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Variation in Caffeine Concentration among Different Weight Loss Supplements Containing Green Tea and Green Coffee Extracts

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Summary

Many dietary supplements have caffeine as an active ingredient. Because they are supplements, they are not required by the FDA to state the concentration of caffeine they contain. The purpose of this study was to find the actual amount of caffeine in various supplements, specifically those that include green tea and green coffee extract. We analyzed the dietary supplements, green coffee, and green tea by high-pressure liquid chromatography to compare the amounts of caffeine in each. We found that the amount of caffeine in all of the supplements tested was higher than the predicted value. This was expected as extracts should be more concentrated than their original substance. In addition, the supplements differed from each other in their caffeine content, with a range of 5.33 to 67.2 mg/serving in green coffee–based supplements and a range of 2.97 to 12.85 mg/serving in green tea–based supplements. An ANOVA test revealed significant differences between brands in most pairings and a t-test revealed significant differences between expected caffeine content and actual caffeine content found by HPLC. This supports our hypothesis that the resulting extracts from different preparation methods can vary significantly in caffeine levels. This variation may raise health and safety concerns as the ingredients list does not tell consumers how much caffeine they are ingesting.

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Introduction

Caffeine is a central nervous system stimulant that has powerful effects on the body. It blocks adenosine receptors, preventing adenosine from binding and calming us down. As a result, caffeine promotes wakefulness and improves performance function in low doses. Caffeine is generally considered safe if recommended consumption amounts are adhered to. The 'safe' daily dosage of caffeine is up to 400 mg (1). Higher amounts of caffeine can have dangerous effects on the human body, and excess levels can lead to irritability, insomnia, overstimulation, and, in extreme cases, death (1).

Caffeine has long been an active ingredient in many over-the-counter weight loss pills. The purpose of caffeine is to increase energy and suppress appetite. However, there is little evidence to support that caffeine is effective in promoting weight loss (2). Because diet pills are considered to be a dietary supplement, the Food and Drug Administration (FDA) does not impose restrictions on their caffeine content nor does it require that the caffeine content be reported on the label (3). Supplements differ from prescription drugs in that they are considered safe until proven otherwise (4). The purpose of this study was to find the actual amount of caffeine in various weight loss supplements, specifically weight loss supplements that contain green coffee and green tea extracts. Because many supplements contain these components as their main source of caffeine, and because brewed coffee and tea are common beverages, we also aimed to determine whether there is a correlation between the amount of coffee or tea extract in the supplements and the amount of caffeine found in unprocessed coffee or tea.

Green coffee extracts are made using unroasted seeds. Traditionally, they are made using alcohol as a solvent (5). Green tea extracts are made using a variety of methods, including strong infusion, soft extracts, dry extracts, and partly purified extracts. Extracts made by only strong infusion are the least concentrated and those that become partly purified are the most concentrated (6). The actual preparation methods for green coffee or tea extracts used in dietary supplements is not stated on the labels and therefore is unknown to the average consumer.

In order to compare the caffeine content of green coffee and green tea to the content in dietary supplements, we tested samples of Costa Rica green coffee, Lipton green tea, and the dietary supplements using high-pressure liquid chromatography (HPLC). These commercial tea and coffee products were chosen due to their accessibility and the fact that they are representative of green coffee and green tea drinks. We hypothesized that the dietary supplements containing extracts of green coffee or

green tea would have a higher amount of caffeine than predicted by the weight of extract reported on the label and by the amount of caffeine found in green coffee or tea, because of the more concentrated nature of the extracts.

We chose four different brands of green coffee–based supplements and three different brands of green tea–based supplements to test based on affordability and accessibility. A one-way ANOVA test and a one-sample t-test revealed significant differences in caffeine levels between different brands of supplements, as well as significant differences between the amount of actual caffeine found and the expected levels of caffeine.

Product	Caffeine Found ± SE (mg/g)	Amount from literature (mg/g)
Lipton Green Tea	22.29 ± 0.53	19.44 (product label) 26.7 – 29.5 (Reference 7)
Costa Rica Dota Estate: Unroasted Green coffee	10.81 ± 0.30	10-12 (Reference 8)

Table 1: Caffeine concentration in green tea and green coffee.

