Analysis of Similarities Between Five Indigenous Bio-Catalyst Extracts used by Several Communities in Kenya

Bakari Chaka,
Osano Aloys,
Department of Mathematics and Physical sciences, School of Science and Information sciences, Maasai Mara University, Narok, Kenya
Magu Martin,
Department of Chemistry, Faculty of Science and Technology, Multimedia University of Kenya, Nairobi, Kenya

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Abstract

Commercial fermentation additives are quite expensive leading to increased production costs. Several communities in Kenya have continuously enjoyed fast saccharification of biomass during cooking and fermentation of porridge and ethanol using bio-catalysts. Infusion of these bio-catalysts into modern-day science is crucial in optimization of yields and reduction of production costs in food, alcohol, pharmaceuticals and energy industries. This study aimed at characterizing five extracts (*Terminalia b.*, *Acanthaceae spp.*, *Osyris lanceolata*, *Santaraseae spp.* and *Kigelia africana*) for possible similarities in physical-chemical parameters, elemental composition and ionic stability. Water extracts were preferred as natively done by these communities. Wet chemistry was used for physical-chemistry parameters and elemental composition while spectroscopy was used to analyze presence of bio-metals, functional groups, conjugation and ligands present. Lab simulation studies were conducted to evaluate ionic stabilities. The results indicated the samples had similar pH, conductivity and volatile solids with slight variations in alkalinity and fatty acids. There were similarities in the functional group peaks and conjugation patterns. The ions of $Cu(OH)_2$, $Fe(OH)^{2+}$, $ZnOH^{+}$, $Cr(OH)^{2+}$ and Co^{2+} were found to be dominant in all biocatalyst extracts. *Terminalia b.* and *Osyris lanceolata* were found to have relatively higher sulfate, phosphate levels while nitrate levels were similar in all the samples except for *Terminalia b.* biocatalysts. These compounds extracted from these herbs can be commercialized for industrial fermentation processes.

Keywords: Indigenous Bio-Catalysts, Similarities, Fermentation

Introduction

One of the most vital bio-chemical reactions is respiration. Anaerobic respiration majorly occur as fermentation of biomass. Fermentation is the precursor reaction to several significant industrial and domestic reactions. Fermentation is applied in manufacture of food, beverages, pharmaceuticals, chemicals and reagents, alcohol as well as in energy industry (Mandenius and Gustavsson, 2015). Home-baking and preparation of porridge also require fermentation. Fermentation process is spontaneous under anaerobic environment (Minarrieta *et al.* 2017). Unfortunately, fermentation process is very slow and more energy and/or catalysts have to be used for feasible industrial and domestic reactions. While energy demand is on the rise due to increased human needs, bio-chemical reaction catalysts are rare and expensive (Panagiotopoulou *et al.* 2014).

Saccharification of biomass is a key step in fermentation (Sridevi *et al.* 2015). Actually, most of the energy and time is used in saccharification due to the nature of bonds involved. Strong glycosidic bonds found in ligno-cellulose and amide bonds found in proteins require stronger forces to break down (Yuki *et al.* 2008. A lot of energy is thus used to heat reactors for optimal biomass hydrolysis and to increase regioselectivity of reactions. On the other hand, a bulging human population and increased needs associated with this increment dictates for the need for more energy sources and optimization of the current ones. Optimization of renewable energy sources for more production and safer combustion of carbon-based fuels should thus be emphasized. While both options are feasible even at industrial scales, the former is greener and more appealing.

The success of waste-to-energy processes is questionable in regards to the efforts and time consumed (Li *et al*., 2014; Don *et al*., 2012). Biofuel conversion from ligno-cellulosic biomass rates are still annoyingly low (Lillebo *et al.* 2013). Natural conversion pathways are not only slow but also lack regioselectivity which introduce more costs for separation of the products (Behenna *et al*. 2011). Use of catalysts to hasten biomass hydrolysis using less energy and increase product specificity is thus pertinent. Many catalysts have been employed in hydrolysis of biomass to fermentation products (Kalyani *et al*. 2017). It is however unfortunate that most of these catalysts are expensive, require specific conditions and are tailored for specific reactions only (Wright, & Solar Energy Research Institute, Golden, CO (US), 1988).

Chemical catalysts used in fermentation of biomass involve acids such as formic acid (Marzialetti, 2011) and salts of transition metal origin (Ouyang *et al*. 2012). Ferric and cobalt salts have been successfully used for fast hydrolysis of ligno-cellulose to furfuric acid (Sparks, 2015); a major precursor in this degradation pathways. These chemicals are expensive at industrial scales. On the other hand, biological catalysts used include fungi such as yeast

as well as genetically engineered enzymes (Bychkov *et al*. 2016). The natural anaerobic archea involving acidogenic, acetic and methanogenic bacteria in a natural biomass setup do not always suffice for fast biomass degradation pathways. Synthetic archea, enzymes and fungi all require very specific substrate and conditions to culture in large scale (Stockmann *et al*. 2014). Preparation of these catalysts have thus been left to only a few companies worldwide (Lin *et al*. 2014) accruing monopolistic disadvantages to these catalysts. There is thus need for a cheaper and sustainable means to generate and propagate bio-chemical catalysts.

