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Research paper

Investigation of some useful chemical parameters in Shea tree (*Vitelaria paradoxa*) nut wastewater collected after extraction of the butter: prospects of Shea butter waste valorization

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Abstract

Shea butter is extracted the nut of *Vitellaria paradoxa* for both domestic and commercial purposes. Growing extraction and utilization of the butter especially in pharmaceutical, food and cosmetics industries result in large shea butter extraction with resultant large volumes of waste product of unknown content. Analyses of the wastewater/byproduct was carried out to review possibilities of its value addition in production of new products, or otherwise appropriate disposal. Therefore, Atomic Absorption Spectroscopy, physicochemical, and phyto-chemical screening methods, and extraction were used in the current study to assess antioxidant activity, BOD, calcium, iron, magnesium, lead, nickel, zinc, copper, and phytochemical content of samples of the material from Northern Ghana. The amount of residual oil was also determined. The BOD ranged from 168.00±0.00 to 86.25±6.25 mg O_2/kg; Levels of magnesium in the samples ranged from 16.65 - 206.65 mg/kg, calcium from 4.72 - 19.60 mg/kg, iron was 7.75 - 14.0 mg/kg, copper from 0.02 - 0.08 mg/kg, lead from 0.33 - 1.22 mg/kg, nickel from 0.04 - 0.23 mg/kg and zinc from 3.36 - 5.80 mg/kg. Secondary metabolites present included alkaloids, phenolic, saponins, tannins and flavonoids. while residual oil extracted from the sample was between 14.67-6.46 % of the material. Mean pH was 5.95±0.05 - 6.10±0.005, and the mean temperature of the samples ranged from 24.00±0.05 to 25.25±0.05 ^oC at the time of analyses. From the findings the material has good and diverse content and could be used in cosmetics, pharmaceutical products or organic manure. Otherwise it should be disposed appropriately to safeguard environmental and water pollution.

Keywords: *Shea Nut; Extraction-Waste; Minerals; BOD; Phytochemicals; Antioxidants.*

1. Introduction

Vitellaria paradoxa is a tree that grows in the Sudanian, Sudano-Sahelian, and Guinean savanna zones between the rainforests and the dry land Sahel region (Hall et al., 1996). They are deciduous trees of medium size, with a spherical crown and often reach heights of 10-15 meters (Maydell, 1990). The fruit of *Vitellaria* contains one large oval to slightly round, red brown to dark brown seed, which is usually referred to as the “Shea nut”. This seed comprises about 50% of the weight of the fresh fruit, and is extracted to produce Shea butter (Golden coloured in Figure 1 (Maranz and Wiesman, 2003; Mégnanou et al., 2007). When the nut extract is boiled to obtain the fat, a thick black sludge settles at the bottom of the container under the fat (Dei et al., 2008; Jibreel et al., 2013). The deep brown liquid and black sticky residue that remains after clarification of the butter is referred to as shea butter “wastewater” (Brown coloured in Figure 1).
Shea butter is an edible fat and is used in food preparation in Africa and also as a prophylactic. It has high free fatty acids (Yidana, 2004) and a large constitution of chemicals (Abagale et al., 2016). It is a complex fat largely containing triglycerides such as oleic acid, stearic acid, linoleic acid, palmitic acid, arachidic acid (Figure 1) as well as many non-saponifiable components (Maranz et al., 2004; Honfo et al., 2014). It has a lot of properties (Abagale et al., 2016; Garti et al., 2019) that make it vastly applied in the pharmaceutical, food, cosmetics and personal care companies, traditional medicine and other uses in many rural areas (Hemet, 2003; Goreja, 2004; Dei et al., 2008; Nooratiqah et al., 2013; Nooratiqah et al., 2013; Olife et al., 2013; Abagale et al., 2016). It has also been used in the manufacture of chocolate and confectioneries, and has functional properties due to its stearic content which contribute to a higher melting point than that of cocoa

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butter (Abagale et al., 2016). In West Africa the raw/crude butter is used as lotion to protect the human skin during the dry harmattan season in Africa (Goreja, 2004).

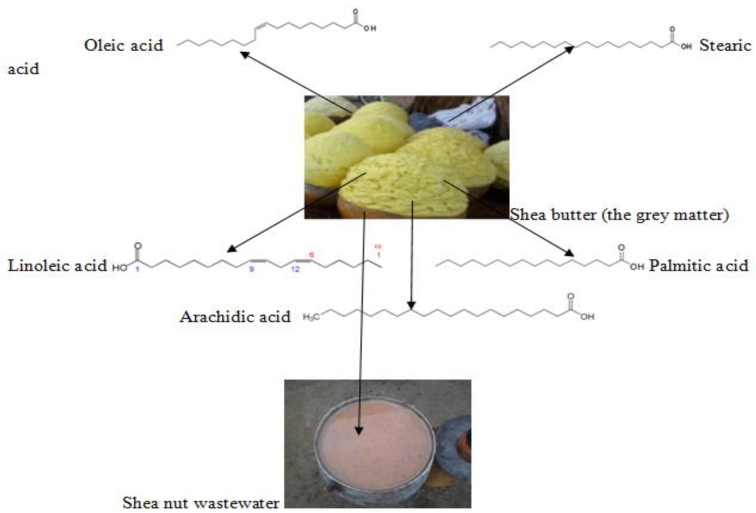

Fig. 1: Shea Butter and Some of Its Components and Byproduct (Shea Nut Wastewater).

