*, *Yersinia enterocolitica* and *Bacillus cereus,* with the exception of *Escherichia coli*. In the case of *Hafnia alvei,* the DMF extract proved to be inactive, opening the possibility of comparing the profile of this extract with the others to attempt to isolate the active compound/s. Interestingly, in this study, total polyphenolic content was determined (Folin-Ciocalteau) and radical scavenging and reducing activity was investigated (DPPH) and in no case was the result correlated to the antimicrobial activity. No MIC values were determined in this case and antimicrobial activity was measured using the disk diffusion method.

In coincidence with results obtained in this study, De Biasi *et al.* (2009) performed tests with ethanolic extracts of *I. paraguariensis* leaves and stems (separately) dried in the sun and in the shade, reporting that all exhibited some inhibition of cultures of *Candida albicans, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Staphylococcus epidermidis* but no activity against *Escherichia coli.* In this study, *I. paraguariensis* leaves were collected; stems and lamina were separated and dried in the shade and in the sun. Ethanol extracts were prepared

(no information of drug: solvent ratio is provided), taken to dryness and the residue was redissolved to obtain concentrations of 100 mg/mL and 50 mg/mL in water. Stems proved to be most effective (those dried in the shade slightly more than sun-dried ones) in all cases in a dose: dependent manner. This is quite notable because stems have little or no caffeine (Mazzafera, 2004) and less saponins and polyphenols (De Biasi, 2009). The activity of stems against bacteria was less affected by exposition to the sun than that of leaves. Again in this case, no MIC values were determined and antimicrobial testing was done with the disk diffusion method. It is also important to note that a concentration of 100 mg/ml implies that the antimicrobial activity is quite low.

These results coincided only partially with a previous report in which a tincture of *I. paraguariensis* leaves (prepared by maceration at room temperature of a ratio of 1:10 dried leaves in 70% ethanol) was tested for its activity against *Escherichia coli*, *Enterobacter aerogenes*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Providencia* spp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Shigella sonnei*, *Staphylococcus aureus* and *Staphylococcus* spp. (Gonçalvez, 2005). For the disk diffusion test, 100 ml of the tincture was applied per disc and tested against the bacteria at a concentration of $1x10^{-6}$ CFU/ml. The *I. paraguariensis* tincture exhibited strong inhibition of *S. aureus* (24 mm) and weaker inhibition of *Staphylococcus spp* (10mm) and *S. sonnei* (14mm). In this study the activity detected for *P. aeruginosa* and *P. mirabilis* by De Biasi (2009) was not detected. However, the process by which the extract was prepared was very different in each case and in the second case (tincture), though conservative, maceration at room temperature during 21 days is most likely less efficient than methods in which heat is used.

A comparative study of the inhibitory activity of *Helicobacter pylori*, a bacterium associated to gastritis, ulcers and stomach cancer (Kusters *et al.*, 2006) by *I. paraguariensis* and other plants with a record of traditional use for gastrointestinal disorders was carried by Cogo *et al.* (2010). They tested the anti-infective potential of extracts of *I. paraguariensis* dried and roasted leaves, *Bixa orellana* L. (annatto), *Chamomilla recutita* L. (chamomile), *Malva sylvestris* L.(mallow), *Plantago major* L. (plantain) and *Rheum rhaponticum* L. (rhubarb) . The extracts were prepared by exhaustive extraction with 96% ethanol at room temperature, followed by filtration, evaporation of the solvent and resuspension in dimethylsulphoxide (DMSO) for MIC determination or water for disk-diffusion tests. The final concentration of DMSO in the dilutions used for MIC was never above 1 %. A total of eleven clinical isolates of *H. pylori* were used for the tests and all extracts that exhibited some inhibition in the disk-diffusion tests were evaluated to determine their MIC_{50} and MIC_{90}. Of the tested extracts *I. paraguariensis* green leaf and *C. recutita*

extracts (DMSO) proved to inhibit the growth of all cultures with a MIC_{50} value < 0.625 mg/ml while roasted *I.paraguariensis* leaves had a MIC_{50} of 1.25 mg/ml. In the case of MIC_{90} tests, they concluded that [2] *I. paraguariensis* was able to inhibit a high number of clinical isolates although the green leaves (MIC_{50}: 5.0 mg/ml) were slightly less active than the roasted leaf extracts in this case (MIC_{90}: 2.5 mg/ml).

It is important to note, however, that a MIC_{50} > 100 µg/ml is not really considered active for mixtures (extracts). On the other hand this study was carried out with an infective dose of 1 x 10^8 CFU, which is a lot higher than the recommended value of 1 x 10^5 CFU/ml (Cos *et al.*, 2006).

Another study was carried out with aqueous or 80% ethanol: water extracts (prepared with 10 g of plant material, no details on volume) that were tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa A, Bacillus cereus* and *Bacillus subtillis*. The aqueous extract showed no inhibition of any of the bacteria while the ethanolic extract inhibited all except *B. cereus*. In the same study, the antioxidant activity according to Folin-Ciocalteau was evaluated and the ethanol extract exhibited a lower value than the aqueous extract. Thus, in this case, the antibiotic activity of the extracts had an inverse relation to their antioxidant capacity (Asolini *et al.*, 2006).

Yerba mate methanolic extracts (prepared by extraction of 10 g of commercial yerba mate with 100 ml of methanol, evaporation of the solvent and redissolution with dimethylsulfoxide to a concentration of 400 mg/ml) have also proved to moderately inhibit the growth of *Propionibacterium acnes* with a minimum inhibitory concentration (MIC) of 1 mg/ml (Tsai *et al.*, 2010). This bacteria plays an important role not only in the inflammatory processes typical of acne but also in the formation of comedones by inducing monocytes to secrete pro-inflammatory cytokines including interleukin (IL)-1b, IL-8, and tumour necrosis factor (TNF)-a (Kim *et al.*, 2005). Apart from the antimicrobial activity, yerba mate extracts also exhibited antiinflammatory activity, since it suppressed the secretion of TNFα, one of the most important pro-inflammatory cytokines, in dose-dependent manners and inhibited IL-1b and IL-8 secretion. In order to evaluate the potential of the extract as an ingredient for topical use, its cytotoxicity on skin cells was tested. After exposure to 1 mg/ml of the previously described extract, viability of both human skin keratinocytes and fibroblasts was significantly reduced. No work was done in this case neither on the mechanisms of action nor the possible components in the extract that could be responsible for this activity. Once again, MIC described for these extracts is very high, showing that *I. paraguariensis* has, actually, a very weak activity at least in the described experimental conditions.

- Antifungal

The activity of an aqueous extract of green *Ilex paraguariensis* leaves was tested against *Malassezia furfur*, a lipodependent, dimorphic and saprophyte fungus that causes pityriasis versicolor, dandruff and seborrheic dermatitis in humans. Though authors claim that *I. paraguariensis* shows antifungal potential, results showed an inhibitory activity of *I. paraguariensis* at a concentration of 1000 mg/ml against *M. furfur* equivalent to 2.7 μg/ml of ketoconazole, which is indeed an extremely high concentration (Filip *et al.*, 2010).

7.3.2 Antiparasitic activity

There are several severe endemic infections caused by parasites in Central Argentina and towards the North. One of the most life- threatening is Chagas- Mazza, caused by the protozoa *Trypanozoma cruzi*. Taketa *et al.* (2004) tested the antitrypanosidal activity of some saponins from diverse *Ilex* species, among them *I. paraguariensis*. They found that matesaponin 1, matesaponins 3 and 4, exhibited IC50 < 32 μμM against both *T. brucei* and *T. cruzi*, while ursolic acid had an IC50 of 4 μM.

7.3.3 Lipid metabolism, antioxidant and cell-protective properties

The leaves of *I. paraguariensis* and yerba mate (the roasted leaves) contain large amounts of CQAs and a low content of flavonoids, specifically rutin and lower amounts kaempferol-3-O-glycoside, quercetin-3-O-rhamnoside, rutin, quercetin-3-O-glycoside (hexoside), kaempferol-3-O-rhamnoglucoside, luteolin-O-glycoside (Rostagno *et al.,* 2011, Dugo *et al.*, 2009, Bravo *et al.,* 2007, Carini *et al.*, 1998). There are some differences regarding the presence of kaempferol, and Carini *et al.* (1998) reports luteolin and not kaempferol.

As was mentioned before, up to 42 different caffeoylquinic, caffeoylshikimate or caffeic acid derivatives, many of which exhibit antioxidant properties (Jaiswal *et al.*, 2010). Thus, in order to have an estimate of the polyphenolic content of herbs and their extracts, the Folin-Ciocalteau reaction, based on the reducing power of compounds is used (Chandra and Mejía, 2004; Bravo *et al.*, 2007). Apart from this, their activity related to more specific antioxidant activity has been assessed using assays which allow the evaluation of their scavenging activities of radicals such as DDPH (1,1-Diphenyl-2-picryl-hydrazyl) free radical (Carini *et al.*, 1998), ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid) free radical and their FRAP (Ferric Reducing Antioxidant Power)(Bravo *et al.*, 2007), comparing it to green or black tea or wine, some of the most popular foodstuffs with powerful antioxidant properties. Mate extracts and

tea samples have also been tested for their ORAC (Oxygen Radical Absorbance Capacity) against Trolox (Chandra and Mejía, 2004). Another study used three different methods: TRAP (inhibition of the luminol-induced chemiluminescence assay); TBARS (inhibition of 2.2′-thiobarbituric-reactive substances formation in liposomes by fluorescence) and the protection of Jurkat cells from AMVN-induced oxidation, measuring the oxidation of 5-(and-6)-carboxy-2′7′-dichlorodihydrofluorescein diacetate to a fluorescent derivative (Actis-Goretta *et al.*, 2002) finding that mate infusions had a higher antioxidant activity than different types of wine and only less than green tea at equal total polyphenolic contents.