Several communities in Kenya used indigenous salts to hasten saccharification of cellulose during food preparation (Osano *et al.* 2015). Other communities also used plant extracts to hasten saccharification and fermentation of biomass during porridge fermentation and preparation of alcohol used for traditional events. While use of these extracts have been outdated by modern-day chemicals and enzymes in industries and homes, their use has silently but successfully continued in the so called 'backward communities.' Not only are they cheap but they also enjoy a lot of atom economy with very little by-products formed.

This study purposed to characterize five plant extracts anciently used for saccharification and fermentation of biomass for possible similarities. *Santaraseae spp*. and *Osyris lanceolata* leaves and bark extracts were used by the Aembu community to hasten porridge preparation and fermentation of milk (sour milk is a communal delicacy). *Terminalia brownii* leave extracts were used by the Aandia community of western slopes of Mt. Kenya to hasten ethanol fermentation. *Kigelia Africana* (sausage tree) has continuously been used by many central Kenya natives to prepare traditional alcohol known as '*muratina*.' *Acanthaceae spp*. bark extracts are used by the Maasai community of Kenya to prepare traditional alcohol. Water extracts were preferred, as anciently done by the communities for actualization of findings. Evaluation of similar elements, ionic state, structure or morphology would go a long way in opening doors for propagation of the bio-catalysts or optimize on extraction of 'the common compounds.'

Materials and Methods

Design of Experiment

Five indigenous bio-catalysts were separately characterized for possible similarities in composition and morphology. An independent measures experimental design was followed with all samples being subjected to similar experimental treatments. Solvent extraction was done using water as natively done in the traditional setups of these bio-catalysts. $5\%^{w}/_{v}$ concentrations were used. Physical-chemical parameters including pH, electrical conductivity, total and volatile solids as well as anaerobic digestion

parameters such as dissolved oxygen, volatile fatty acids and alkalinity were measured. Five bio-metals (copper, chromium, cobalt, zinc and iron) composition were analyzed using atomic absorption spectroscopy and ionic speciation by PHREEQC simulation software. Functional group analysis and conjugation were done using FTIR and UV VIS spectroscopy respectively. Phosphates, nitrates and sulfates were also analyzed by UV spectroscopy.

Materials

Chemicals

All chemicals used were lab grade unless otherwise stated. The chemicals used were obtained from Sigma-Aldrich.

Sodium hydroxide pellets, potassium hydroxide pellets, nitric acid, sulfuric acid, hydrochloric acid, absolute ethanol, methyl red indicator, Analytical grade reagents; sodium chloride, glycerol, ammonium metavanadate, hydrazine sulphate, salicyclic acid, potassium bromide and ascorbic acid.

Equipment

Access to Shimadzu Fourier Transform Infrared (FTIR), Shimadzu-1800 ultraviolet visible spectrophotometer (UV-VIS), Atomic absorption spectrophotometer (PG-990 AAS), Hanna G-114 pH meter.

Methods

Sample extraction

Terminalia b. and *Santaraseae spp*. fresh leaves were squeezed and 5ml of the resulting crude extracts soaked in distilled water to make 100ml solution. The solution was left to macerate completely for 24 hours at room temperature away from direct light. The mixture was then serially filtered using Whatman no. 42 filter papers and the resulting solution preserved. For *Osyris lanceolata* and *Acanthaceae spp.,* the barks of these samples were ground to fine powder. 5g of these powders were soaked in 100ml distilled water and the procedure done for *Terminalia b*. and *Santaraseae spp*. repeated. For *Kigelia africana,* wet tubers were air dried away from direct light at room temperature before crushing the fibers to obtain fine powder. The procedures used for *Osyris lanceolate* and *Acanthaceae spp.* were then followed. About a liter of each of the sample solutions were made for all the tests done.

Sample characterization

pH, conductivity and dissolved oxygen

pH, electrical conductivity and dissolved oxygen were measured using a pH meter, conductivity meter and oxygen meter respectively.

Total solids and volatile solids content

100.0ml of sample solution was weighed, M_1 and placed in an oven conditioned at 105°C for 6 hours before removing, cooling (in a desiccator) and reweighing. The new mass was recorded as M_2.

$$\%TS = \frac{M2}{M1} \times 100\%$$

100.0ml of another sample solution was also weighed, M_1 and placed in an oven conditioned at 540°C for 1 hour before removing, cooling and reweighing. The new mass was recorded as M_2.

$$\%VS = \frac{M2}{M1} \times 100\%$$

Sample alkalinity

A raw sample was distilled in water (1:1) and the distillate titrated against standard 0.05N H_2SO_4 solution up to pH 4.0. The volume of sample solution used was used to determine the concentration of Alkalinity in the sample.

Sample volatile acids

A raw sample was distilled in water (1:1) and the distillate titrated against standard 0.1N NaOH solution up to pH 8.3. The volume of sample solution used was used to determine the concentration of VFAs in the sample.

Total dissolved solids and total suspended solids

For these procedures, sample concoction that had not been serially filtered were used. For the total suspended solids, the mass of 100.0 ml extract solution was weighed. The solution was then passed through a pre-weighed Whatman #41 filter paper. The used filter paper was then dried in an oven at 105°C for 1 hour, cooled in a desiccator before reweighing the filter paper and solution again. The difference in weight of the filter paper is the Total Suspended Solids (TSS).

Total dissolved solids were obtained by subtracting total suspended solids from the total solids values.

IR Functional Groups

The extracts were heated slowly at 60°C until all the water was dried. The samples were then cast into pellets using KBr pellet before analyzing for functional groups using IR Spectrometer.

