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Urinary excretion of ginkgolide terpene lactones following acute consumption of *Ginkgo biloba* extract

Running Title: Dose dependent urinary excretion of ginkgolides

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Abstract

Urinary biomarkers of plant food supplement (PFS) exposure/ intake represent an accurate, non-subjective tool for determining PFS consumption in humans with applications ranging from epidemiology to subject compliance in clinical trials. *Ginkgo biloba* remains one of the worlds most popular PFS, yet few studies have investigated the uptake and metabolism of its primary unique bioactives: the terpene lactones. To this end, we conducted a dual-dose, acute crossover intervention using *Ginkgo biloba* supplements in healthy participants (n=12). Pooled 24-hr urine samples were analysed by triple quadrupole LC-MS-MS. We observed that bilobalide, and ginkgolides A and B were passed into urine intact and in a dose-dependent manner. Low levels of intact ginkgolides C and J were also excreted. To our knowledge, this is the first study to report intact ginkgolide J in urine following oral consumption of ginkgo supplements, and is also the first to account for excreted terpene lactones as a proportion of dose.

Introduction

Ginkgo biloba (ginkgo, also referred to as Maidenhair tree) has a long history of phyto-medicinal use, and is of high commercial significance to the worldwide plant food supplement (PFS) market. It has an association with Chinese herbal medicine dating back 5000 years, with its use as a medical treatment for senility being first recorded over 500 years ago (1). More recent evidence links ginkgo principals to several promising properties, including anti-platelet activity (2), improved nitric oxide availability (3), wound healing (4), anti-asthmatic activity (5) and neuroprotection (6). However the evidence from randomized controlled

human interventions is less clear, and recent systematic reviews and meta-analyses of Ginkgo efficacy in the areas of cognition in dementia (e.g. Weinmann *et al.* (7); vs. Birks & Evans (8)) and tinnitus management (e.g. von Boetticher (9); vs. Hilton *et al.* (10)) are contradictory in their conclusions. Deficiencies in intervention design are a major driver of this controversy. Nevertheless, Ginkgo remains one of the worlds most popular PFS, with combined sales from France, Germany, UK, US, Japan and China worth approximately half a billion dollars (US) in 2011 alone (11).

The accurate determination of individual PFS intake is vital for related efficacy research. Urinary biomarkers of PFS exposure/ intake can be used to rapidly (and often retrospectively) gauge the intake of large cohorts, assess subject compliance in clinical trials, and quantify the bioavailability of active principals from different PFS preparations. This typically involves collecting urine, either over a 24-hour period or “spot” urine as a single sample, and measuring one or more representative metabolites in the sample using appropriate methodology. However, the identification of specific biomarkers of intake can be complicated by wide inter-individual variation in the uptake and metabolism of ingested compounds from the PFS of interest, and by the occurrence of the same compounds in other foods, beverages and supplements.

High quality Ginkgo PFS products are commonly based on standardised leaf extracts, such as EGb 761 (Dr. Wilmar Schwabe GmbH & Co.) or LI 1370 (Lichtwer Pharma AG). The German Commission E monograph on Ginkgo requires that such extracts contain less than 5ppm ginkolic acids, a flavonol

glycoside content of 22-27% and a terpene lactone content of 5-7%. Capillary LC-ITMS fingerprint analysis of commercial Ginkgo extracts showed that of 72 tentatively identified flavonoids, 42 were glycosides of the flavonols quercetin, isorhamnetin and kampferol (12). Following hydrolysis to the aglycone forms, these 3 compounds can easily be quantified by HPLC-UV, however, routine use of flavonol aglycone content as a raw material quality indicator may facilitate adulteration (13). The terpene lactones, including the Ginkgolides (A,B, C and J, see figure 1) and Bilobalide (figure 2), can be observed at 220 nm, however response and selectivity is too poor for HPLC analysis (14). Whilst a wide range of improved analytical techniques for Ginkgo terpenes are now available (for review see (15)), LC-MS has become a well-utilised technique for analysis in biological matrices. Several authors have reported on the pharmacokinetics of terpene lactones in plasma following Ginkgo consumption (16-18) however to date the majority of studies considering urinary derivatives have focused on the flavonols (19) and their common downstream metabolites (20).

Quercetin, isorhamnetin and kampferol are not unique to *Ginkgo biloba*, being widely found in foods such as onions, red wine, tomatoes and tea. As such, the specificity of these flavonols would be insufficient to quantitatively determine ginkgo intake within participants consuming an open diet. Therefore, we investigated the utility of terpene lactones as urinary biomarkers of ginkgo intake via an acute randomized, 2-dose crossover intervention study.

