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PHYTOCHEMICAL COMPOSITION OF COCOA TREE (*THEOBROMA CACAO*) ROOTS AND THEIR POTENTIAL APPLICATION IN ALCOHOLIC BITTERS

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Abstract

Most research reports on the phytochemical constituents of cocoa are on the bean and powder with very little on the importance of the vegetative parts of the cocoa tree. To identify and promote the utilization of these tree parts, the study evaluated the chemical components of cocoa roots and their application in alcoholic bitters production. Dry cocoa roots were screened for phytochemicals including volatile organic compounds (VOCs). In an attempt to develop alcoholic bitters, dry cocoa roots were soaked in two alcoholic solutions with concentrations 85 and 43% (v) using three alcohol/root ratios of 9:1, 8:2 and 7:3 for each concentration. A total of 8 phytochemicals which included alkaloids, tannins, cardiac glycosides as well as 60 VOCs such as lauryl alcohol and undecanoic acid were identified in the roots. Physicochemical analysis of the bitters revealed no significant differences among the bitters in terms of pH, Specific gravity (SG), and Brix for each alcohol solution. Sensory analysis revealed significant differences in terms of colour, brightness and bitter taste among experimental products developed ($p < 0.05$). However, the differences in their aromatic flavours were insignificant with 43% alcoholic bitters and 7:3 alcohol/root ratio having the highest acceptability score of 6.72. With an LD50 above 5000 mg/kg (b.w.), cocoa tree roots have the potential of being utilized in the beverage industry.

Introduction

Phytochemicals are bioactive and naturally occurring compounds found in plants. They protect plants from diseases and damages from environmental stress such as drought and pathogenic attack. They are believed to possess therapeutic properties useful for treatment of various ailments such as respiratory and urinary complications, hepatic and cardiovascular diseases (Prior and Cao, 2000). Phytochemicals are deposited in the different parts of plants, including the roots, stems, leaves, flowers, fruits and seeds (Shi et al., 2022). They are responsible for the colour, aroma and flavour of plants. They comprise primary metabolites such as carbohydrates, amino acids, ethanol and organic acids (Kumar et al., 2017) as well as secondary metabolites including volatile organic compounds (VOCs) (Siddiqui and Moid, 2022).

Cocoa fruit is made up of a husk filled with sweet, whitish mucilage surrounding seeds (beans). The beans contain appreciable amounts of various phytochemicals such as phenols, tannins, steroids and flavonoids (Kim et al., 2011). Ishaq and Jafri (2017) selected and studied about 73 research articles and reviews on the biomedical importance of cocoa. These articles gave authentic information on the nutritional, phytochemical and biological activities of cocoa. Most of the researches were done using extracts from the cocoa bean, cocoa powder and chocolate. They concluded that flavanols in cocoa bean extracts have high antioxidant activity and are useful for skin health. Studies on anti-cancer activities of polyphenols from non-edible cocoa plant parts such as cocoa leaf, bark, husk and shell revealed that the cocoa leaf polyphenols presented the highest antiproliferative activity against breast cancer cell line (Ishaq and Jafri, 2017). Nwokonkwo and Okeke (2014) have also studied the chemical constituents and biological activities of extracts from cocoa stem bark and reported on the presence of seven different phytochemicals in the stem extract. They found out that the extract was active against four human pathogens, Escherichia coli, Pseudomonas aeruginosa, Streptococcus pnemoniae and Staphylococcus aureus. However, not much has been done with the roots of the cocoa tree despite the fact that it is known to be traditionally chewed to treat common colds, cough and chest problems. Therefore, the need for the assessment of phytochemicals in cocoa tree roots concerning their application is important.

