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Original Research Article

Chemical composition of *Theobroma cacao* L (*sterculiaceae*) and *Sorghum bicolor* (L) Moench, Syn. *Sorghum vulgare* Pers (*Poaceae*)

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ABSTRACT

Theobroma cacao L (*sterculiaceae*) and *Sorghum bicolor* (L) Moench, Syn. *Sorghum vulgare*. Pers (Poaceae) are plants with well-known medicinal properties. Aqueous extracts of the plant blend are being used in the treatment of anaemia caused by the shortage of iron in patients. It is therefore important to investigate the synergetic effects of the blends on their chemical composition. The proximate, phytochemical and mineral composition of the plants and the blend samples containing 50% (w/w) were evaluated using standard procedures. The proximate composition showed that samples contained a high fraction of carbohydrate compared to other parameters; 36.70% for *T. cacao*, 48.66% for *S. bicolor* and 73.59% for the blended sample, respectively. The phytochemical composition of the samples showed the presence of medicinally active constituents like alkaloids, tannins, saponins, phlobatannin, flavonoids and cardiac glucosides. The samples showed moderate phytate composition of 18.53 mg/g, 11.74 mg/g and 14.0 mg/g for *T. cacao*, *S. bicolor*, and the blended samples, respectively. The study further revealed that the composition of the phytochemicals/antinutrients were temperature dependent. The mineral analysis of the samples showed the presence of Na, K, Ca, Mg, Zn, Fe, Cu, and Mn with a significant amount of Mg (68.20 mg/100g) and Fe (55.10 mg/100g) observed in the blended sample. Our findings support the ethnomedicinal use of the plants in the treatment of anaemia.

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1. Introduction

Plants with medicinal properties have continued to significantly impact the health care systems and drug development in both developed and developing countries. The traditional medicine system in countries like China and India has been based on materials from plants for thousands of years (Newman et al., 2000). According to an estimate by the World Health Organization, 80% of the world population depend majorly on traditional medicine for their basic health care (Ojong et al., 2016). A medicinal plant has been described as any plant containing substances that are used for therapeutic purposes or can serve as precursors for pharmaceutical development of drugs (WHO). Raw materials obtained from naturally occurring sources such as the seeds, roots, stems, leaves, and fruits of plants have become the backbone for modern day drug development (Nwokonkwo and Okeke, 2014). These active plant products have been employed in the treatment of various disease conditions, with increased efficiency and reduced side effects achieved by the development of the synthetic analogues of the active components (Asuk et al., 2015). In sub-Saharan Africa, easy access to medicinal plants and their relatively low cost make traditional medicine more appealing compared to modern medicine (Agbor and Ngogang, 2005).

Apart from the application of medicinal plants in drug development, numerous applications of these plants have also been explored. For instance, *Artemisia* species and *Origanum onites* L have been reported to be potent pesticidal and insecticidal agents against larvae of *Anopheles* insect and *Sitophilus* pests,

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respectively (Mohammadhosseini et al., 2017; Erenler et al., 2018). Moghaddam et al. (2011) reported on the use of *Ocimum ciliatumas* for culinary purposes. A recent review of the chemical compositions and biological activities of *Achillea* species, detailed the herbicidal, larvicidal and insecticidal activities of their essential oils and extracts (Mohammadhosseini et al., 2017).

Sorghum, the fifth most important cereal crop after wheat; rice, corn, and barley is well-known for its high phytochemical composition. The majority of cultivated *Sorghum* is used in animal feed and industrial products, while about 35% is directly consumed by humans. Interest in the phytochemical composition of *Sorghum* has increased due to its effectiveness in cholesterol control, antioxidant activity and many health benefits (Awika and Rooney, 2004). The stem has been reportedly used in the preparation of local medicine to treat anaemia and blood related ailments. It is also used as an anti-malarial and anthelminthic agent (Oluwalana and Adedeji, 2013).

Theobroma cacao shown in Fig. 1, tree is the main source of chocolate. The chocolate obtained from the seed of the tree has been found to contain a high content of important compounds. For example, theobromine, which is an important antioxidant (Martínez-Pinilla et al., 2015). The use of chocolate was found useful in stimulating the healthy function of the spleen and other digestive functions. In the 17th and 18th centuries, chocolate was regularly recommended or mixed into medications for all sorts of ailments and diseases from colds and coughs, to promote digestion, fertility, reinforce mental performance and as an anti-depressant remedy (Abbe and Amin, 2008).

