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# Mixture design analysis of solvent extractor effects on epicatechin, epigallocatechin gallate, epigallocatechin and antioxidant activities of the *Camellia sinensis* L. leaves

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ABSTRACT

This paper reports an RP-HPLC-UV-DAD study of the effects of four solvents and their mixtures on the extraction and antioxidant activities of three main catechins, epicatechin (EC), epigallocatechin gallate (EGCG) and epigallocatechin (EGC) of *Camellia sinensis* L. leaves for two harvests. The extraction efficiency solvent was measured by the chromatographic peak areas. The results showed that the relative abundance of the catechins in the second harvest is somewhat larger than in the first one, although there is no significant difference at the 95% level. The relative abundance found for EGCG is greater than for EGC which is greater than EC for all solvent mixtures. According to the mixture models, the maximum values of relative abundances of EGCG, EGC and EC can be obtained with a (70:30 v/v) ethanol:ethyl acetate binary mixture and the antioxidant activities with a (55:25:20 v/v/v) ethanol:ethyl acetate:dichloromethane ternary mixture.

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## 1. Introduction

It is well known that tea is produced from *Camellia sinensis* leaves and generally is divided into three groups, green, black and oolong tea, according to the manufacturing process. Green tea is known as unfermented tea, because enzyme inactivation, of polyphenol oxidase for example, occurs in the manufacturing process, ensuring that fermentation does not take place. Black tea is the product of the fermentation of polyphenols by the oxidizing enzyme present in green tea leaves, while oolong tea is half fermented by an enzyme present in the leaves.

Tea production begins by picking the leaves and buds from the *C. sinensis* shrubs. A variation in tea quality occurs depending on the picking procedure, hand or mechanical. Hand picking is the most desirable and common for high quality teas, but mechanical harvesting equipment is also used to collect tea leaves and shoots generally producing a lower quality tea because it can incorporate plant stems. There are two main types of green tea, Sencha and Bancha. Sencha is prepared from young buds of tea leaves grown with exposure to plenty of sunshine that are picked, steamed and completely processed, while Bancha is produced from the leaves remaining after the young buds of the tea leaves have been picked including the hard leaves and stalks left over from Sencha production.

In Brazil, tea harvesting and full production begins in September and continues for eight months until the month of April while it is winter in Japan and there is no harvest. Also, the weather in Brazil is milder than in Japan so the development of shoots requires less time and can be collected at a spacing of two weeks, allowing about twelve to fourteen crops per year.

In recent years, interest in green tea has increased due to its medicinal and pharmacological effects attributed to the polyphenols especially catechins and phenolic acids. The main catechin compounds from *C. sinensis* (L.) leaves are epigallocatechin gallate (EGCG) epicatechin gallate (ECG), epicatechin (EC) and epigallocatechin (EGC) [1]. These catechins are responsible for the astringent and bitter taste of green tea and have received considerable attention due to their reported beneficial health properties such as the prevention of cardiovascular diseases [2] and cancer [3–5] as well as their anti-hypertensive [6,7], anti-inflammatory [8], anti-obesity [9] and anti-aging effects [10], among others.

Many methods, such as ultra-high performance liquid chromatography (UHPLC) [11], Fourier transform near infrared reflectance

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spectroscopy (FT-NIR) [12-15], capillary electrophoresis (CE) [16,17], thin-layer chromatography [18], centrifugal precipitation chromatography [19], high-speed counter-current chromatography [20,21], potentiometric flow injection [22], among others have been reported for the analysis of catechin contents in green tea.

Several studies on catechins in green tea have been reported including their antioxidant activities [17–23], tea qualities [24] and health benefits [25]. Different solvent systems have been used for extraction of polyphenols from their plant materials but for catechins the most widely used is boiling water. This is expected, because most research is related to the effects of polyphenols on human health, but the catechins are also used to evaluate tea quality, as reference substances, as well as for their potential cosmetic resources. In these cases organic solvents can be used but there is a lack of studies in the literature comparing different solvents for extraction as well as evaluations of the catechin antioxidant activities. In this case it is important to optimize the extractor solvent to obtain optimum yields.

The objective of this research was to investigate the effects of different extracting solvents on epicatechin (EC), epigallocatechin gallate (EGCG) and epigallocatechin (EGC) as well as on their antioxidant activities in two sets of *C. sinensis* (L.) leaves using a simplex centroid mixture design for the ethanol, dichloromethane, ethyl acetate and chloroform solvents. The first and second sample sets correspond to the first and second harvests after pruning. An experimental mixture design involving water with different organic modifiers was also used to investigate different mobile phases for peak separation in Reversed-Phase High-Performance Liquid Chromatography depending on the solvent strength and polarity. Principal component analysis (PCA) was then used to compare the chromatographic fingerprints for the different extracts.