De Morais *et al.* (2009) conducted a single-blind controlled trial with 120 participants with the purpose of studying the effect of the ingestion of mate infusions on lipid metabolism. Normolipidemic (n = 15), dyslipidemic (n = 57), and hypercholesterolemic subjects on long-term statin therapy (n = 30) ingested 330 mL, 3 times/day, of green or roasted yerba mate infusions for 40 days. In normolipidemic subjects, yerba mate consumption reduced LDL-cholesterol by 8.7% ($p < 0.05$). Compared with the baseline period, yerba mate intake by dyslipidemic individuals for 20 and 40 days lowered LDL-cholesterol by 8.1 and 8.6% ($p < 0.001$) and non-HDL cholesterol by 5.4 and 6.5% ($p < 0.01$). After 20 days of yerba mate intake, apolipoprotein B was reduced by 6.0% ($p < 0.05$) and HDL-cholesterol was increased by 4.4% ($p < 0.01$). In all participants triglyceride levels remained unchanged. The consumption of yerba mate by hypercholesterolemic individuals on statin therapy promoted additional 10.0 and 13.1% reductions in LDL-C after 20 and 40 days, respectively ($p < 0.001$) and increased HDL-cholesterol by 6.2% after 40 days ($p < 0.05$). It was thus concluded that intake of yerba mate infusion improved the lipid parameters in normolipidemic and dyslipidemic subjects and provided an additional LDL-cholesterol reduction in hypercholesterolemic subjects on statin treatment, which may reduce the risk for cardiovascular diseases.

Working with Wistar rats submitted to different types of high-fat diets (with cholesterol, animal fat or vegetable oil) followed by treatment with mate tea, Melo *et al.* (2007) reported a tendency of lower weight gain, increase in HDL-c, reduction in glucose level, liver weight and transaminases only in rats fed with saturated fat in the animals treated with mate tea, suggesting a possible protective effect of *Ilex paraguariensis* on the metabolic profile. Similar results were reported by Przygodda *et al.* (2010), who also tested rats fed with HFD (high-fat diet) and others with high sugar diets. In all cases the administration of mate tea decrease body weight gain, visceral fat and plasmatic glucose, cholesterol and triacylglyceride levels.

Another study described the *in vitro* inhibition of porcine pancreatic lipase of several saponins: matesaponin I, nudicaucin C and 3-O-α-L-rhamnopyranosyl (1—2)-α-L-arabinopyranosyloleanolic acid 28-O-β-D-glucopyranosyl(1—6)-β-D-glucopyranoside and the monoterpene oligoglycoside, (R)-linalyl-6-O-α-L-arabinopyranosyl-β-D-glucoside isolated from a methanolic extract of *I. paraguariensis* green leaves (Sugimoto *et al.*, 2009).

Sample	Analytical method	Activity	Reference
Mate infusion	TBARS production (*in vitro)*	Inhibit LDL oxidation	Gugliucci & Stahl (1995)
Mate infusion	TBARS production (*ex-vivo)*	Inhibit LDL oxidation in healthy human plasma	Gugliucci (1996)
Mate infusion	TBARS production, diene conjugates formation and total polyphenols (*ex-vivo)*	Inhibit LDL oxidation in healthy human plasma	Gugliucci and Menini (2002)
Ip methanolic extract	TBARS production *in-vivo*	Inhibit LDL oxidation in liposomes	Filip *et al.* *(*2000)
Ip aqueous extract	Lipid peroxidation- induced - erythrocyte membrane peroxidation and free radical generation (*in vivo)*	Inhibit LDL oxidation; free radical scavenging properties in rat liver and erythrocytes.	Schinella *et al.* (2000)
Ip aqueous extract (infusion)	Double strand breaks detn. (TAFE), TBARS production, diene conjugates formation and DPPH assay. Human plasma and *S. cerevisiae*	Decrease DNA fractures Inhibit LDL oxidation	Brasesco *et al.* (2003)
Ip aqueous extract (maceration)	Cytotoxicity, TPA-induced ornithine decarboxylase (ODC) and quinone reductase (QR) activities. *In vitro* (HepG2 cells). Topoisomerase- *S. cerevisiae*	Cytotoxic activity No QR nor ODC inhibition activity. Inhibition of topoisomerase	Ramirez-Mares *et al.* (2004)
YM aqueous extract (maceration)	Total antioxidant capacity (ORAC) - Quinine reductase assay (*in vitro*- HepG2 cells)	No QR inhibition activity. Antioxidant activities.	Chandra and Mejia (2004)
YM infusion	DPPH; nitration of BSA, LDH cytotoxicity. (*In vitro* -murine RAW264.7 macrophages	Inhibition of protein nitration and cytoprotective effects	Bixby *et al.* (2005)

Sample	Analytical method	Activity	Reference
YM infusion	Lipid profile; TBARS production and antioxidant enzymes (*in vivo*-cholesterol fed rabbits)	No effect on lipid profile nor anti- oxidant liver enzymes. Reduced atherosclerotic lesions and aortic cholesterol.	Mossiman *et al.* (2006)
Ip infusion	Luminol-induced hemi-luminescence assay (TRAP); TBARS formation in liposomes by fluorescence; 2,2-azobis-(2,4-valeronitrile).	Antioxidant activity	Actis-Goretta *et al.* (200)
	In vitro- Jukart cells (human leukemia T cells)	Protection from oxidation	
YM infusion	FRAP/ABTS	Antioxidant activity	Bravo *et al.* (2007)
YM infusion	FRAP/ copper ion- or AAPH-induced lipid peroxidation of LDL / platelet aggregation/ blood coagulation(*ex vivo* human plasma)	Inhibition of LDL oxidation. No effect on platelet agreggation nor blood coagulation	Da Silva (2008)
YM infusion	LDL/HDL/Triglyceride detn. (Clinical trial;healthy and statin-treated patients).	Decrease LDL/triglycerides Increase HDL	De Morais (2009)
YM infusion	Plasma total antioxidant status (TAS), diene conjugate generation, (TBARS) mRNA levels of antioxidant gluthatione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT)- *Ex vivo* (human plasma)	Decrease in lipid peroxidation	Matsumuto *et al.* (2009)

Table 7-4: Summary of the principal reports of the activities of Yerba mate on lipid metabolism and antioxidant and cell-protective effects. Abbreviations: Ip= Ilex paraguariensis; YM= yerba mate (Modified from Bastos et al. -2005).

The role of " Antioxidants" in humans

When reviewing the bioactivity of food and medicinal plants, it is very frequent to find that these activities, that may range from flu protection to prevention or protection from life-threateing diseases such as cancer, Alzheimer's disease, diabetes, etc., are attributed to the presence of compounds such as polyphenols or compounds that have high *in vitro* or *in vivo* antioxidant activities as measured by their free ROS scavenging activities or Fe(III), thiobarbituric acid reducing capability, ORAC, among others.

There are hundreds of claims in literature on the antioxidant properties of CQAs, for example and even though they are ubiquitous in nature, it would seem that in some plants their presence, associated to that of other compounds, generate a strong at least *in vitro* antioxidant activity (Carini *et al.*, 1998).

Whether these antioxidant compounds can reach their targets in pharmacologically active doses and act on them in a similar manner to that observed in *in vitro* studies has not been really demonstrated to date. In the first place, the absorption and pharmokinetics of their metabolism and excretion were not clear until the last decade. Olthof *et al.* (2001) studied their absorption in experiments on colostomised subjects that showed that around 33% of CQAs are absorbed in the small intestine as such. Later on, experiments carried out on rats showed the rest of the CQAs were hydrolysed by gut microflora in the colon to quinic acid and caffeic acid that then suffer further transformations. Quinic acid is metabolised to hippuric acid and caffeic acid to *m*-coumaric acid or first to 3,4 hydroxyphenylpropionic acid which can then be metabolised to hippuric or 3-hydroxyhippuric acid (Gonthier *et al.,* 2003). These conclusions coincided with experiments done by Olthof *et al.* (2003) who performed studies on humans with a functioning colon and concluded that the caffeic acid moiety could be further dehydroxylated by the intestinal flora and absorbed after which it could be β-oxidysed to benzoic acid. The quinic acid moiety is dehydroxylated into cyclohexane carboxylic acid and then aromatized into benzoic acid by the colonic microflora or after absorption in body tissues. The benzoic acid formed is conjugated with glycine and excreted in urine as hippuric acid. This is important since it leads to reflect on the real antioxidant capacity of these polyphenols, since most of the studies carried out are *in vitro*, that is, the samples are subjected to pure CQAs for example, while in the body, a very low proportion of the consumed polyphenols will reach the target organs or tissues or more so, given their hydrophillicity might not even reach certain cells.

Hollman (2010) also made another interesting point that should be reflected on when automatically attributing activities of polyphenols to their *in vitro* antioxidant capacity. According to this "polyphenol" specialist, while the pharmacological properties

or bioactivities reported for polyphenols by many researchers should not be put in doubt, some authors consider that these activities cannot be explained by their antioxidant properties alone since the cellular defence mechanisms against oxidative damage are much more powerful than the contribution that can be made by polyphenols or their metabolites. This is of course difficult to predict since the damage that might lead to disorders at a cellular level, could be precisely due to failures in these mechanisms. Referring to their activity on cardiovascular health, Hollman *et al.* (2011) were of the opinion that a direct antioxidant effect of polyphenols *in vivo* is questionable, because their concentrations in blood are usually low compared to other antioxidants and that their extensive metabolism following ingestion lowered their antioxidant activity. They concluded that while some polyphenol-rich foods exerted beneficial effects on some biomarkers of cardiovascular health, there was no evidence to justify connecting these with improvements in antioxidant function biomarkers (oxidative damage or antioxidant capacity).