Some studies have examined the contributions of the flavonoids in cocoa and cocoa products towards health benefits. Cocoa and their products are also rich in methylxanthines, namely caffeine, theobromine and theophylline. Methylxanthines have been shown to possess both positive and negative health effects. For instance, caffeine intake has been reported to have negative effects on reproductive health while, its supplementation enhanced net hepatic glucose uptake through increment of glucose-6- phosphate production in the liver (Abbe and Amin, 2008).

In Nigeria, aqueous extracts of the blends of *S. bicolor* sheath and *T. cacao* stem bark are used for the treatment of anaemia. Iron deficiency is a major problem in both developed and the developing nations. Insufficient iron in the diet causes iron deficiency anaemia, a condition in which the red blood cells are low in hemoglobin and correspondingly carry less oxygen. The various symptoms of anaemia include fatigue, listlessness and decreased resistance to infection (Eubanks et al., 2006).

This study will, therefore, evaluate the effect of blending of *S. bicolor* and *T. cacao* on the proximate, phytochemical and mineral composition of the aqueous extract to confirm their folkloric use in the treatment of anaemia.

2. Materials and Mthods

2.1. Collection and identification of plant materials

The bark of *Theobroma cacao* was obtained from a cocoa plantation in Igbajo, Osun State, Nigeria, while the *Sorghum bicolor* L stalk/stems were purchased in the dry forms at Oja Oba market in Ado-Ekiti, Ekiti State, Nigeria. The plant materials were identified and authenticated by Mr. Femi Omotayo of the Department of Plant Science and Biotechnology, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria where voucher specimen was deposited. The voucher specimen numbers were UHAE 2018027 (*T. cacao*), and UHAE 2018028 for *S. bicolor*. All reagents used were of analytical grade and were used as purchased.

2.2. Sample preparation

The leaf stalk and bark were washed in distilled water, chopped into smaller sizes and air dried at room

A B

Fig. 1. The photograph of *Theobroma cacao* L (*sterculiaceae*) (A) and *Sorghum bicolor* (L) Moench, Syn. *Sorghum vulgare*. Pers (*Poaceae*) (B).

temperature in the absence of sunlight for about 20 days. The samples were then pulverized, sieved and stored in dry containers pending analysis. Cold extracts of the samples were prepared by soaking 2.0 g of the samples in 50 mL of distilled water at room temperature for 3 h, while warm extracts were prepared by heating the soaked samples at 60 °C for 1 h. The blended sample was obtained by mixing *S. bicolor* and *T. cacao* in 1:1 ratio.

2.3. Proximate analysis

The proximate analysis carried out on the samples include crude protein, crude fat, moisture content, ash, soluble carbohydrates and crude fibre. The analyses were carried out using standard procedures (AOAC, 2005).

2.4. Mineral analysis

Mineral composition of the samples was determined by following procedures described by the official AOAC (2005) methods. The weighed samples (5.0 g) were ashed for 24 h in an electric furnace at 550 °C. The obtained ashes were allowed to cool in a desiccator and weighed. The weighed ash was then dissolved in 2 mL concentrated HCl, followed by the addition of a few drops of concentrated HNO_3. The resultant solution was heated in a water bath until dryness. The obtained residue was then transferred into a 100 mL volumetric flask and diluted with deionized water to volume. The solution was analyzed by an Atomic Absorption Spectrometer (AAS). For each of the metal determined, serial dilutions of each metal standard were prepared and calibration curves obtained.

2.5. Phytochemical analysis

Phytochemical analysis for tannins, alkaloids, flavonoids, saponins, phlobatannins, terpenoids, steroids and cardiac glucosides were carried out according to known and standard methods described by De et al. (2010) and Yadav and Agarwala (2011).

2.6. Antinutrient determination

Tannin, oxalate, and phytin were determined using the methods described by Makkar and Goodchild (1996), Agbaire (2011) and Fasuyi et al. (2007), respectively. On the other hand, saponin, flavonoid and alkaloid content of the materials were determined by the methods described by Obadoni and Ochuko (2001), Böhm and Kocipai-Abyazan (1994) and Harborne (1973), respectively.

