High performance liquid chromatography analysis of tea-infused alcohol - a potential substance of abuse in Nigeria

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Abstract

In Nigeria, tea infused with locally distilled gin is often sold as a tea-alcohol blend and consumed as a recreational beverage, which has been linked to substance abuse. The purpose of this research is to assess the effects of solvents on the phytochemical compositions of tea-infused gin and hot water tea infusion. A commercial tea was extracted with locally distilled gin as well as hot distilled water. After drying, the extracts were subjected to HPLC fingerprinting analysis at a UV detection wavelength of 254 nm. HPLC analysis showed that hot water extracted 28 metabolites and locally distilled gin extracted 24 metabolites. Both extracts had two significant main peaks with similar retention times. The results of this study show that hot water tea contains similar phytocomponents as alcoholic blended tea. This implies that aqueous tea infusions and tea-alcohol blends may both provide health benefits from tea. Those with alcohol addiction and alcohol-related health problems should avoid tea-alcohol mixtures and enjoy aqueous tea infusions instead.

Key words: Tea, alcohol, tea-infused gin, aqueous tea infusion, HPLC

Introduction

Tea is considered the second most consumed beverage after water and is known to be rich in phytochemicals with antioxidant properties (Ochanda *et al*., 2016). Tea is made from the leaves of the *Camellia sinensis* plant, which originated in Southeast Asia and now cultivated in more than 30 countries around the world. The green, black and oolong teas are the result of different manufacturing processes of the tea plant (Hayat *et al*., 2015).

Tea is known to contain polyphenols and other components that may reduce the risk of developing chronic diseases such as cancer, cardiovascular disease, arthritis, and diabetes. Tea consumption has been shown to be useful in preventing many debilitating human diseases including cardiovascular and metabolic diseases (Khan and Mukhtar, 2013).

Alcohol, on the other hand, is part of many cultural, religious, and social practices and provides perceived pleasure for many consumers. However, harmful alcohol use is a global problem causing millions of deaths, including hundreds of thousands of young

people. It is not only a causative factor in many diseases, but also a precursor to injury and violence (WHO, 2011; WHO, 2018).

Alcohol consumption is the world's third leading cause of disease and disability, with middle-income countries bearing the greatest risk (WHO, 2011). According to the WHO (2018), Nigeria (along with Gabon, Seychelles, and Equatorial Guinea) has the highest per capita consumption of alcohol (10 liters or more) among people aged 15 and older in the WHO African Region. The prevalence of alcohol use disorders (harmful alcohol use and alcohol dependence) in Nigeria is estimated to be 0.6% of the total adult population (15+ years) (WHO, 2018).

The hazardous and harmful use of alcohol is a major global contributing factor to death, disease, and injury through health impacts such as alcohol dependence, liver cirrhosis, cancers, and injuries; as well as dangerous actions of intoxicated people, such as drink-driving and violence; or through the impact on fetus and child development. Considering the estimated beneficial impact of low levels of alcohol use on some diseases in certain population groups, harmful alcohol use causes approximately 2.5 million deaths per year, with a net loss of 2.25 million lives (WHO, 2011).

Alcohol consumption and metabolism generate acetaldehyde and free radicals in the body, leaving the liver vulnerable to damage from these by-products (Ochanda *et al*., 2016). To reduce intoxication, alcoholic beverages are often accompanied by non-alcoholic beverages such as herbal infusions, tea, and carbonated beverages (Li *et al*., 2014).

Tea which is normally consumed by soaking in hot or cold water, is now consumed as gin-tea blends. This tea-alcohol mixture has become popular beverage in Nigeria. Locally produced gins are a common alcoholic beverage in Nigeria that are made by distilling the fermented sap of raphia palms (*Raphia hookeri*), coconut palm (*Cocos nucifera*), and oil palm (*Elaeis guineensis*) (Idonije *et al*., 2012). These locally distilled gins are sold in street shops in a variety of blends, including tea-gin infusions. Green and black teas are estimated to contain 35 mg and 70 mg of caffeine per 180 ml respectively.