Conjugation Analysis

Extracts were diluted serially using distilled water until a clear spectrum could be seen on the UV VIS monitor. A scan was then run between 190 – 900 nm wavelength.

Bio-metal analysis

Extracts obtained were triple filtered using Whatman # 42 filter papers before analyzing for Zn, Cu, Co, Fe and Cr using Flame Atomic Absorption Spectrometer (AAS).

The conditions for the AAS are as summarized in table 1 below;

Table 1: AAS conditions used to analyze the bio-metals

Bio-metal	Wavelength	Bandwidth	Lamp current	Flame	Sensitivity
Cr	357.9nm	0.4nm	5.0ma	Air/Acetylene	0.05mg/L
Co	240.7nm	0.4nm	5.0ma	Air/Acetylene	0.05mg/L
Cu	324.7nm	0.4nm	5.0ma	Air/Acetylene	0.03mg/L
Fe	248.3nm	0.2nm	5.0ma	Air/Acetylene	0.05mg/L
Zn	213.9nm	0.4nm	4.0ma	Air/Acetylene	0.01mg/L

Bio-metal speciation was done by combining the concentrations of the bio-metals with their electrical conductivity, pH and temperature of solution. These simulations were done with the help of PHREEQC software.

Ligands analysis

Nitrates

Nitrate standards were prepared by dissolving 8.0g of salicyclic acid in 100ml of 1M H_2SO_4 acid then swirling to fully dissolve. 10ml of this solution was added to 90ml of aliquot sample solution. Acidification using 1M HCl was done to minimize interference by other ligands. Absorbance was checked in the range of 520-560nm.

Sulfates

For standard preparation, 10g of NaCl and 10ml of conc HCl acid were added to 40ml of glycerol solution. A yellowish color was formed. 5ml of this solution was added to 45ml of analyte solution and the absorbance read at 410-430nm.

Phosphates

A conditioning reagent was added. It was made by dissolving 1.7081g of ammonium metavanadate and ascorbic acid (5.82g/300ml distilled water) in 150ml warm water. The solution was cooled before diluting to 250ml. Exactly 0.125g of hydrazine sulphate in 100ml distilled water was added.

Analyte samples were diluted by a factor of 10 and added the conditioning reagent before measuring the absorbances at 420-450nm against those of the blank and standards.

Data analysis

Statistical tools used include mean and median to test the appearance of the data sets while standard deviation and variance monitored the spread of the data. Correlation and regression to check on relation between absorbance and concentration during AAS and UV VIS spectroscopy analysis were also done. f-test was used to check the significance in the variances while t-test was used to assess how the means of the data are related. Statistical packages used include Microsoft Excel, PHREEQC and OriginLab applications.

Results and Discussions

Physical-chemical and bio-chemical analysis

The pH of sample solutions is a function of several parameters, including the volatile acids present and composition of other metals and ligands (Schultz *et al*. 2015). The bio-catalysts pH ranged between 5.950 ± 1.001 (*Osyris lanceolata) to* 7.160 ± 0.006 (*Acanthaceae spp.).* In general, the samples were slightly acidic. Bio-catalyst pH is a key factor in determining the existence of crucial micro-organisms necessary for biomass saccharification and fermentation (Berthelot *et al.,* 2019). Most hydrolytic enzymes thrive in acidic media (Amend and Shock, 2001) thus *Osyris lanceolata* specie could harbor the most population of these enzymes. Kaldor and Woodin, (1982); proposes that a good bio-catalyst should lie in the pH range of 6.0-7.0 to increase its availability and applicability. This pH range is quite neutral thus can easily accommodate both acidic and alkaline microorganisms. Chude-Okonkwo *et al.* (2017); reports that the stability of crucial polymer-protein complexes involved in bio-catalysis processes depend on pH and ionic strengths.

Ionic strengths of solutes in solutions were measured as electrical conductivities in this study. From the results, there was direct correlation between the electrical conductivity of the samples and their pH. *Acanthaceae spp*. sample with the highest pH value had the highest conductivity value (57.472 ± 0.012mS). *Osyris lanceolata* specie had the lowest pH and conductivity value ($5.950 \pm 1.001 mS$). Several enzymatic reactions are hydrophobic in nature (Duijnhoven *et al*. 2015); thus making bio-catalyst samples with more electrical conductivity in a better position to allow for these reactions. Marriott *et al*. (2016); reports that presence of a polar medium supports existence of important enzymes fundamental in catalyzing biomass. The total solids and total dissolved solids content of solutions are also related to the electrical conductivity. The same order was witnessed in the bio-catalysts analyzed.

The average values of the pH, total solids (TS), volatile solids (VS), total suspended and dissolved solids (TSS and TDS), electrical conductivity (EC),

dissolved oxygen (DO), volatile fatty acids (VFAs) and alkalinity (ALK) are analyzed and summarized in table 2 below.