## 2. Experimental

### 2.1. Chemicals and reagents

HPLC grade acetonitrile and methanol were purchased from J.T. Baker (Phillipsburg, USA). HPLC grade water (18.2 MΩ cm) was prepared using a Millipore Milli-Q Gradient purification system (Bedford, USA) and used for the mobile phases. For plant extraction, all organic solvents were of analytical grade and obtained from F. Maia (São Paulo, Brazil). All crude extracts were filtered with 25 mm PTFE 0.2 μm syringe filters purchased from chromafil (Macherey-Nagel, Düren, Germany). For the free radical scavenging activities 2,2-diphenyl-1-picrylhydrazyl (Sigma Aldrich, St. Louis, USA) was used. Reference substances, (−)-Epigallocatechin gallate (⩾80% (HPLC), from green tea and (−)-Epicatequina (⩾90% (HPLC) were purchased from Sigma Chemical. Caffeine was purchased from Fluka.

### 2.2. Plant materials

Two set of leaf samples from *C. sinensis* (L.) Kuntze were kindly provided by the Agrochá Boa Vista farm (Araucária, PR, Brazil). The first sample set was collected in September 2011 and corresponds to the first harvest after pruning. Eighteen days later, the second set of samples was collected, when the new shoots were ready to be picked. Since the tea leaves contain oxidizing enzymes, they were subjected to steaming prior to oxidation, thus disabling oxidizing enzymes, while retaining the color of chlorophyll as well as the active components contained in tea. A voucher specimen catalogued as FUEL 49288 has been stored in the Herbarium of the Universidade Estadual de Londrina (UEL). The specie was identified by A.O. Vieira, Departamento de Biologia, UEL.

### 2.3. Extract preparation

To investigate the effect of different extracting solvents on epicatechin (EC), epigallocatechin gallate (EGCG) and epigallocatechin (EGC) as well as the antioxidant activity of *C. sinensis* leaves, a simplex-centroid design was used, Fig. 1. The solvents were (e) ethanol, (a) ethyl acetate, (d) dichloromethane and (c) chloroform, resulting in 15 different mixtures with a triplicate at the center point to calculate the experimental error. The selection of each solvent was made considering Snyder's solvent selectivity triangle, since solvents from different groups in the triangle have different selectivity characteristics [26].

The samples were previously crushed and sieved. Each extract was prepared by weighing 2.0 g of green tea sample and adding 6.0 mL of the solvent mixtures listed in Table 1. Each mixture was placed in an ultrasonic bath (Unique, model Ultracleaner 1400) for 30 min, and filtered to separate the solution from the leaves. This procedure was repeated fourteen more times, so the total volume of pure or mixture solvent added to the leaves was 90 mL. The extracts were left at rest under forced ventilation until reaching constant weight. For HPLC analysis, 2.00 mg of the extracts were dissolved in 2.0 mL of methanol and 100 μL of this solution was diluted in 400 μL of mobile phase and filtered through a 0.2 μm polytetrafluoroethylene membrane (PTFE, Chromafil). Twenty microliters of this diluted solution was used in the HPLC for obtaining the chromatograms.

### 2.4. HPLC analysis

HPLC analysis was conducted on a Finnigan Surveyor 61607 system coupled with Finnigan Surveyor PDA Plus photodiode array detector (PDA) and manual sample injector with a 20 μL loop. A Gemini $C_{18}$ from Phenomenex (250 × 4.6 mm) column and a guard column (4 × 3 mm d.i.) with 5 μm nominal particle size were used. The flow rate was 1 mL $min^{-1}$. UV detection was monitored at 210, 254 and 280 nm. Satisfactory separation was achieved at 210 nm. The data were processed using ChromQuest 4.2 software. The effect of the mobile phase strength on chromatographic separation was investigated by an experimental mixture design consisting of water, acetonitrile and methanol as illustrated in Fig. 2. The solvent proportions were calculated considering the total solvent strength ($S_T$), and the values of the polarity ($P$) [27].

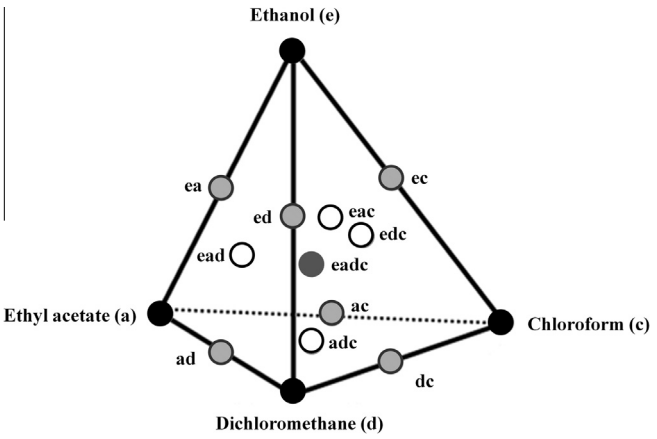


**Fig. 1.** Experimental design for mobile phases involving mixtures of water, acetonitrile and methanol according to the strength and polarity total performed for each crude extract obtained by simplex centroid mixture design for the pure, binary, ternary and quaternary mixtures of ethanol, ethyl acetate, dichloromethane and chloroform solvents.