Halliwell, another antioxidant specialist, also puts the contribution of dietary antioxidants in doubt. Considering that living organisms, as aerobes, synthesize reactive oxygen species (ROS), they also make antioxidants to modulate their activity. In this way, the activity of these reactive species that fulfill some very necessary roles in the organism is modulated, producing the so-called *redox balance*. The presence of oxidative damage triggers the protective effects of the immune system that are very powerful. Thus, in his opinion, we are perhaps fortunate that diet-derived 'antioxidants' do not markedly decrease oxidative damage in humans –because otherwise they might sometimes have caused harm rather than good. He also disagrees with the antioxidant free-radical scavenging assays as a measure for *in vitro* antioxidant activity, with the exception of the HPLC TBA assay (Haliwell, 2011). As regards the assays with cell-lines used to assess biological activities of the so-called antioxidants, he puts their validity in doubt for various reasons. On one hand, cell-cultures as performed, suffer oxidative stress; on the other hand, the reaction of the tested antioxidants with cell-culture components are ignored or disregarded, when in fact, they often react and the observed activities are probably due to their oxidation or degradation products (Haliwell, 2008; Long *et al.*, 2010). But perhaps the worst news lies in the reports of the misidentification of the cell-lines used for the tests, as described by the American Type Culture Collection Standards Development Organization Workgroup ASN-0002 (2010), leading to artefactual reports not only in this field of course.

One of the most popular antioxidants, quercetin, a flavonol and its glycoside, rutin, are widely distributed in a great number of higher plants and have been held

responsible for the positive effects observed for these plants or their derivatives, in all types of disorders. A few years ago, evaluating the prevention of H_2O_2 induced damage to DNA in rat lung epithelial cells (RLE), Boots *et al.* (2007) found that the oxidation product of quercetin was thiol reactive, arylating GSH (reduced glutathione), which naturally protects against protein arylation and also increasing levels of cystolic free calcium. Thus, the positive effect observed by quercetin in reducing DNA damage was in fact counteracted by the oxidation of quercetin itself, which produced a highly toxic compound. This paradoxical effect is not, apparently, limited to quercetin, as shown by reports of similar effects with other known dietary antioxidants such as vitamin A, E and β-carotene (Bjelakovic *et al.* (2007).

There is no doubt, thus, that further work should be done to fully explain the real mechanism of action of these compounds.

In the case of yerba mate, as in so many other cases, the *in vitro and ex vivo* antioxidant properties of mate have been extensively reviewed (Mejia *et al.,* 2007; Bastos *et al.,* 2007; Ranilla *et al.,* 2010; Bravo *et al.*, 2006; Brasesco *et al.*, 2010; among others) and have often been used to explain many of the biological activities observed.

7.3.4 Obesity

In the last 10 years, obesity has come to be considered as a disease or at least as a serious condition that can cause a vast range of life-threatening disorders such as metabolic syndrome, cardiovascular disease, and diabetes among others. Research into the causes and mechanisms behind obesity has intensified, as has the search for drugs or preparations that can help to control it. There are no real ethnopharmacological references to antiobesity activities because it was never considered to be a sickness on one hand, but on the other, it is clearly a product of modern lifestyle, characterised by high fat and sugar diets and scarce physical activity. Because of the complex biochemistry behind obesity when installed as a disorder and its recognition as an addiction, a serious search for compounds that can assist in its control has now been established and plants, both medicinal or those incorporated in daily diet as food are being investigated a possible source.

Thus, researchers have begun to screen herbs, both medicinal and aromatic, which contain compounds with antiobesity activities to test their potential use in the treatment of this disorder. Considering that obesity results as an unbalance between input and output of energy, the main ways of approaching the condition are either any of the following: a reduction of energy intake by appetite suppression; inhibition of nutrient absorption; increase of energy expenditure or the modulation of fat (6). Yuliana

et al. (2011) screened a number of spices used in Asia cuisine, such as nutmeg, mace, black pepper and turmeric but and medicinal plants some of the interesting findings regarding plants used as spices.

Yerba mate extracts and infusions have been tested for antiobesity activities, in some cases with very interesting results. The treatment of obesity is usually focused on any of the following targets: a decrease of absorption of energy from the food, an increase in the rate of metabolisation of food or an increase in the expenditure of energy. A recent paper (Martins *et al.,* 2009) described the activity of a yerba mate aqueous extract (MT) as an inhibitor of pancreatic lipase, the enzyme which is involved in the hydrolysis of α- and α' positions of tryglicerides, releasing the fatty acids and leaving the β-monoglycerides which are later absorbed. The inhibition of pancreatic lipase had been observed for green and oolong tea (*Camelia sinensis*) that are rich in condensed tannins and flavonoids. Yerba mate has scarce or no tannins and only low amounts of the flavonoids, rutin and luteolin but a high amount of CQAs, caffeine and saponins, many of which have been identified, as described above. In this case, an instant yerba mate tea preparation that contained 348.80 $\pm$ 16.35 mg/g of phenolic compounds as determined by Folin–Ciocalteau method using 5-caffeoylquinic acid as the standard for the calibration curve ($y = 4.843x + 0.0149$); 5.82 $\pm$ 0.17mg/g of caffeine, 32.25 $\pm$ 0.50mg/g of 5-caffeoylquinic acid, 0.58 $\pm$ 0.01mg/g of caffeic acid, and 3.30 $\pm$ 0.35 mg/g of theobromine, was tested for its *in vitro* inhibitory activity against porcine and human pancreatic lipases. The results showed that a concentration of 3 mg/ml inhibited pancreatic lipases similarly to black and oolong tea but more than green tea. In this study no relation between the polyphenols present in the samples and the activity could be established. In the same experiment, mice that were fed a high-fat-diet (HFD) containing instant yerba mate powder did not exhibit an increase in body weight and showed a decrease in serum triglyceride, cholesterol, and LDL-cholesterol concentrations after they had been increased by HFD. These effects did not depend on decreased food or energy intakes because there were no significant differences between the HFD and HFD plus MT groups. The results thus suggested that MT is able to suppress dietary fat absorption from the small intestine of mice by inhibiting pancreatic activity.

Sugimoto *et al. (*2009) also observed an *in vitro* inhibitory effect of porcine pancreatic lipase with a methanolic extract of green *I. paraguariensis* leaves. Having isolated a great number of compounds from the extract, the authors reported a high inhibitory effect of three saponins and a monoterpene glycoside while caffeine, rutin and CQA derivatives had low activities, leading to the conclusion that they could contribute a little but not decisively to the overall activity of the methanolic extract.

Another *in vivo* experiment (Pang *et al.,* 2008) consisted in the administration of a Yerba mate extract obtained from Frutarom (Switzerland) to mice, concomittantly with a high-fat diet (HFD) during 60 days. According to the manufacturers, the extract is obtained by the extraction of dried leaves of *I. paraguariensis* with 15% ethanol at 50 °C for 10 h. The resulting extract is filtered and evaporated, and the remaining paste extract is spray-dried at 160– 170 °C. The yield of the *I. paraguariensis* extract from the dried leaves is 20%. The specifications of the product are 24-30% CQAs, 2–4% caffeine, 0.3–1.2% theobromine, and >1.0% triterpenic saponins as analysed by HPLC. However, the authors do not clarify the exact chemical composition of the tested extract. The results indicated that the yerba mate extract was very active as an appetite suppresant, causing significant decreases in the body weight gain, visceral fat-pad weights, adipocyte size, blood and hepatic lipid concentrations, and blood levels of glucose, insulin, and leptin in a rodent model with HFD-induced obesity. The authors concluded that this could be through the β-oxidation of fatty acids, increasing AMPK activation in visceral adipose tissue and subsequently reducing ACC activity. Activated AMPK phosphorylates (inactivates) ACC and lowers levels of intracellular malonyl-CoA, which is the fatty acid synthesis substrate. At the same time, malonyl-CoA inhibits CPT-1, the rate-limiting enzyme in mitochondrial fatty acid oxidation. Accordingly, these processes lead to the promotion of fatty acid oxidation (Yun *et al.,* 2010).

In an *ex vivo* experiment, Paganini Stein *et al.* (2005) investigated the vasorelaxant properties of the aqueous and acid n-butanolic fractions from *I. paraguariensis* leaves. The effect was evaluated using isolated and perfused mesenteric arterial beds (MABs) from rats fed hypercholesterolemic and standard diets. They observed that the administration of these fractions resulted in a significant reduction in serum levels of cholesterol and triglycerides of hypercholesterolemic rats.