Extraction of plant materials using alcohol dates back to the Hippocratic wine of the Greeks (Tonutti and Liddle, 2010). These extractions were mostly for medicinal purposes and a typical example is Stoughton's Great Cordial Elixir, an alcohol based herbal extraction using gentian roots in 1690 (Johnson et al., 2015). This is the closest ancestor to what is termed bitters today. The production of bitters started in 1800s (Parsons, 2011) and now several types are being produced commercially with many of them being used to add flavour to alcoholic cocktail drinks. Recently, there has been a proliferation of alcoholic herbal bitters on the Ghanaian market (Kyeremeh et al., 2013). With the current interest in producing new products from unusual raw materials that enable acquisition of different flavors to attract new markets, several researches have been focused in finding potential raw materials for the production of alcoholic beverages. Recent works have also shown the presence and appreciable amounts of phytochemicals in the roots of medicinal plants (Benarba and Pandiella, 2018). Thus, in order to diversify the uses of the cocoa tree, this work was aimed at identifying phytochemicals in the roots and their potential application in bitters production. Particularly, roots from unproductive trees which are normally cut down and destroyed during rehabilitation of old cocoa farms could serve as a source of raw materials for bitters production.

Materials and Methods

Preparation of cocoa roots

Roots were obtained from unproductive cocoa trees in old experimental plots which were undergoing rehabilitation at the Cocoa Research Institute of Ghana (CRIG). After cutting down of the trees, their roots were dug out and cut into small pieces. These were then washed with clean running water and dried in the open air for 5 days. The dried roots were collected and packaged in transparent polyethylene bags and stored under room temperature. Samples of dry cocoa roots were then milled into powder for experimental purposes.

Determination of total phenol content

This was done using the Folin-Ciocalteau assay. Crude methanolic extract of dry cocoa root powder was diluted 10 times with distilled water, then 1 ml Folin-Ciocalteau reagent was added. The mixture was vortexed and left to stand for a few minutes, after which 1 ml of sodium carbonate (7.5% w/v) was added and incubated for 30 mins. The absorbance of the mixture was read at 760 nm using a Cary 60 UV-VIS spectrophotometer. A standard calibration curve for gallic acid was constructed and used to determine the total phenol concentration in the cocoa root extract. This was done in three replicates.

Phytochemical screening of dry cocoa roots

Extracts from powdered cocoa roots were screened for the presence of phytochemicals using standard coloration tests (Sasidharan et al., 2011). Chemicals screened for were flavonoids, tannins, alkaloids, saponins, steroids, reducing sugars, anthraquinone, cardiac glycosides, phenol, phlobatannin, terpenoids and volatile oil.

Qualitative analysis of volatile organic compounds (VOCs) in dry cocoa roots

Extraction of VOCs in dry cocoa roots using different solvents

Two types of extraction solvents, polar and non-polar, were used. The polar solvents used were absolute ethanol, 70% ethanol in water (70:30), 50% ethanol in water (50:50), absolute methanol and 80% methanol in water (80:20). For each solvent, 10 g of the powdered sample was taken into 250 ml conical flask and 100 ml solvent was added. Maceration was done for 72 hours after which the solution was filtered through a cheese cloth and then filtered again through Whatman no. 41 filter paper. This was followed by taking 10 mL of the filtrate and adding to 10ml hexane in a separating funnel. The mixture was agitated for about 5 minutes and the organic layer (hexane) separated into another flask. Anhydrous sodium sulfate was then added to get rid of any traces of water in the solution, after which 2 mL of each extract was taken into GC-MS vials for analysis.

The non-polar solvents used were hexane and acetonitrile, with 1 g of sample weighed into 10 ml of each solvent and microwaved for 2 minutes. This was followed by filtering through cheese cloth and no. 41 filter paper. Then 2 mL of the filtrate was taken into GC-MS vials for analysis. These extractions were done in two replicates.

Detection of VOCs through GC-MS analysis

This was done using a Shimadzu GC-MS 2010 Plus equipment with a Varian VF-5MS column, with the dimensions of 30 m in length x 0.25mm in diameter x 0.25µm in thickness of film. Dry cocoa root extracts from the various solvents were injected into the GC-MS using pure helium gas as the carrier at a constant flow rate of 1 ml/min. The oven temperature was maintained at 60 oC for 2 minutes and then was raised to a final temperature of 300 oC at a rate of 10^{o}C/min. The injector temperature was maintained at 260^{o}C and the extracts were auto injected at a volume of 1µl with a split ratio of 30:1 for the detection VOCs. The detector was operated at an electronic impact mode of 70 eV. GC-MS library version used was NIST 2020 with a match score above 80%.