3. Results and Discussion

3.1. Proximate analysis of *S. bicolor*, *T. cacao* and blend

Table 1 shows the proximate compositions of *S. bicolor*, *T. cacao* and blend. The obtained results showed that the samples total ash, crude fat, crude protein, crude fibre and carbohydrates and moisture contents are in an appreciable amount. The moisture content for *S. bicolor*, *T. cacao* and the blend were found to be 4.88% ± 0.12%, 15.56% ± 0.01 and 10.67% ± 0.02%, respectively, while the values for *T. cacao* and blend were within the acceptable limits of about 6%-15% for most vegetable drugs, the value of 4.88% in *S. bicolor* was below the lower acceptable limits (Ehiabhi et al., 2012). High moisture content value reduces the shelf life of vegetables due to bacterial and fungal action leading to spoilage (Akinwunmi and Omotayo, 2016).

The total ash content is a measure of the mineral content of the samples. Total ash represents both physiological and non-physiological ash. The non-physiological ash has its source from the environment, while physiological ash is due to biochemical processes (Asuk et al., 2015). The total ash content for *S. bicolor* and *T. cacao* were 19.07 ± 0.02% and 12.64 ± 0.01% respectively, while the value for blend was 13.39 ± 0.01%. The high ash value obtained is an indicator of the rich organic content of the material, which on heating are convertible to oxides and water.

The crude fat contents of the samples were 16.39 ± 0.08%, 6.75 ± 0.01% and 9.26 ± 0.01% for *S. bicolor*, *T. cacao* and the blend, respectively. The values show that the samples can serve as a very poor source of dietary fat when compared to oil seeds, but are higher sources of dietary fat when compared to leafy vegetables

Table 1
Proximate composition of *Sorghum bicolor*, *Theobroma cacao* and blend samples.

Parameter (%)	*S.bicolor*	*T.cacao*	Blend
Moisture content	4.88±0.12	15.56±0.01	10.67±0.02
Total Ash	19.07±0.02	12.64±0.01	13.39±0.01
Crude Fat	16.39±0.08	6.75±0.01	9.26±0.01
Crude Protein	14.21±0.53	10.06±0.01	8.95±0.87
Crude Fibre	8.76±0.02	6.34±0.01	10.25±0.01
Carbohydrates	36.70±0.49	48.66±0.02	47.49±0.90

Table 2

Phytochemical screening of *S. bicolor*, *T. cacao* and blend.

Phytochemicals	*S. bicolor*	*T. cacao*	Blend
Alkaloids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Steroids	+	-	+
Phlobatannins	+	+	+
Terpenoids	-	+	+
Flavonoids	+	+	+
Cardiac glycosides	+	+	+

Table 3

Phytochemical /antinutritional composition of *S. bicolor*, *T. cacao* and blend.

Phytochemicals	*S.bicolor*	*T. cacao*	Blend	
			Cold	Warm
Tannic acid	4.06±0.22	0.87±0.10	3.42±0.05	5.28±0.03
Phenol	1.75±0.04	0.27±0.03	1.14±0.05	3.53±0.03
Phytate	18.53±0.41	11.74±0.62	14.40±0.82	19.15±0.47
Phytin phosphorus	5.22±0.12	3.31±0.18	3.95±0.24	5.40±0.14
Oxalate	2.34±0.09	1.58±0.05	2.79±0.09	6.18±0.03
Saponin	0.74±0.01	0.54±0.01	1.19±0.03	4.34±0.02
Alkaloids	1.25±0.01	0.87±0.02	1.51±0.02	5.09±0.03
Flavonoids	0.39±0.01	0.32±0.01	0.56±0.01	2.36±0.02

(Adeyeye et al., 2016).

Amino acid forms of proteins are requirements for body maintenance and growth such as maintenance and growth of connective tissue, bone marrow, skin, muscle, and body vital organs (Nelson and Cox, 2005; Lal, 2008). The crude protein contents of the samples were 14.21 ±0.53%, 10.06 ± 0.01%, and 8.95 ± 0.87% for *S. bicolor*, *T. cacao* and blend samples, respectively. This shows that the samples are poor sources of protein.

The crude fibre content of the blend sample (8.76 ± 0.02%) is slightly higher than that of *S. bicolor* (6.34 ± 0.01%) and *T. cacao* (10.25 ± 0.01%). Fibre is significant in the prevention of diverticulosis, adsorption of trace elements in the gut and in the elimination of undigested food materials (Chiba et al., 2015). The samples showed high carbohydrate composition with a value ranging from 36.70-48.66%. Carbohydrates are good energy sources and also aid in the assimilation of other nutrients.