Results

This study was designed to evaluate the differences in caffeine concentration between dietary supplements containing green tea or coffee bean extracts, green tea, and green coffee. Green tea and green coffee were used as a basis for comparison because the extracts were not diluted and are as, if not more, concentrated than pure green coffee and tea. They serve as a reference to help determine how much more concentrated the extract is. **Table 1** summarizes the caffeine concentration found in Lipton Green Tea and Costa Rica Green Coffee. The results are consistent with literature values (7, 8) and the values reported on the label. The amount of caffeine found in green tea and green coffee by HPLC analysis was 22.29 mg/g and 10.81 mg/g, with a standard error of 0.53 mg/g and 0.30 mg/g, respectively.

The dietary supplements used in this study and the corresponding results are summarized for green coffee bean extract and green tea extract (**Tables 2 & 3**). The amount of green tea or green coffee extract and other ingredients that may contain caffeine was obtained from the label on the bottle of each supplement and is listed (**Tables 2 & 3**). Values in the tables are reported in units of mg caffeine per gram supplement. The weight of each serving listed was used to calculate the milligrams caffeine found per serving. Because Hydroxycut Drops is in liquid form, it was assumed that 1 mL of product is equal to 1 gram for the calculations. We discovered that concentrations of caffeine found in the green coffee–based supplements ranged from 6.65 mg/g to 26.66 mg/g, while the concentrations of caffeine found in the green tea based supplements ranged from 6.25 mg/g to 24.95 mg/g. Because the product label ingredients and the amount consumed is based on serving size, the amount of caffeine per serving was calculated. In this study, one serving represents one capsule, one tablet, or the recommended volume of liquid. The amount of caffeine per serving of green coffee based supplements ranged from 5.33 mg to 67.2 mg, and the amount of caffeine per serving of green tea-based supplements ranged from 2.97 mg to 12.85 mg. A one-way ANOVA with Tukey's multiple comparison test was carried out on the data to determine whether differences in the means were statistically significant ($p \leq 0.05$) (**Table 4**). The analysis showed that all pairs of supplements have significantly different amounts of caffeine, except for the MaritzMayer/Nature's Measure, MaritzMayer/ Swanson Grapeseed, and Nature's Measure/ Swanson Grapeseed pairs.

The amount of caffeine that would be in each serving of supplement if coffee or tea were added directly was calculated by multiplying the amount of extract in the supplement by the amount of caffeine

Table 2: Summary and results for dietary supplements containing green coffee bean extract.

Product	Caffeine Found ± SE (mg/g)	Caffeine Found per Serving ± SE (mg)	[1]Calculated Caffeine per Serving (mg)	Difference per Serving (mg)	[2]Amount of Green Coffee Extract per Serving (g)	[3]Weight of Serving (g)	Form	Other Sources of Caffeine
MaritzMayer: Pure Green Coffee Bean Extract Cleanse	6.65 ± 0.35	5.33 ± 0.35	4.32	1.01	0.400	0.801	Capsule	
Good State Health Solution: Green Coffee Bean Extract	26.39 ± 0.24	19.90 ± 0.24	8.64	11.26	0.800	0.754	Capsule	
Finest Nutrition: Green Coffee Bean Extract	26.66 ± 2.22	9.01 ± 2.22	4.32	4.69	0.400	0.338	Capsule	120 mg Yerba Mate (standardized for 20% caffeine = 24 mg)
Hydroxycut Drops	19.76 ± 0.12	67.20 ± 0.12	2.16	65.04	0.200	3.4	Liquid	50 mg pure caffeine

[1] Calculated Caffeine per Serving (mg) = 10.81 mg caffeine per gram green coffee x grams of Green Coffee Extract per serving
[2] One serving is one capsule, tablet or recommended liquid volume. 1 mL = 1 g for Hydroxycut
[3] Weight does not include weight of empty capsule.

