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Evaluation of the essential oils and teas produced from the Bahia and Pará cocoa almond husks

Avaliação dos óleos essenciais e das infusões produzidos a partir das cascas das amêndoas de cacau provenientes da Bahia e do Pará

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Brazil is one of the largest producers of cocoa in the world and, consequently, of the husks of cocoa almonds. This material is an industrial food waste from which it is possible to produce an aromatic tea. The aim of this work was to evaluate the chemical composition of the essential oils of the husks of cocoa almond samples from Bahia and Pará states. Total phenolic (Folin-Ciocalteu) and flavonoid ($AlCl_3$) contents, antioxidant capacity (DPPH assay) and toxicity (*Artemia salina* bioassay) of its infusions were also evaluated. Essential oils were isolated by hydrodistillation and analyzed by GC techniques. Only tetradecanoic acid, n-hexadecanoic acid, ethyl tetradecanoate and methyl oleate were detected in all samples. Pyrazines are probably produced during the roasting of the seeds. The infusions presented moderate antioxidant potentials that were highly correlated with their total phenolic and flavonoid contents, and were classified as non-toxic.
Keywords: husks, cocoa, teas.

O Brasil é um dos maiores produtores de cacau do mundo e, consequentemente, de cascas de amêndoas de cacau. Esse material é um resíduo da indústria de alimentos a partir do qual é possível produzir uma infusão aromática. O objetivo desse trabalho foi avaliar a composição química dos óleos essenciais das cascas das amêndoas de cacau provenientes dos estados da Bahia e do Pará. Os teores totais de compostos fenólicos (Folin-Ciocalteu) e de flavonoides ($AlCl_3$), a capacidade antioxidante (ensaio do DPPH) e a toxicidade (bioensaio com *Artemia salina*) das infusões produzidas a partir dessas cascas também foram avaliados. Os óleos essenciais foram isolados por hidrodestilação e analisados por técnicas de cromatografia gasosa. Apenas o ácido tetradecanóico, o ácido n-hexadecanóico, o etil tetradecanoato e o metil oleato foram detectados em todas as amostras. As pirazinas foram produzidas, provavelmente, durante a torrefação das sementes. As infusões apresentaram potenciais antioxidantes moderados altamente correlacionados com os teores totais de compostos fenólicos e de flavonoides e foram classificadas como atóxicas.
Palavras-chave: cascas, cacau, infusões.

1. INTRODUCTION

Brazil is one of the largest producers of cocoa (*Theobroma cacao*) in the world and, in this context, the Brazilian states of Bahia (BA) and Pará (PA) stand out. The state of Bahia produced approximately 122.568 tons of cocoa in 2019, followed by the state of Pará, accounted for the production of 116.110 tons of cocoa in the same year [1].

Cocoa seeds represent about 20% of the fruit's mass and are considered the major marketed products (after fermentation and drying) for food manufacturing. The cocoa seed consists of an almond and its husk (commonly known as forehead or cocoa bran) [2, 3]. Owing to the high acidity of these husks, the seeds can only be used to produce “cocoa butter” after removal of the husks. Therefore, these husks are an important residue of the cocoa industry [4].

Pieces of the almond are often found on the husks, making their composition quite variable. This variation in the composition of husks may also be associated with factors such as: cocoa origin, type of roasting, fruit ripening stage, etc [5]. The cocoa bran (husks) can be used as boiler

fuel, in the flavoring of craft beers, in fertilizer preparations and in animal feed production [4]. In the latter case, husks should be used carefully, since they contain the alkaloid known as theobromine, which can be toxic to some animal species [6].

Few studies have been developed to offer a better destination to this material, which has some nutritional value, expressive fat content, and several bioactive compounds [5, 7, 8]. Recently, a new proposal has emerged for the use of these husks: its use for the preparation of aromatic teas. However, there is virtually no information in the scientific literature on the toxicological potential, antioxidant capacity and chemical composition of the volatile and non-volatile fractions of this new beverage. This type of knowledge has the potential to encourage the use of this beverage, making the cocoa industry more sustainable, adding economic value to this industrial waste and allowing a more enjoyable, conscious and efficient use of this product as a potential nutraceutical agent.