Experimental Procedures

Reagents and Reference Materials

All solvents (methanol, ethanol, ethyl acetate) were of HPLC grade or higher, obtained from VWR. LC-MS grade water was produced using a combination Elix/MilliQ purification system (Millipore, UK). Sodium azide (>99%) and EDTA (>98.5%) were obtained from Sigma (UK). Reagent grade ascorbic acid was obtained from Extrasynthese (France)

All reference standards were of HPLC grade (>95%). Taxifolin was obtained from Extrasynthese (France). Ethyl gallate was obtained from Apin Chemicals (UK). Reference standards for bilobalide and ginkgolides A, B, C and J were kindly provided by Phytolab (Germany).

Participants

This open oral intervention was carried out within the School of Food Science and Nutrition, University of Leeds, UK. Approval was granted by the University ethics committee (MEEC 09-019), and the study adhered to Declaration of Helsinki principles. Volunteers were required to sign a consent form, and complete a medical questionnaire. A total of 12 healthy volunteers were recruited, aged 23-26 years, comprising 5 males and 7 females, with an average BMI of 23.5 ± 7 Kg/m². Exclusion criteria included i) any significant medical complaint, ii) history of surgery of the gastrointestinal tract, iii) any diseases that might influence the rate/degree of PFS uptake, iv) drug allergies pertinent to the PFS of interest, v) routine use of medication, traditional herbal medicine or other plant-based supplements.

Study Design

The intervention was a dual dose, crossover study, in which participants were randomly allocated to consume either 1 or 2 x 500 mg tablets at a single sitting (in line with manufacturers dosage instructions). Each intervention stage required participants keep to a polyphenol-free diet for 48 hrs, avoiding tea, coffee, alcohol, fruit and vegetable derived foods, chocolate, and high bran cereal derived foods such as brown bread, whole grain breakfast cereals and brown rice. At the end of the first 24 hrs, a urine sample was collected as a compliance measure. The intervention dose was then consumed, and urine was collected as a 24hr pool. After completion of the 24 hr pool, participants were free to cross over to the next intervention stage.

Urine was collected in clean, dry, 3 L polypropylene vessels (Urisafe, obtained from VWR, UK), each containing a 3 g aliquot of ascorbic acid powder to act as a preservative. Filled vessels were kept cool and dark, and collected the day after use. The weight and volume of urine was noted, and following homogenisation, 3 x 10 ml aliquots were placed in 15 ml centrifuge tubes (BD Biosciences, UK). A 100 µl aliquot of sodium azide solution (10% w/v) was added to each tube as an anti-bacterial agent. The tubes were vortexed (10 sec) then stored at -20°C until extraction.

Analysis of Ginkgo Supplements

Supplements were from Boots UK Ltd., branded “Ginkgo Biloba Brain Health” tablets. Each disk shaped compressed 500 mg tablet was stated to contain 120 mg of ginkgo biloba extract, standardised to provide 28.8 mg of flavonol

glycosides and 7.2 mg of terpene lactones. Tablets were obtained from a local pharmacy, and 2 distinct batches were utilized during the human intervention, designated batch 1 (#141015), and 2 (#141941). From each batch, 2 tablets were weighed and then individually crushed using a pestle and mortar. From each powdered tablet an aliquot of approximately 10 mg was accurately weighed into a 1.5 ml centrifuge tube (Eppendorf, UK). To each aliquot 1 ml of extraction solvent was added, comprising aqueous ethanol (50% v/v), ascorbic acid (0.1% w/v) and taxifolin as internal standard (10 µg/ml). The mixture was vortexed (30 sec), placed in an ultrasonic water bath (30 min) then vortexed again to extract soluble materials. The tubes were then centrifuged at 17,000 R.C.F. (10 min; Thermo Scientific, UK) and the supernatant removed. A second 1 ml aliquot of extraction solvent was added to the pellet, and the process extraction repeated. A 1 in 10 dilution of the 1st extract was made using extraction solvent, and all 3 samples (per tablet) were analysed via LC-MS. Typically 96.6% of target analytes were present in the first extract.