Product	Caffeine Found ± SE (mg/g)	Caffeine Found per Serving ± SE (mg)	[1]Calculated Caffeine per Serving (mg)	Difference per Serving (mg)	[2]Amount of Green Tea Extract per Serving (g)	[3]Weight of Serving (g)	Form	Other Sources of Caffeine
Nature's Measure: Green Tea Slim	7.11 ± 0.05	3.61± 0.05	2.29	1.32	0.100	0.508	Tablet	25 mg yerba mate
Swanson: Grapeseed. Green Tea & Pine Bark Complex	6.25 ± 0.31	2.97 ± 0.31	2.79	0.18	0.125	0.475	Capsule	
Swanson: Green Tea	24.95 ± 1.87	12.85 ± 1.87	11.15	1.7	0.500	0.515	Capsule	

[1] Calculated Caffeine per Serving (mg) = 22.29 mg caffeine per gram green coffee x grams of Green Coffee Extract per serving
[2] One serving is one capsule, tablet or recommended liquid form
[3] Weight does not include weight of empty capsule.

Table 3: Summary and results for dietary supplements containing green tea extract.

found in unprocessed tea or coffee (**Tables 2 & 3**). The calculated results and actual caffeine found by HPLC were then compared (**Figure 1**). For all supplements tested, the amount of caffeine in the supplements was greater than or equal to the amount initially predicted by using the amount of caffeine found in green tea and coffee (**Tables 2 & 3**).

A one-sample t-test (two-tailed) was carried out between the actual and predicted amount of caffeine per serving for each supplement to determine whether the difference between them was significant (**Figure 1**). The analysis showed that the predicted values of caffeine in the following supplements were significantly different ($p \leq 0.05$) from the determined / measured values: Good State Health, Finest Nutrition, Hydroxycut, and Nature's Measure. However, the remaining three supplements' predicted values were not significantly different from the measured values.

Discussion

Although the amount of caffeine found in the supplements in this study are not dangerous, the variability in the caffeine concentrations in green tea or green coffee bean extracts may be unhealthy as there is no way for those taking the supplements to know how much caffeine they are consuming. With the countless number of options for weight loss supplements, it is difficult to say which ones have too much caffeine and which are safe.

The green coffee and green tea tested served as an indicator for how much caffeine was expected to be found in the supplements. In some cases, the difference between the expected and actual amount was significant, as indicated in the one-sample t-test results shown in **Figure 1**. Possible reasons for the discrepancy between predicted and actual amounts of caffeine include added pure caffeine in the case of Hydroxycut, added Yerba Mate extract in the cases of Nature's Measure and Finest Nutrition, as well as other included ingredients not listed on the label. Additionally, differences in preparation of the extracts may have led to unregulated caffeine levels. Since companies do not report how their extracts are made, it is difficult to predict how concentrated each is. Furthermore, the amount in which the supplements differed varied between brands. The ANOVA test results indicate that almost all pairings between brands showed significant differences in concentration (**Table 4**). This supports the fact that the way in which the extracts were prepared may differ between companies. Extraction methods are not regulated, so the amount of extract reported bears little correspondence to actual caffeine amount.

There are several different extraction methods used by various companies. For example, to make green tea extracts, the most common method is strong infusion during which the green tea leaves are soaked in an aqueous solution of alcohol. But after the strong infusion, many companies go on to obtain soft extracts or dry extracts to make more concentrated substances. Additionally, new techniques such as membrane extraction are being developed to acquire higher

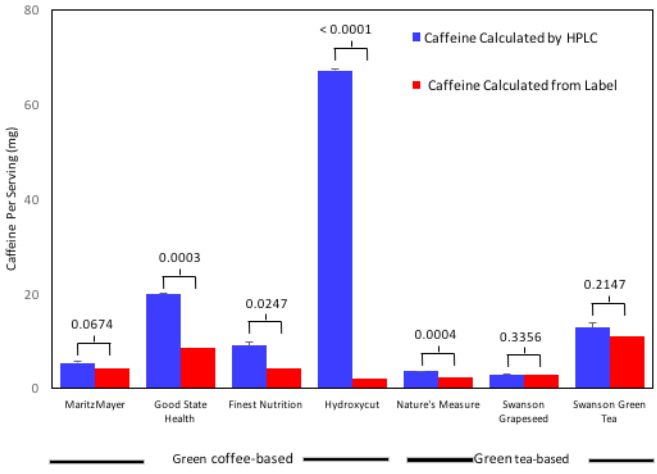

Figure 1: Actual Versus Expected Caffeine Amounts. Found by HPLC analysis of different dietary supplements. *P*-values from a one-sample t-test (two tailed) are listed above each pair of bars.