An *in vitro* experiment using lipopolysaccharide-induced RAW 264.7 macrophages was carried out to evaluate the anti-inflammatory responses of the major *I. paraguariensis* components and aqueous extracts, through the inhibition of COX-2/PGE2 and iNOS/NO pathways - well-known mediators in inflammatory processes (Puangpraphant and Mejía, 2009). Testing an aqueous extract of yerba mate (prepared as a tea), a decaffeinated yerba mate extract, a hydrolysed extract of saponins from Yerba mate (78% purity), caffeine and ursolic acid, they found that while quercetin exhibited a high inhibitory activity at concentrations 10 times lower than the other tested compounds (caffeine, saponins), this activity was greatly increased in presence of the hydrolysed saponins. The molar ratio of the mixture was 0.001:0.004 of quercetin and sapogenins respectively, which is roughly equivalent to that found in a cup of tea

(prepared with 1.5 g of yerba mate /150 ml of water). Interestingly, this did not occur with combinations of caffeine or CQAs with sapogenins that actually resulted in an antagonistic inhibition of NO and PGE2 production. The absence of significant anti-inflammatory effects of the aqueous yerba mate extract effect may be due to the antagonistic effect of some of its compounds. The authors concluded that the synergistic or antagonistic effects of the mixtures maybe depended on the formation of stable intermolecular complexes. In this paper there are two issues that seem a bit misleading, i.e., their consistent mention of caffeine as matein (which does not exist as a chemical entity different to caffeine) and the "mate saponins", that are actually a 78% pure mixture of mate sapogenins, namely ursolic and oleanolic acid and a minor content of one other sapogenins as described in section 7.2.2. The interesting aspect of this, not discussed by the authors, is that when tested as isolated compounds, neither ursolic nor oleanolic acid exhibited any significant activities.

A study of the effects of the administration of lyophilysed mate instant tea to HFD fed mice demonstrated that the obese mice exhibited marked attenuation of weight gain adiposity, a decrease in epididymal fat-pad weight, and a restoration of the serum levels of cholesterol, triglycerides, LDL cholesterol and glucose (Arcari *et al.,* 2009). In the study, the expression of diverse adipokines (TNF-α, IL-6, leptin, CCR2, CCL2, angiotensinogen, PAI-1, adiponectin, PPAR-γ2, PGC-1α, and UCP1) secreted by the adipose tissue that play a fundamental role in the regulation of metabolism and homeostasis were found to be directly regulated by the high-fat diet. Studying the levels of these proteins in obese mice which were given a dose of 1 mg/kg of yerba mate extract during 8 weeks, the authors observed that the expression levels of cytokines (TNF-α, IL-6, and leptin), chemo- attractant proteins (CCR2 and CCL2), and genes involved in the regulation of blood pressure, vascular homeostasis or angiogenesis (angiotensinogen and PAI-1) were significantly reduced. On the other hand, the downregulation of genes implicated in adipogenesis (PPAR-γ2) and glucose and lipid metabolism (adiponectin) were reversed. In addition, the yerba mate treatment recovered the expression of genes implicated in thermogenesis (PGC-1α and UCP1) in BAT. The authors hypothesized about the compounds present in yerba mate extract that could potentially be responsible for these activities, concluding that the activity of caffeine, CQAs and saponins could all have some participation in the biological effects. The extract contained 348.80 $\pm$ 16.35 mg/g of phenolic compounds, 5.82 $\pm$ 0.17 mg/g of caffeine, 32.25 $\pm$ 0.50 mg/g of 5-caffeyolquinic acid, 0.58 $\pm$ 0.01 mg/g of caffeic acid, and 3.30 $\pm$ 0.35 mg/g of theobromine (the saponin content was not examined).

The same group of researchers recently published another paper in which they describe the results of their investigation into the effect of the same yerba mate extract on markers of insulin resistance and inflammatory markers in mice with high fat diet-induced obesity (Arcari *et al.,* 2011). After 8 weeks of treatment with 1.0 mg/kg of the roasted yerba mate lyophilysed extract, the mice showed a weight reduction of 20% as compared to untreated HFD fed mice that was not related to a reduction in food intake. Similarly, their blood glucose level and insulin resistance markers were reduced to those of the control standard diet-fed mice. The authors explained this to be a consequence of the down-regulation of TNF-αB, a major pro-inflammatory factor, which mediates insulin resistance among other things through the activation of the NF-αB pathway. The increase of NF-αB is known to activate a battery of genes related to inflammatory proteins such as IL-6 and iNOS, which play a critical role in obesity-related inflammation and metabolic pathologies. TNFα and NF-αB translocation to the liver that had been increased in HFD-fed obese mice were found to be decreased in the yerba mate treated mice. Additionally, these data demonstrate, for the first time, that yerba mate can inhibit hepatic TNF-αB and restore hepatic and muscle insulin signaling in mice with high fat diet-induced obesity.

There are other papers that report results from diverse *in vitro* experiments describing diverse biological activities of *I. paraguariensis* extracts or infusions related to aspects of obesity prevention. Ranilla *et al.* (2010) compared aqueous extracts of spices, medicinal herbs and herbal teas from S. America for their associated phenolic profiles, antioxidant activity and potential for managing early stages of Type 2 diabetes such as hyperglycemia relevant α-glucosidase and α-amylase and hypertension relevant angiotensin I-converting enzyme (ACE). Results revealed that *I. paraguariensis* had a moderate α-glucosidase inhibitor capacity and no effect on α-amylase and ACE, having the highest polyphenolic content among the studied herbs including Boldo (*Peumus boldus*), Cedron or Lemon verbena (*Aloysia triphylla)*, Linden (*Tillia platyphyllos*) among others.

There are very few clinical studies done with Yerba mate. One of them was conducted in Denmark on patients receiving a mixed herbal preparation 'YGD' containing Yerba Mate (leaves of *I. paraguariensis*), Guarana (seeds of *Paullinia cupana*) and Damiana (leaves of *Turnera diffusa* var. *aphrodisiaca*) to determine their effect on gastric emptying and weight loss over a 10 - and 45 day period and weight maintenance over 12 months. Results showed a significant delay in gastric emptying and reduced the time required to perceive gastric fullness. A significant weight loss was induced over 45 days in overweight patients treated in a primary health care context.

Maintenance treatment given in an uncontrolled context resulted in no further weight loss, nor weight regain in the group as a whole (Andersen and Foch, 2001).

In another case, 12 commercial herbal preparations that claimed to have antiobesity activity were tested on healthy non-obese women and men. The thermogenic capacity was evaluated through the measurement of the respiratory quotient (RQ) that showed that the only preparation in which the RQ dropped, indicating an increase in lipid oxidation was with *I. paraguariensis* - yerba mate - extract (Martinet *et al.*, 1999).

7.3.5 Antidiabetes activities

Yerba mate has been shown to inhibit the formation of advanced glycation end-products (AGEs), with an effect comparable to that of two pharmaceutical grade AGE inhibitor drugs. Lunceford and Gugliucci (2005) reported that polyphenol-rich *I. paraguariensis* extracts are capable of inhibiting AGEs (or Maillard reaction products) on a protein model *in vitro*, whereas green tea displays no significant effect. The AGEs, which are irreversibly formed, accumulate with aging, atherosclerosis, and diabetes mellitus (Wiemsperger 2004). The authors related this activity to phenolics, such as chlorogenic acids, since they have been claimed to modulate the activity of glucose-6-phosphatase involved in glucose metabolism (Hemmerle *et al.,* 1997), but no experimental data was obtained in this case. Oliveira (2008), however, did not find any reduction in glycemia when investigating the influence of "erva-mate" on parameters related to diabetes mellitus and metabolism of glucose.

Ranilla *et al.* (2010) studied a number of S. American medicinal plants, herbal teas and spices, among which Yerba Mate was evaluated as a herbal tea. They found that *I. paraguariensis* had the highest total phenolic contents and antioxidant activity, and that it showed moderate α-glucosidase inhibitory activity but no effect on α-amylase and ACE (angiotensin I-converting enzyme). Such a combination would be helpful to manage glucose uptake and the glucose-induced increased levels of mitochondrial ROS (reactive oxygen species) linked to hyperglycemia.

7.3.6 Anticancer activities

A great amount of research into the anticancer activities of *I. paraguariensis* leaves and yerba mate have been undertaken in the last 15 years. Most of these have been *in vitro* experiments while only a few *in vivo* or *ex-vivo* experiments have been reported. Unfortunately no real epidemiological studies have been performed. Several reviews

have been published recently that cover these activities extensively (Bastos *et al.,* 2007; Heck and Mejía, 2008 and more recently Bracesco *et al*., 2011).

Antimutagenic and DNA protective properties have also been observed in cell cultures by Bracesco *et al*., (2003) and in mice by Miranda *et al.* (2008), who related the activity to its CGAs and flavonoid content (rutin).

A recent study showed that saponin-rich fractions of *I. paraguariensis* inhibit colon cancer cell (HT-29) proliferation through the activation of a specific intracellular apoptosis pathway in HT-29 cells. This saponin fraction also increased the expression of the pro-apoptotic protein Bax, decreased the expression of anti-apoptotic protein Bcl-2; and subsequently activated caspase-3. These findings suggest that apoptosis induction in matesaponins-treated HT-29 cells could be associated with a caspase-dependent cascade that involves the activation of the mitochondrial pathway, initiated by the inhibition of Bcl-2 and the activation of Bax (Puangpraphant *et al*., 2011).

Along with the effects described above, a recent article suggests some deleterious effects of *I. paraguariensis* extracts on lymphocytes. In an *in vitro* study using human lymphocytes the authors show a cytotoxic activity of the extracts against these cells that was due mostly to caffeine and therefore it is not unique to maté beverages (Alves *et al*., 2008; Wnuk *et al*., 2009).

One of the activities involved in cancer biology is angiogenesis, though it is also known to play a key role in inflammation and repair. Treatments performed with caffeine and aqueous extracts made with leaves of different ages, containing 0.15 mg/ml of polyphenols and caffeine in the case of young leaves and 0.14 and 0.24 mg/ml of polyphenols and caffeine respecitively in the case of mature leaves. The tests which were carried out on the vascular membranes of chick embryos yolk sac revealed pro-vasculo- and angiogenic properties as well as embryonic growth enhancement. The authors related this activity to the presence of methylxanthines but considered that other constituents of the yerba mate extracts should be studied (Strassmann *et al*., 2008).