Table 2: Characterization of indigenous biocatalyst extracts

Parameter	Samples				
	Terminalia b.	Acanthaceae spp.	Santaraseae spp.	Osyris lanceolata	Kigelia africana
pH	6.713±0.045	7.160 ±0.006	6.880±0.123	5.950±1.001	6.810±0.187
EC (mS)	48.428±0.998	57.472±0.012	34.600±1.225	31.200 ± 2.436	41.200 ± 1.168
TS (g/L)	9.000±0.000	11.000±0.000	11.000±0.000	11.670±0.000	10.667±0.000
VS (g/L)	7.333±0.000	8.330±0.000	7.000±0.000	8.000±0.000	3.000±0.000
TSS (g/L)	5.333±0.000	2.667±0.000	7.000±0.000	6.670±0.000	4.267±0.000
TDS (g/L)	3.670 ± 0.000	8.333±0.000	4.000±0.000	5.000±0.000	6.400±0.000
DO (%)	10.467±0.306	6.600±0.100	10.400±1.010	13.100±2.115	8.800±0.986
VFA (mg/L)	32.738±2.335	28.389±1.386	39.684±1.212	32.738±1.111	29.667±4.154
ALK (mg/L)	7.200±0.000	9.720±0.000	6.100±0.000	7.520±0.000	9.160±0.000

From the data in figure 2 above, about 81% of the solid content in *Terminalia b.* were volatile while *Acanthaceae spp*., *Santaraseae spp.* and *Osyris lanceolata* all had more than 63% of their solids volatile. Only *Kigelia africana* had little volatile solids (28%). Most of organic matter associated with biocatalytic activity are enzymes, some fungi strains and acids, all volatile in nature (Kinderlerer *et al.* 1989). Reduction in volatile solids imply high abundance in fixed solids. The biocatalytic nature of *Kigelia africana* might thus be induced by transition metals, also widely known as catalysts. *Acanthaceae spp*. and *Kigelia africana* species had more dissolved solids than the other samples. These findings are in tandem with their total solids, electrical conductivity and pH values. Kuzyakov and Xu (2013); reports that biocatalytic microorganisms prefer an ecological niche with more organic content with abundance or polymeric groups and protruded functional groups on their surface for enhanced activities (Yao *et al*. 2019). Such an environment is more profound in samples with more volatile solids and total suspended solids. The variation in volatile fatty acids was affected by the volatile and suspended solids. Therefore, *Acanthaceae spp*. and *Kigelia africana* had less volatile acids and more alkalinity. Alkalinity of biocatalysts represents the total buffer capacity of the samples against excessively acidic matter for optimal production of alkaline microorganisms (Soria *et al.* 2011). Alkalinity is crucial especially in biofuels (such as in biogas production). The alkalinity of biocatalysts should compose of soft ligands such as bicarbonates (Jakubowska and Normant-Saremba, 2015). *Acanthaceae spp., Osyris*

lanceolata and *Kigelia africana* had more alkalinity levels thus more suitable in biogas production.

Functional group of biocatalyst samples

The FT-IR spectra of the bio-catalysts showed concise resemblance in positioning and intensity of most of the peaks. The collated FT-IR spectra of the biocatalysts are as illustrated in figure 1 below.

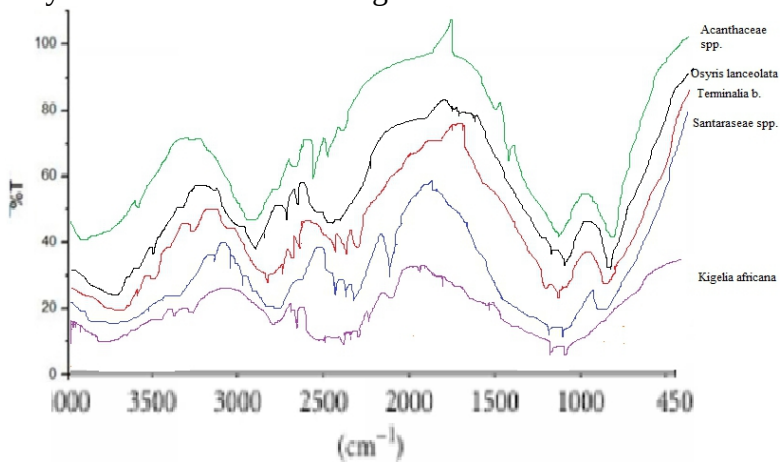

Figure 1; FT-IR spectra of the five biocatalysts analyzed

The pattern was more severe before 1200cm^{-1} wavenumber. The peaks had a broad O-H_{ROH} peaks between 3100-3600cm^{-1} indicating presence of alcohols. The FT-IR spectra of *Aspergillus niger* lipase enzyme (a biocatalyst) was found to exhibit dominant peaks at 3420–3150 cm^{-1} (–OH stretching vibrations and –NH stretching vibrations) (Harvey, 2015). There were several vibrational -CH_2 stretches at about 2850cm^{-1} which were also observed in *Nigel sativa* biocatalytic extracts (Mihoubi *et al*. 2017). There were peaks at around 2250cm^{-1} illustrating presence of amide groups and aryl compounds (Hasan *et al*. 2018). The lignin-based biocatalysts also had a sharp peak at around 1162cm^{-1} which was also present in all the samples except the *Acanthaceae spp.* specie. There were two close peaks at around 1500cm^{-1}, possible due to trans alkenes and a further broad peak at 1050cm^{-1} depicting presence of C-OH peaks. Goacher *et al*. (2018); reported such peaks to be abundant in lignin biocatalysts analyzed. Several enzymes characterized were also found to have these peaks, especially 1080cm^{-1} (C–O bond stretching vibrations) (Kristoffersen *et al*. 2019).

Conjugation analysis of the bio-catalysts by UV VIS

All samples were found to have a broad absorption peak with several multiplicities for *Terminalia b., Osyris lanceolata* and *Santaraseae spp.*

samples. The peaks were found to range between 300 to 600nm. The UV VIS profiles of the five biocatalysts analyzed are illustrated in figure 2 below.