However, shea waste material has largely been used only ethnobotanically. It is used for filling cracks in mud hut walls, and also as a substitute for kerosene when lighting firewood (Garti et al., 2019). The sludge is added to herbs for treatment of animal wounds, and a dry cake obtained from the sludge is used in producing livestock feed (Dei et al., 2008). It also serves as waterproofing material used in plastering wall of mud buildings (Olife et al., 2013). It is suggested to have pesticidal properties and has been used as poison against termites. Farmers also use it to fertilize crops in organic agriculture (Honfo et al., 2014; Otaiku, 2016).

Earlier scientific studies indicated that the wastewater is oily and contain high iodine number, acid number and free fatty acids which result in a pungent odour or taste within a short period of time when stored (Adomako, 1985). The wastewater is also reported to have crude protein, ether extract, crude fibre, ash and nitrogen-free extract nutrient compositions, as well as anti-nutritive factors such as saponins, tannins and theobromine (Dei et al., 2008). It has been investigated for use as poultry feed, biofertilizer, and in making of fired clay bricks (Dei et al., 2008; Otaiku, 2016; Adazabra and Viruthagiri, 2017). The current study will therefore provide novel results since these aspects of analyses have never been done on the sample.

In West Africa, water residue of the waste is available in large quantities (Dei et al., 2008), and Otaiku et al. (2016) indicated that 388 MT of the waste is produced annually. As a result of increasing demand and use of shea butter due to its useful properties, increasing volumes of the wastewater is anticipated (Dei et al., 2008; Nooratiqah et al., 2013; Olife et al., 2013; Abagale et al., 2016). But most of the wastewater is widely disposed (Jibreel et al., 2013) because it does not have a definite use. Disposed wastewater could run into streams and rivers, resulting in pollution and can affect fishes, and water quality. It also has potential effects on soil structure and properties inhibit plant germination and growth (Jibreel et al., 2013), as well as disrupting environmental microbes.

Many crop waste are rich in biologically active compounds and have the potential to become important raw materials and promising sources of valuable phytochemicals having antioxidant, antimicrobial, anti-inflammatory, anti-cancer, and cardiovascular protection activities (Kasapidou et al., 2018). It is very important to valorize them because agro-industrial residues of plant materials are a cheap and natural source of bioactive compounds, which can be used in the prevention and treatment of various diseases (Oleszek et al., 2023). The current investigation of shea wastewater will promote utilization of the material and contribute to its value addition. There are no previous characterization studies of the shea wastewater.

Drug related complications of antioxidant medicines or synthetics is a major medical challenge (Mohammedi and Atik, 2011). Hence, there has been an intense growing interest in exploring plant materials for natural antioxidants which are sometimes very effective compared to synthetic antioxidants (Sharma et. al., 2011). Reactive Oxygen Species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide are highly reactive and potentially damaging transient chemical species. Decreased or damaged scavenging systems lead to a variety of disorders including degenerative disorders (Mantle et al., 2000).

Research has shown the immense potential of plant materials in treatment of unhealthy systems (Manjula and Mamidala, 2013). Thus, plants have been used to treat infectious diseases and to avoid side effects associated with synthetic antimicrobials (Perulmalsamy and Ignacimuthu, 2000). Other advantages of plant based medicine include safety, cost effectiveness (Koche et al., 2011) and avoidance of the challenge of adulteration of synthetic drugs (Shariff, 2001).

Resistance of microbes to drugs is becoming increasingly important (Lagnika et al., 2012), and so finding alternative treatment, especially in unconventional sources, has become urgent. According to some reports, antimicrobials from plant origin have been found to have great therapeutic potentials (Werner et al., 1999), due to flavonoids, saponins, tannins and phenolic compounds according to some reports (Mohammedi and Atik, 2011; Sarang and Ameeta, 2012; Dariusz et al., 2013).

Therefore, the current study investigated specific characteristics of the shea wastewater to assess some useful chemical parameters for application in production of cosmetics, pharmaceuticals, and/or fertlizer. Otherwise the findings provide leads for safe disposal of the wastewater. Thus, we investigated antioxidant activity using hydrogen peroxide decomposition, levels of calcium, iron, magnesium, lead, copper, zinc and nickel as well as tannins, terpenoids, saponins, flavonoids, alkaloids and general phenolics content. In addition, biochemical oxygen demand (BOD), and amount of residual oil were determined in the samples. From the outcome of the analyses, shea wastewater could serve as a 3byproduct for production of some industrial products.

2. Materials

2.1. Samples

Shea nut “waste water” collected after extraction of the butter in Northern Ghana.

2.2. Reagents

Conc. Nitric acid, hydrochloric acid, distilled water, 1ml buffer, sodium hydroxide, ferroVer reagent, murex ide (ammonium purpurate), 0.2M EDTA, Eriochrome Black T indicator, Bromothymol blue solution.

2.3. Glassware and equipment

Conical flask, Beakers, Funnels, Atomic adsorption spectrometer, Electronic balance, 25ml test tubes, 100ml Conical flask, Burrete, Beakers, Digester, 250ml test tubes, Calibrated thermometer, Digital meter, pH comparator, Incubator, Magnetic stirrer, Rubber quiver, Pippete, Cell, OxiTop.