**Table 1**
Volume proportions of the extraction mixtures.

| Extract | Solvents | | | |
|---|---|---|---|---|
| | Ethanol | Ethyl acetate | Dichloromethane | Chloroform |
| *e* | 1 | 0 | 0 | 0 |
| *a* | 0 | 1 | 0 | 0 |
| *d* | 0 | 0 | 1 | 0 |
| *c* | 0 | 0 | 0 | 1 |
| *ea* | 1/2 | 1/2 | 0 | 0 |
| *ed* | 1/2 | 0 | 1/2 | 0 |
| *ec* | 1/2 | 0 | 0 | 1/2 |
| *ad* | 0 | 1/2 | 1/2 | 0 |
| *ac* | 0 | 1/2 | 0 | 1/2 |
| *dc* | 0 | 0 | 1/2 | 1/2 |
| *ead* | 1/3 | 1/3 | 1/3 | 0 |
| *eac* | 1/3 | 1/3 | 0 | 1/3 |
| *edc* | 1/3 | 0 | 1/3 | 1/3 |
| *adc* | 0 | 1/3 | 1/3 | 1/3 |
| *eadc*1 | 1/4 | 1/4 | 1/4 | 1/4 |
| *eadc*2 | 1/4 | 1/4 | 1/4 | 1/4 |
| *eadc*3 | 1/4 | 1/4 | 1/4 | 1/4 |

### 2.5. Free radical scavenging activity assay

The radical scavenging activities of crude extracts of *C. sinensis*, Table 1, were measured by using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Stock solutions of each extract (Table 1) were prepared by dissolving 7.5 mg of the extract in 25 mL of ethanol. From these stock solutions, work solutions were prepared at different masses (1–40 μg $mL^{-1}$) and dissolved in 3 mL of ethanol and then added to an ethanolic solution of 0.1 mL of free radical DPPH (1 mM). After 30 min incubation, spectra of the resultant solutions were recorded in an Evolution 60 S spectrophotometer (Thermo Scientific) with absorbance read at 517 nm [28]. Tests were carried out in triplicate. The results are reported as μg $mL^{-1}$ of 50% inhibition ($IC_{50}$) that is, mass of crude extract required to cause 50% inhibition of DPPH.

### 2.6. Software

Mixture response surfaces and principal component analysis calculations were carried out using the Statistica 8.0 software (Tulsa, OK (USA)).

## 3. Results and discussion

Separation and identification of the catechins was carried out by RP-HPLC-DAD. The effect of the extractor solvent as well as the mobile phases in the catechin analyses were investigated using the mixture design in Table 1 crossed with the mixture design in