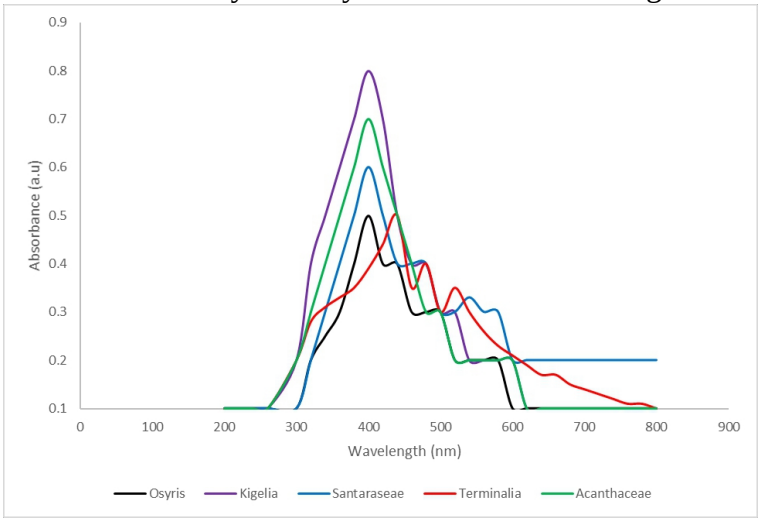

Figure 2; UV VIS spectra of the indigenous biocatalysts

All spectra except *Terminalia b*. sample had maximum absorption at 400nm. Niknejad *et al*. (2015); obtained similar spectra when analyzing yeast cells impregnated with silver nanoparticles. Similar findings were also reported by (Whitcombe *et al*. 2014) who obtained λ_{max} at 425nm wavelength for yeast particles. *Terminalia b.* and *Santaraseae spp*. samples were found to have an absorption band at 460nm, citing presence of conjugated pigments such as carotenoids or xanthophylls, halo-alkanes and sulphate groups (Priyanka *et al*. 2015). These two biocatalysts also portrayed more peaks indicating more conjugation levels. Presence of halides in the *Terminalia b.* sample was confirmed by the peak at 540nm. A similar peak was present at 560nm in the *Santaraseae spp.* sample. *Kigelia africana* and *Acanthaceae spp.* samples exhibited stronger absorption peaks at 400nm but had fewer multiplicities.

Bio-metal analysis of the biocatalysts

Five bio-metals were analyzed for their concentrations in the biocatalytic extracts. Their presence is crucial in catalyzing biochemical reactions. Both iron and cobalt ions were found to be quite abundant in the test samples whereas chromium ions were the least concentrated. Table 3 below summarizes the concentrations of these bio-metals in mg/Kg of wet plant sample.

Table 3: Concentrations of bio-metals in the indigenous biocatalyst samples

Sample	Copper (mg/Kg)	Zinc (mg/Kg)	Iron (mg/Kg)	Chromium(mg/Kg)	Cobalt (mg/Kg)
Terminalia b.	72.020 ± 0.037	69.285 ± 0.006	242.645 ± 1.308	12.295±0.103	244.370 ± 0.303
Acanthaceae spp.	75.855±0.202	75.105±0.097	459.665 ± 0.827	24.595±0.154	487.755 ± 0.446
Santaraseae spp.	102.620±1.229	61.221±1.002	400.000 ± 0.000	15.169±1.028	403.600±12.108
Osyris lanceolata	106.752±3.457	63.155±2.011	396.000 ± 2.215	10.113±0.015	403.321±1.240
Kigelia africana	97.155±6.120	71.255±0.075	400.000 ± 1.000	16.199±0.276	401.440±2.500

Acanthaceae spp. was found to have the most abundant bio-metals except copper (most abundant in *Osyris lanceolata*). Only *Santaraseae spp*. and *Osyris lanceolata* had more than 100mg/Kg of copper ions per wet sample. Vianello *et al.* (2014); reported that similar concentrations of copper ions deposited alongside histidine in laccase enzymes. Abundance of copper ions was attributed to enhanced enzymatic roles of ligninolytic enzymes (Kostadinova *et al*. 2018). The abundance of copper and zinc ions in plant samples is a function of several parameters including, the pH, conductivity and levels of other compounds such as phosphates present (Chaka and Osano, 2019). The concentrations of these bio-metals are however largely affected by their immediate soil and water environment (Zhao *et al*. 2015). Together with zinc and manganese ions, copper ions have been used to enrich yeast (*Saccharomyces cerevisae*) in catalyzing degradation of biomass (Marjamaa and Kruus, 2018). Iron, zinc and cobalt in various concentrations have all been used as cofactors in biomass degradation enzymes (Haferburg and Kothe, 2007). The levels of chromium ions were all less than 25mg/Kg of wet sample. Only *Acanthaceae spp*. had more than 20 mg/Kg ($24.595 \pm 0.154 mg/Kg$) while *Osyris lanceolata* had as little as 10.113 ± 0.015 mg/Kg of the wet sample. Stanley *et al*. (2014) reported that increased chromium (vi) concentration in enzymes lead to death of the enzymes. Chromium (vi) levels of higher than 4.0mg/Kg of dry plant sample were proven to be toxic. The exact type of chromium ion and other bio-metal ions present are discussed under bio-metal speciation below.