3. Methods

3.1. Sample collection

Samples of shea butter wastewater were collected from shea butter producers in seven communities of three regions of Northern Ghana in West Africa. The samples were collected in duplicates from the Northern region, Upper East region, and Upper West region into separate clean clearly labelled bottles grouped as samples 1, 2, 3 and 4. The samples from the Northern region were of two kinds; those obtained after manual extraction (Megnanou et al., 2007; Maranz and Wiesman, 2003) of butter (sample 1), and those obtained after mechanical extraction of butter (sample 2). All samples from the Upper East and Upper West regions were also pooled respectively on regional basis to constitute sample 3 and sample 4. The collected samples were stored at 4°C until laboratory analyses were done. Before the analyses, the samples were air dried under shade four one week, powdered, and sieved to obtain uniform powder and then used for analyses.

3.2. Determination of pH, temperature and Biochemical Oxygen Demand (BOD)

Temperature was measured using the Fisherbrand Traceable Digital Thermometer with Short Sensors. The instrument was suspended such that the reservoir (the tip) of the thermometer was below the surface of the wastewater, but the tip was not allowed to touch the walls or bottom of the container.
pH was determined using a pH meter comparator. After recording the temperature, about 10.0 ml of sample were filled into the comparator tube, and 0.5 ml of Bromothymol blue solution was added to the mixture, swirled gently, and then placed in the right hand compartment. The pH comparator was held before a uniform source of white light. The colour produced in the test solution was compared with colours in the standard disc by rotating the disc until the colour match was obtained and identified for recording (Kukulka et al., 2011).
BOD was determined using 1L of each sample. The prepared sample was placed in opaque glass bottle containing a magnet, and basified. Optimum pH range between 6.5-7.5 was maintained, and incubation was done at 20 °C for 5 days on the magnetic stirrer to accelerate carbon dioxide and oxygen transfer. The carbonate content of absorption solution was determined using a turbidity meter, with barium chloride as a suspension reagent. The BOD value was calculated from a calibration curve using the concentration of carbonate. BOD values of the samples were high and so were analyzed after dilution (Rezvani Pour et al., 2014).

3.3. Determination of hydrogen peroxide decomposition

Hydrogen peroxide decomposition was investigated using a modification iodometric method, of the approach described in Al-Amiery et al. (2015). A mass of 10.0g of dried shea waste was dissolved in distilled water to make 2g/ml solution and used to prepare an assay mixture with 8ml H_2O_2 solution (80mM) and 2ml of each sample. Mixing was done by gentle swirling, at room temperature. 1ml of the reaction mixture was allowed to run into a large volume (25ml) of water in order to slow down the consumption of H_2O_2 by the extract and for a period of 4 minutes, samples were taken at 60s intervals for analyses. 5.5g of KI were added to each sample analyte and the resulting iodine (I_2) solution (Equation 1) titrated with a standard solution of sodium thiosulphate (0.05 M $Na_2S_2O_3$) to the end point (Equation 2).

$$H_2O_2 + 2KI + 2H\ I_{2(aq)} + 2H_2O \longrightarrow \quad (1)$$

$$I_2 + 2S_2O_3^{2-} \longrightarrow S_4O_6^{2-} + 2I^- \quad (2)$$

The following precautions were taken:
Time (≥ 30 minutes) was allowed after the addition of KI to get the maximum generation of I_2; the conical flask was covered with watch glass while waiting; and the whole system was acidified with 5ml of 3M HCl.
The process was repeated for 4 and 8 ml of each shea waste sample, each mixing with 8ml of 80 mM H_2O_2 solution. The decomposition of H_2O_2 was back calculated, and the effect of the shea waste on the decomposition of H_2O_2 was then examined by plotting the Time of Collection of sample aliquots at each interval against the amount of H_2O_2. The effect of change in volume (amount) of each sample on decomposition of H_2O_2 with time was also investigated for 2, 4 and 8 ml of each sample.

3.4. Determination of residual oil content of shea butter waste

80g of shea butter waste sample was mixed thoroughly with 0.5L of diethyl ether. The mixture was allowed to stand for a minimum of 12 hours overnight. The solution was filtered over Whatman No. 1 filter paper into a pre-weighed conical flask, and the filtrate placed in a steam bath to evaporate the diethyl ether. The conical flask containing the oil was dried in the oven for 2 hours at 100 ^{0}C and placed in a desiccator to cool. The weight of the cooled conical flask was used to determine the percentage of recovered oil, applying equation (3).

$$[(W3 - W2) \div W1] * 100\% \quad (3)$$

Where W1 = Weight of waste sample used
W2 = Weight of empty conical flask
W3 = Weight of conical flask + oil after evaporation of diethyl ether.
The process was repeated using all the other samples.