Thus, this study aims to characterize the essential oil (volatile fraction) of the cocoa almond husks from the Brazilian states of Bahia (BA) and Pará (PA). Total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity (AC) and toxicity of its infusions were also evaluated.

2. MATERIAL AND METHODS

2.1. Samples

Samples of cocoa almond husks from the states of BA and PA were evaluated. These samples were supplied by Quetzal Chocolate de Origem (Bonsucesso, R.J., Brazil). For each origin, the wastes (cocoa almond husks), which had resulted from chocolate preparation process for three months, were collected, mixed and homogenized to produce enough material to allow for the development of all analyses.

2.2. Chemicals

Ethyl acetate (purity grade = 99.5%) was purchased from Aldrich (Milwaukee, WI, USA). The C_7-C_{26} alkane mixture used as a retention-index marker probe was obtained from Supelco (Bellefonte, PA, USA). All other reagents (Folin-Ciocalteu solution, $AlCl_3$ salt, gallic acid, rutin, and DPPH solution) were of analytical grade.

2.3. Essential Oil (Volatile Fraction) Analysis

A methodology similar to the one employed by other researchers [9, 10] was used to extract the essential oils from cocoa almond husks by hydrodistillation. This isolation process was carried out for 4 hours at a temperature of 100°C. The essential oils were removed from the Clevenger's apparatus by flushing the system with 10 mL of ethyl acetate. The residual water was eliminated from this organic extract with the aid of anhydrous sodium sulfate. After filtration, the solvent was removed from the essential oil by evaporation over a gas nitrogen flow. Essential oils were stored at a temperature of -18ºC until the beginning of the chromatographic analyses, which were carried out according to a previous approach [9], but with some modifications.

The oven was programmed as follows: the temperature was kept at 50ºC for one minute. Then, the temperature increased from 50ºC to 110ºC at a constant rate of 3ºC minute^{-1}. This latter temperature was maintained for one minute. Subsequently, the temperature increased at a rate of 2ºC minute^{-1} until it reached 200ºC (maintained for one more minute). Finally, the temperature increased to 230ºC at a rate of 10ºC minute^{-1}, and was kept at this level for thirty minutes. Injections of the essential oils were performed in a split ratio of 1:20.

Quantification of the compounds was based on the normalization technique using the GC/FID apparatus. On the other hand, the identification process was carried out with the aid of GC/MS equipment, comparing the mass spectra of the compounds with data available in the NIST12.lib

and NIST62.lib libraries. External standard compounds and modified Kovats indices [11] were also used to allow for the identification of the essential oil constituents. As a matter of prevention, a solvent (residual ethyl acetate) delay of two minutes was used during the GC/MS analysis.

2.4. Evaluation of the Teas Prepared from the Cocoa Almond Husks

2.4.1. Preparation of the Teas

An amount corresponding to 1 g of the cocoa almond husks was infused in 50 mL of boiling distilled water for 5 minutes with manual shaking. This extract was then cooled, filtered, and adjusted to 100 mL.

2.4.2. Total Phenolic and Flavonoid Content Assays

Total phenolic content (TPC) and total flavonoid content (TFC) of the teas were evaluated by the spectrophotometric methods of Folin-Ciocalteu and $AlCl_3$, respectively [9]. TPC was calculated using a calibration curve of gallic acid ($y = 44.198x$; $R^2 = 0.9947$), and the results were expressed as mg of gallic acid equivalents (GAE) L^{-1}. TFC was calculated using a calibration curve of rutin ($y = 0.0054x - 0.0991$, $R^2 = 0.9834$), and the results were expressed in mg of rutin equivalents (RE) L^{-1}.