Analysis was conducted on a Shimadzu LC-MS system comprising a Nexera UHPLC (with binary pump, autosampler set to 8°C, and column oven set to 35°C) and an LC-2020 single quadrupole mass spectrometer. A 5 µl sample aliquot was injected onto a Kinetix C18 XDB 150x2.1 2.6 µm column (Phenomenex UK) fitted with a low volume “Krud catcher” pre-filter. Samples were separated on a binary gradient of aqueous LC-MS grade methanol (5 versus 95% v/v, solvents A and B respectively) plus 0.1% formic acid (v/v) running at a flow rate of 0.3 ml/min. The gradient was held at 8% solvent B for 5 min, then increased linearly to 40% over 15 min, up to 90% in 2 min, was held for a further 2 min, then decreased to

8% over 2 min and was re-equilibrated over 8 min. The first 3 min of each run was diverted to waste. The MS ran with an interface temperature set to 350°C, using nebuliser and drying gas flow rates of 1.5- and 15L/min, respectively. The analysis was performed in negative SIM mode, optimized using reference standards. Bilobalide and taxifolin internal standard were followed on m/z 325.1 and 303.1 respectively. In addition to m/z equivalent to [M-1]⁻, the ginkgolides also formed persistent formate adducts, i.e. [M + 45]⁻, which were also followed. Hence ginkgolides A, B, C and J were quantified / qualified on m/z 453.2/407.2, 423.1/469.1, 439.1/485.1 and 469.1/423.1, respectively. Peak areas were normalized to internal standard, and analytes quantified via a log:log standard curve plot ranging 0.1-10 µg/ml.

Analysis of Participant Urine

Urine samples were defrosted at room temperature immediately before extraction, placed in an ultrasonic water bath (5 min) then vortexed (30 sec) to ensure homogeneity. Each urine sample was extracted in triplicate. Each 200 µL aliquot of urine was combined with 20 µL of an aqueous solution of ascorbic acid (1% w/v) and EDTA (0.1% w/v), 100 µL of ethanol containing 2 µg/ml ethyl gallate (IS1) and 1 ml of ethyl acetate in a 1.5 ml centrifuge tube. The solution was vortexed, placed in an ultrasonic water bath and vortexed again to extract soluble material. The tubes were centrifuged at 17,000 R.C.F. (10 min) and the upper layer removed. A second 1 ml aliquot of ethyl acetate was added to the pellet, and the extraction procedure repeated. All extracts were dried under nitrogen at room temperature and frozen at -20°C. On the day of analysis, the second extracts were reconstituted with 70 µl of ethanol, vortexed, placed in an

ultrasonic water bath and vortexed again. A 50 μ l aliquot of the reconstituted second extract was combined with the corresponding first (dried) extract, which was similarly vortexed and sonicated. A 50 μ l aliquot of ascorbic acid solution (0.2% w/v) containing taxifolin (IS2) at 8 μ g/ml was added to the combined first and second extract, which was then vortexed and centrifuged. The supernatant was placed in an amber 300 μ l HPLC vial, which was capped and kept at 8°C until analysis.

A modified sample preparation method was developed to prepare polar material for pilot analyses, wherein ethyl acetate was substituted for methanol. Methanol was removed from the samples using an EZ2+ centrifugal evaporator (Genevac, UK) using the HPLC drying program for 1 hr. Water was subsequently removed from the samples via freeze drying at -50°C for 24 hrs. Reconstitution occurred as described above.

Analysis was conducted on an Agilent LC-MS-MS system comprising a 1200 SL binary pump, well plate autosampler set to 8°C, and column oven set to 35°C, connected to a 6410a triple quadrupole mass spectrometer. Each 5 μ l sample was separated via the same chromatographic method detailed above. The first 3 min of each run was diverted to waste, and thereafter directed into the MS interface set to 350°C, using nebuliser pressure of 30 psi and drying gas flow rates of 11 L/min. The analysis was performed in negative MRM mode, optimized using reference standards. The following m/z transitions were used for quantification / qualification purposes: ethyl gallate (IS1), 197.1>124.0/169.1; taxifolin (IS2), 303.1>285.0/125.0; bilobalide,

325.1>163.1/251.1; ginkgolide B, 423.2>367.2; ginkgolide C, 439.2>383.2. Ginkgolide A formed a persistent formate adduct and was followed on a transition of 453.2>351.2/407.1. Ginkgolide J did not fragment in the collision cell, and was thus followed on a SIM of m/z 423.2. The standard curve was produced by spiking blank urine (made from a 12 participant homogenate of baseline urine) with reference standards to a final concentration range of 0.01-100 μ M. Each sample was then handled as for the ethyl acetate preparation protocol described above, and each curve point was produced in triplicate. Peak areas were normalized to IS2, and analytes quantified via a log:log standard curve plot. The limit of quantification (LOQ) was below 500 nM for bilobalide and ginkgolide J, below 50 nM for ginkgolide A and below 10 nM for ginkgolides B and C. Total terpene lactones excreted post-supplementation was calculated as μ mol in total volume of 24 hr pooled urine.