Development of cocoa root bitters

Production of alcohol

Cocoa pulp juice was collected from fresh beans using wooden fermentation boxes. The juice was allowed to undergo spontaneous fermentation after being naturally inoculated with wild yeasts. After 4 days of fermentation, the juice was distilled into 85% (v) alcohol (ethanol) using a Jacob Carl J.C. Super 300 fractional distillation unit. A portion of the alcohol obtained was diluted with distilled water to 43% (v) based on protocols developed at CRIG for alcoholic beverage production.

Bitters preparation

Two bitters preparations were made using 85% and 43% alcohol. This was done by soaking dry cocoa roots in the two alcoholic solutions for 72 hours, using three alcohol/root (A/R) ratios of 9:1 (5 kg alcohol:0.5 kg roots), 8:2 (5 kg alcohol:1.0 kg roots) and 7:3 (5 kg alcohol:1.5 kg roots). A total of six (6) experimental products were obtained.

Determination of physicochemical characteristics of cocoa root bitters

The specific gravity (SG) of the bitters obtained were determined using a hydrometer, pH and brix were measured using a Mettler Toledo FE20/EL20 pH meter and a hand-held ATC refractometer respectively. These measurements were repeated for 85 and 43% alcoholic solutions without cocoa roots to serve as standards. The total soluble solids (TSS) were also determined by drying samples of the bitters in a vacuum oven for 2 hours at 70 oC. All determinations were done in triplicates.

Identification of microorganisms in bitters

Identification of Escherichia coli, Salmonella spp. and Staphylococcus spp. was done using methods described by AOAC International (1995) and American Public Health Association (1998).

Acute toxicity test

Twelve Institute of Cancer Research (ICR) male mice divided into two groups were used for the toxicity test. They were fed with food and hygienic water and their initial weights measured (20-25 g). Samples of the cocoa root bitters were freeze-dried and a limit dose of 5,000 mg per kg body weight (b.w.) was administered to the two groups of mice orally. The dose was prepared by dissolving the freeze-dried root extract in water. A control group was given water. The mice were then observed continuously for 14 days for changes in movement, appetite, water intake, salivation, diarrhea, and urination.

The above two sections were conducted at the Centre for Plant Medicine Research, and accredited institution for toxicity determinations.

Sensory analysis

This was done by presenting a set of coded samples of bitters to a panel of thirty (30) untrained panellists. They were then asked to rank the samples in order of intensity for appearance, taste and aroma on 9-point hedonic scale. In terms of appearance, colour and brightness were the attributes assessed. The panel was asked to determine their overall acceptability with 1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely.

Sensory and physicochemical data obtained for the products was analysed using Genstat (9th Edition) for analysis of variance (ANOVA) with LSD test to determine differences among the products at a significance level of 5%. Correlation analysis was also done to determine the relationship between sensory and physico-chemical qualities of the bitters.

Results and Discussion

Phenols are a class of secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens. Plant phenols may contribute to the sensory properties and oxidative stability in plant products and they include flavonoids, tannins and phenolic acids (Ma et al., 2014). The total phenol content (TPC) of the dry cocoa roots was 33.75 ± 0.4 mg GAE/g dry root and this compares well with the 22.0 ± 1.1 g GAE/100 g extract obtained by Baharum et al. (2014) for cocoa roots in Malaysia. They also found the TPCs of cherelle, bark, leaf, pod husk, fermented and unfermented shell to be 19.6 ± 0.3, 15.4 ± 0.1, 13.3 ± 0.2, 8.7 ± 0.4, 4.3 ± 0.3 and 1.5 ± 0.1 g GAE/100 g extract respectively. It is therefore apparent that the distribution of phenolics in different plant parts is not uniform (Pandey and Rizvi, 2009). Unroasted cocoa beans from Ghana have been reported to have a TPC of 105.18 ± 0.6 mg GAE/g dry beans (Oracz and Nebesny, 2016), making it the part with the highest phenolic content followed by the roots.