3.5. Determination of calcium, iron, magnesium, lead, nickel, copper and zinc

About 0.5 g of each shea butter waste sample was weighed into labeled 250 ml test tubes on the electronic balance. Digestion and analysis were done using the procedure (with slight modification) in Singh et al. (2015). 20 ml of a mixture of nitric acid and hydrochloric acid (9:4 ratio) were poured on the sample and placed on the hot plate, using a holder, for 15-20 minutes at a temperature of 100 ^{o}C for digestion. Appearance of a brown color for some time before turning light yellow indicated completion of the digestion. The sample was then allowed to cool, then transferred into separate 100 ml conical flask, and topped with distilled water to the 50 ml mark. The digested sample was used to determine the levels of iron, nickel, copper, zinc and lead using the Atomic Adsorption Spectrometer, Buck scientific, model 210 VGP. The process was repeated using each sample. Duplicate analyses were done in each case.
Calcium was estimated through determination of hardness of the samples. 100 ml of each sample was measured and 1ml of 1N sodium hydroxide solution added. A small mass of powdered ammonium murixide indicator were then added and the mixture titrated against 0.02M of EDTA solution until the colour changed from pink to purple. Triplicate titre values were recorded and used to calculate the calcium hardness. The calcium hardness of each sample was then multiplied by a constant factor of 0.40 to obtain the actual calcium content (Nielsen, 2017). The process was repeated for each sample.
To obtain the magnesium content of the samples, the titre values obtained were used to calculate the total hardness. Magnesium hardness was obtained by subtracting calcium hardness from total hardness, and the result multiplied by a standard factor of 0.243 to obtain the magnesium content (Nielsen, 2017). The process was repeated for each sample.

3.6. Phytochemicals screening

Tests were done using standard procedures for determination of tannins, flavonoids, terpenoids, saponins, alkaloids, phenolics and steroids (Sofowora, 1993; Trease and Evans, 2002; Jothi and Jayachitra, 2019).
Test for saponins
Test for frothing; in a test tube, 2ml of the extract and 3ml of distilled water were mixed together and vigorously agitated for two minutes. Foam (bubbles) formation suggested the presence of saponin.

3.7. Test for tannins

About 2ml of each portion of fruits extracts were stirred with 10ml of distilled water, and 0.5ml ferric chloride was then added to the extract. The appearance of a greenish colour indicated the presence of tannin.

3.8. Test for alkaloids

To 1ml of extract was mixed with 1% dilute sulpheric acid and Dragendoff reagent was added to give a reddish-yellow colour which indicate the presence of alkaloids.

3.9. Test for flavonoids

To 1ml of extract, a few drops of dilute sodium hydroxide was added. Appearance of intense yellow colour in the extract which became colourless on the addition of few drops of diluted sulphuric acid was indicative of the presence of flavonoids.

3.10. Test for terpenoids

A 2ml of the extract was mixed with 2ml of chloroform and 1ml of concentrated sulphuric acid was carefully added to form a layer. Formation of reddish-brown interphase between two clear layers indicated a positive result.

3.11. Test for phenols

2ml of the extract was mixed with few drops of 10% ferric chloride solution. The formation of greenish blue or violet or blue-black coloration indicated a positive result.

3.12. Test for steroids

0.5ml of a mixture of acetic anhydride and acetic acid was added to about 4mg of each extract in separate tubes. Conc. H_2SO_4 was slowly added to the mixtures in each test tube. The presence of steroids was revealed in the form of a reddish-brown coloration.

4. Statistical analyses

The data obtained were expressed in terms of mean (M) values, and standard errors of mean (M±SEM) using Microsoft Excel processing.

5. Results

Table 1: pH., Temperature and BOD of the Shea Butter Wastewater

	Mean (M±SEM)		
Sample	pH	Temperature/ºC	BOD/mg O_2/L
1	6.10±0.00	25.25±0.05	168.00±0.00
2	6.00±0.00	24.60±0.03	86.25±6.25
3	5.95±0.05	24.65±0.13	155.00±0.00
4	6.00±0.00	24.00±0.05	138.00±3.25

Table 2: Levels of Analysed Macro and Micro Nutrients in the Shea Butter Wastewater

Metals	Mean concentration of the metals in each sample (Mean±SEM)/mg/kg			
	1	2	3	4
Magnesium	88.35±8.3	159.20±0.8	206.65±4.8	16.65±0.20
Calcium	15.00±5.0	19.60±0.4	14.60±4.6	4.72±5.20
Iron	7.75±6.2	9.50±0.8	10.75±5.2	14.0±0.60
Copper	0.08±0.0	0.03±0.0	0.02±0.0	0.04±0.00
Lead	1.22±0.0	1.00±0.76	0.33±0.29	0.58±0.02
Nickel	0.09±0.01	0.17±0.12	0.23±0.05	0.04±0.12
Zinc	3.36±0.04	5.80±0.80	4.49±0.73	4.40±0.08

Table 3: Residual Oil Extracted from 80g Shea Butter Wastewater

Sample	Mean mass of oil (Mean±SEM)/g	Percent of oil/%
1	11.23±0.04	14.03
2	5.17±0.11	6.46
3	11.74±0.02	14.67
4	7.20±0.11	9.00

Table 4: Amount of H_2O_2 Consumed by Samples of Shea Butter Wastewater

Time/sec	Mean amount of H_2O_2 consumed (Mean±SEM)/moldm^3			
	1	2	3	4
60	0.35±0.03	0.42±0.00	0.46±0.02	0.57±0.11
120	0.41±0.07	0.47±0.06	0.62±0.10	0.40±0.07
180	0.46±0.12	0.43±0.06	0.59±0.07	0.57±0.06
240	0.083±0.08	0.43±0.24	0.80±0.25	0.53±0.07