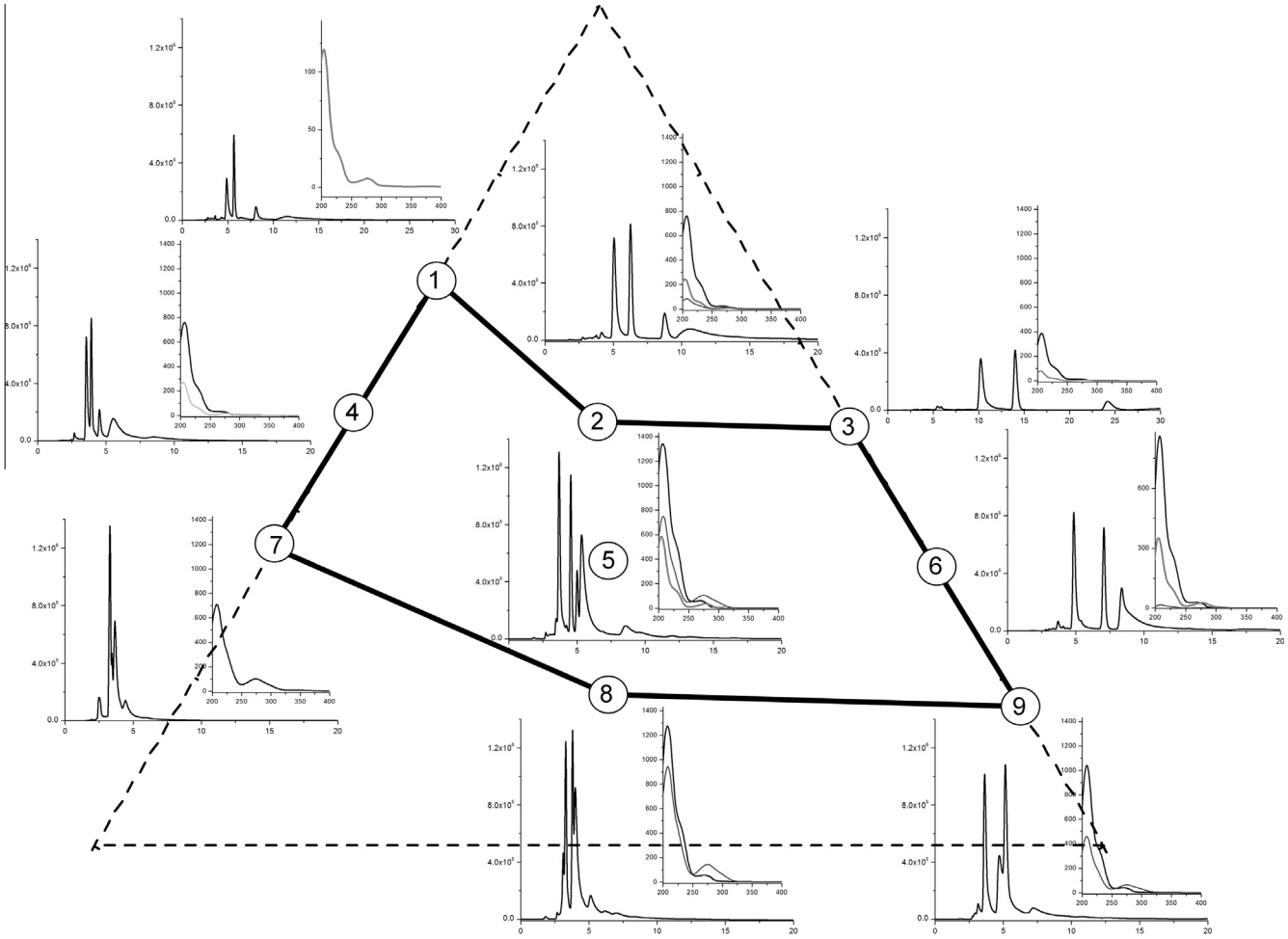


**Fig. 2.** Experimental design for mobile phases with their respective chromatograms and the spectra of the catechins in the ethanol extract. Mobile phase: **(1)** 83.9:16.1 (v/v) $H_2O$:ACN ($S$ = 0.50 and $P$ = 9.48); **(2)** 75.4:12.3:12.3 (v/v/v) $H_2O$:ACN:MeOH ($S$ = 0.75 and $P$ = 9.03); **(3)** 75.0:25.0 (v/v) $H_2O$:MeOH ($S$ = 0.75 and $P$ = 8.93); **(4)** 75.8 $H_2O$:24.5 (v/v) $H_2O$:ACN ($S$ = 0.75 and $P$ = 9.14); **(5)** 67.0:16.0:17.0 (v/v/v) $H_2O$:ACN:MeOH ($S$ = 1.00 and $P$ = 8.63); **(6)** 66.5:33.5 (v/v) $H_2O$:MeOH ($S$ = 1.00 and $P$ = 8.49); **(7)** 68.0 $H_2O$:32.0 (v/v) $H_2O$:ACN ($S$ = 1.00 and $P$ = 8.79); **(8)** 59.0:20.0:21.0 (v/v/v) $H_2O$:ACN:MeOH ($S$ = 1.25 and $P$ = 8.25); **(9)** 58.3:41.7 (v/v) $H_2O$:MeOH ($S$ = 1.25 and $P$ = 8.07), where $S$ and $P$ represents the solvent strength and polarity, respectively.

Fig. 2, that represents not only the relative proportions of the two modifying solvents in the mobile phases but also the total solvent strength, *S*, and polarity (*P*). The extraction efficiency of each solvent was compared using relative abundances determined from chromatographic peak areas. Fig. 2 shows the chromatograms and DAD-spectra of the chromatographic peak of the catechins in the ethanol extract. These results show that using the same extract but changing the mobile phase causes overlapping peaks. In other words, the variation in strength of the mobile phase can cause overlapping peaks limiting the desired separation on the extract.

Fig. 2 shows that the highest overall resolution was obtained using the mobile phase prepared with (67:16:17 v/v/v) water: acetonitrile:methanol, because only this mobile phase separated the spectra of the epicatechin (EC), epigallocatechin gallate (EGCG) and epigallocatechin (EGC) peaks. Therefore this mobile phase was used for all subsequent analysis.

To explore the effect of the extraction solvent on the *C. sinensis* leaf fingerprint the chromatographic data were subjected to a principal components analysis. These data were arranged in a $34 \times 616$ matrix, corresponding to 15 different solvent proportions with a triplicate at the central point for each harvest

Fig. 3 shows the scores plot for PC2 against PC3 which accounts for 11.0% of total variance. It may be noted that extracts without ethanol have positive PC2 scores while those prepared in ethanol and its mixtures have negative values. Extracts prepared in ethanol, dichloromethane and a binary mixture of ethanol: ethyl acetate is on the upper left side with negative PC2 and positive PC3 scores. From the PC2 and PC3 loadings (Fig. 4) one can observe that the samples in the more positive region of CP2 contain higher relative abundances of caffeine (tr = 4.53 min) whereas those in the negative part have greater relative abundances of EGC (tr = 3.66 min). Those extracts that are negative on the PC2, but more positive on PC3 have greater relative abundances of EGCG (tr = 5.28 min) whereas negative on both PC2 and PC3 has greater relative abundance of EC (tr = 5.02 min). From this figure, it can be concluded that the ethanol: ethyl acetate mixture extracts larger amounts of EGCG followed by dichloromethane and pure ethanol, while EC and EGC can be extracted with the other mixtures containing ethanol. To confirm these results mixture models were developed for the three catechins EC, EGCG and EGC.