2.4.3. DPPH Assay

Antioxidant activity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [9, 12]. $IC_{50(DPPH)}$ was calculated using the antioxidant curve produced after testing the teas at the following concentrations: 0.10 mg mL^{-1}, 0.30 mg mL^{-1}, 0.60 mg mL^{-1}, and 1.00 mg mL^{-1}. Rutin and gallic acid were tested as positive controls.

2.4.4. Artemia Salina Bioassay

The acute toxicological evaluation of the teas was carried out by the *Artemia salina* bioassay [9, 13]. Aliquots of the teas were directly added to the sea water to produce final solutions of 100, 250, 500, 1,000, 1,500, and 2,000 ppm. Triplicate experiments were conducted with each concentration. Mortality curves were plotted to establish the LD_{50} of the teas.

2.5. Statistical Analysis

Statistical analyses were carried out with the aid of the Graph Pad Prism 6.0 software. The parameters were submitted to the normality test of D'Agostino & Pearson. For those parameters that passed the normality test (TPC, TFC, $IC_{50(DPPH)}$, and LD_{50}), significant statistical differences between the groups were evaluated by using the parametric t test. For those parameters that did not pass the normality test (mean yields of the extraction process and the concentrations (%) of the compounds in the essential oils), the non-parametric Mann-Whitney test was performed to detect differences between the groups. In all of these analyses, the probability levels of $p < 0.05$ were considered to be statistically significant. Correlations between antioxidant capacity and TPC, and TFC were determined by using Pearson's Correlation Coefficient Test.

3. RESULTS AND DISCUSSION

3.1. Essential Oils from BA And PA Cocoa Almond Husks

The hydrodistillation process yielded small amounts of the essential oils obtained from the cocoa almond husks of both origins (see Table 1). There was no significant statistical difference ($p > 0.05$) between the mean extraction yield values of the samples originating from BA and those originating from PA. The low yields of the extraction processes of these essential oils should be considered as a limiting factor for the development of studies on the composition and phytotherapeutic potential of this kind of fraction (essential oil) from this natural product (cocoa almond husk). This may explain the lack of scientific studies published so far about the volatile fraction of this residue from the cocoa industry.

Table 1. Mean yields of the extraction process of the essential oils from the cocoa almond husks from BA and PA.

Samples	Mean yield (g of essential oil / 100 g of sample)
BA	0.14 ± 0.10
PA	0.07 ± 0.05

BA – Bahia; PA – Pará. The average values that were shown in this Table were obtained from triplicate analysis of the samples of each origin.

The volatile compounds found in the essential oils of BA and PA cocoa almond husks are shown in Table 2. This is the first time that the volatile fraction composition of this kind of matrix has been evaluated. The diversity of compounds of different chemical classes was high in the samples from both origins (see Table 2).

On the other hand, some chemical classes were exclusively found in BA samples (halogenated compounds, sulfur compounds, ethers and pyrazoles), while others only belonged to PA samples (epoxides, oxygenated monoterpenes and derivatives, oxygenated sesquiterpenes, and oxygenated dipertenes). Indeed, among the 75 identified compounds, 31 were found exclusively in group 1 (corresponding to the samples from BA), while 34 compounds were found only in group 2 (corresponding to the samples from PA). Clearly, there is a large difference in the essential oil composition of these two groups (BA and PA) (see Table 2). These variations in the composition of these essential oils are not unexpected and can be associated with several factors such as the genetic group in which the samples are classified, the time of year in which the harvest was performed, the time of collection (owing to the circadian cycle of the plants), the incidence of solar radiation, water availability, etc [14, 15].

Table 2. Chemical composition of the essential oils isolated from BA and PA cocoa almond husks.