Table 5: Levels of Some Phytochemical Components the Shea Butter Wastewater

PHYTOCHEMICAL	Indicative level in each sample			
	1	2	3	4
Alkaloids	+++	+++	+++	+++
Saponins	++	++	+	+
Steroids	-	-	-	-
Tannins	++	++	++	++
Flavonoids	+	+	-	-
Phenolics	+++	+++	+++	+++
Terpenoids	-	-	-	-

Key: +++ = Appreciable amount; ++ = Moderate amount; + = trace amount; – = absent

6. Discussion

Agro-industrial by-products have caught special attention from the scientific community for being an available, cost-effective and sustainable source of a wide array of bioactive compounds (Reguengo et al., 2022). They have interesting applications mainly in pharmaceuticals and food production, but also in agriculture and wastewater remediation, as well as metal and steel industries (Oleszek et al., 2023).

Mean pH (Table 1) of the samples were from 5.95±0.05 to 6.10±0.00. Thus, the shea wastewater was weakly acidic, at the time of analyses, as indicated by the pH (Keith et al., 2008). Also, mean temperature of the samples during analyses fluctuated from 24.6±0.03ºC to 25.25±0.05ºC (Table 1). BOD of the samples was also in the range of 86.25±6.25 to 168.00±0.00 mgO_2/kg. BOD has been used as an indicator of amount of organic pollutants in most aquatic systems, and especially a good indicator for biodegradable organic compounds (Kwak et al., 2013). Factors such as pH, temperature, hardness, and total organic content impact on the accumulation of chemicals in aquatic environment (Ionescu et al., 2019).

From the analyses, various levels of iron, calcium, magnesium, copper, nickel, zinc, and lead were found in the shea wastewater (Table 2). The results indicate that higher levels of calcium, magnesium and iron, ranging from 206.65±4.8 to 4.72±5.2 mg/kg were present in the samples. Magnesium was the most abundant, with a concentration of 206.65±4.8 mg/kg among the metals investigated while the least was calcium (4.72±5.2 mg/kg). The least amount of nickel occurrence in the wastewater samples analysed was 0.04±0.12 mg/kg, and that of zinc was 3.36±0.04 mg/kg. These elements are all important nutrients that have also been reported in shea butter (Fernande et al., 2014). Most of these elements could also be contaminants that do not easily degrade (Ionescu et al., 2019). Inappropriate disposal of the wastewater would therefore result in transfer of metals from the waste into soil and subsequent leaching (Keith et al., 2008). These metals found in limited amounts can easily accumulate (Mustapha and Muhammad, 2020).

Generally, these minerals and trace elements such as found in the shea waste have already been reported in shea butter (Alhassan et al., 2010; Mégnanou and Niamke, 2015), therefore, they could be found in the waste. Some of the metals present in the sample can activate enzymes, be incorporated into metalloenzymes and also influence substrates. It has been reported that shea butter wastewater is used by farmers as manure and so minerals of these elements present in the samples may have been beneficial to plants as micro and macro nutrients properties (Kataba-Pendias and Pendias, 2001). Fe and Zn are trace elements of the transition metal group, while Ca and Mg are macro elements that support plant growth. Also, calcium is an essential plant nutrient that helps in protecting plants against heat stress and diseases, while zinc and copper are essential minerals for plant growth, promotion of seed production, and formation of plant chlorophyll (Hafeez et al., 2013). Copper is an essential micronutrient in all living organisms necessary for growth and metabolism, and also supports reduction of pH in soils. This means a fertilizer based on the shea wastewater will be useful in managing extremely alkaline soils and provide essential plant nutrients.

The largest amount of copper and lead in the different samples were 0.08±0.00mg/kg and 1.22±0.00 mg/kg respectively, while that of nickel and zinc were also respectfully 0.23±0.05mg/kg and 5.80±0.80mg/kg in the samples. Lead and nickel, though toxic, are known to have high fire and water-resistive properties (Valko et al., 2005; Briffa et al., 2020). However, at high concentrations it becomes one of the most toxic elements (Ionescu et al., 2019). Zinc is indispensable for plant growth and development and thus very essential in microorganisms, plants and animals. Excess zinc is however toxic (Ionescu et al., 2019; Mustapha and Muhammad, 2020). Concentration of dissolved calcium and magnesium ions in water contributes to hardness, a very important property for life processes (Mustapha and Muhammad, 2020) and some industrial processes.

While some accumulated metals are useful in biochemical and energy transformations in living tissues, others are highly toxic even at very low concentrations (Xingfeng et al., 2014). Aquatic organisms are constantly exposed to when the waste is not appropriately disposed, metals found in the material could accumulate in soil and then transfer into and become detrimental to living things (Mustapha and Muhammad, 2020). toxic chemicals through contaminated water (Ionescu et al., 2019). Thus toxicity resulting from metals in the shea waste may transfer into aquatic environments thereby into aquatic food chains and eventually affect human health.