3.2. Phytochemical composition of *S. bicolor*, *T. cacao* and the blend

The phytochemical screening of cold aqueous extract of the samples as presented in Table 2 showed the presence of alkaloids, tannins, saponins, phlobatannins, terpenoids, flavonoids and cardiac glycosides in all the three samples while steroids was absent in *T. cacao*. Steroids was present in *S. bicolor* and the blended sample. Table 3 summarizes the phytochemical and antinutritional composition of the cold and warm aqueous extracts of the sample and the blended sample. It was observed that the phytochemical composition of the blended sample varied with temperature. This might be due to the increase in solubility of the phytochemicals with an increase in temperature.

On the other hand, the phytochemical composition is an indicator of the medicinal and nutritional potential of the samples, antinutrients are substances presents in human or animal foods which may reduce the digestion, utilization and absorption of nutrients. The effective use of plant materials as antimicrobial, anti-proliferative, antitumor and anticancer agents have been well-documented (Monteiro et al., 2014). The flavonoid composition of the samples is 0.39 ± 0.01, 0.32 ± 0.01 for *S. bicolor* and *T. cacao*, respectively. For the blend sample, the flavonoid composition was 0.56 ± 0.01 and 2.36 ± 0.02 for the cold and warm extracts, respectively. Flavonoids have been reported to exert anti-oxidative activities, hepatic toxicity and platelet

Table 4
Mineral composition of *S. bicolor*, *T. cacao* and blend.

Minerals (mg/100g)	*Sorghum*	*Theobroma*	Blend
Na	47.36	37.55	33.00
K	66.04	28.14	46.78
Ca	9.14	12.13	23.91
Mg	46.59	22.51	68.20
Zn	61.37	28.52	44.58
Fe	83.16	41.14	55.10
Mn	0.49	0.29	0.67
Cu	ND	4.85	0.96
Pb	ND	ND	ND

ND: Not Detected

aggregation (Syed et al., 2013; Georgiev et al., 2014).

The alkaloid content of the samples were 1.25 ± 0.01%, 0.87 ± 0.02% for *S. bicolor* and *T. cacao*, while the values for the cold and hot extract for the blend samples are 1.51 ± 0.02%, 5.09 ± 0.03%, respectively. Alkaloids are important due to their biological activity and are active components of various drugs due to their antispasmodic, analgesic and antibacterial properties (Manske et al., 2007; Ahmad et al., 2013).

Saponins have been found to have a beneficial effect on bone health, cancer and stimulation of the immune system. The saponin value ranges from 0.54-4.34% for the samples. With a level of < 10 %, the saponin level is considered safe as higher values have been found to lead to gastroenteritis (Awe and Sodipo, 2001). The phenol component of the samples are 1.75 ± 0.04, 0.27 ± 0.03, 1.14 ± 0.05 and 3.53 ± 0.03 for *S. bicolor*, *T. cacao*, cold and warm blend extracts respectively. Phenols are natural antioxidants with anti-inflammatory and anti-cancer activities (Karker et al., 2016).

Tannins have been associated with different positive and negative impacts on human health. Tannins negatively affect the productivity of livestock by reducing the digestibility of various feed nutrients (Awika and Rooney, 2004). Tannin-plants are used in the formulation of treatments for diseases like diarrhea, leucorrhoea, and rhinorrhoea (Doughari, 2012). *S. bicolor* (4.06 ± 0.22) shows a significantly higher tannin content compared to *T. cacao* (0.87 ± 0.10). The blended sample showed a reasonable amount of tannin for the cold extract (3.42 ± 0.05) and warm extract (5.28 ± 0.03), respectively.