The relative abundances from chromatographic peak area data for two harvests are given in Fig. 5. These results showed that the relative abundance of the catechins in the second harvest is

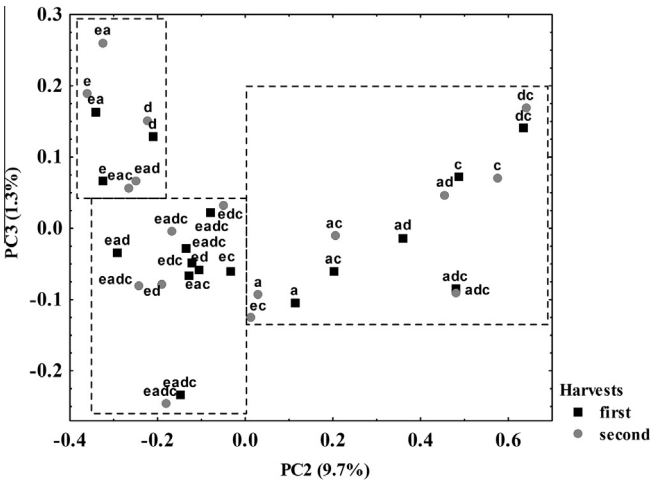


**Fig. 3.** Principal component score graph of the chromatograms of the extracts obtained from the simplex centroid design mixtures. *e*, *a*, *d* and *c* represent the ethanol, ethyl acetate, dichloromethane and chloroform solvents, respectively.

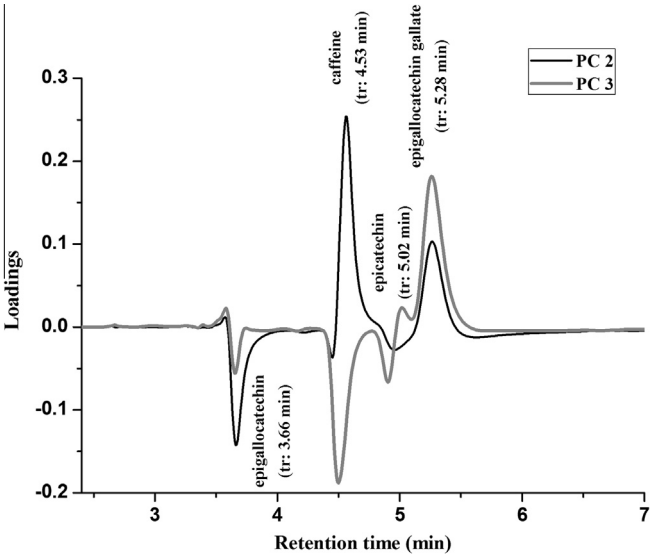


**Fig. 4.** Loading plots of the second (black) and third (gray) principal components of the chromatograms of extracts obtained using the simplex centroid design mixtures.

somewhat larger than in the first one and there are also differences in the relative abundances of the catechins extracted by ethanol, dichloromethane and mixtures containing ethanol in relation to the other solvent extractors. The relative abundance of EGCG is greater than EGC which is greater than EC for all solvent mixtures extracting more than minimal amounts of catechins. The individual values of the second crop were higher than those of the first harvest and a paired *t*-test was used to verify if the differences between relative abundances of the two harvests are indeed significant. The results of the paired *t*-test showed no significant difference at the 95% level of the relative abundances from chromatographic peak area data for the three catechins of these two harvests. Therefore, all their values were analyzed together as function of the extraction solvent composition to optimize the catechin extractions.

Linear, quadratic and special cubic models were fit to the chromatographic peak area data of the three catechins. Model quality was determined by ANOVA of the regression results. Special cubic models for EC and EGC.

$$EC = \underset{(\pm 6.23)}{49.16}e + \underset{(\pm 6.69)}{25.78}a + \underset{(\pm 6.69)}{47.38}d + \underset{(\pm 6.69)}{9.89}c + \underset{(\pm 31.08)}{98.4}ea - \underset{(\pm 31.08)}{68.56}ad - \underset{(\pm 32.91)}{107.63}dc + \underset{(\pm 195.27)}{668.59}edc$$

and

$$EGC = \underset{(\pm 10.23)}{109.34}e + \underset{(\pm 10.98)}{41.17}a + \underset{(\pm 10.98)}{108.23}d + \underset{(\pm 9.97)}{15.34}c + \underset{(\pm 51.03)}{240.21}ea - \underset{(\pm 51.04)}{210.66}ad - \underset{(\pm 54.05)}{223.59}dc + \underset{(\pm 320.66)}{876.39}edc$$

and a quadratic model for EGCG were found to be most the adequate

$$EGCG = \underset{(\pm 16.29)}{152.61}e + \underset{(\pm 16.29)}{37.61}a + \underset{(\pm 17.76)}{167.76}d + \underset{(\pm 14.78)}{12.94}c + \underset{(\pm 75.74)}{447.19}ea - \underset{(\pm 75.70)}{171.93}ed - \underset{(\pm 75.70)}{320.17}ad - \underset{(\pm 75.74)}{291.08}dc$$

In these equations only the significant binary and ternary coefficients are retained and *e*, *a*, *d* and *c* represent the ethanol, ethyl acetate, dichloromethane and chloroform proportions, respectively. Standard error estimates are given in parentheses below the corresponding model coefficients.