Compounds	LRI1	LRI2	Avg ± SD (BA)	Avg ± SD (PA)
Non-terpenic compounds				
Fatty acids				
Isobutyric acid	755	793	nd	1.07 ± 1.51
3-Methyl-butanoic acid	836	834	nd	0.98 ± 1.39
2-Methyl-butanoic acid	848	811	nd	1.38 ± 1.75
Nonanoic acid	1259	1272	0.11 ± 0.08	nd
Dodecanoic acid	1566	1570	2.14 ± 0.16	0.50 ± 0.70
Tridecanoic acid	1623	1660	nd	0.71 ± 0.90
Tetradecanoic acid	1758	1769	1.61 ± 0.12	1.78 ± 0.78
Pentadecanoic acid	1848	1869	0.20 ± 0.05	0.18 ± 0.25
cis-9-Hexadecenoic acid	1947	1976	1.13 ± 0.04	nd
n-Hexadecanoic acid	1977	1968	24.27 ± 8.00	35.91 ± 2.0

Heptadecanoic acid	2050	2067	nd	0.86 ± 0.95
17-octadecynoic acid	2143	2165	14.33 ± 4.41	nd
Octadecanoic acid	2169	2167	1.11 ± 0.18	0.84 ± 1.18
Oleic acid	2130	2175	nd	3.35 ± 4.73
Linoleic acid	2137	2183	nd	2.89 ± 4.08
Alcohols				
2-Heptanol	882	879	0.33 ± 0.13	nd
2-Ethyl-1-hexanol	1003	995	1.67 ± 0.53	nd
3,7-Dimethyl-1,6-octadien-3-ol	1080	1082	0.21 ± 0.06	nd
2-Nonanol	1082	1078	0.10 ± 0.04	nd
3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	1539	1564	nd	0.10 ± 0.03
1-Octadecanol	2050	2050	nd	0.93 ± 1.31
(Z,Z)-9,12-Octadecadien-1-ol	2069	2069	nd	2.46 ± 3.32
(Z,Z,Z)- 9,12,15-Octadecatrien-1-ol	2072	2077	nd	0.34 ± 0.07
Aldehydes				
Benzaldehyde	922	982	0.46 ± 0.12	nd
Benzene acetaldehyde	1002	1002	nd	2.35 ± 1.46
Nonanal	1077	1104	0.11 ± 0.03	nd
5-Methyl-2-phenyl-2-hexenal	1486	1499	0.10 ± 0.03	0.50 ± 0.71
Ketones				
6,10,14-Trimethyl-2-pentadecanone	1778	1754	nd	0.68 ± 0.91
2-Heptadecanone	1874	1847	0.21 ± 0.01	0.04 ± 0.06
Oxacyclohexadecan-2-one	2149	2144	5.98 ± 3.54	nd
2-Hydroxy-cyclopentadecanone	2135	2158	nd	1.75 ± 2.47
Furan compounds				
1-(2-Furanyl)-ethanone	875	878	nd	0.07 ± 0.08
Tetrahydro-2,2-dimethyl-5-(1-methyl-1-propenyl)furan	1084	1068	0.18 ± 0.05	nd
Halogenated compound				
(Iodomethyl)benzene	1205	1208	0.12 ± 0.03	nd
Sulfur compound				
sec-Butyl-pentyl-disulfide	1353	1353	0.14 ± 0.08	nd
Epoxide				
cis-9,10-Epoxy-octadecan-1-ol	2054	2015	nd	0.57 ± 0.80
Esters				
1-methylethyl 2-methylpropanoate	755	756	nd	2.20 ± 2.91
2-methyl butanoate	848	811	nd	1.35 ± 1.70
2-phenylethyl acetate	1217	1259	0.68 ± 0.13	nd
Butyl benzoate	1359	1359	0.44 ± 0	nd
3-methylbut-2-enoic acid, 3,5-dimethylphenyl ester	1586	1570	nd	0.59 ± 0.83
Ethyl dodecanoate	1572	1580	0.32 ± 0.04	nd
Ethyl tetradecanoate	1778	1779	0.24 ± 0.01	0.75 ± 0.81
Methyl palmitate	1856	1878	nd	0.60 ± 0.69
Ethyl pentadecanoate	1882	1878	nd	0.10 ± 0.04
Ethyl palmitate	1921	1978	nd	5.53 ± 2.28
Isopropyl palmitate	2010	2013	27.67 ± 8.49	nd
Methyl oleate	2078	2085	0.11 ± 0.01	0.68 ± 0.66
Ethyl linoleate	2137	2141	nd	4.06 ± 1.74
Ethyl oleate	2147	2149	nd	4.43 ± 1.31
Ethyl octadecanoate	2178	2177	0.35 ± 0.01	0.20 ± 0.28
Ether				
8-Methoxy-10-methyl-7-azabicyclo[4.2.2]deca-2,4,7,9-tetraene	1331	1323	0.27 ± 0.05	nd
Hydrocarbons				
2-Propenylidene-cyclobutene	744	735	nd	0.57 ± 0.81
n-Propylbenzene	995	992	nd	2.42 ± 1.29
Pentadecane	1493	1512	0.13 ± 0.01	nd
Heneicosane	2096	2109	0.25 ± 0.01	nd
Tricosane	2294	2307	0.40 ± 0.02	nd
7-Butylbicyclo[4.2.1]nona-2,4,7-triene	1331	1316	nd	0.13 ± 0.11