7.3.7 Skin antiageing activity

Yerba mate extracts are increasingly used in cosmetic preparations. Natura, a leading Brazilian cosmetic firm markets a line of products based on yerba mate extracts. A possible anti-wrinkle activity was described for two neostigmanes newly isolated from *I. paraguariensis* leaves, matenosides A and B (Xu *et al.,* 201*0).* One of the causes of wrinkle appearance is the decrease in the amount of elastin, a minor component of the dermis that plays an important role in sustaining the elasticity of the skin. With age,

and particularly in people above 40, the elasticity of the skin is decreased significantly due to the activity of elastase (Robert, 2001). Inhibition of the elastase activity thus can also be a useful method for protecting against skin aging. Matenosides were found to inhibit Human neutrophil elastase (HNE, EC 3.4.21.37), a serine protease that plays a role in the degradation of a wide range of extracellular matrix proteins, including fibronectin, laminin, proteoglycans, collagens and elastin (Steinbrecher et al., 2008).

Previously, the same group had observed a strong HNE- inhibitor activity with a methanolic extract of *I. paraguariensis* leaves. Bioguided assay led to the isolation of a new pyrrazolidinic alkaloid and several CQAs. The only compounds, however that exhibited an interesting HNE- inhibitor activity were 3, 4- dicaffeoylquinic acid methyl ester; 3, 5-dicaffeoyl- quinic acid; 4, 5-dicaffeoylquinic acid methyl ester and 3, 4-dicaffeoylquinic acid (Xu *et al*., 2009).

7.4 Anticancer or carcinogenic?

The fact that yerba mate is drunk so intensively by a large population, throughout most of their lives led to investigations into its potential toxicity. In certain provinces in Argentina for example, children drink mate since a very early age, in amounts that exceed 1 litre/day.

The results of these studies showed a possible connection between mate-drinking and oesophageal, oral, lung, bladder, renal, as well as other cancers of the head and neck (Vasallo *et al.* 1985*;* Victora *et al.* 1987, Pintos *et al.* 1994; De Stefani *et al.* 1996, 1998; Goldenberg *et al.* 2003; Bates *et al.* 2007).

The IARC (International Agency for Research on Cancer), however, included a monograph on Mate in 1991 and other caffeine containing teas (coffee, tea) apart from caffeine itself. After the publication of one of the first studies on oesophagal cancer by Vasallo *et al.* in 1985, the IARC coordinated two clinical studies in S. American countries with results that differed from those cited above (Victora, 1987; De Stefani, 1990 and Casteletto *et al*., 1994). In the monograph, IARC evaluated the carcinogenic risk of *mate* and concluded that there is: (a) "limited evidence for carcinogenicity of hot *mate* drinking in humans;" (b) "no data available on the drinking of cold *mate*;" and, (c) "no data on its carcinogenicity in experimental animals" (3). Overall, IARC classified drinking hot *mate* as "probably carcinogenic to humans (Group 2A)" and *mate* as "not classifiable as to its carcinogenicity to humans (Group 3)." Most of the studies on which the IARC evaluation was based were similar in methodology and shared part of the enrolled subjects and some of the researchers. As of August 2008, the IARC has not released an updated evaluation (IARC, 1991).

Loria *et al*., (2008), researchers from the "Instituto de Oncología Dr. Angel Roffo" (National Oncology Institute, Buenos Aires, Argentina), published a review of the situtation, in which all the available bibliography was analysed. They found that in general, almost all epidemiological studies shared a similar methodology: hospital-based, case-control studies where participants were personally interviewed on the main risk factors, using similar questionnaires. Controls were recruited from among hospital patients suffering from diseases apparently not related with the risk factors in question. In the opinion of Loria, the choice of hospitalised controls was not entirely appropriate since from a methodological aspect, it would have been better to use subjects from the general population in the control groups. Two risk factors were identified: the temperature of the ingested mate, which could act by damaging the mucosa of the oesophagus or accelerating metabolic reactions, and the PAHs (polyaromatic hydrocarbons) and tar content of mate. As far back as 1941, research by Dr. Angel Roffo referred to the tar content of *yerba mate* extracts and the occurrence of skin tumors in experimental animals painted with a *yerba mate* mixture (Roffo *et al.,* 1941).

In 1985, the presence of benzo-α-pyrene (BP) was assessed in samples of commercial brands of *yerba mate* (Ruschenburg 1985). Concentrations of BP as high as 461 μg/1 Kg dry *mate* were found; lower concentrations were found in the prepared beverage. Zuin *et al.* (2005) reported an effective and simple procedure to analyze PAHs in *mate* infusions. The method detected 15 PAHs in 11 *mate* brands available in the market in Brazil. In 2008, another study measured PAHs in *mate* brands in the market in Brazil. The researchers found that *mate* leaves contained PAHs in concentrations ranging from 2–11 times that of green tea leaves. High mass fractions of carcinogenic PAHs were found, not only in dry leaves, but also in hot and cold *mate* infusions.

Clearly, the most concerning aspect of yerba mate toxicity is related to the presence of PAHs. These appear mostly during the roasting of the leaves, when they are exposed to direct fire. As a consequence, there are companies that are incorporating different technological processes to avoid this step without changing the typical yerba mate taste, which is undoubtedly produced by the smoking stage.

The European Community monograph of *Mate folium* includes a comment on the need to control and restrict PAH content of mate leaves.

7.5 Conclusion

The above review was made with the purpose of showing the complexity and variability of *I. paraguariensis* chemical composition and the incidence this has on the sensorial attributes and even biological activities of this herbal drug. Both green and roasted leaves have a content of caffeine that is comparable to that of coffee and tea. Alongside with this, it is a good source of chlorogenic acid and its saponin content assists in the solubility of these and other phytochemicals. But above this, the "mate culture", leads to the consumption of above 3 L /day of a concentrated mate beverage, providing considerable amounts of all these phytochemicals. And this consumption is growing steadily as mate drinking becomes remarkably increasingly popular. With a content of 5 g/L of polyphenols and 0.35 g/L of saponins (Bracesco *et al.*, 2011) ingested probably in the course of one hour or less, it cannot be underrated as a source of these phytochemicals and even when this content is comparable to that of coffee, it would be practically impossible to drink this amount of coffee in less than one hour, much less consider drinking above 3 litres as a daily habit. Similar considerations apply for other high polyphenolic content beverages such as wine.

As has been discussed, a great deal of the scientific literature related to its biological activity is lacking in scientific rigour and serious epidemiological studies should be made to get trustworthy information. Its marketing as a stimulant is obviously correct due to its high caffeine content, but its antiobesity and lipidic profile improvement properties have still to be better documented. The same applies for its antimicrobial, anticancer activities. It undoubtedly scores well in all antioxidant capability tests, but as discussed above, this should not be used indiscriminately to justify and explain all biological activities.

What is important in this context thus, is to ensure at least, its atoxicity on one hand and find ways of guaranteeing that what reaches the consumer is in fact *I. paraguariensis* leaf with a consistent flavour and aroma. This is also important in order to contribute to the search for better processing methods that could both reduce time and especially decrease the content of the carcinogenic PAHs.

The chromatographic profiles of the polyphenolic content and caffeine or saponin content have not proved to be sufficient to control the quality of yerba mate, nor has it been possible to relate these profiles to the presence of adulterants or poor quality raw material. Detecting saponins that are not common to *I. paraguariensis* for example is not an easy task for a QC lab.

Thus, the possibility of using a more holistic approach as mentioned in Chapter 6, to discover the metabolites common and characterisitic of *I. paraguariensis* can be a real contribution to its quality control.

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REFERENCES

- **Actis-Goretta L, Mackenzie GG, Oteiza PI, Fraga CG** (2002). Comparative Study on the Antioxidant Capacity of Wines and Other Plant-Derived Beverages. Issue Annals of the New York Academy of Sciences, 957, Alcohol And Wine In Health And Disease pages 279–283.

- American Type Culture Collection Standards DevelopmentOrganization Workgroup ASN-0002 (2010) Cell line misidentification: the beginning of the end. Nat. Rev. Cancer 10, 441–448

- **Andersen T, Fogh J** (2001). Weight loss and delayed gastric emptying following a South American herbal preparation in overweight patients. J Hum Nutr Diet, 14: 243–250.

- **Araújo HC, Lacerda MEG, Lopes D, Bizzo HR, Kaplan MAC** (2007), Studies on the aroma of maté (*Ilex paraguariensis* St. Hil.) using headspace solid-phase microextraction. Phytochem Analysis, 18: 469–474. doi: 10.1002/pca.1002

- **Arçari DP, Bartchewsky W, dos Santos TW, Oliveira KA, Funck A, Pedrazzoli J, de Souza MFF, Saad MJ, Bastos DHM, Gambero A, Carvalho P, Ribeiro ML** (2009). Antiobesity Effects of *yerba maté* Extract (*Ilex paraguariensis*) in High-fat Diet-induced Obese Mice. Obesity 17, 2127–2133

- **Arçari DP, Bartchewsky W, dos Santos TW, Oliveira KA, de Oliveira CC , Gotardo EM, Pedrazzoli Jr., Gambero A, Ferraz LFC, Carvalho P, Ribeiro ML** (2011). Anti-inflammatory effects of yerba maté extract (*Ilex paraguariensis*) ameliorate insulin resistance in mice with high fat diet-induced obesity. Mol Cell Endocrinol 335. 110–115

- **Ashihara H, Kubota H** (1986) Patterns of adenine metabolism and caffeine biosynthesis in different parts of tea seedlings. Physiol Plant 68:275–28

- **Ashihara H, Gillies FM, Crozier A** (1997). Metabolism of caffeine and related purine alkaloids in leaves of tea (Camellia sinensis L.). Plant Cell Physiol 38:413–419

- **Ashihara H and T Suzuki** (2004). Distribution and biosynthesis of caffeine in plants. Front Biosci 9, 1864-1876.