Li *et al.* (2014) pointed out that while some beverages should not be consumed after excessive alcohol consumption, others may be potential dietary supplements for the prevention and treatment of problems associated with excessive alcohol consumption. Combining tea and alcohol is believed to have some benefits (Ding et al., 2022; Ochanda *et al*., 2016; Li *et al.*, 2014), but some potential risks have been reported (Hayat *et al*., 2015). Consuming alcohol and caffeine are known to produce overlapping effects (Cappelletti *et al.*, 2015). The severity of the risks associated with tea-alcohol combinations varies depending on factors such as the type and amount of alcohol consumed, the type of tea consumed, and the individual's health conditions.

The purpose of this research is to assess the effects of solvents on the phytochemical compositions of tea-infused gin and hot water tea infusion. It is hoped that this study will provide new information on whether or not mixing alcohol with tea is necessary, as well as the potential risks of these tea-alcohol combinations.

Materials and Methods

Sample collection

The study was conducted using a commercially available tea from Eke Awka Market, Awka, and a locally produced gin from Atani, Ogbaru Local Government Area, both in Anambra State, Nigeria.

Extraction of Aqueous Tea Infusion and Tea-infused Gin

Four tea bags with 9.4 g net weight of the tea were placed in a conical flask and 65 ml of

distilled water (for aqueous tea infusion) or gin (for tea-infused gin) was added. For the aqueous tea infusion, the flask was covered with aluminium foil and the mixture in the flask was boiled for 5 min on a heated magnetic stirrer (100ºC) with constant agitation. For the tea-infused gin, the conical flask was covered with aluminium foil and the mixture allowed to stand for 24 h with constant agitation. Using Whatman filter papers (No. 1), the mixtures were then filtered into respective beakers. The filtrates (extracts) were collected and concentrated in a water bath at 50℃ for 24 h. The percentage yield of the extracts was also calculated and recorded.

HPLC Analysis

The HPLC analysis was carried out on a Shimadzu HPLC system by preparing 10 mg/mL solution of the extracts, weighing 10 mg of each sample, dissolving it in 0.5 mL of 50 % aqueous methanol solution and then making up to 1 mL with 50 % aqueous methanol solution. The final solution was filtered with a 0.45 µm Millipore membrane filter prior to use. An aliquot of 10 µl of each sample solution was injected into the HPLC system for analysis. A binary gradient elution system composed of 0.1 % formic acid in HPLC grade water as solvent A, and HPLC grade acetonitrile as solvent B was applied for the fingerprint analysis with the gradient elution as follows: 10 mins,15% of B; 20 min, 30% of B; 35 min, 50% of B; 40 min, 25% of B; 50 min, 15% of B; 60 min, 15% of B. The mobile phase flow rate was 0.6 mL/min, and the column [Shimadzu VP-ODS 5 µm and dimensions (150 x 4.6 mm)] was maintained at 40℃. The UV detection wavelength was set at 254 nm (Fatokun *et al*., 2020).

Data analysis

Data analysis was performed using Microsoft® Excel version 16.72 (23040990). Using two-way analysis of variance (ANOVA) at 95% confidence level, the peak areas (mAU) of the constituents of the "aqueous tea infusion" detected by HPLC analysis were compared to those of the "tea-infused gin". Indicator of statistical significance is $P \leq 0.05$.