1,5,5-Trimethyl-6-[(1E)-3-methyl-1,3-butadienyl]-1-cyclohexene	1368	1360	0.22 ± 0.05	nd
1,2,3,6,7,8,8a,8b-octahydro-4,5-dimethyl-biphenylene	1423	1443	0.20 ± 0.04	nd
1,2,3,4-tetrahydro-2-(1,1-dimethylethyl)-naphthalene	1447	1441	1.75 ± 0.37	nd
Pyrazines				
2,6-Dimethylpyrazine	887	894	nd	0.10 ± 0.11
2-Ethyl-5-methylpyrazine	970	994	0.4 ± 0.10	nd
2-ethyl-3,5-dimethylpyrazine	1048	1107	nd	0.66 ± 0.93
3-isopropyl-2,5-dimethylpyrazine	1131	1142	0.29 ± 0.11	nd
3-Butyl-2,5-dimethylpyrazine	1284	1306	0.24 ± 0.30	nd
2-(2-Methylpropyl)-3,5,6-trimethylpyrazine	1363	1355	0.09 ± 0.02	nd
Pyrazoles				
1,4-Dimethylpyrazole	799	804	0.14 ± 0.06	nd
1-vinyl-3,5-dimethylpyrazole	972	983	0.09 ± 0.02	nd
1-allyl-3,5-dimethylpyrazole	1049	1083	0.20 ± 0.25	nd
Terpenic compounds				
Oxygenated monoterpenes and derivatives				
Myrcenol	1051	1064	nd	0.42 ± 0.59
Neryl acetone	1420	1420	nd	0.16 ± 0.10
Oxygenated sesquiterpene				
Ar-Tumerone	1586	1611	nd	0.54 ± 0.76
Farnesyl acetate	1835	1834	nd	0.72 ± 0.64
Oxygenated diterpene				
Phytol	2096	2104	nd	0.71 ± 1.00
Total			88.86	87.99

LRI1 – modified Kovats index calculated using C_7-C_{26} alkanes [11]; LRI2 – data available in the NIST libraries of the GC/MS software; Avg – average value; SD – standard deviation; The average values that were shown in this Table were obtained from triplicate analysis of the samples of each origin; nd – not detected, BA – Bahia; PA – Pará.