- **Ashihara H, Kato M, Crozier A** (2011). "Distribution, Biosynthesis and Catabolism of Methylxanthines in Plants" in B.B. Fredholm (ed.), Methylxanthines, Handbook of Experimental Pharmacology. DOI 10.1007/978-3-642-13443-2_2, # Springer-Verlag Berlin Heidelberg 2011.

- **Asolini FC, Tedesco AM, Carpes ST** (2006). Antioxidant and Antibacterial Activities of Phenolic Compounds from Extracts of Plants Used as Tea. Braz J Food Technol, v.9, n.3, p. 209-215.

- **Athayde ML, Schenkel EP, Gossman G, Guillaume D** (1999). Triterpenoid saponins from Leaves of *I. theezans* Martius ex Reiss. Acta Farm Bonaerense 18 *(1): 49-52.*

- **Athayde ML, Schenkel EP, Gnoatto SB, Gosmann G, Giberti GC, Guillaume D** (2001) Triterpenes and Saponins from *Ilex* argentina Leaves. Acta Farm Bonaerense, 20(*1):* 13-6.

- **Athayde ML, Coelho GC, Schenkel EP** (2000). Caffeine and theobromine in epicuticular wax of *Ilex paraguariensis* A. St.-Hil. Phytochemistry, 55, 7, Pages 853-85.

- **Badin P, Deulofeu V, Galmarini OL** (1962). Chlorogenic and chlorogenic-like acids in Mat6 *(Ilex paraguariensis* St H1.). Chem Ind-London, 257-8.

- **Baltassat F, Darbour N, Ferry S** (1984). Study On The Purine Content Of Caffeine Containing Drugs. I. Mate: *Ilex paraguariensis* Lamb. Plant Med Phytother, 18 4: 195-203

- **Bastos DHM, Fornari AC, De Queiroz YS, Soares RAM, Torres EAFS** (2005). The Chlorogenic Acid and Caffeine Content of Yerba Maté *(Ilex paraguariensis)* Beverages. *Acta Farm. Bonaerense* 24 (1): 91-5.

- **Bastos DHM, de Oliveira DM , Matsumoto LTR, de Oliveira Carvalho P, Lima Ribeiro M** (2007). *Yerba maté*: Pharmacological Properties, Research and Biotechnology. In "Medicinal and Aromatic Plant Science and Biotechnology". ©2007 Global Science Books

- **Bonpland Amadeo** (1897) " Notas sobre la utilidad de trabajar los yerbales" Revista Farmacéutica T:V: Buenos Aires, Argentina.

- **Bjelakovic G, Nikolova D, Gluud LI, Simonetti RG, Gluud C** (2007). Mortality in randomized trials of antioxidant supplements for primary and secondary prevention. JAMA 28, 842–857.

- **Borille AMW, Reissmann CB, Freitas RJS** (2005). Relação entre compostos fitoquímicos e o nitrogênio em morfotipos de erva-mate (*Ilex paraguariensis* St. Hil.). Boletim CEPPA, v. 23, n. 1, p. 183-198.

- **Boots AW, Li H, Schins RPF, Duffin R, Heemskerk JWM, Bast A, Haenen GRMM** (2007). The quercetin paradox. Toxicology and Applied Pharmacology 222, 89–96.

- **Bracesco N, Sanchez AG, Contreras V, Menini T, Gugliucci A** (2011). Recent advances on *Ilex paraguariensis* research: Minireview. J Ethnopharmacol. 136(3): 378-84.

- **Bravo L, Goya L, Lecumberri E** (2007). LC/MS characterization of phenolic constituents of mate (*Ilex paraguariensis*, St. Hil.) and its antioxidant activity compared to commonly consumed beverages. Food Res Int, 40,393–405.

- **Cardozo Jr. EL.' Ferrarese-Filho O, Cardozo Filho L, Lucio Ferrarese, ML, Donaduzzi CM, Sturion JA** (2007). Methyl-xanthines and phenolic compounds in mate (*Ilex paraguariensis* St. Hil.) progenies grown in Brazil J Food Compos Anal, 20, 7, 553-558

- **Carini M, Maffei Facino R, Aldini G, Calloni M, Colombo L** (1998). Characterization of Phenolic Antioxidants from Maté (*Ilex Paraguayensis)* by Liquid Chromatography/Mass Spectrometry and Liquid Chromatography / Tandem Mass Spectrometry. Rapid Commun Mass Spectrom, 12, 1813–1819.

- **Castelletto R, Castellsague X, Muñoz N, Iscovich J, Chopita N, Jmelnitsky A** (1994). Alcohol, tobacco, diet, mate drinking, and esophageal cancer in Argentina. Cancer Epidemiol Biom Prev, 1994; 3:557–64.

- **Chandra S, Mejía EG** (2004). Polyphenolic Compounds, Antioxidant Capacity, and Quinone Reductase Activity of an Aqueous Extract of Ardisia compressa in Comparison to Mate (Ilex paraguariensis) and Green (Camellia sinensis) Teas. J Agr Food Chem, 52 (11), pp 3583–3589

- **Choi YH, Sertic S, Kim HY, Wilson EG, Michopoulos F, Lefeber AWM, Erkelens C, Prat Kricun SD, Verpoorte R** (2005). Classification of *Ilex* Species Based on Metabolomic Fingerprinting Using Nuclear Magnetic Resonance and Multivariate Data Analysis. J Agr Food Chem, 53 (4), 1237-1245

- **Clifford MN, Ramirez-Martinez** JR (1990). Chlorogenic acids and purine alkaloids contents of maté (Ilex paraguariensis) leaf and beverage. Food Chem, 35, 13-21

- **Constantin MB** (1997). Analysis Of The Saponins From *Ilex integerrima* Reissek And Comparison With *Ilex theezans* Martius. Caderno De Farmácia, v. 13, n. 2, p. 182 – 182. Faculdade de Farmácia da Universidade Federal do Rio Grande do Sul (UFRGS).

- **Coelho GC, Athayde ML, Schenkel EP** (2001). Methyxanthines of *Ilex paraguariensis* A. St.-Hil. *var. vestita* Loes. and *var. paraguariensis*. Revista Brasileira de Ciências Farmacéuticas / Brazilian Journal of Pharm Sci, 37,2.

- **Coelho GC, Gnoatto SB, Bassani V, Schenkel EP** (2010). Quantification of Saponins in Extractive Solution of Mate Leaves (*Ilex paraguariensis* A. St. Hil.). J Med Food, 13(2): 439-443. doi:10.1089/jmf.2009.0046

- **Cogo LL, Bastos Monteiro CL, Miguel MD, Gomes Miguel O, Machado Cunico M, Lima Ribeiro M, Ramalho de Camargo E, Botão Kussen GM, da Silva Nogueira K, Dalla Costa LM** (2010). Anti-*Helicobacter pylori* activity of plant extracts traditionally used for the treatment of gastrointestinal disorders. Braz J Microbiol, 41, 2.

- **Cos P, Vlietinck AJ, Vanden Berghe D, Maes L** (2006). Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. J Ethnophar, 106 -290–302

- **De Biasi B, Grazziotin NA, Hofmann Jr AE**. (2009). Antimicrobial activity of extracts of the leaves and branches of *Ilex paraguariensis* (erva-mate). Rev Bras Farmacogn,19 ,2b .

- **De Morais EC, Stefanuto A, Klein GA, Boaventura BCB, Andrade F, Wazlawik E, Di Pietro PF, Maraschin M, da Silva EL** (2009). Consumption of yerba mate (*Ilex paraguariensis*) improves serum lipid parameters in healthy dyslipidemic subjects and provides an additional LDL-cholesterol reduction in individuals on statin therapy. J Agric Food Chem, 57, 8316–8324.

- **da Silva EL, Neiva TJC , Shirai M, Terao J,Abdalla DSP** (2008). Acute ingestion of yerba mate infusion (*Ilex paraguariensis*) inhibits plasma and lipoprotein oxidation. Food Res Int, 41 , 973–979

- **Descartes R, de Garcia P** (1956). Alkaloids in Brazilian mate. Boletin de Instituto National de Tecnologia *7 (15), 3-7.*

- **Descartes R, de Garcia P** (1958). The alkaloids of mate. Revista Quimica Znd. (Rio de Janeiro*) 27 (318), 21-22.*

- **Descartes R, de Garcia P** (1962). Alkaloids of mate. Revista Brasileira de Quimica (Sao Paulo) *54,492-494.*

- **De Stefani E, Correa P, Oreggia E, Leiva J, Rivero S, Femandez G, Deneo-Pellegrini H, Zavala D, Fontham E** (1987). Risk factors for laryngeal cancer. Cancer, 60, 3087-3091

- **De Stefani E, Correa P, Oreggia E, Deneo-Pellegrini H, Femandez G, Zavala D,Carzoglio J, Leiva J, Fontham E, Rivero S** (1988). Black tobacco, wine and mate in

oropharygeal cancer. A case-control study from Uruguay. Rev Epidémiol. Santé publ., 36, 389-394

- **De Stefani E, Muñoz N, Estéve J, Vassallo A, Victora CG, Teuchmann S** (1990). Mate drinking, alcohol, tobacc, diet, and esophageal cancer in Uruguay. Cancer Res, 50,426-431

- **Deulofeu V, Diaz H, Fondovilla ME, Mendive JR** (1943). The so-called tannin of yerba mate. Anales de la Asociacion Quimica Argentina 31, 99-108.