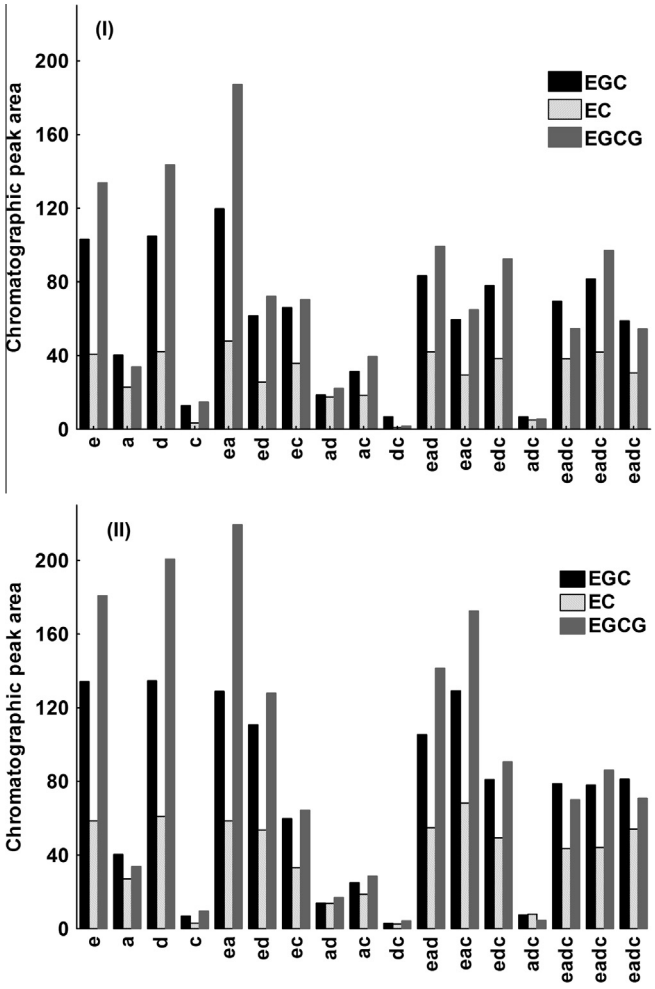


**Fig. 5.** Variation of chromatographic peak area of epicatechin (EC), epigallocatechin gallate (EGCG) and epigallocatechin (EGC) for first (I) and second (II) harvest.

The ANOVA results are presented in Table 2. As can be seen there on observing the calculated *F* values and the probability values the above models do not suffer from lack of fit and are highly significant.

On examining the above three equations in detail it can be seen that the chromatographic peak areas have very similar dependencies on the extraction solvent compositions. For all three equations the linear ethanol and dichloromethane coefficients are approximately the same and larger than the ethyl acetate coefficient which is larger than the chloroform one. All three equations have positive and significant binary synergic coefficients between ethanol and ethyl acetate and negative antagonistic ones involving ethyl acetate and dichloromethane and dichloromethane and chloroform. The only major difference occurs for the EGCG model. The EC and EGC models have synergic ternary interactions involving ethanol–dichloromethane and chloroform whereas EGCG has an antagonistic interaction between ethanol and dichloromethane. Also the EGCG coefficients are normally larger than their corresponding EGC ones and the EC coefficients are usually the smallest. This is to be expected after visualization of Fig. 5 where for most extraction solvent mixtures the EGCG chromatographic peak area was larger than the EGC one and the EC one was the smallest.

Owing to the similarities in the above models one might expect their response surfaces for the chromatographic peak areas to very similar. This can be seen in Fig. 6 where the EC, EGC and EGCG response surfaces are shown as functions of the ethanol, ethyl acetate and dichloromethane proportions. According to the above equations and these response surfaces a maximum value of relative abundance for these three catechins can be obtained with a (70:30 v/v) ethanol:ethyl acetate binary mixture. It is not surprising that the same solvent mixture extracts maximum amounts of each of these catechins owing to their similar structures. Furthermore ethanol and ethyl acetate can be expected to form hydrogen bonds with the hydroxyl groups and the oxygen ring atom of the catechins that could enhance extraction results.

Considering that phenolic compounds are responsible for the antioxidant activity of green tea, the mixture model was applied to the extracts of the *C. sinensis* leaves.