Only ten compounds were simultaneously found in BA and PA essential oils. In this context, tetradecanoic acid, n-hexadecanoic acid, ethyl tetradecanoate, and methyl oleate were detected in all samples analyzed (three samples of each origin). There are no significant statistical differences between BA and PA essential oils for the concentrations of these four above mentioned compounds ($p > 0.05$). Dodecanoic acid, pentadecanoic acid, octadecanoic acid, 5-methyl-2-phenyl-2-hexenal, 2-heptadecanone, and ethyl octadecanoate were also found in the husks of both origins, but not in all the samples analyzed. The major compounds of the BA essential oils were isopropyl palmitate, which is used in the cosmetic industry in the production of creams and moisturizers [16]; n-hexadecanoic acid, also known as palmitic acid, and 17-octadecynoic acid. Together, these three compounds account for 66.27% of the total content of these BA essential oils. With respect to PA samples, n-hexadecanoic acid, ethyl palmitate and ethyl oleate were classified as the major compounds; together, they account for 45.87% of the total content of their essential oils.

The pyrazines found in the essential oils of both groups (BA and PA) are indicative that these samples were subjected to heat treatment, probably during the roasting of the seeds [17]. The content of alkyl pyrazines is proportional to the degree of roasting. This content increases to a certain limit as the roasting temperature increases. When this roasting is prolonged, degradation of these pyrazines begins to occur. Pyrazines can be generated directly by the Maillard reaction, Strecker degradation or by hydroxy-amino acid pyrolysis. Some pyrazines markedly contribute to the sensory characteristics of the foods in which they are present. 2,6-Dimethylpyrazine, for instance, has a characteristic ether odor, 2-ethyl-5-methylpyrazine has a grass odor, and 2-ethyl-3,5-dimethyl-pyrazine often plays an important role in roasted coffee and coffee beverage aromas [17].

3.2. Teas Produced from BA and PA Cocoa Almond Husks

The mean TPC of BA teas was (33.40 ± 2.19) mg of gallic acid equivalents (GAE) g^{-1} of sample, while the mean TPC value of PA teas was (26.70 ± 12.83) mg GAE g^{-1} (see Table 3). There were no statistical differences ($p > 0.05$) between BA and PA for this parameter. To our knowledge, there was no information to date about the TPC of infusions produced from cocoa almond husks. However, when comparing these cocoa almond husk teas with infusions usually consumed by the Brazilian population, such as yerba mate and green tea, it can be seen that the cocoa almond husk teas have low levels of phenolic compounds. The mean TPC value of yerba mate infusions was calculated as 58.37 mg GAE g^{-1} [18]. In the analysis of green tea, the TPC values ranged from 30.13 to 63.99 mg GAE g^{-1} [19]. On the other hand, the mean TPC value of chamomile teas was estimated as 12.90 mg GAE g^{-1} [18], a value approximately 2.3 times lower than the one found for the cocoa almond husk infusions analyzed in the present study.

Table 3. Phenol and flavonoid contents, antioxidant capacity and toxicological potential of teas produced from BA and PA cocoa almond husks.

Analyses	BA (Avg ± SD)	PA (Avg ± SD)
TPC (mg GAE g^{-1})	33.40 ± 2.19	26.70 ± 12.83
TFC (mg RE g^{-1})	25.41 ± 2.17	22.34 ± 15.39
Antioxidant capacity ($IC_{50(DPPH)}$) (μg mL^{-1})	838.72 ± 339.13	1,235.35 ± 635.96
ASB (LD_{50}) (ppm)	6,739	1,497

BA - Bahia; PA - Pará; TPC – total phenolic content; GAE – gallic acid equivalent; TFC – total flavonoid content; RE – rutin equivalent; IC_{50} – the concentration of an antioxidant which reduces the free radical *DPPH•* by about 50%; ASB – *Artemia salina* bioassay; LD_{50} – lethal dose capable of killing 50% of the tested population; Avg – average value; SD – standard deviation. The average values that were shown in this Table were obtained from triplicate analysis of the samples of each origin.

The TFC of the tested cocoa almond husk infusions can also be seen in Table 3. With respect to this parameter, the samples from BA do not differ statistically ($p > 0.05$) from the samples from PA. TFC of green teas ranged from 9.41 to 28.62 mg of rutin equivalents (RE) g^{-1} of sample [19]. The TFC values found for the cocoa almond husk teas analyzed in the present study are within this range found for green teas.