- **Deulofeu V, Diaz H, Fondovilla ME, Mendive JR** (1944). The so-called tannin of yerba mate. Boletin del Instituto de Bacteriologia "Carlos G. Malbran" 12, 205-214.

- **Dugo P, Cacciola F, Donatoa P, Assis Jacques R, Bastos Caramão E, Mondello** L(2009). High efficiency liquid chromatography techniques coupled to mass spectrometry for the characterization of mate extracts. J Chromatogr A, 1216 - 7213–7221

- **Filip R, Lopez P, Coussio J, Ferraro G** (1988). Mate substitutes or adulterants: study of xanthine content. Phytother Res, 12: 129–131.

- **Filip R, Lotito SB, Ferraro G, Fraga CG** (2000). Antioxidant activity of *Ilex paraguariensis* and related species. Nutr Res, 20, 10, 1437-1446.

- **Filip R, Lopez P, Giberti G, Coussio JD, Ferraro G** (2001). Phenolic compounds in seven South American *Ilex* species. Fitoterapia, Volume 72, Issue 7, Pages 774-778.

- **Gnoatto SCB, Schenkel EP, Bassani VL** (2005). HPLC Method to Assay Total Saponins in *Ilex paraguariensis* Aqueous Extract. J Braz Chem Soc, Vol. 16, No. 4, 723-726.

- **Gonçalves AL, Filho AA, Menezes H** (2005). Estudo comparativo da atividade antimicrobiana de extratos de algumas árvores nativas. Arq Inst Biol, 3: 353-358

- **Gonthier MP, Verny MA, Besson C, Rémésy C, Scalbert A** (2003). Chlorogenic Acid Bioavailability Largely Depends on Its Metabolism by the Gut Microflora in Rats. J Nutr, 133:1853-1859.

- **Gosmann G, Schenkel EP, Seligman OP** (1989). A New Saponin From Mate, *Ilex paraguariensis*. J Nat Prod, 52- 6: 1367-1370

- **Gosmann G, Guillaume D, Taketa AT, Schenkel EP** (1995). Triterpenoid saponins from *I. paraguariensis*. J Nat Prod, 58 (3), pp 438–441

- **Gutteridge JMC, Halliwell B** (2010). Antioxidants: Molecules, medicines, and myths. Biochem Biophys Res Co, 393 (2010) 561–564

- **Halliwell B** (2011). Free radicals and antioxidants –*quo vadis?* Trends Pharmacol Sci, 32, 3,125-130

- **Hamada S, Slade HD** (1980). Biology, immunology and cariogenicity of *Streptococcus mutans*. Microbiol Rev, 44: 331-384

- **Heck CI, Mejía EG** (2007). Yerba Mate Tea (*Ilex paraguariensis*): A Comprehensive Review on Chemistry, HealthImplications, and Technological Considerations. J Food Sci, Vol. 72, Nr. 9, 2007

- **Heck CI, Schmalko M, Mejia EG** (2008). Effect of Growing and Drying Conditions on the Phenolic Composition of Mate Teas *(Ilex paraguariensis).* J Agric Food Chem, *56 (18), pp 8394–8403*

- **Heinrichs R, Malavolta E** (2001). Mineral Composition Of A Commercial Product From Mate-Herb (*Ilex paraguariensis* St. Hil.). Ciência Rural, Santa Maria, v.31, n.5, p.781-785.

- **Heinzmann E , Schenkel EP**(1995). Saponins from *Ilex dumosa*. J Nat Prod ,Vol.58, 9,pp. 1419-1422.

- **Hauschild W**(1935). Untersuchung iiber die Bestandteile des Mate. Mitteilungen Lebensmittel Hygien Bern, 26, 329-59.

- **Hollman PCH** (2010). Lecture at the conference "Antioxidants 2010"- Brussels, Belgium. Available at: http://www.nutraingredients.com/Research/Veteran-researcher-Polyphenols-don-t-work-as-antioxidants-in-vivo-but

- **Hollman PCH, Cassidy A, Comte B, Heinonen M, Richelle M, Richling E, Serafini M, Scalbert A, Sies H, Vidry S** (2011). The Biological Relevance of Direct Antioxidant Effects of Polyphenols for Cardiovascular Health in Humans Is Not Established. J Nutr, 141: 5 989S-1009S.

- **Ito E, Crozier A, Ashihara H** (1997). Theophylline metabolism in higher plants. Biochim Biophys Acta, 1336:323–330

- **Kamangar F, Scantz MM, Abnet CC, Fagundes RB, Dawsey SM** (2008). High levels of carcinogenic polycyclic aromatic hydrocarbons in mate drinks. Cancer Epidemiol Biomarkers Prev; 17:1262–8

- **Kraemer, KH, Taketa ATC, Schenkel EP, Gosmann G, Guillaume D** (1996). Matesaponin 5, a highly polar saponin from *Ilex paraguariensis*. Phytochemistry, 42, 1119-1122.

- **Kusters JG, Van Vliet AH, Kuipers EJ** (2006). Pathogenesis of *Helicobacter pylori* infection. Clin Microbiol Rev, 19 (3), 449-490

- **Kim HK, Saifullah. Saifullah Khan, Wilson EG, Prat Kricun SD, Meissner AM, Goraler S, Deelder A, Choi YH, Verpoorte R** (2010). Metabolic classification of South American *Ilex* species by NMR-based metabolomics. Phytochemistry, 71, 773–784.

- **Kraemer KH, Taketa AT, Schenkel EP, Gosmann G, Guillaume D** (1996) Matesaponin 5, a highly polar saponin from *Ilex paraguariensis* . Phytochemistry, 42(4): 1119-22

- **Kubo I, Muroi H, Masaki H** (1993). Antibacterial activity against *Streptococcus mutans* of mate tea flavor components. J Agr Food Chem, 41: 107-111.

- **Long LH, Long Hoi A, Halliwell B** (2010). Instability of, and generation of hydrogen peroxide by, phenolic compounds in cell culture media. Arch. Biochem. Biophys. 501, 162–169

- **Loria D, Barrios E, Zanetti R** (2009) Cancer and *yerba mate* consumption: a review of possible associations. Rev Panam Salud Publica, 25(6):530–9.

- **Lozano PR, Cadwallader KR, Gonzalez de Mejia E** (2007). Identification of characteristic aroma components of Mate (*Ilex paraguariensis*) Tea. In: Tunick MH, de Mejia EG, editors. Hispanic foods: chemistry and flavor. Washington, D.C.: Amer Chem Soc. p 143–50.

- **Lunceford N, Gugliucci A** (2005). *Ilex paraguariensis* extracts inhibit AGE formation more efficiently than green tea. Fitoterapia, 76, 419-427.

- **Martinet A, Hostettmann K, Schutz Y** (1999). Thermogenic effects of commercially available plant preparations aimed at treating human obesity. Phytomedicine, 6(4): 231-8.

- **Martinet A, Ndjoko K, Terreaux C, Marston A, Hostettmann K, Schutz** Y (2001). NMR and LC-MSn characterisation of two minor saponins from *Ilex paraguariensis*. Phytochem Anal, 12(1):48-52.

- **Martins F, Noso TM, Porto VB, Curiel A, Gambero A, Bastos DHM, Ribeiro ML, Carvalho PO** (2009). Maté Tea Inhibits *In Vitro* Pancreatic Lipase activity and Has Hypolipidemic Effect on High-fat Diet-induced Obese Mice. Obesity 18, 42–47.

- **Mazzafera P** (1994). Caffeine, Theobromine And Theophylline Distribution In *Ilex* paraguaryensis. R Bras Fisiol Veg, 6(2): 149-151,

- **Mazzafera P** (1996). Maté drinking. Studies on caffeine and phenolic intake. Food Chem, 60, 1, 67-71.

- **Mazzafera P** (2004). Catabolism of caffeine in plants and microorganisms. Front Biosc 9, 1348-1359.

- **Matsumoto RLT, Bastos DHM, Mendonça S, Nunes VS, Bartchewsky Jr W, Ribeiro ML, Oliveira Carvalho P** (2009). Effects of Maté Tea (*Ilex paraguariensis*) Ingestion on mRNA Expression of Antioxidant Enzymes, Lipid Peroxidation, and Total Antioxidant Status in Healthy Young Women. J Agric Food Chem, *57* (5), 1775-1780

- **Melo SS, Nunes NSI, Baumgarten C, Tressoldi C, Faccin G, Zanuzo K, Michels MK, Cunha N, Specht S, Silva MW** (2007). Effect of mate tea (*Ilex paraguariensis* A. St. Hil.) in rats fed on hyperlipidic diets. Alim. Nutr., Araraquara, p. 439-447.

- **Nagata T, Sakai S**(1985). Purine Base Pattern Of Camellia Irrawadiensis. Phytochemistry, 24 10: 2271-2272.

- **Nishimura K, Miyase T, Nogushi H** (1999). Triterpenoid saponins from *Ilex* kudincha. J Nat Prod, 62, 1128.

- **Müller V, Chávez JH, Reginatto FH, Zucolotto SM, Niero R, Navarro D, Yunes RA, Schenkel EP, Barardi CRM, Zanetti CR Simões CMO** (2007). Evaluation of antiviral activity of South American plant extracts against herpes simplex virus type 1 and rabies virus. Phytother Res, 21: 970–974. doi: 10.1002/ptr.2198

- **Olthof MR, Hollman PCH, Katan MB** (2001). Chlorogenic acid and caffeic acid are absorbed in humans. J Nutr, 131: 66–71.