Speciation of bio-metals present

Different biochemical functions of enzymes and other biocatalysts are only achieved by metals at specific concentration ranges and ionic form. Elucidation of the exact form and abundance of bio-metal is detrimental in predicting the nature of reactions to be expected. Tables 4, 5, 6, 7 and 8 below summarize the speciation of these bio-metals when analyzed at their specific pH, electrical conductivity and temperature. Only the most abundant ions species were recorded. Most of the species were found to be oxides and hydroxides.

Copper

Table 4 below summarizes the speciation of copper ions in the five indigenous biocatalysts analyzed.

Table 4: Speciation of copper ions in the biocatalysts

Sample	Cu^+	$Cu(OH)_2$	$CuOH^+$	$Cu_2(OH)_2$
Terminalia b. (%)	1.573	95.283	3.069	0.075
Acanthaceae spp. (%)	1.560	94.617	3.043	0.078
Santaraseae spp. (%)	2.653	55.852	4.032	37.464
Osyris lanceolata (%)	11.836	1.571	1.836	84.757
Kigelia africana (%)	1.749	76.959	2.291	18.127

From table 4 above, $Cu(OH)_2$ was the prevalent ion in all biocatalysts except in *Osyris lanceolata* sample. *Osyris lanceolata* had an extremely low pH value (5.950 ± 1.001) and conductivity ($31.200 \pm 2.436 mS$) which might have contributed to this variance. *Santaraseae spp.* sample which also had low pH and conductivity had less $Cu(OH)_2$ ions. Copper (ii) ions have been found in various oxidase and reductase enzymes (Gromov *et al.* 1999) in plant samples. Copper (ii) hydroxide ions in ammonia solution form Schweizer's reagent which have been proven to dissolve cellulose (Tomczyńska-Mleko *et al.* 2015). Natural occurrence of these ions in the bio-catalysts position them as potential cellulose degrading extracts. Only *Osyris lanceolata* had more than 10% of copper (i) ions present.

Iron

Iron and cobalt were found to be abundant in all the bio-metals analyzed. Several hydroxides of iron were recorded as being the most abundant. These findings were recorded in table 5 below.

Table 5: Speciation of the most abundant iron species in the biocatalysts

Sample	$FeOH^{+}$	$Fe(OH)_3$	$Fe(OH)^{2+}$	$Fe(OH)^{-}_4$	$FeOH^{+2}$
Terminalia b. (%)	77.830	11.739	10.329	0.098	0.004
Acanthaceae spp. (%)	78.139	11.549	10.213	0.097	0.004
Santaraseae spp. (%)	1.950	38.709	59.060	0.237	0.044
Osyris lanceolata (%)	21.982	3.167	73.575	0.004	1.272
Kigelia africana (%)	2.741	33.387	63.648	0.165	0.050

Hydroxides of ferric ($Fe(OH)^{2+}$) ions were prevalent in *Santaraseae spp.*, *Osyris lanceolata* and *Kigelia africana* while ferrous hydroxides ($FeOH^{+}$) dominated in *Terminalia b*. and *Acanthaceae spp*. extracts. Both ferric and ferrous ions in plants have been reported to catalyze movement of electrons in plants during biochemical reactions (Lindh, 2007). Such movements in biocatalytic plants increase the rate of fermentation of cellulose. Ferric ions have been reported to be abundant in citric and oxalic acids, all used during fermentation process (Barbeau, 2006). Kimata *et al*. (2018); found out that ferric ions in bio-metals participate in redox biochemical reactions. Ferric ions have also been reported as key factors for the growth of yeast (a biocatalyst) (Sreenivasan *et al*., 1993). Ranawat and Rawat, (2018); also reported ferric ions to be crucial electron acceptors in anaerobic respiration process.

Zinc

There are over 300 zinc enzymes covering all the six classes of enzymes (Garingrdestedt et al. 2009). Elucidation of the exact specie of zinc ions in the biocatalysts was summarized in table 6 below.

Table 6: Speciation of zinc ions in biocatalysts

Sample	$ZnOH^{+}$	$Zn(OH)_2$
Terminalia b. (%)	99.882	0.1172
Acanthaceae spp. (%)	99.880	0.115
Santaraseae spp. (%)	89.107	10.893
Osyris lanceolata (%)	98.957	1.043
Kigelia africana (%)	89.673	10.327

All the zinc ions were found to be divalent in the biocatalytic samples. Several researchers have found out that zinc is not readily oxidized or reduced from its +2-oxidation state (Ahmad *et al.* 2012). From table 6 above, most of the biocatalysts zinc ions were $ZnOH^{+}$ for all the test samples. This implied that

zinc (ii) ions with one hydroxide group and a vacant coordination site were prevalent in the biocatalysts at slightly acidic pH and low temperatures (about 17-19°C). Krezel and Maret (2016); McCall *et al.* (2000); showed that according to Pourbaix diagram, in the absence of other coordinating ligands, zinc ions are only present in biological compounds as zinc (ii) ions. Zinc is thus redox-inert in biocatalysts and all other biological compounds. Zastrow and Pecoraro, (2014); proved that hydrolytic zinc (ii) ions were the dominant ions in hydrase enzymes.

Chromium

Chromium toxicity has previously been cited as one of the factors leading to death of enzymes (Stanley *et al*. 2014). This phenomenon is caused by chromium (vi) ions. On the other hand, chromium (iii) ions are important catalysts with the ability to hasten organic and inorganic processes (Soheili *et al*. 2018). The variations of chromium ions in the biocatalyst samples analyzed are summarized in table 7 below. Most of the species were found to have hydroxide ligands.