The MS parameters of the above method were adapted to enable investigation into possible phase 2 terpene lactone conjugates. MRM parameters matching anticipated compounds can be observed in tables 1 and 2.

Results

Supplement Analysis

On average, each tablet weighed 517.0 ± 5.5 mg. Tablets in batch 1 delivered 7.9 ± 0.1 mg of target ginkgolides and bilobalide, whilst batch 2 tablets delivered 8.0 ± 0.2 mg, equivalent to 20.3 ± 0.3 and 20.7 ± 0.4 μ mol. This marginally exceeded the 7.2 mg of terpene lactones per tablet stated by the manufacture. As can be

seen in figure 3, on a molar basis bilobalide was the single largest contributor to the terpene lactone profile at $7.8 \pm 0.2 \mu\text{mol}$ per capsule, with ginkgolides A B and C making roughly equal contributions. Ginkgolide J was a comparatively minor contributor to the overall terpene lactone profile.

Urinary biomarker analysis

Separation of target analytes in urine can be seen in figure 4. Elevated levels of terpene lactones compared to baseline indicated 100% compliance in all participants, regardless of randomization order. However, it is possible that the minimum 24 hr wash out was slightly too short to ensure complete clearance of all target compounds in all participants, as very low levels of terpene lactones could be observed in the baseline urine of 7 volunteers at the start of the second dose. In the majority of cases this was judged not to influence the outcome of the study: total terpene lactone concentrations at baseline ranged just 0.01-0.3% that of the post-supplement concentrations. However in 3 individuals, baseline terpene lactone concentrations equated 2-6% that of the corresponding post-supplement urine. As such, statistical analysis was performed both including and excluding these 3 individuals.

For the full $n=12$ group, average total terpene lactones excreted were $7.5 \pm 2.6 \mu\text{mol}$ following low dose ginkgo, and $17.1 \pm 3.6 \mu\text{mol}$ following high dose supplementation (see figure 5) indicating a dose-dependent excretion of analytes. Average low versus high dose excretion of individual compounds were as follows: bilobalide, $2.4 \pm 0.8 \mu\text{mol}$ vs $5.3 \pm 2.1 \mu\text{mol}$; ginkgolide J $21.6 \pm 29.3 \text{ nmol}$ vs. $171.7 \pm 85.7 \text{ nmol}$; ginkgolide C, $119.8 \pm 45.7 \text{ nmol}$ vs. $318.2 \pm 124.0 \text{ nmol}$;

ginkgolide A, $3.8 \pm 0.4 \mu\text{mol}$ vs. $8.7 \pm 2.1 \mu\text{mol}$; ginkgolide B, $1.1 \pm 0.4 \mu\text{mol}$ vs. $2.7 \pm 0.7 \mu\text{mol}$. All increases for individual terpene lactone excretion with dose were statistically significant, returning p-values of >0.01 for both paired 2-tail t-tests and single factor anova tests. Removal of data from the 3 flagged washout subjects did not diminish the strength of this statistical significance, except in the case that a paired 2-tail t-test for bilobalide excretion returned $p=0.011$.

In line with a dose-dependent excretion of compounds, the percentage of the initial dose recovered in urine was similar between low and high dosing, with total terpene lactone recoveries of $37 \pm 13 \%$ and $42 \pm 9\%$, respectively. However, there was a marked difference between the recoveries of each individual compound (see figure 6). At high dose, $34 \pm 14\%$ and $36 \pm 10\%$ of bilobalide and ginkgolide B were recovered intact in urine, respectively. However, only $8 \pm 4 \%$ and $4 \pm 2 \%$ of the high dose were recovered for ginkgolides J and C. The calculated recovery of ginkgolide A was $109 \pm 26\%$, ranging from 57% to 149%.

Pilot phase 2 conjugate identification

Following successful identification of intact ginkgolides in urine, a further exploratory investigation was performed to seek classical phase 2 metabolites, namely sulfates, glucuronides and methylated forms. However, MRM experiments, based on anticipated post-collision compound fragmentation, did not identify any potential conjugates produced at a level approaching that of the intact ginkgolides, with no signals observed in non-polar solvent-extracted urine. However, in urine prepared via a polar inclusive method, a small transition return was observed corresponding to a possible methylated bilobalide

(339>163, retention time 20.4 min) with approx 30% relative peak area of bilobalide itself. Whilst methylation would increase non-polarity and thus retention on a C18 bonded phase, the retention of this analyte is almost double that of proposed parent (10.9 min). The identity of this relatively minor compound therefore requires further clarification.