Results and Discussion

Table 1: *Percentage Yield of Hot Water and Gin Extracts of Tea*

Extract	Initial Weight of Tea (g)	Final Weight of Extract (g)	Percentage Yield (%)
Hot Water	9.4	1.4	14.9
Gin	9.4	2.7	28.7

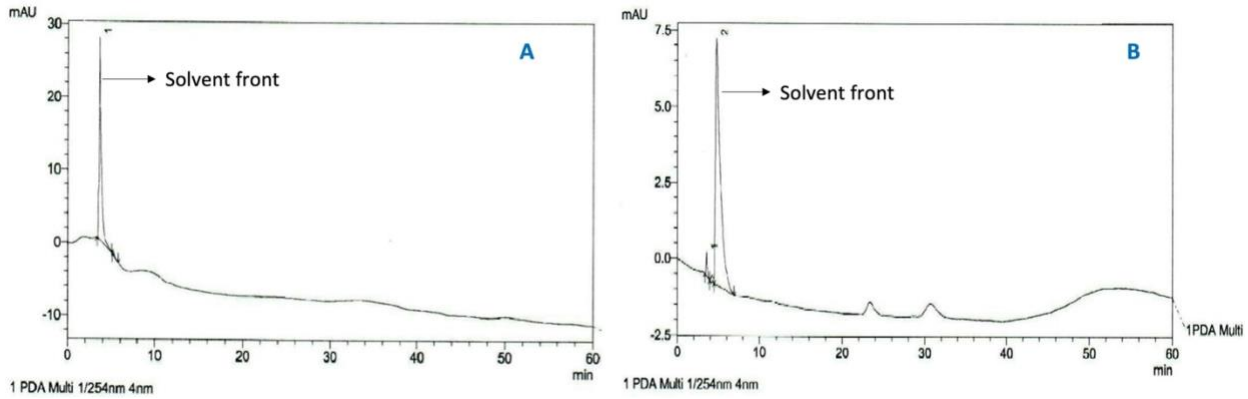


Fig 1: *HPLC Chromatogram of the extraction Solvents*

The extraction solvents were subjected to HPLC analysis as a control to confirm the quality of the extraction solvent and to avoid the detection of impurities or substances that may not have arisen from the extracts but from an adulterated solvent. Fig 1 shows the HPLC chromatograms of the extraction solvents, distilled water (A) and gin (B), with solvent fronts at 3.581 min and 4.744 min, respectively.

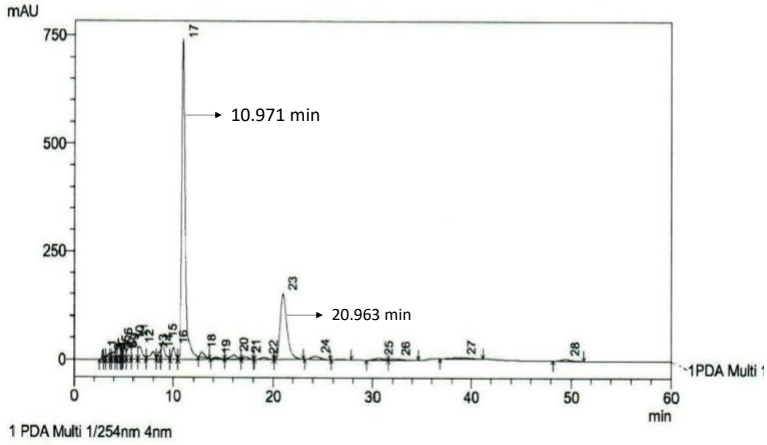


Fig 2*: HPLC Chromatogram of the Hot Water Tea Infusion*

It can be observed in Fig 2 that the HPLC chromatogram of aqueous tea infusion shows the presence of 28 compounds, with two major compounds: compound 17 (RT=10.971 min, PA=17981358 mAU) and compound 23 (RT=20.963 min, PA=7071745 mAU).