High level of oxalate has been found to interfere with carbohydrate metabolism, due to the inhibition of succinic dehydrogenase and may also cause stone formation in the urinary tract (Han et al., 2015). The negative effect of oxalates on calcium and iron absorption is a major concern in the consumption of high oxalate foods because oxalate binds calcium and other minerals. The oxalate formation for the samples are 2.34 ± 0.09, 1.58 ± 0.05, 2.79 ± 0.09 and 6.18 ± 0.03 for *S. bicolor*, *T. cacao*, cold and warm blended extracts, respectively. The phytate composition of the samples was relatively high compared to other phytochemicals with a value of 18.53 ± 0.41, 11.74 ± 0.62, 14.40 ± 0.82 and 19.15 ± 0.47 for *S. bicolor*, *T. cacao*, cold and warm blended extracts, respectively. In seed plants; inositol and phosphate are stored as phytates. Phytate is responsible for some mineral related deficiencies, due to its formation of complexes with dietary minerals and it also impacts lipid and protein utilization negatively (Kumar et al., 2010). However, phytate has been found to be good anti-oxidant, anti-cancer and also lower blood glucose by reducing starch digestion rate (Shamsuddin, 2002).

3.3. Mineral composition of *S. bicolor*, *T. cacao* and blend

The mineral composition of the samples are shown in Table 4 below. The samples showed the presence of significant amount of Na, K, Ca, Mg, Zn and Fe. Cu and Mn composition of the samples were relatively low compared to other minerals while Pb was detected in the three samples.

Though minerals composition of the human body is only 4-6%, dietary minerals are important in the prevention of several diseases. Minerals with > 100 mg per day requirements are referred to as major minerals and they include phosphorus, potassium, chloride, calcium, sulphur and sodium. Minerals with < 100 mg per day daily requirement such as iodine, zinc, manganese, iron and chromium are referred to as trace minerals (Imelouane et al., 2011). The intake of elements like calcium, iodine, copper, sodium, potassium, zinc and manganese has been reported to reduce individual risk factors to cardiovascular diseases in both animals and humans (Rahmatollah and Mahbobeh, 2010).

Extracellular fluid is composed majorly of sodium and it is thus important in body fluid retention. The electrical potential created by sodium in conjunction with potassium helps in nerve impulse conduction which enables the contraction of muscles. In the small intestine, the absorption of nutrients like amino acid and proteins are facilitated by glucose (Asuk et al., 2015). Despite its many benefits, high level of sodium in the body is associated with high blood pressure and hypertension. The significant amount of calcium, magnessium and potassium which collectively act in hypertension and blood pressure reduction and treatment attest to the nutritional and medicinal value of the samples.

The samples showed very high iron composition (41.14-83.16 mg/100g), which may account for their use in treating blood shortage. Iron is essential in energy metabolism, cognitive development, immune function, haemoglobin and myoglobin synthesis and temperature regulation (Wardlaw et al., 2004). It has also been shown that iron is important for the prevention of anaemia and other diseases in pregnant and nursing women, adults and children (Geissler and Singh, 2011).

The magnessium content of the samples ranged from 22.51-68.20 mg/100mg. Magnessium is an important element in maintaining optimum heart operation by dilating arteries and preventing abnormal heart rhythm. It also helps in maintaining the insulin level of the body (Wardlaw et al., 2004). Its deficiency may result in convulsion, irritability and death. Calcium and magnessium are important in nucleic acids, photosynthesis, carbohydrate and binding agents of the cell wall (Imelouane et al., 2011). The heavy metal analysis of the samples (Pb and Mn), showed that they are present in insignificant amount and the samples are therefore safe and may exhibit no toxic effect when taken.

4. Concluding remarks

The search for natural products from plants having important physiological properties is on the increase and cuts across the globe. Extracts from plants can be given singly or as concoctions for various ailments. Many people rely on herbs to meet their various health needs since they are safe, and may be consumed with little or no side effects. In addition, medicinal herbs are easily accessible and very cheap. With the advancement of process formulation and production technology, various formulations can be made available using plant extracts in form of tablets, capsules, granules, oral liquid, injections which can be useful in the treatment of various diseases and ailments. In this study, the proximate analysis, phytochemical and mineral compositions of *S. bicolor*, *T. cacao* and blended mixture of the samples have been evaluated. The analysis showed a synergistic effect in the composition of the blended sample, especially in the mineral composition. This study affirms the nutritional and medicinal value of the samples and shows that blending of the samples together leads to the enhancement in the composition of the samples and their usefulness in the fight against anaemia. To the best of our knowledge, this is the first report in the literature on the enhanced properties of the blend samples; thus making it an important contribution to scientific knowledge.

Our future prospects include isolating the active components of the plant materials and the blends.

Conflict of interest

The authors declare that there is no conflict of interest.

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