- **Olthof MR, Hollman PCH, Buijsman MNCP, van Amelsvoort JMM and Katan MB** (2003). Chlorogenic Acid, Quercetin-3-Rutinoside and Black Tea Phenols Are Extensively Metabolized in Humans. J Nutr, 133:1806-14

- **Pang J, Choi Y, Park T** (2008). *Ilex paraguariensis* extract ameliorates obesity induced by high-fat diet: Potential role of AMPK in the visceral adipose tissue. Arch Biochem Biophys, 476 (2008) 178–185

- **Paganini Stein FL, Schmidt B, Furlong E B, Souza Soares LA, Flores Soares MC, Cezar Vaz MR, Muccillo Baisch AL** (2005). Vascular Responses to Extractable Fractions of *Ilex paraguariensis* in Rats Fed Standard and High-Cholesterol Diets. Biol Res Nurs, 7: 146-156

- **Pezzuto de Andrade FD, Piacente S, Pizza C, Vilegas W** (2002). Studies on the Constituents of a Brazilian Folk Infusion. Isolation and Structure Elucidation of New Triterpene Saponins from *Ilex amara* Leaves. J Agric Food Chem, 50 (2), pp 255–261.

- **Pires V, Guillaume D, Gosmann G, Schenkel EP** (1997). Saponins from *Ilex* dumosa, an Erva Maté (*Ilex paraguariensis*) Adulterating Plant. J Agric Food Chem, 45, 1027–1031

- **Pomilio A, Trajtemberg S, Vitale A** (2002). High-Performance Capillary Electrophoresis Analysis of mate infusions prepared from stems and leaves of *Ilex paraguariensis* using Automated Micellar Electrokinetic Capillary Chromatography. Phytochem Anal, 13, 235–241.

- **Przygodda F, Martins ZN, Castaldelli APA, Minella TV, Vieira LP, Cantelli K, Fronza J, Padoin MJ** (2010). Effect of erva-mate (*Ilex paraguariensis* A. St.-Hil., Aquifoliaceae) on serum cholesterol, triacylglycerides and glucose in Wistar rats fed a diet supplemented with fat and sugar. Braz J Pharmacogn, 20(6): 956-961.

- **Puangpraphant S, Mejia EG** (2009). Saponins in Yerba Mate Tea (*Ilex paraguariensis* A. St.-Hil) and Quercetin Synergistically Inhibit iNOS and COX-2 in Lipopolysaccharide-Induced Macrophages through NFKB Pathways.J Agric Food Chem, 2009, 57, 8873–8883

- **Puangpraphant S,. Berhow MA, Mejia EG** (2011). Mate (*Ilex paraguariensis* St. Hilaire) saponins induce caspase-3-dependent apoptosis in human colon cancer cells in vitro. Food Chem, 125,1171–117.

- **Ranilla GL, Kwon YI, Apostolidis E, Shetty K** (2010). Phenolic compounds, antioxidant activity and in vitro inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. Bioresource Technol, 101, 4676–4689

- **Reginatto FH, Athayde ML, Gosmann G, Schenkel EP** (1999). Methylxanthines Accumulation in *Ilex* Species - Caffeine and Theobromine in Erva-Mate (*Ilex paraguariensis*) and Other *Ilex* Species. J Braz Chem Soc, Vol. 10, No. 6, 443-446.

- **Roffo AH** (1941) Cáncer producido por el alquitrán del mate. Bol Inst Med Expt Cancer. 18(56): 5–20

- **Rostagno MA, Manchóna N, D'Arrigoa M, Guillamóna E, Villaresa A, García-Lafuentea A, Ramosa A, Martínez JA** (2011). Fast and simultaneous determination of phenolic compounds and caffeine in teas, mate, instant coffee, soft drink and energetic drink by high-performance liquid chromatography using a fused-core column. Anal Chim Acta 685, 204–211.

- **Ruschenburg U** (1985). Benzo[α]pyrene content of coffee and some other foodstuffs. In: 11e Colloque Scientifique International sur le Café. Paris: Association Internationale du Café. Pp. 205–212.

- **Sari F, Turkmen N, Polat G, Sedat Velioglu Y** (2007). Total Polyphenol, Antioxidant and Antibacterial Activities of Black Mate Tea. Food Sci Technol Res, (3), 265-269.

- **Schenkel P, Gosmann G, Montanha Jarbas A, Heizmann M, Athayde L, Taketa ATC, Pires VS, Guillaume D** (1997). Saponins from maté (*Ilex paraguariensis*) and other South American *Ilex* species: ten years research on Ilex saponins. Ciènc. cult. (Sao Paulo); 49 (5/6): 359-63.

- **Schenkel EP, Athayde MI, Giberti G, Guillaume D** (1995). A New Saponin From *Ilex* Argentina. Acta Farm. Bonaerense 14(1): 5-10.

- **Souza MFF** (2009). Chá-mate (*Ilex paraguariensis*): compostos bioativos e relação com atividade antioxidante. Dissertação (Mestrado em Nutrição em Saúde Pública) - Faculdade de Saúde Pública -USP, Fundação de Amparo À Pesquisa do Estado de São Paulo.

- **Strassmann BB, Vieira AR, Pedrotti EL, Morais HN, Dias PF** (2008). Quantitation of methylxanthinic alkaloids and phenolic compounds in mate (*Ilex paraguariensis*) and their effects on blood vessel formation in chick embryos. J Agric Food Chem, 56(18):8348-53.

- **Sugimoto S, Nakamura S, Yamamoto S, Yamashita C, Oda Y, Matsuda H, Yoshikawa M** (2009). Brazilian Natural Medicines. III.1) Structures of Triterpene Oligoglycosides and Lipase Inhibitors from Mate, Leaves of *Ilex paraguariensis*. Chem Pharm Bull, 57(3), 257-261.

- **Suzuki T, Waller GR** (1984). Biosynthesis and biodegradation of theobromine and theophylline in *Coffea arabica* fruits. J Agr Food Chem, 32, 845-848.

- **Suzuki T and Waller GR** (1984). Biodegradation of caffeine: Formation of theophylline and theobromine from caffeine in mature *Coffea Arabica* fruits. J Sci Food Agric, 35, 66-70

- **Taketa ATC, Schenkel EP** (1995). Saponins from *Ilex pseudobuxus*. Acta Farm. Bonaerense 13 (3): 159-64.

- **Taketa ATC, Schmittmann-Schlager T, Guillaume D, Gosmann G, Schenkel EP** (2000). Triterpenoid glycosides and a triterpene from *Ilex* brevicuspis. Phytochemistry, 53(8): 901-4.

- **Taketa ATC, Gondorf M, Breitmaier E, Schenkel EP** (2002). New triterpene and triterpenoid glycosides from *Ilex* brevicuspis. *Rev Bras Cienc Farm* [online]., vol.38, n.2 [cited 2011-03-09], pp. 155-161 . Available from: <http://www.scielo.br/scielo.php?

- **Taketa ATL, Gnoatto SCB, Gosmann G, Pires VS, P. Schenkel EP, Guillaume D** (2004). Triterpenoids from Brazilian *Ilex* Species and Their in Vitro Antitrypanosomal Activity. J Nat Prod, *67*, 1697-1700

- **Tenorio Sanz MD, Torija Isasa, ME** (1991). Elementos minerales en la yerba mate (*Ilex paraguariensis* St. H.) / Mineral elements in of mate (*Ilex paraguariensis* St. H.). Arch Latinoam Nutr; 41(3): 441-54

- **Vassallo A, Correa P, De Stefani E, Cendán M, Zavala D, Chen V, Carzoglio J, Ceneo-Pellegrini H** (1985). Esophageal cancer in Uruguay: a case-control study. J Natl Cancer Inst, 75, 1005-1

- **Victora CG, Muñoz N, Day NE, Barcelos LB, Peccin DA, Braga NM** (1987). Hot beverages and oesophageal cancer in southern Brazil: a case-control study. Int J Cancer, 39, 710-716

- **Vazquez A, Moyna P** (1986). Studies On Mate Drinking. J Ethnopharmacol, 18 3: 267-272

- **Weevers T** (1930). The function of Xanthine Derivatives in Plant Materials, Archives Neerlandaises, Series III, 5, 111- 195

- **Wilson EG, Rondina RVD, Coussio JD** (1981). Valoración de las xantinas presentes en el mate cebado al estilo rioplatense. Rev. Farmacéutica 124, 5-8, 41-56.

- **Woodard WA, Cowland AN** (1935). The question of tannin in mate. Analyst 60, 135-146.

- **Wróbel K, Wróbel K, Colunga Urbina EM** (2000). Determination of total aluminum, chromium, copper, iron, manganese, and nickel and their fractions leached to the infusions of black tea, green tea, *Hibiscus sabdariffa*, and *Ilex paraguariensis* (mate) by ETA-AAS. Biol Trace Elem Res, 78:271–80

- **Xu GH, Kim YH, Choo SJ, Ryoo IJ, Yoo JK, Ahn JS, Yoo ID** (2009). Chemical Constituents from the Leaves of *Ilex paraguariensis* Inhibit Human Neutrophil Elastase. Arch Pharm Res, Vol 32, No 9, 1215-1220, 2

- **Xu GH, Kim YH, Choo SJ, Ryoo IJ, Yoo JK, Ahn JS, Yoo ID** (2010). Two acetylated megastigmane glycosides from the leaves of *Ilex paraguariensis*. Arch Pharm Res. 33(3): 369-73.

- **Yun YW** (2010). Possible anti-obesity therapeutics from nature – A review. Phytochemistry 71, 1625–1641.

- **Zuin VG, Montero L, Bauer C, Popp P** (2005). Stir bar sorptive extraction and high-performance liquid chromatography–fluorescence detection for the determination of polycyclic aromatic hydrocarbons in Mate teas. J Chromatogr;1091:2–10.