Phytochemical screening of the dry roots showed the presence of 8 out of the 12 chemicals screened. These were cardiac glycosides, saponins, terpenoids, reducing sugars, anthraquinones, phenols, tannins and alkaloids. Nwokonkwo and Okeke (2014) reported on the presence of alkaloids, saponins, phenols, tannins, glycosides, flavonoids and carboxylic acid in cocoa stem bark while work done by Baharum et al. (2014) showed the presence of saponins and tannins in cocoa beans, leaves, bark as well as root extracts. Alkaloids have also been reported to be present in aqueous cocoa pod husk extract (Santos et al., 2014). These phytochemicals are known to have a long history of medicinal use. Alkaloids and saponins have anti-inflammatory, anti-cancer, and pain relief properties (Sparg et al., 2004; Tava and Avato, 2006). They also have antibacterial and antifungal properties and are useful as diet ingredients, supplements as well as in pharmaceuticals. Anthraquinones are potent laxatives and can be irritating to both the upper and lower parts of the gastrointestinal tract (Fouillaud et al., 2016). Cardiac glycosides have the ability to exert powerful action on the cardiac muscle (Morsy, 2017). A very small amount can exert a beneficial simulation on a diseased heart.

The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage (Chung et al., 1998). The antimicrobial activities of tannins are also well documented. Terpenoids are one of the largest classes of natural products and they form a major constituent of essential oils produced by plants (Agatonovic-Kustrin and Morton, 2018). Phenols are a class of secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens. Therefore, the presence of these chemicals in cocoa roots suggests that cocoa roots could be used for therapeutic purposes and this may also explain why they are traditionally chewed to treat common colds, cough and chest problems.

Results on the GC-MS showed that the VOCs were identified based on retention time, peak area and peak height. A total of 60 VOCs were detected in dry cocoa roots from the different extraction solvents used Table 1 with esters, secondary alcohols and organic acids being the major plant volatiles detected. This agrees with the Rosenkranz and Schnitzler (2016) report that plant volatiles are usually complex mixtures of a wide range of organic compounds. These compounds are mostly present in minute quantities but are very important to plants because they form the major components of flavour and aromatic properties of plants. Studies on VOCs in cocoa pulp juice from Indonesia, Vietnam, Cameroon, and Nicaragua identified a total of 66 compounds with juice from Vietnam having the highest number of compounds (57) and that from Cameroon having the least of 43 compounds (Bickel Haase et al., 2021). In other studies, a total of 74, 66 and 60 VOCs were identified in fresh, UHT-treated and pasteurized cocoa

Table 1. Volatile organic compounds detected in the different extraction solvents

No.	Name of compound	No.	Name of compound
1	3,3-Dimethylbutane-2-ol	31	Succinic acid, dodec-2-en-1-yl 2-methoxyphenyl ester
2	Propane, 2-methyl-1-nitro-	32	1,2-Benzenedicarboxylic acid, didodecyl ester
3	Pentane, 2,2,3-trimethyl-	33	Heptacosanoic acid, 25-methyl-, methyl ester
4	1,3-Propanediamine	34	6,10-Dimethyl-4-undecanol
5	4-Heptyn-2-ol	35	Undecanoic acid
6	Acetic acid, trifluoro-, 2,2-dimethylpropyl ester	36	Lauroyl peroxide
7	cis-2-Nitro-4-t-butylcyclohexanone	37	3-Ethylheptanoic acid
8	tert-Butyl cyclopropylmethyl sulfoxide	38	Palmitic acid, ethyl ester
9	1-Butanol, 3,3-dimethyl-	39	Tetradecanoic acid, 12-methyl-, methyl ester
10	Oxalic acid, allyl butyl ester	40	Chloroacetic acid, 4-pentadecyl ester
11	Trifluoromethyl peroxynitrate	41	9-Octadecenoic acid, methyl ester
12	Propargyl alcohol or Ethynyl carbinol	42	17-Pentatriacontene
13	Borane, ethylisopropylmethyl-	43	Octadecanoic acid, methyl ester
14	4-Trifluoroacetoxyoctane	44	9-Eicosenoic acid
15	But-3-enyl isobutyl carbonate	45	Ethyl 14-methyl-hexadecanoate
16	Isovaline, 3-hydroxy-	46	Heptadecanoic acid, heptadecyl ester
17	Silane, dodecyltriethoxy-	47	Pentadecanoic acid
18	5-Hexenoic acid	48	Arachic acid
19	Phthalic acid, cyclobutyl tridecyl ester	49	Heneicosanoic acid, methyl ester
20	4-Octanol	50	Oleic Acid
21	3-Hexanol, 2-methyl-	51	Acetic acid, trichloro-, nonyl ester
22	4-Dodecanol or lauryl alcohol	52	18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetradehydro-16-(hydroxymethyl)-, methyl ester, (15.beta,16E)-
23	2-Propenoic acid, propyl ester	53	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester or methyl palmitate
24	8-Methylnonanoic acid	54	Docosanoic acid, 1,2,3-propanetriyl ester
25	Eicosanoic acid or Arachic acid	55	9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione
26	Cyclohexanol, 1,1'-dioxybis-	56	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
27	4-Heptanol or Dipropylcarbinol	57	Triarachine
28	n-Propyl acrylate or 2-Propenoic acid, propyl ester	58	Isopropyl hexacosyl ether
29	1-Isopropyl-1-butanol; 3-Hexanol, 2-methyl-	59	Docosanoic acid, ethyl ester
30	2-Butanol, 3-(1-methyl-2-phenylethoxy)-	60	Dotriacontyl isopropyl ether