Discussion

The uptake and metabolism of flavonols from plant foods is well established. For example, quercetin is typically the most abundant flavonol in commercial ginkgo extracts (13), but exists as a variety of glycosides of which rutin (quercetin-3-O-rhamnoglucoside) is a major form. Rutin is not significantly absorbed in the small intestine, requiring cleavage via colonic glycosidases before uptake in the large intestine (21). Upon absorption, quercetin can be metabolised both within the epithelial cells and in the liver to form methylated, glucuronidated and sulphated metabolites, which are the forms present in circulating plasma and urine (22). Typically, the proportion of the dose appearing in urine is quite low. For example following consumption of fried onions by volunteers (n=6), the recovery of all quercetin, kaempferol and isorhamnetin conjugates in 24 hr urine equated to 4.7% of the dose (23). Both quercetin and kaempferol have been identified intact in human urine following ginkgo extract consumption (24, 19). Pietta *et al.* (20) also observed a variety of conjugated phenolic acids in urine following ginkgo extract consumption in humans, of which 3,4-dihydroxybenzoic acid represented the most abundant compound present in post-supplement (but not baseline) urine.

Comparatively fewer studies have addressed the uptake of terpene lactones from *Ginkgo biloba* in humans, the majority of which have focussed on the major forms and concentrations found in plasma. For example, in a pharmacokinetic study by Kressman *et al.* (17), healthy male volunteers (n=12) consumed 2 x ginkgo supplement tablets, each containing 60 mg of EGb761 extract that provided 2.0 ± 0.7 , 1.0 ± 0.2 and 5.8 ± 2.8 $\mu\text{mol}/\text{tablet}$ of ginkgolide A, ginkgolide B and bilobalide respectively, the total dose being similar to that of our low dose condition. The average plasma C_{max} for ginkgolide A, ginkgolide B and bilobalide was 54.4 ± 11.2 , 19.5 ± 4.3 and 166.8 ± 41.7 nM respectively, with t_{max} achieved at 1.2, 1.5 and 1.2 hr (16 hr follow up) indicating all 3 compounds are primarily absorbed in the small intestine. This data is consistent with typical plasma C_{max} and t_{max} values achieved following consumption of 3 commercial ginkgo preparations by 24 volunteers (18). Ginkgolide C has been reported as absent in plasma for both humans (16) and guinea pigs (25) following oral administration of ginkgo extract. The oral bioavailability of ginkgolides from extracts is related to the rate of supplement dissolution at low pH (17) and can be improved via complexation with soy phospholipids (16).

To our knowledge, only one other study has investigated the presence of ginkgo terpene lactones in human urine. Following consumption of a single tablet stated to contain 7.2 mg terpene lactones, average concentrations of ginkgolide A, ginkgolide B and bilobalide were 4.1 ± 0.8 , 1.5 ± 0.3 and 4.8 ± 0.7 μM , respectively in urine collected up to 4 hr post-intervention (n=5, \pm SD; (26)). These values are consistent with typical concentrations in our study from 24 hr pooled urine following low dose ginkgo, with concentrations of 5.9 ± 2.1 , 1.7 ± 0.5 and 4.2 ± 1.6

μM ($n=12$, \pm SD) for ginkgolide A, ginkgolide B and bilobalide, respectively. Ding *et al.*, also detected Ginkgolide C in urine, albeit at a comparatively lower average concentration of $0.2\pm 0.05 \mu\text{M}$, which again is consistent with an observed post low-dose concentration of $0.18\pm 0.06 \mu\text{M}$ from our study.

To our knowledge, ours is the first study to report intact ginkgolide J in urine following oral consumption of ginkgo supplements, and is also the first to account for excreted terpene lactones as a proportion of dose. The high levels of ginkgolide A as a percentage of ingested dose in some individuals, combined with a wide inter-individual variability in output is suggestive of additional production of this compound *in vivo*, possibly through a metabolic conversion of other ginkgolides. Ginkgolides A-J are differentiated only by the level of hydroxylation at positions R1 and R2 (see figure 1). It is therefore feasible that that ginkgolides B, C and J could all be converted to ginkgolide A through dehydroxylation *in vivo*, a common step in xenobiotic metabolism. For example, following oral administration of pure chlorogenic acid to rats (27) and humans (28), subsequent increases in 3-hydroxy cinnamic acid (aka *m*-coumaric acid) were observed in urine. The appearance of this molecule follows a cleavage of chlorogenic acid to quinic and caffeic acids and then to 3,4-dihydroxycinnamic acid, which can then undergo dehydroxylation. Whilst this type of metabolic transformation might explain the apparent low recovery of ginkgolides C and J, further mechanistic studies are required to explore this possibility.