Table 7: Speciation of chromium ions in biocatalyst extracts

Sample	**$Cr(OH)^{2+}$**	**$Cr(OH)_3$**	**$Cr(OH)_2^+$**	**Cr^{+3}**	**$Cr_3(OH)_4^+$**	**$Cr_2(OH)_2^+$**	**$Cr(OH)_4^-$**
Terminalia b. (%)	73.140	17.114	9.701	0.044	0.001	0.000	0.001
Acanthaceae spp. (%)	73.132	17.102	9.720	0.044	0.001	0.000	0.001
Santaraseae spp. (%)	63.509	2.157	5.859	0.012	28.397	0.058	0.007
Osyris lanceolata (%)	0.271	0.001	0.582	0.000	98.965	0.181	0.000
Kigelia africana (%)	58.850	1.698	6.414	0.015	32.948	0.071	0.005

All biocatalysts had chromium (iii) ions with one hydroxide ligand as the dominant specie except *Osyris lanceolata* extracts. The prevalent specie in *Osyris lanceolata* was $Cr_3(OH)_4^+$. These species were also quite abundant in *Santaraseae spp.* and *Kigelia africana* extracts. Oliviera (2012) reported abundance of trivalent chromium in enzymes used in sugar metabolism. The ions also play key roles in sugar tolerance levels in animals (Hua *et al.* 2012). Other trivalent chromium ions with hydroxide groups accounted for up to 20% in the biocatalyst extracts. *Terminalia b.* and *Acanthaceae spp.* samples were the most diverse as far as the abundance of chromium ions were concerned; lacking only $Cr_2(OH)_2^+$ present in the other biocatalysts.

Cobalt

Cobalt has the ability to complex with several enzymes, acting as a co-factor in their processes (Remy *et al.* 2013). Determination of the exact ions and possible number of coordination sites in the cobalt complexes is

fundamental in modelling industrial biocatalysts for fermentation process. The speciation of the abundant cobalt ions in the biocatalysts were summarized in table 8 below.

Table 8: Speciation of cobalt ions in biocatalytic extracts

Sample	$HCoO_2^-$	$Co(OH)_2$	Co^{2+}	Co_2OH^{+3}
Terminalia b. (%)	5.581	0.000	94.419	0.000
Acanthaceae spp. (%)	56.549	0.001	43.451	0.000
Santaraseae spp. (%)	99.997	0.003	0.000	0.000
Osyris lanceolata (%)	0.014	0.000	99.985	0.001
Kigelia africana (%)	10.058	0.000	89.942	0.000

The abundance of cobalt ions in the biocatalysts varied between hydroxy (oxo) cobalt ions ($HCoO_2^-$) and 'other' cobalt (ii) ions (Co^{2+}). The *Acanthaceae spp*. extracts were quite diverse in the two ions, with both having almost equal distribution. Hoppert (2011) reported abundance of cobalt (ii) ions in non-corrin enzymes involved in catalyzing the oxidation of nitriles to amides (nitrile hydratase enzymes). Cobalt (ii) ions were also found to supersede their cobalt (iii) counterparts in speciation studies conducted on methanogenic cells (Paulo *et al.* 2017). The research however found out that the cobalt (ii) ions favored chloride, carbonates and citrate ligands unlike the oxide ligand in *Acanthaceae spp.* and *Santaraseae spp*. above.

Analysis of ligands present

Nitrates

Nitrate concentration in anaerobic digestion substrate is seen as an inhibitor of the whole fermentation process (Świątczak, and Cydzik-Kwiatkowska, 2018). Anaerobic digestion process is proportional to Chemical Oxygen Demand (COD)/Nitrogen ratio. Over anaerobic digestion retention period, nitrates present in the substrate are gradually reduced to nitrites and nitriles (Igamberdiev and Hill, 2004). Viewing the scenario from a biocatalytic angle (nitrates present in the biocatalysts) is however quite complicated. Dyckmans *et al.* (2006); found the growth of anaerobic archea to be affected by presence of nitrates. The fermentation patterns for lactate, glycerol and pyruvate compounds were also inhibited in nitrate media (Patil *et al.,* 2017). Figure 3 below illustrates the UV VIS profiles of the five biocatalysts (λ_{max} = 550nm).

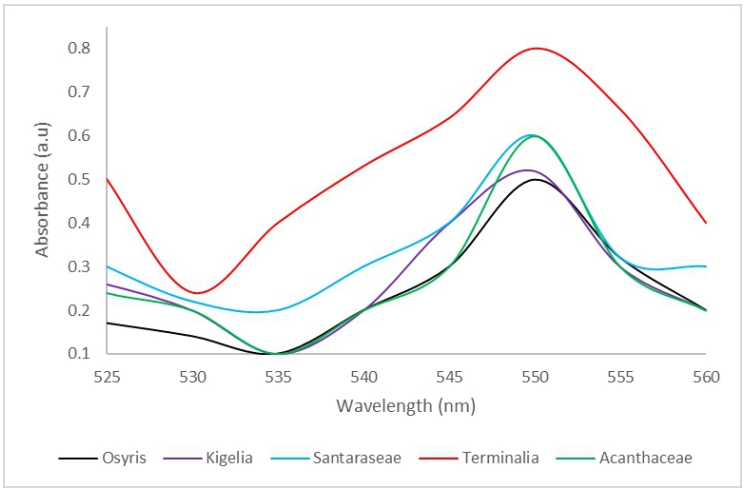

Figure 3; UV VIS spectra of the biocatalyst nitrates (λ_{max} = 550nm)

The levels of nitrate ions were most abundant in *Terminalia b.* samples. Nitrate levels in this biocatalyst was significantly higher and different from the other extracts. Abundance of nitrates in amylase enzymes was found to increase the activity of the enzymes in solid state fermentation of soluble starch (Sethi *et al.* 2016). Increased nitrate and chloride ions concentrations in the enzymes yielded significantly higher products compared to the control.