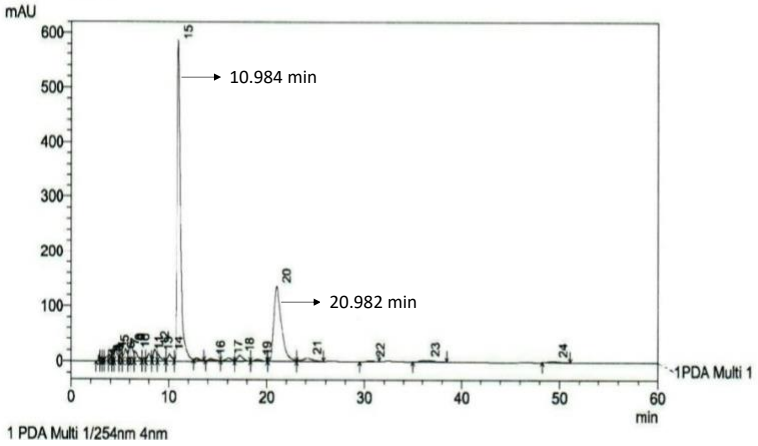


Fig 3: *HPLC Chromatogram of the Tea-Infused Gin*

In Fig 3, the chromatogram for the analysis of the tea-infused gin indicated the presence of 24 compounds with two major compounds: compound 15 having a RT=10.984 min, peak area = 15115236 mAU) and compound 20 (RT=20.982 min, PA=7100146 mAU).

Table 2: *Result of HPLC Analysis of Aqueous Tea Infusion and Tea-Infused Gin*

Aqueous Tea Infusion			**Tea-Infused Gin**		
Peak no.	**Retention Time (min)**	**Area (mAU)**	**Peak no.**	**Retention Time (min)**	**Area (mAU)**
1	2.829	130039	-	-	-
*2	3.456	227993	1	3.275	49915
*3	3.692	159208	2	3.700	192241
*4	3.967	273280	3	3.926	174149
*5	4.250	359217	4	4.241	196386
*6	4.481	647898	5	4.506	516971
7	4.732	132934	-	-	-
8	4.879	134266	-	-	-
*9	5.134	443552	6	5.181	214539
*10	5.622	837887	7	5.617	492518
*11	6.060	1429553	8	6.059	365505
12	6.614	819133	-	-	-
-	-	-	9	6.204	584860
-	-	-	10	6.619	424082
*13	8.000	637959	11	8.017	325554
*14	8.570	317802	12	8.575	456796
*15	8.957	920556	13	8.932	278069
*16	10.022	681041	14	10.056	328796
*†17	10.971	17981358	15	10.984	15115236
18	12.812	322655	-	-	-
*19	14.287	101294	16	14.310	129199
*20	16.068	404906	17	16.097	188618
*21	17.264	194227	18	17.274	357026
*22	18.988	192976	19	19.041	112517
*†23	20.963	7071745	20	20.982	7100146
*24	24.206	392959	21	24.186	395990
*25	30.580	260813	22	30.638	72454
*26	32.344	252040	23	36.336	233134
*27	39.015	375775	24	49.369	163808
28	49.386	243848		-	-
***p-value**	0.03				
†p-value	0.51				

In Table 2, the HPLC data of the respective phytoconstituents of the "aqueous tea infusion" were aligned with those of the "tea-infused gin" based on RT. Both tea extracts showed similar HPLC fingerprints, and their major phytoconstituents were identified as peaks 17 and 23 (aqueous tea infusion) and peaks 15 and 20 (tea-infused gin). There was no significant difference ($\dagger p \geq 0.05$) in the peak area of these major components in both tea extracts. In addition, a general comparison was made for similar phytoconstituents (with similar RT) detected in the extracts of the aqueous tea infusion and the tea-infused gin. The PA of similar phytoconstituents in one extract differed significantly from the other ($*p \leq 0.05$).

It is well understood that chromatographic peak heights and areas are proportional to concentration. In principle, quantitative analysis can be performed on the basis of either peak height or peak area (Guiochon and

Guillemin, 1988; Elpa *et al.,* 2020). PA in this study is therefore a measure of the concentration of the compound it represents.