Ding *et al.* (26) previously observed that β -glucuronidase and sulfatase enzymes had no influence on apparent levels of urinary terpene lactones, which is in line

with our lack of response for signals relating to classical phase 2 metabolites via MRM and also the high excretion of these compounds relative to dose. Similarly we did not observe methylated ginkgolide C, previously reported as a new species in plasma by Mauri *et al.* (25) after oral ginkgo supplementation in both guinea pigs and human subjects. It is noted, however, that this new species, quantified via SIM on m/z 453, co-eluted exactly with ginkgolide A. In our study and that of Woelkart *et al.* (18), ginkgolide A predominantly formed a formate adduct on LC-MS analysis, which also has $m/z = 453$. Whilst Mauri *et al.* validated the identity of methylated ginkgolide C via its absence in the plasma of animals treated with pure bilobalide and ginkgolide B, critically the same validation test was not performed for pure ginkgolide A. Therefore, we propose that the identity of methylated ginkgolide C is in question.

In conclusion, following the consumption of ginkgo biloba supplements at doses relevant to typical consumer use, it is apparent that bilobalide, and ginkgolides A and B are i) excreted in urine at sufficient levels to be routinely detectable via LC- triple quadrupole analysis and ii) are excreted in a dose-dependent manner. Therefore, these compounds are recommended as biomarkers of *Ginkgo biloba* consumption.

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TABLES AND FIGURES

TABLE 1: MRM parameters of anticipated glucuronide/sulfate terpene lactone conjugates

Compound	MRM Parent ions					MRM Daughter ions
	<i>Mono sulfate</i>	<i>Di-sulfate</i>	<i>Mono glucuronide</i>	<i>Di-glucuronide</i>	<i>Sulfate-glucuronoide</i>	
<i>GA</i>	533.2	613.2	629.2	805.2	709.2	453.2
	487	567	583	759	663	407
<i>GB</i>	503.2	583.2	599.2	775.2	679.2	423.2
<i>GC</i>	51932	599.2	615.2	791.2	695.2	439.2
<i>GJ</i>	503.2	583.2	599.2	775.2	679.2	423.2
<i>BIL</i>	405.1	485.1	501.1	677.1	581.1	325.1

TABLE 2: MRM parameters of anticipated methylated glucuronide/sulfate terpene lactone conjugates

Compound	MRM Parent ions		MRM Daughter ions	MRM Parent ions	MRM Daughter ions
	<i>Methylated mono-sulfate</i>	<i>Methylated mono-glucuronide</i>		<i>Mono-methyl conjugate</i>	
<i>GA</i>	547.2	643.2	467.2	467.2	351/365/421/407
	501	597	421	421	351/365
<i>GB</i>	517.2	613.2	437.2	437.2	367/395/381/409
<i>GC</i>	533.2	629.2	453.2	453.2	383/397
<i>GJ</i>	517.2	613.2	437.2	437.2	367/395/381/409
<i>BIL</i>	419.1	515.1	339.1	339.1	163/251/177/265

FIGURE 1: Structure of Ginkgolides

	R1	R2	R3
<i>Ginkgolide A</i>	H	H	OH
<i>Ginkgolide B</i>	OH	H	OH
<i>Ginkgolide C</i>	OH	OH	OH
<i>Ginkgolide J</i>	H	OH	OH

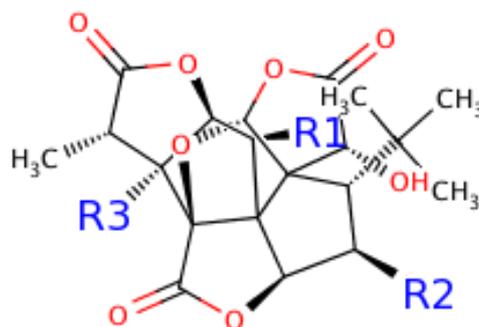


FIGURE 2: Structure of Bilobalide

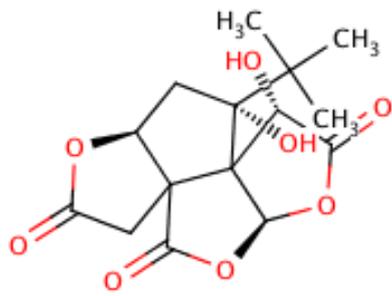


FIGURE 3: Profile of average terpene lactone content (μmol per capsule, \pm S.D.)