**Table 2**
Analysis of variance (ANOVA) results for the mixture models obtained from the chromatographic peak areas of EC, EGCG and EGC for the first and second harvests of green tea.

| Variation source | Sum of squares | Degrees of freedom | Mean square | Calculated *F*-value | Probability |
|---|---|---|---|---|---|
| *EC* | | | | | |
| Model | 9274.72 | 7 | 1324.96 | 12.064 | 0.000 |
| Total error | 2855.39 | 26 | 109.82 | | |
| Lack of fit | 849.94 | 7 | 121.42 | 1.150[a] | 0.375 |
| Pure error | 2005.45 | 19 | 105.55 | | |
| Total | 12130.12 | 33 | 367.58 | | |
| *EGCG* | | | | | |
| Model | 111578.7 | 7 | 15939.81 | 24.448 | 0.000 |
| Total error | 16951.8 | 26 | 651.99 | | |
| Lack of fit | 3905.2 | 7 | 557.89 | 0.812[a] | 0.588 |
| Pure error | 13046.6 | 19 | 686.66 | | |
| Total | 128530.5 | 33 | 3894.86 | | |
| *EGC* | | | | | |
| Model | 50414.33 | 7 | 7202.046 | 24.320 | 0.000 |
| Total error | 7699.53 | 26 | 296.136 | | |
| Lack of fit | 2374.57 | 7 | 339.225 | 1.2104 | 0.344 |
| Pure error | 5324.96 | 19 | 280.261 | | |
| Total | 58113.86 | 33 | 1761.026 | | |
| $IC_{50}$ | | | | | |
| Model | 6612.21 | 13 | 508.631 | 198.430 | 0.000 |
| Total error | 51.266 | 20 | 2.563 | | |
| Lack of fit | 0.168 | 1 | 0.168 | 0.062[a] | 0.805 |
| Pure error | 51.097 | 19 | 2.689 | | |
| Total | 6663.472 | 33 | 201.923 | | |

[a] Lack of fit mean square/pure error mean square ratio. Corresponding critical *F* values at the 95% confidence level is $F_{7,19,0.05} = 2.6$.

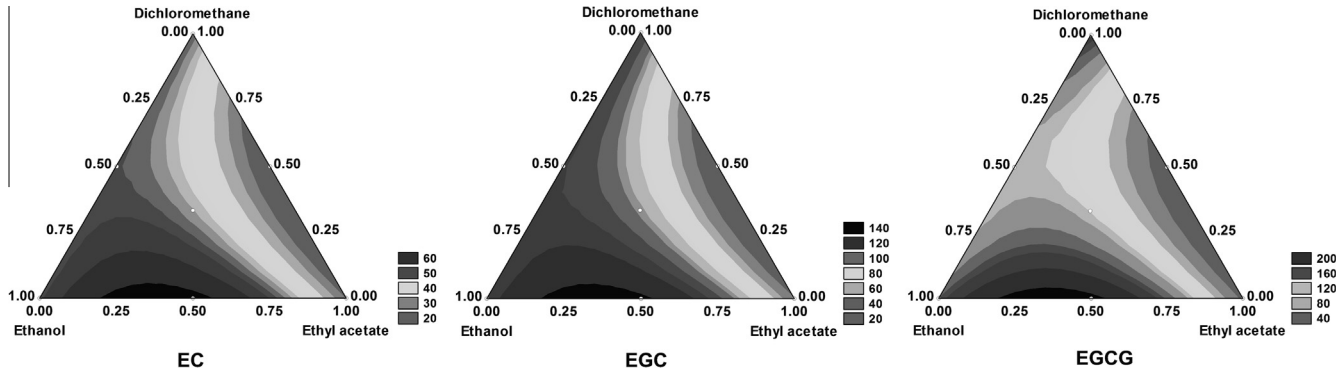


**Fig. 6.** Response surfaces for the chromatographic peak areas of epicatechin (EC), epigallocatechin (EGC) and epigallocatechin gallate (EGCG) for both harvests of *C. sinensis* leaves as a function of ternary ethanol, ethyl acetate and dichloromethane mixture composition.

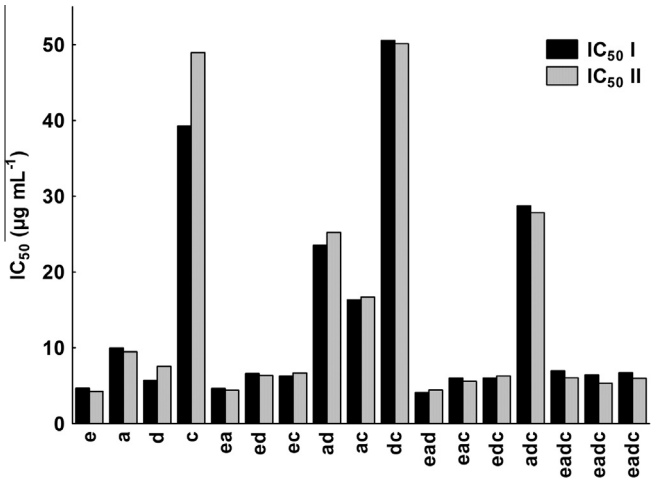


**Fig. 7.** Concentrations causing 50% inhibition of DPPH ($IC_{50}$) for each crude extract for the first (I) and second (II) harvests of green tea for the experimental design mixtures.