pulp respectively, with a few of them being common to the three juices (Bickel Haase et al., 2023).

Out of the 8 extraction solvents used, the highest and least numbers of VOCs were detected in acetonitrile and water respectively (Figure 1) and this may be due to the low solubility of VOCs in water (Abrahão et al., 2013). However, in the aqueous solvents (water/solvent mixtures), more VOCs were detected than in water alone. In 80% methanol, 70 and 50% ethanol extracts, 24, 19 and 21 VOCs were detected respectively. Ramirez et al. (2010) reported that, although solvent extraction method for VOCs detection is simple and compatible with both high molecular mass and thermally unstable compounds, it has several limitations. Its major limitation is the dilution of the compounds in the test sample by the extraction solvent and therefore increasing the limit of detection. This renders some of the compounds in the test sample undetectable (Guzowska et al., 2022). Additionally, some polar compounds tend to have poor desorption efficiencies with the presence of other polar compounds in the same solvent (Ramirez et al., 2010). Such compounds may react with the other compounds in the solvent to form different products, making them undetectable as well. This may also explain why only 4 (4-trifluoroacetoxyoctane, borane ethyl(isopropyl)methyl-, 4-dodecanol, methyl palmitate and triarachine) out of the 60 VOCs were detected in all 8 solvents. Trifluoromethyl peroxynitrate, 4-octanol and undecanoic acid were detected in 7 of the solvents with propargyl alcohol, 4-heptanol and heptadecanoic acid, heptadecyl ester, detected in 6 solvents. The remaining VOCs were detected in 5 or less extraction solvents.

Apart from the aromatic properties of VOCs, some of them are known to have antibacterial, antiviral and antioxidant properties (Akoh and Mac-Kalunta, 2021). Triarachine, which was detected in all the solvents, is reported to be biologically active against bacteria, virus and fungi while undecanoic acid has antifungal properties (Akoh and Mac-Kalunta, 2021). The esters,

Table 2. Performance metrics for each machine learning algorithm without SMOTE

Bitters	Yield (%)	SG	pH	Brix (%)	TSS (%)
Cocoa alcohol (85% v)					
0	–	0.85 ± *0.00*	8.04 ± *0.10*	19.0	–
9:1	81.3 ± *3.32*	0.86 ± *0.01*	6.51 ± *0.15*	18.5	0.26 ± *0.01*
8:2	63.8 ± *2.83*	0.86 ± *0.01*	6.71 ± *0.12*	18.5	0.57 ± *0.01*
7:3	40.4 ± *3.32*	0.86 ± *0.01*	6.82 ± *0.03*	18.5	0.52 ± *0.17*
Cocoa alcohol (43% v)					
0	–	0.94 ± *0.02*	5.90 ± *0.07*	13.5	–
10	83.1 ± *0.35*	0.90 ± *0.01*	5.70 ± *0.13*	13.0	0.60 ± *0.01*
20	62.5 ± *0.00*	0.92 ± *0.01*	5.65 ± *0.12*	13.0	1.4 ± *0.03*
30	40.6 ± *3.25*	0.93 ± *0.02*	5.61 ± *0.06*	13.0	0.57 ± *0.00*