The amount of residual oil in the waste was quite substantial. The largest amount of residual oil found in any one sample was a mean of 11.74±0.02 g obtained from 80 g of sample 3, while the least mean amount of recovered oil was 5.17±0.11 g (Table 3). The largest amount of residual oil obtained from the waste is a little over 14.0 % portion of the waste. From literature, 47 % and 25-40 % shea nut oil yields have been reported from nuts using solvent and traditional extraction methods respectively (Shea nut processing, 2008; Ikya et al., 2013; Nooratiqah et al., 2013). Taking into consideration the cost of processing involved in obtaining butter from shea nuts (Shea nut processing, 2008), industrial processing of the waste alternatives and cheap raw material with be worthwhile. Some of these products could be feed supplement for poultry and ruminants.

6.1. Peroxide decomposition (antioxidant) analyses

Plant materials could contain potent antioxidant activity because they contain antioxidant compounds. This is shown in the phytochemical makeup of the samples (Table 5). Some plant metabolites react with free radicals to moderate the extent of oxidative deterioration (Valko et al., 2005). Antioxidant activity of phenolic compounds in plants is directly related to their role in neutralizing free radicals, chelating transition metals and quenching singlet and triplet oxygen through delocalization or decomposition of peroxides (Briffa et al., 2020).

The waste samples exhibited moderate capacity to breakdown oxidative species. The amount of H_2O_2 decomposed by the wastewater (Table 5) was used in estimating the antioxidant activity. Breakdown of the oxidant (Figures 2–4), and the level of decomposition of the oxidant by varying amount of shea wastewater over time (Figures 5–8) have been analysed. Generally, the breakdown of hydrogen peroxide by the shea wastewater samples was found to be dose-dependent. This was indicated by the increasing amount of H_2O_2 decomposed with time by increasing amount shea wastewater. Tests of 2, 4 and 8 ml of the shea wastewater found that amount of H_2O_2 decomposed generally increased at least up to four minutes as shown in figures 2-4. Numerous studies have demonstrated that agricultural residues are rich in bioactive compounds, particularly phenolic compounds, with antioxidant properties. Also, the scavenging and protective effects of antioxidant compounds have shown a connection and synergistic effect with other biological properties, such as anti-inflammatory, anti-tumor, anti-aging, neuroprotective, cardio-protective, or antidiabetic. These compounds can be applied in several fields, including food, cosmetic, and pharmaceutical industry (Carpena et al., 2022). The current material under study is therefore of potential use.

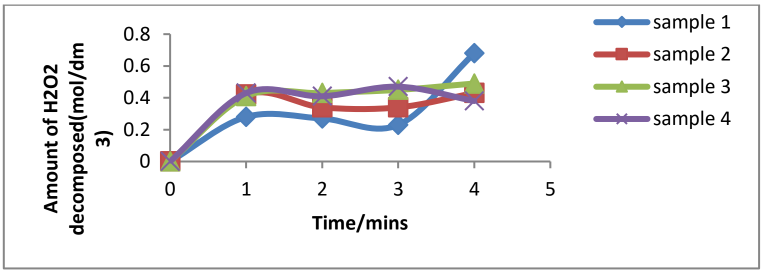

Fig. 2: A Plot of Concentration of H_2O_2 against Time for 2mls Shea Wastewater.

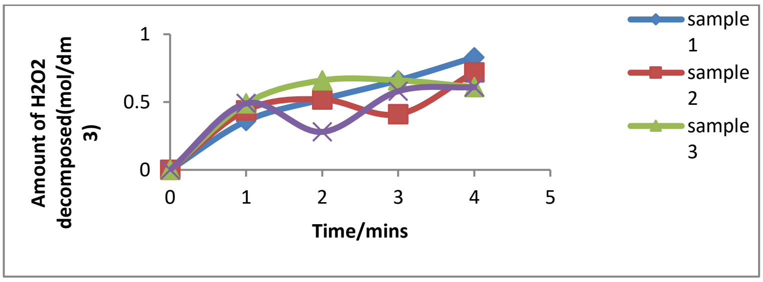

Fig. 3: A Plot of Concentration of H_2O_2 against Time for 4mls Shea Wastewater.

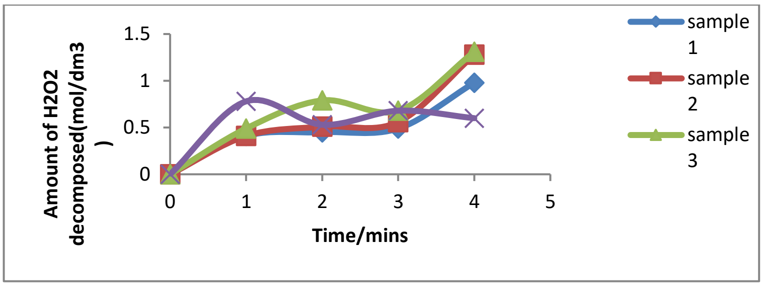

Fig. 4: A Plot of Concentration of H_2O_2 against Time for 8mls Shea Wastewater.