	MaritzMayer	Good State Health Solution	Finest Nutrition	Hydroxycut	Nature's Measure	Swanson Grapeseed	Swanson Green Tea
MaritzMayer		****	**	****	ns	ns	****
Good State Health Solution			****	****	****	****	****
Finest Nutrition				****	****	****	**
Hydroxycut					****	****	****
Nature's Measure						ns	****
Swanson Grapeseed							****
Swanson Green Tea							

ns = not significant
* $p \leq 0.05$
** $p < 0.01$
*** $p < 0.001$
**** $p < 0.0001$

Table 4: One-way ANOVA test, followed by Tukey's multiple comparison test of amount caffeine found per serving of supplement.

concentrations. Green coffee extracts are also made in a variety of ways (5). Though most are soaked in alcohol, the ways in which they are further concentrated can vary greatly (6).

It is important to note that not all of the caffeine found in the dietary supplements was derived from green tea or green coffee extracts. Yerba Mate extract is also a popular ingredient in weight loss pills and is included in Finest Nutrition and Nature's Measure supplements. It may explain the difference between the calculated and the actual amount of caffeine found in these two supplements. The caffeine content of Yerba Mate is unknown because of the nature of the extract preparation. Additionally, some supplements contain pure caffeine, which can easily bring up the caffeine level to a dangerous amount. The most notable difference can be found in the Hydroxycut drops and Good State Health supplements. The product label of Hydroxycut states that 50 mg of pure caffeine was added, so if this added amount is subtracted from 67.20 mg, the amount of caffeine arising from green tea extract should be about 17 mg, which is still higher than the 2.16 mg that was calculated using the results for green coffee.

Our study was limited by financial constraints so the samples tested were biased towards cheaper dietary supplements and do not accurately reflect all the supplements being used today. Further research should be conducted to test more popular supplements, such as Hydroxycut regular pills and Zantrex-3, which is notorious for having high caffeine content (9). Other caffeine-containing extracts, such as Yerba Mate, also need to be tested. While there are some limitations to the study, the results of this experiment indicate that green coffee and green tea extracts in dietary supplements vary in caffeine levels between brands. The lack of labeling and regulation in extraction methods prompt us to question the safety of consuming dietary supplements.

Methods

High-Pressure Liquid Chromatography (HPLC)

The Agilent 1100 HPLC with UV-Vis diode array detector and Inertsil ODS-3, 5 micron, 250×4.6 mm column (MetaChem) were used for this study. The mobile phase was methanol/water (30:70) with a flow rate of 1.0 mL/min and injection volume of 20 µL. The detection wavelength was 270 nm and the column temperature was 40°C.

Standard Curve

Ten milligrams of caffeine powder (Sigma-Aldrich) was dissolved in 100 mL of water to prepare a 0.1 mg/mL stock. The stock was then used to make 9 standards ranging from 2 µg/mL to 100 µg/mL in water. The samples were injected onto the HPLC and the peak area was plotted against concentration to create a standard curve.

Sample Preparation

The contents of three capsules or three tablets were each weighed separately then combined before processing. The supplements in tablet form, as well as the green coffee and green tea, were ground into powder. Approximately 0.3 grams of the composite sample was weighed then transferred to an Erlenmeyer flask and 30 mL of near-boiling water was added. The flask was stoppered and placed in a hot water bath (95°C) with agitation for 20 min. Three replicates were prepared for each sample. Once the solutions were stirred and cooled, they were filtered through Whatman 41 filter paper. Each solution was then filtered and transferred to an HPLC vial using a syringe and 0.45-µm syringe filter (PALL Life Sciences). As necessary, dilutions were made so that the peak areas were within the range of the standard curve. The samples were then injected onto the HPLC.

Calculations Using Standard Curve

The total milligrams caffeine per capsule was calculated using the following equation:

$$\frac{\frac{\text{Peak area} - \text{intercept}}{\text{slope}} \times \text{dilution factor} \times 30\ \text{mL volume of extract}}{1000\ \mu\text{g/g} \times \text{actual weight of 0.3 g powder}} \times (\text{average weight of one capsule})$$

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