Table 2 shows the variation in the number of phytocompounds detected in tea extracts. Twenty-four (24) phytocompounds were detected in the tea-infused gin, while 28 phytocompounds were detected in the aqueous tea infusion. Although the amount of extract recovered after alcohol extraction was higher (% yield= 28.7) than with hot water (% yield= 14.9) (Table 1), hot water extracted more of the major compounds (as indicated by higher peak areas), as well as five (2) additional compounds (peaks 7, 8, 12, 18, and 28) that were not detected in the alcohol extract (Table 2). The alcohol extract however showed 2 compounds (peaks 9 and 10) that were not detected in the aqueous extract.

Phytocompounds detected by HPLC analysis of the extracts of the tea-infused gin and aqueous tea infusion showed that several of these compounds eluted in the same order with similar retention times, suggesting they could be closely related. The two major peaks (compounds) in the tea-infused gin (peaks 15 and 20) and aqueous tea infusion (peaks 17 and 23). The peak areas for these major compounds were higher in the aqueous tea infusion, than in the tea-infused gin. However, they were not statistically different (†$p \geq 0.05$) (see Table 2).

The results of this study show that hot water tea contains similar phytocomponents as alcoholic blended tea. This implies that there is no advantage in tea-alcohol blends. Those with alcohol addiction and alcohol-related health problems should avoid tea-alcohol mixtures and enjoy aqueous tea infusions instead.

Tea consumption after alcohol consumption is believed to be generally beneficial to health because of their ability to accelerate ethanol metabolism and prevent liver injuries caused by alcohol. Tea contains polyphenols, which are potent antioxidants that boost antioxidant levels in the blood, reducing inflammation, damage, and abnormal proliferation in animal cells.

Although it is believed that tea and alcohol mixtures are beneficial and capable of reducing alcohol toxicity, some reports have highlighted the possibility of harm when tea is consumed with alcohol. According to Hayat *et al*. (2015), the harmful effects of tea overconsumption (black or green) are primarily due to its caffeine content, as well as other factors such as the presence of aluminium and the effects of tea polyphenols on iron bioavailability. Caffeine has been linked to nervousness, restlessness, tremors, palpitations, sleep disorders, vomiting, diarrhea, headaches, epigastric pain, and tachycardia, making it unsuitable for patients with heart conditions or major cardiovascular problems (Hayat *et al.,* 2015). According to studies, mixing alcohol with caffeinated beverages increases the desire to drink alcohol. Caffeine has been found to mask the effects of alcohol in a dose-dependent manner, thereby increasing the amount of alcohol consumed (Marczinski *et al.*, 2016). Because the effects of various beverages on alcohol dehydrogenase and aldehyde dehydrogenase activity differ, it is critical that information on the effects of non-alcoholic beverages on alcohol metabolism be provided. This information is especially important for nutritionists and the public to reduce the harm caused by excessive alcohol consumption (Li *et al.,* 2014).

Many people can responsibly enjoy alcohol without becoming addicted or suffering negative consequences. Additionally, it is understandable that moderate alcohol consumption (within recommended limits) and moderate tea consumption are generally considered safe for most healthy people. Tea-alcohol blends can provide certain benefits and unique flavors when consumed responsibly

and in moderation. However, it is important for people to consider their health status, alcohol tolerance, and possible drug interactions before mixing tea and alcohol.

Conclusion

The results of this study show that hot water tea contains similar phytocomponents as alcoholic blended tea. This implies that aqueous tea infusions and tea-alcohol blends may both provide health benefits from tea. Those with alcohol addiction and alcohol-related health problems should avoid tea-alcohol mixtures and enjoy aqueous tea infusions instead. Given the negative effects of tea and alcohol on the health of individuals with predisposing health complications, excessive consumption of tea and alcohol blends exposes those individuals to the health risks resulting from the overlapping effects of tea and alcohol. It is hoped that this study will help nutritionists and the general public understand the potential risks of tea and alcohol blends and work towards reducing the problems associated with excessive alcohol consumption.

Conflict of Interest

The authors wish to declare that there is no conflict of interest.

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