Removal or decomposition of H_2O_2 occurs through antioxidant reactions resulting in generation of H_2O (Sasikumar and Kalaisezhiyen, 2014). Activities of reactive oxygen species (such as H_2O_2) have positive roles (Packer et al., 2008), and impact on the physiology of living things (Jung et al., 2009). Studies indicate that reactive oxygen species influence many degenerative diseases, which relate to aging (Packer et al., 2008). Any increase/decrease in either oxidant/antioxidant species disrupts the balance and could result in oxidative stress and tissue damage (Wells et al., 2009). This biochemical property of the samples should be explored by industry for production of feed supplements or medical products for humans and animals. The antioxidant capacity exhibited by samples probably inform some of the ethnobotanical applications (Dei et al., 2008; Olife et al., 2013; Honfo et al., 2014; Otaiku, 2016) of the material. Hydrogen peroxide (H_2O_2) contributes to cell dysfunction and transformation. In the Fenton and the Haber-Weiss reactions H_2O_2 serves an intermediate and substrate respectively, for the production of OH• radicals which is responsible for oxidative stress and cellular damages. The OH• radical is the most reactive member of the ROS because of its short-lived half-life. The destructive action of this unstable molecule (OH•) is implicated in series of cell dysfunction, transformation, and even death (Kehrer, 2000; Sindhi et al., 2013; Anraku et al., 2018; Gulcin, 2020; Chigozie et al., 2021).

In all cases from figures 5-8, 4 and 8 ml shea wastewater had higher peroxide decomposition levels compared to the 2ml. But for a given time H_2O_2 consumed increased slightly up to 8ml of sample added. This outcome further established that the antioxidant activity of the waste is dose-dependent. Increasing volume of the sample increases antioxidant activity. But at the 4^{th} minute, sample 2 and 3 had higher peroxide decomposition than samples 1 and 4 (Figures 5-8). Therefore, samples 1 and 2 showed clear peroxide decomposition with amount of shea wastewater and the time lapse. Our findings are similar to the report by Keser et al. (2012) who indicated that extracts of C. monogyna were capable of scavenging hydrogen peroxide in an amount dependent manner. Hence, the antioxidant activity of the samples is attributable to the metabolism.

6.2. Anti-oxidative influence of increasing volume of shea wastewater

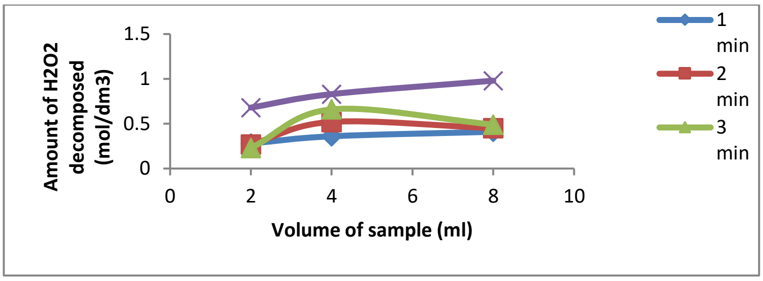

Fig. 5: Plot of H_2o_2 Decomposition against Volume of Samples 1.

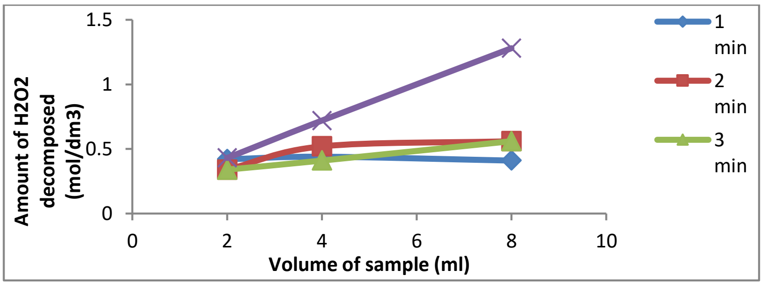

Fig. 6: Plot of H_2O_2 Decomposition against Volume of Sample 2.

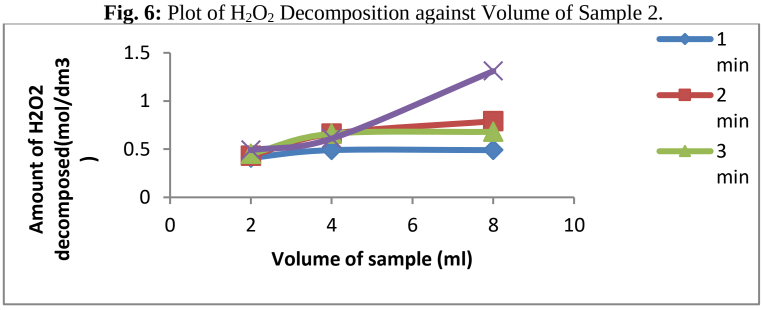

Fig. 7: Plot of H_2O_2 Decomposition against Volume of Sample 3.

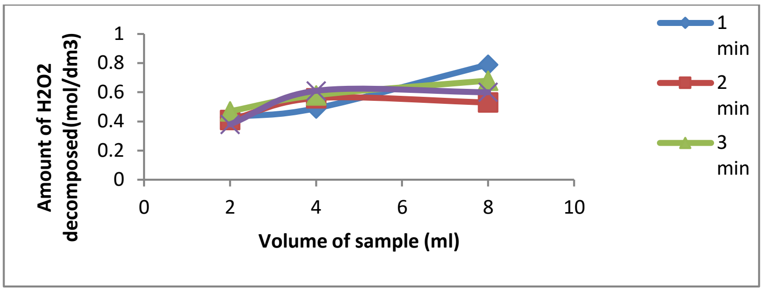

Fig. 8: Plot of H_2O_2 Decomposition against Volume of Sample 4.