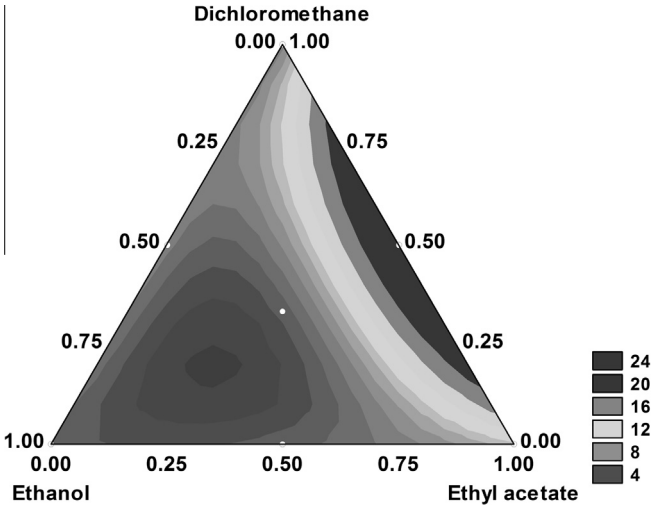


**Fig. 8.** Mixture response surface for extractor mixture compositions causing 50% inhibition of DPPH ($IC_{50}$) for both harvests of *C. sinensis* leaves. The chloroform proportion was held at the zero proportion level.

Fig. 7 shows the effect of the solvent compositions on the $IC_{50}$ of crude extracts of *C. sinensis* for the two harvests. By comparing the results, one can notice that pure chloroform and the dichloromethane:chloroform (50:50 v/v) binary mixture showed higher $IC_{50}$ values, followed by the ethyl acetate:dichloromethane:chloroform ternary mixture and binary mixtures of ethyl acetate:dichloromethane and ethyl acetate:chloroform. Since a lower value of $IC_{50}$ indicates a higher antioxidant activity, this result shows that the extracts prepared in chloroform and dichloromethane:chloroform mixtures showed the worst antioxidant activities. Mixtures not containing ethanol also showed higher $IC_{50}$ values, or lower antioxidant capacities. It is interesting to notice that high antioxidant activities were also obtained with the ternary and quaternary solvent mixtures containing ethanol.

The results of a paired *t*-test showed no significant difference at the 95% confidence level between the $IC_{50}$ values for the two harvests. Therefore, the $IC_{50}$ values as a function of the extraction solvent compositions were analyzed together to determine the best solvent extractor composition to optimize the antioxidant activity. The special cubic model showed no significant lack of fit at the 95% confidence level, as can be seen with the ANOVA results in Table 2, and is given by Eq. 4, where only the significant higher order coefficients are retained.

$$IC_{50} = \underset{(\pm 1.13)}{4.48}e + \underset{(\pm 1.13)}{9.73}a + \underset{(\pm 1.13)}{6.62}d + \underset{(\pm 1.13)}{44.10}c - \underset{(\pm 5.51)}{71.36}ec + \underset{(\pm 5.51)}{64.67}ad - \underset{(\pm 5.51)}{41.72}ac + \underset{(\pm 5.51)}{99.73}dc - \underset{(\pm 35.12)}{242.52}ead - \underset{(\pm 35.12)}{423.13}edc - \underset{(\pm 35.12)}{133.30}adc$$

The linear coefficient for chloroform is much larger than the other pure solvents producing much smaller antioxidant activities. On the other hand the ethanol coefficient is smallest but significant at the 95% confidence level, indicating the highest antioxidant activities. As can be seen from the model coefficient values in the equation synergic and antagonistic binary and ternary effects are significant. The (1:1:1 v/v/v) ethanol:dichloromethane:chloroform interaction is the most important antagonistic effect having the largest ternary values, following by the (1:1:1 v/v/v) ethanol:ethyl acetate:dichloromethane and the (1:1:1 v/v/v) ethyl acetate: dichloromethane:chloroform ternary mixtures.

The response surfaces for $IC_{50}$ of Eq. 4 are shown in Fig. 8 as a function of solvent proportions. The lower $IC_{50}$ value on the response surface graph is seen to occur with a 55:25:20 ternary ethanol–ethyl acetate–dichloromethane mixture.

## 4. Conclusions

These results showed that the extractor solvent composition affects the extraction efficiency of catechins. The relative abundance in the second harvest is somewhat larger than in the first but the difference is not significant at the 95% confidence level. EGCG abundance is greater than the EGC which is greater than for EC for all solvent mixtures extracting significant quantities of these compounds. Higher relative abundances of EG, EGCG and

EGC were obtained with (70:30 v/v) ethanol:ethyl acetate binary mixture. The lowest $IC_{50}$ values are seen to occur with a (55:25:20 v/v/v) ethanol:ethyl acetate:dichoromethane ternary mixture.

## Conflict of interest

The authors declare there is no conflict of interest.

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