*Std dev. In italics

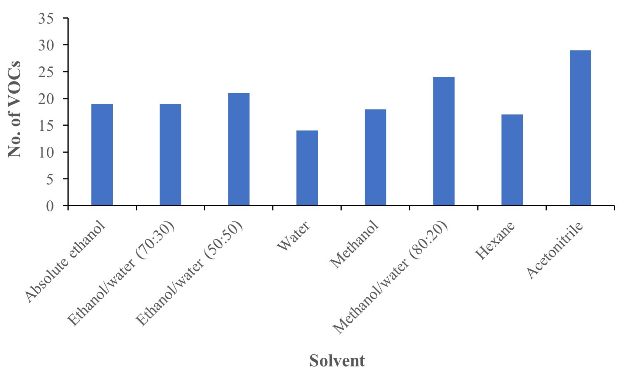

Figure 1. Number of VOCs detected in the different solvents

which formed the majority of the VOCs detected are mostly known to be associated with pleasant aroma (Mostafa et al., 2022). Oxalic acid, allyl butyl ester has a fruity smell and palmitic acid, ethyl ester has a wax-like odour. Amino acid derivatives such as 1,3-propanediamine have fishy odour (Ma et al., 2014). The alcohols and organic acids provide wine-like odour and sour taste respectively (Shi et al., 2022). However, 4-dodecanol has a floral scent while 4-heptanol has a pungent alcoholic odour. Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester has anti-inflammatory and antioxidant activities while oleic acid has anticancer, antifungal and antibacterial properties (Al-Marzoqi et al., 2015; Arora and Kumar, 2017).

In an attempt to develop alcoholic bitters with dry cocoa roots, it was observed that the yield of bitters produced depended on the alcohol/root ratio. Thus, the greater the A:R ratio, the better the yield (Table 2). However, it has been reported that, a solvent/solid ratio that is too high will cause excessive extraction which will require a long time for concentration (Qing-Wen et al., 2018). Although all the bitters produced were brown in colour and clear in appearance, 43% alcohol bitters were deeper in colour than the 85% alcohol. This coincides with the GC-MS results for the different ethanolic extracts where a slightly higher number of VOCs were extracted in 50% ethanol than the 70% and absolute ethanol solutions (Figure 1). All the six experimental products were also found to be aromatic in odour, confirming reports that most of the VOCs detected in the roots had aromatic properties.

Physicochemical analysis of the products showed a significant drop in pH of raw 85% alcohol after the addition of cocoa roots (Table 2). This may be due to the presence of organic acids and phenols in the roots. The reduction in pH of raw 43% alcohol was however not significant. Standard alcoholic beverages such as gin, vodka and whiskey, which usually contain 40-50% ethanol have pH values between 3.3-6.0 (Bass, 2021). The pH for raw 43% alcohol as well as its bitters fall within this range. Bitters from both 85 and 43% alcohol were significantly different from each other ($p \leq 0.05$) in terms of pH, SG and brix (Table 2). Bitters of 85% alcohol had higher pH and brix values than 43% alcoholic bitters. However, bitters obtained at the three different alcohol/root ratios (9:1, 8:2 and 7:3) for both 43 and 85% alcohol did not show any significant differences within each group. Generally, TSS for 43% alcoholic bitters was higher than that of 85% alcoholic bitters, suggesting that mixing water with alcohol is likely to have extracted more solutes from the dry roots. However, the differences in TSS were not significant. Reports by Washburn (1937) also showed that SG of ethanol/water mixtures at 25^{o}C for 85 and 40% ethanol were 0.82 and 0.93 respectively.