From results of the phytochemical analyses (Table 5), alkaloids, saponins, tannins, flavonoids and general phenolics were found in the shea wastewater. Secondary metabolites occur in different parts of various plant species (Shankar, 2013; Al-Amiery et al., 2015), and those found in the wastewater would have been from the shea nuts from which the wastewater was generated. Alkaloids were found to be the most abundant while saponins and flavonoids were least. Alkaloids, saponins, tannins and general phenolics were found in all the shea wastewater samples, while flavonoids were found only in two samples. Plant based antioxidant activity can significantly be attributed to activity of flavonoids and related polyphenols (Honfo et al., 2014; Esmaeili et al., Ekin et al., 2017). Phytochemical extracts from crops are important for the health and food industry, making valorization of such waste possible to strengthen local economy and help mitigate environmental issues. Phytochemical content in by-products sometimes exceeds the main edible parts making them useful for functional food and nutraceutical production (Martillanes et al., 2018). Some crop by-products can be added directly in diet, or used as food additives or supplements. Phytochemicals are known to lower the risk of developing many diseases such as cancer, cataracts, Alzheimer, and Parkinson, etc. mainly due to their antioxidant and radical scavenging activities which can delay or inhibit the oxidation of DNA, proteins, and lipids (Martillanes et al., 2018).

Agri-waste can potentially be exploited as alternative to energy-intensive chemical fertilizers in organic production systems. Furthermore, value-added agri-waste can be a potential feedstock for livestock and industrial products (Subhash et al., 2022). It has high energy conversion efficiency compared to fossil fuel-based energy generation materials and can be processed for the production of second-generation biofuels such as biodiesel (Rao and Rathod, 2019).

Some natural antioxidants, which are secondary metabolites, currently found in the shea wastewater, have diverse biochemical activities [80]. Known phenolic antioxidants include tocopherols, tocotrienols, carotenoids, and polyphenols (Honfo et al., 2014) some of which have been reported to have molluscicidal, anthelmintic, hypnotic and insecticidal, medicinal and free radical scavenging activities. From the analyses and reviews, shea waste is indicated to have potential for formulating medicaments, supplements and other useful products. No specific regional trends of the presence of any given phytochemical was found pertaining to the different sources of the samples. Thus, it could be inferred that the general properties of all the wastewater obtained from shea nut extraction has close proximity chemical makeup.

7. Conclusion

Simultaneously, non-edible portions of many crops are rich in bioactive compounds with valuable properties. For this reason, developing various methods for utilizing agro-industrial residues as a source of high-value by-products is very important.

The shea wastewater samples were weakly acidic, with mean pH range of 5.95±0.05 – 6.10±0.00, at sample temperature range of 24.00±0.05 to 25.25±0.05 ^oC. Biochemical Oxygen Demand of the samples ranged from 168.00±0.00 to 86.25±6.25 mg O_2/L, indicating its potential hosting of organic matter. The oil content in the waste is quite substantial. Extracted residual oil from the wastewater was 6.46 to 14.67% of the waste. This makes it an important potential energy source to be explored.

Three mineral elements have been found in high abundance amongst those analysed in the samples. Magnesium in the wastewater was present in larger amounts; 206.65±mg/kg, 159.20±mg/kg and 88.35±mg/kg in different samples; on the order hand calcium and iron were lesser with mean concentrations of 19.60±mg/kg, 15.00±mg/kg, 14.60±mg/kg, and 10.75±mg/kg, 9.50±mg/kg, 7.75±mg/kg respectively. Highest mean concentrations of copper and lead were 0.08±0.00mg/kg and 1.22±0.00mg/kg respectively whilst the highest mean amounts of nickel and zinc were respectively 0.23±0.05mg/kg and 5.80±0.08mg/kg. Thus, the samples have both macro and micro nutrients.

The waste samples have appreciable antioxidant activities, exhibited by the high reducing power and decomposition of H_2O_2. The antioxidant activity was dose dependent. Phytochemicals found in the samples were alkaloids, phenolics, saponins, tannins and flavonoids while steroids and terpenoids were absent. However, the presence of phytochemicals was deduced to be related to the biochemical make up of shea nuts as it did not show environmental trends.

Shea wastewater could be a cheap and convenient source of natural antioxidants, and also antimicrobial agents for industrial use. The chemical makeup indicates a potential for use of the material in pharmaceutical, cosmetics and agro-based industries. Otherwise, appropriate disposal techniques have to be developed for the increasing volumes of the waste expected from awareness of shea butter uses, and for that matter nut extraction. The generated shea waste byproduct should be accessed as asset, and not disposed as waste. The possibility of shea wastewater causing environmental pollution indicative from the levels of BOD, mineral content oil, and antioxidant contents and phytochemical composition is obvious since the volume of waste is bound to keep rising.

Processing and application of shea wastewater in the production of biodiesel, environmental remediation, curative medicine, fertilizer, and in the development of biocatalysts is therefore a potential to be exploited.

8. Recommendations

Avenues for industrial applications from the biochemical make-up of shea waste should be explored.

Enhanced ethnobotanical use of the crude shea waste as source of biofuel should be improved and applied.

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