The potential of cocoa almond husk infusions to scavenge the free radical of DPPH was expressed as the concentration of these infusions required to neutralize 50% of the DPPH radical (IC_{50} value expressed in μg mL^{-1}) (see Table 3). An extract with high potential to neutralize free radicals (high antioxidant potential) should have a low $IC_{50(DPPH)}$ value. Based on the IC_{50} values determined by the DPPH assay, the cocoa almond husk infusions from both origins (BA and PA) have similar antioxidant potentials, as there was no significant statistical difference ($p > 0.05$) between these groups in this regard.

As there are no studies about the antioxidant potential of cocoa almond husk infusions using the DPPH radical assay, the data collected in the present study were compared to those available in the literature for other teas (e.g.: yerba mate, fennel and chamomile teas). The yerba mate infusions showed a mean IC_{50} value estimated as 250 μg mL^{-1} [17]. In another study, a mean IC_{50} value for the teas of this kind of herb was estimated as being (12.0 ± 0.2) μg mL^{-1} [20]. Both results indicate that yerba mate tea has a greater antioxidant capacity than the cocoa almond husk infusions. On the other hand, the fennel and chamomile teas were considered to be weaker antioxidants than these husk infusions, with mean IC_{50} values estimated as 2,750 μg mL^{-1} and 2,100 μg mL^{-1}, respectively [18].

The mean $IC_{50(DPPH)}$ of the cocoa almond husk infusions was estimated as 1,037.04 μg mL^{-1}. This IC_{50} value is 11.2 times higher than the one found for rutin (IC_{50} = 93 μg mL^{-1}) and 122 times higher than the one calculated for gallic acid (IC_{50} = 8.5 μg mL^{-1}), both of which were used as positive controls. Thus, these husk infusions must also be considered to be weaker than rutin and gallic acid with respect to their antioxidant powers.

The results indicated a strong correlation between TPC, TFC, and the antioxidant capacity of husk infusions from BA and PA (see Table 4). The correlation coefficients (r) shown in this Table

were negative, and their absolute values can be considered to be high, indicating that the husk infusions with higher amounts of phenolic or flavonoid compounds present lower IC_{50} values and, consequently, better antioxidant capacities. This finding suggests, for instance, that phenolic and flavonoid compounds are relevant for the antioxidant activity of these infusions.

Table 4. Pearson's correlation analysis.

Samples	$IC_{50(DPPH)}$ x TPC	$IC_{50(DPPH)}$ x TFC
PA	r = -0.888	r = -0.918
BA	r = -0.920	r = -0.926

PA - Pará; BA - Bahia; TPC – total phenolic content; TFC – total flavonoid content; $IC_{50(DPPH)}$ – the concentration of an antioxidant which reduces the free radical *DPPH•* about 50%; r = Pearson's correlation coefficient.

According to the brine-shrimp lethality assay, the infusions produced from the cocoa almond husks of BA presented a mean LD_{50} value of 6,739.28 ppm, while the PA infusions showed a mean LD_{50} value of 1,496.55 ppm (see Table 3). In the *Artemia salina* bioassay, LD_{50} values upper than 1,000 ppm must be related to non-toxic extracts [13, 21]. Therefore, these teas from BA and PA were considered to be non-toxic beverages.

4. CONCLUSIONS

The profiles of the essential oils (volatile fractions) of the cocoa almond husks of BA and PA were partially elucidated. There was great diversity of compounds of different chemical classes in the samples from both origins, with fatty acids and its esters being the major compounds. The infusions produced from these husks presented a moderate antioxidant potential and they are non-toxic. This kind of infusion can be considered as an alternative source of natural antioxidants. The marketing of these husks to produce an aromatic, tasty, and bioactive home tea could allow the reduction of the environmental contamination exerted by the cocoa industry.

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