To ensure food safety, all substances must be safe for use before consumption and must therefore undergo risk of exposure or toxicity assessment. Thus, to introduce cocoa roots as an additive to the beverage industry, it must be assessed for toxicity by determining its microbial status and the amount to which humans and animals could be exposed. There was no detection of *Escherichea coli*, *Salmonella spp.* and *Staphylococcus spp.* in all the experimental products, making them microbiologically safe for consumption. It is also possible that, the root bitters were biologically active against the three bacteria as reported by Nwokonkwo and Okeke (2014) on cocoa stem bark.

Acute toxicity studies on bitters developed showed no allergic or adverse effect on rats at 5000 mg/kg b.w., suggesting that the median lethal dose (LD50) for cocoa roots was more than 5000 mg/kg b.w. Kifayatullah et al. (2015) reported that the ethanolic extract of Pericampylus glaucus, a medicinal plant in Malaysia, was non-toxic at a dose of 4000 mg/kg b.w. In South Africa, Ng'unia et al. (2018) also reported that extract from a South African medicinal plant, Galenia Africana, was non-toxic at a dose of 2000 mg/kg b.w.

Distilled beverages are made up of mixtures of compounds derived from their raw materials and production processes and these help in defining the sensory identities of these products (Faria, 2012). The quality of distilled beverages therefore could be defined by a small number of attributes, which are colour, aroma, taste and mouthfeel. The tasting and smelling of these beverages remain very important in their development and production Aumatel (2011). Sensory evaluation of cocoa root bitters from the two alcoholic solutions showed that the higher the proportion of cocoa roots the darker and dimmer, the appearance (Figure 2). Generally, 85% alcoholic bitters were lighter in colour and looked brighter than those in 43% alcohol and this may be due to their lower SG and TSS values in Table 2. The differences in the colour for products with A/R ratios 8:2 and 7:3 for the two alcoholic solutions were significant ($p < 0.05$), with their highest scores being 7.81 and 7.52 for 85% alcohol (7:3 A/R ratio) and 43% alcohol (7:3 A/R ratio) respectively. The significant differences in brightness were observed in products with 9:1 and 8:2 A/R ratios with the highest scores being 6.46 and 5.48 for 85% alcohol (9:1 A/R ratio) and 43% alcohol (9:1 A/R ratio) respectively. In terms of taste, the higher the proportion of roots, the more bitter and woody the taste of the products (Figure 3). However, there was no significant difference in the woody taste of the two alcoholic concentrations. Differences in bitterness were significant for products with 7:3 A/R ratio in both 43% and 85% alcohol. Bitters of 85% alcohol (7:3 A/R ratio) were the most bitter of all the products with a score of 7.73. Woody taste is reported to play a significant role in contributing flavour to distilled spirits (González-Robles et al., 2017; Maga, 1989). The bitter taste was likely contributed by the phenols and tannins extracted into the products (Ma et al., 2014). These two compounds may also contribute to the colour of the extracts since they form part of plant pigments. For aromatic flavour, there was no significant difference in all the products although products with higher proportions of cocoa roots gave a better aromatic (Figure 4). The woody taste may be due to the breakdown of woody lignin by the alcohol (Jahn et al., 2020) while the aromatic flavour may also be due to the VOCs in the cocoa roots. Increasing the proportion of roots in the products increased the overall acceptability of products with 7:3 A/R ratio in 43% alcohol being significantly different from those of 85% alcohol (Figure 4). The low acceptability of a 7:3 A/R ratio in 85% alcohol may be due to its intense bitterness. The most accepted product was 43% alcohol (7:3 A/R ratio) with a score of 6.72. This therefore suggests that cocoa root bitters can be produced with both 43% alcohol up to the level of a 7:3 A/R ratio.