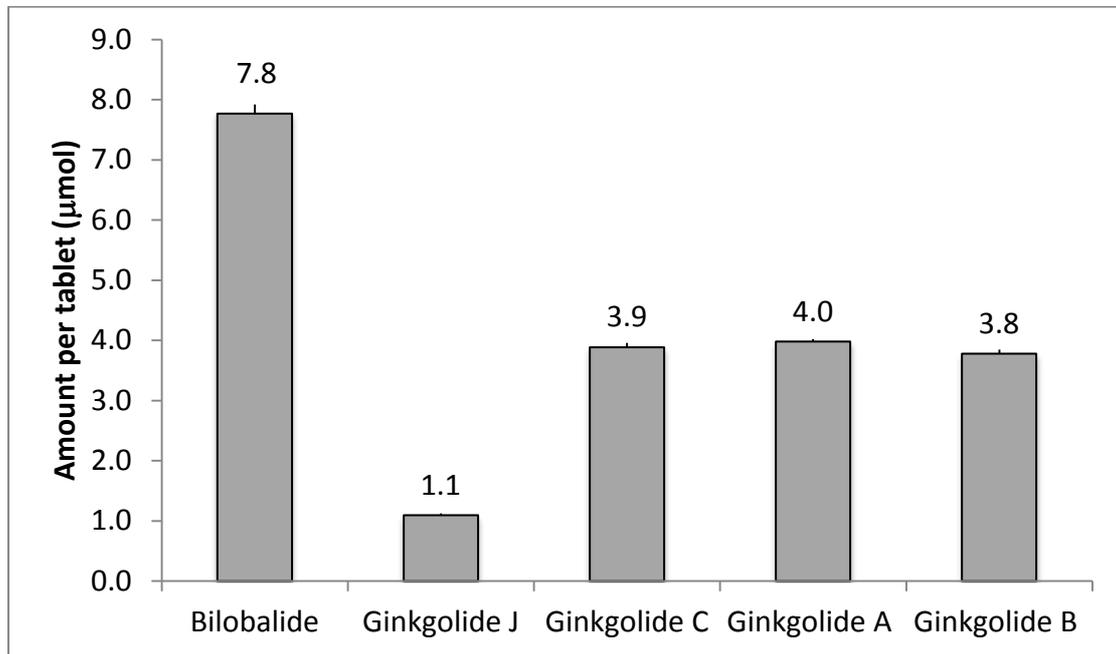
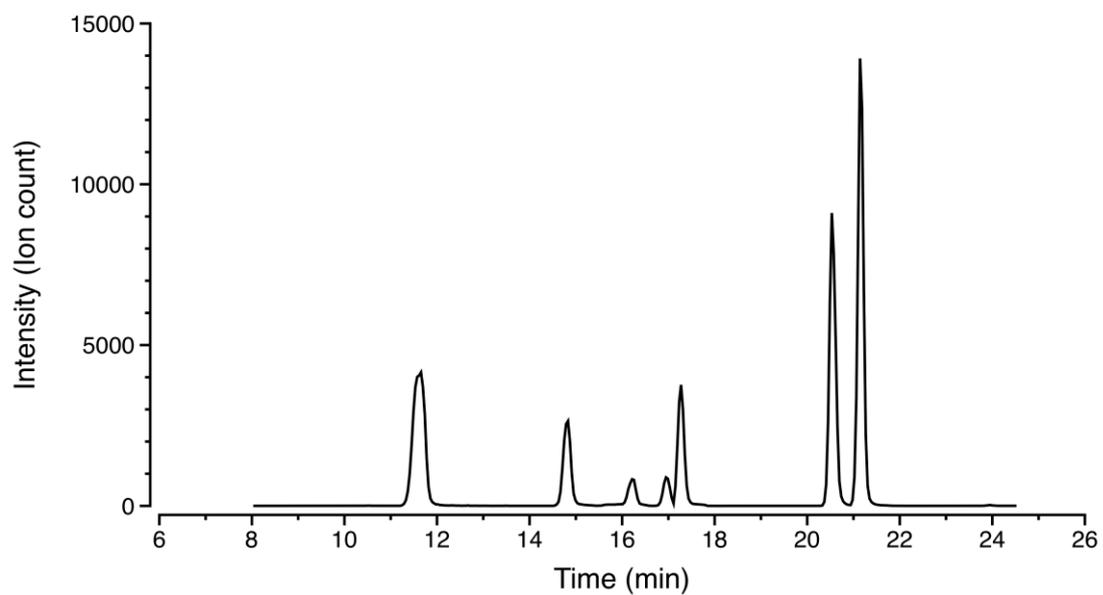


FIGURE 4: LC-MS-MS Total ion count chromatogram of terpene lactones in 24 hr urine following consumption of 240 mg ginkgo extract (high dose)



Retention times (min) of bilobalide (11.7), ethyl gallate (14.9), ginkgolide J (16.2), ginkgolide C (16.9), taxifolin (17.3), ginkgolide A (20.5) and ginkgolide B (21.2)

FIGURE 5: Terpene lactones excreted within 24 hr at low and high ginkgo doses
(μmol , $\pm\text{SD}$; n=12)

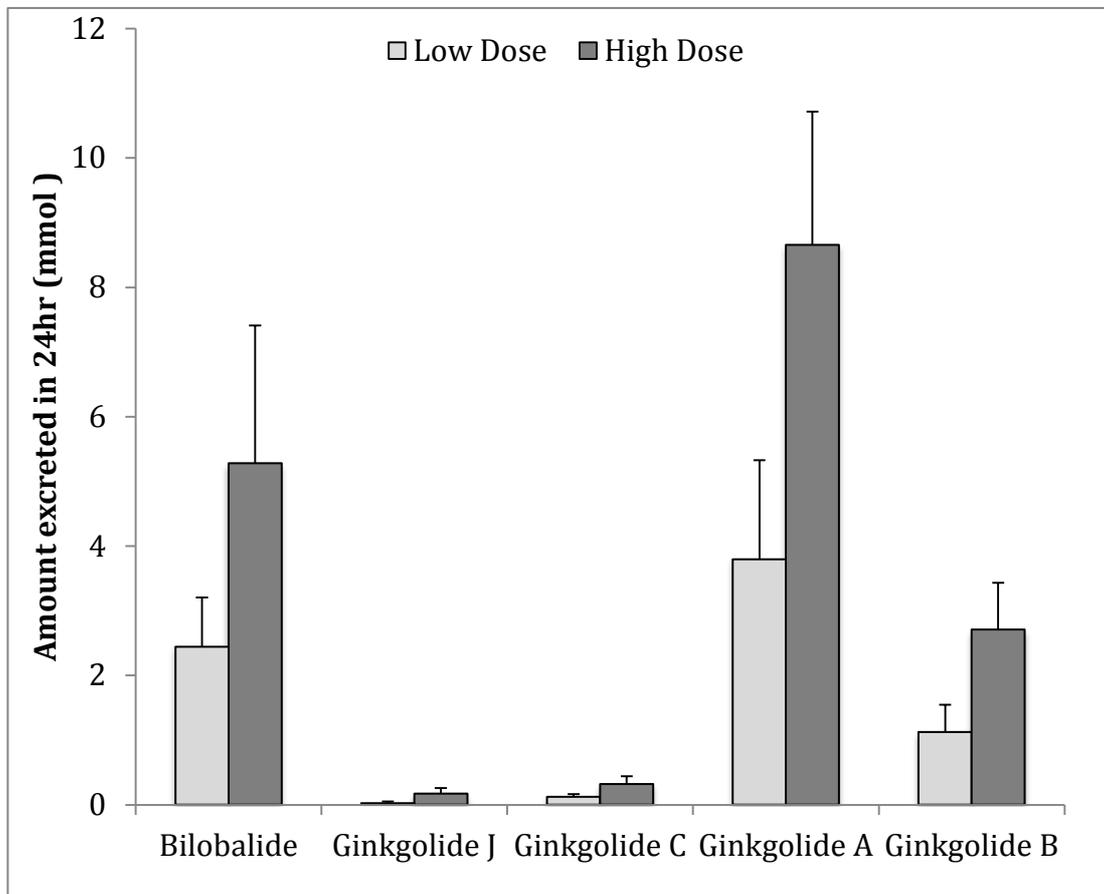


FIGURE 6: Percentage of terpene lactone dose recovered in 24 hr urine following ginkgo consumption (%; \pm SD; n=12)