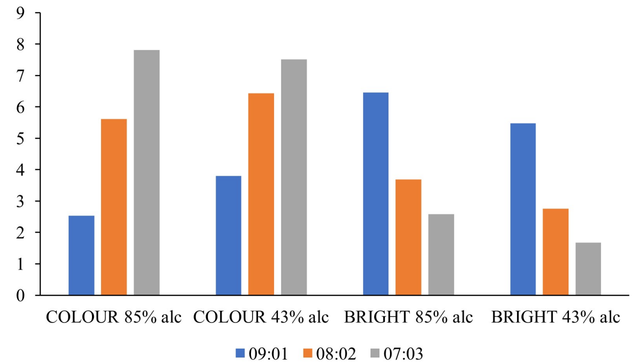

Figure 2. Sensory distributions for appearance

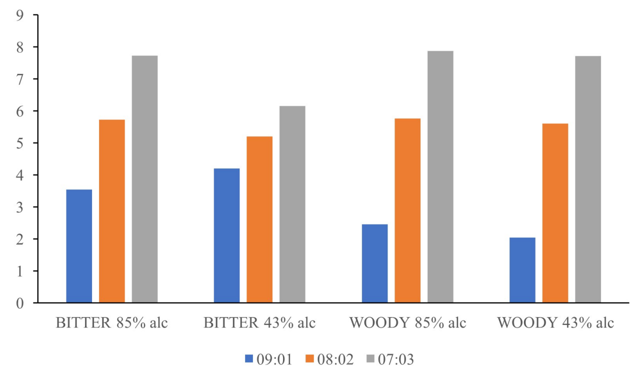

Figure 3. Sensory distributions for taste

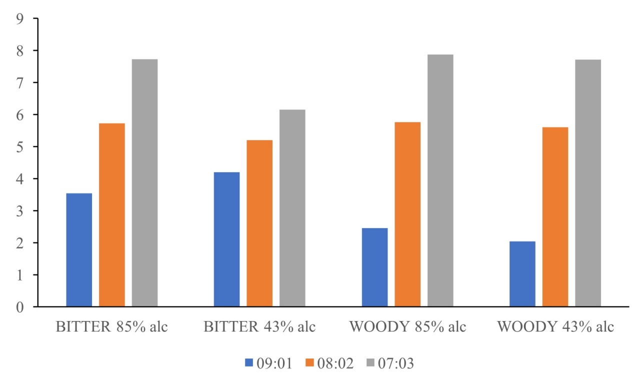

Figure 4. Sensory distributions for aromatic flavour and acceptability

Correlation analysis showed that apart from brightness, all the sensory attributes for 85% alcoholic bitters were positively dependent on pH and TSS (Table 3). They all had correlation coefficients (r) above 0.9. The correlation between pH and colour, brightness as well as woody taste was significant at a p-value of 0.05. However, for 43% alcoholic bitters, all the sensory attributes had a negative relationship with pH, with the exception of brightness (Table 3). There was also a relationship between sensory quality and SG of 43% alcoholic bitters with r values between 0.5 and 0.7. Acceptability was found to be dependent, either positively or negatively, on both sensory and physico-chemical properties of the two types of products ($r > 0.5$).

Table 3. Performance metrics for each machine learning algorithm without SMOTE

	85% Bitters		43% Bitters	
Sensory Attribute	**TSS**	**pH**	**SG**	**pH**
Colour	0.837	0.997*	-0.690	-0.985
Brightness	-0.908	-0.997*	0.695	0.984
Flavour	0.771	0.984	-0.560	-1.000
Bitter	0.797	0.990	-0.510	-0.999*
Woody	0.854	0.999*	-0.620	-0.997
Acceptability	0.986	0.782	-0.530	-1.000*

*Correlation is significant at the 0.05 level

Conclusion

Cocoa tree roots were found to contain phytochemicals such as phenols, alkaloids, cardiac glycosides and saponins and 60 VOCs were detected in the roots using different extraction solvents. Alcoholic bitters developed with dry cocoa roots using both 85 and 43% alcohol, were found to have the characteristic bitter taste and aromatic flavour for herbal products. Sensory quality of the products correlated well with their physico-chemical properties with 43% alcoholic bitters with A/R ratio of 7:3 being the most accepted product. With the numerous health benefits and aromatic properties of some of the phytochemicals detected in the dry roots combined with its high LD50 of more than 5000 mg/kg b.w., the use of cocoa roots, particularly, those from unproductive trees could be explored in the beverage industry.

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