Sulfates

Alongside ferric ions (Fe^{3+}) and nitrates, sulfate ions are fundamental inorganic compounds used as electron acceptors in anaerobic respiration process (Aharon, 2011). Another organic compound with sulfate ions and responsible for altering anaerobic digestion is dimethyl sulfoxide (DMSO) reagent (Ali *et al*. 2014). The levels of sulfate ions in the extracts of the biocatalysts were screened at λ_{max} = 425nm using UV VIS. From the UV VIS spectra, there was a lot of disparity in the levels of sulfates. Figure 4 below illustrates the spectra of sulfates in the biocatalyst samples.

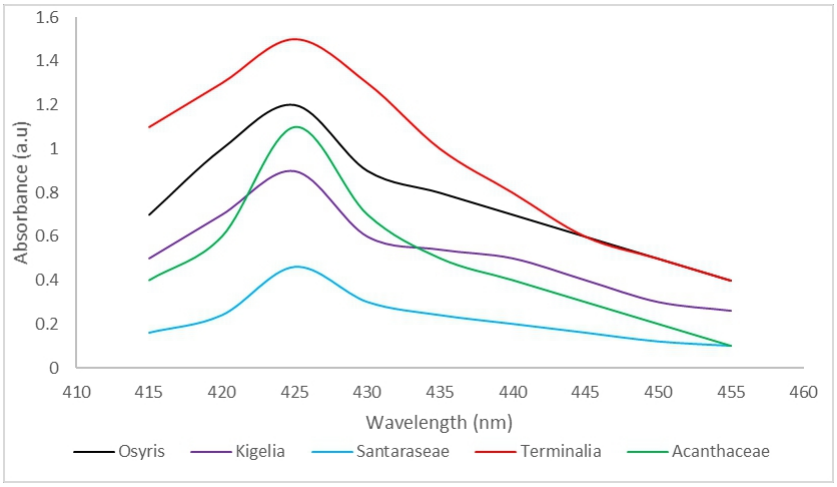

Figure 4; UV VIS spectra showing the sulfate levels in the biocatalysts (λ_{max} = 425nm)

Sulfate levels in the biocatalyst extracts were in the order of *Terminalia b., Osyris lanceolata, Acanthaceae spp., Kigelia africana* and *Santaraseae spp.* The results imply that *Terminalia b.* would be most suitable in electron transfer effectively degrading more glucose molecules in lactate and pyruvate fermentation processes. In such reactions, more sulphide products are likely to be formed due to the reduction processes. Ali *et al.* (2014) also reports δ-proteobacteria used in biomass hydrolysis being more efficient as sulfate levels increased.

Phosphates

Phosphate ions are involved in phosphylation process during breakdown of glucose to produce energy by respiration (Lodish *et al.* 2000). These ions can also be used in fermentation process (anaerobic). From the spectra, *Santaraseae spp.* extracts showed the highest levels of phosphates followed by *Terminalia b., Osyris lanceolata, Acanthaceae spp*. and *Kigelia africana.* Hara *et al.* (2015); reported phosphate levels as being key in catalysis of glyceraldehydes in biomass. Presence of phosphates in pyro-phosphate releasing enzymes was found to trigger rapid degradation of inosines (Suarez *et al.* 2012). The levels of phosphate ions in the biocatalyst samples were analyzed by UV VIS spectroscopy as illustrated in figure 5 below.

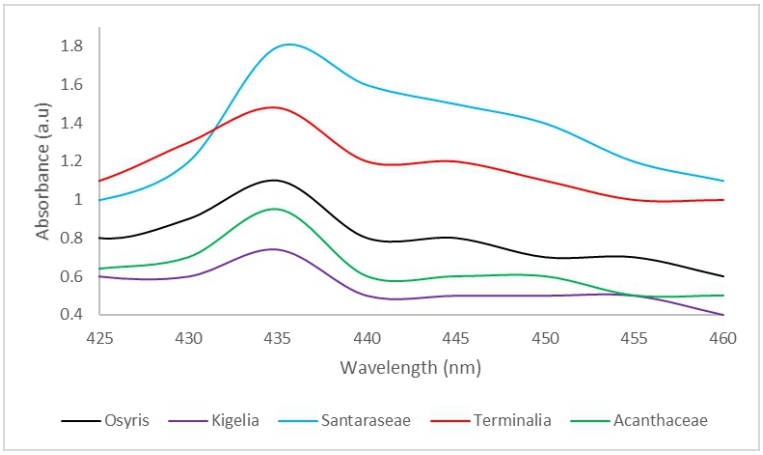

Figure 5; The UV VIS spectra of phosphates in the biocatalyst samples (λ_{max} = 435nm)

From figure 5 above, *Santaraseae spp.* and *Terminalia b.* samples would be more suitable in phosphylation enzymatic processes compared to the other biocatalysts. Increased phosphate levels in enzymes were found to accelerate the functioning of adenosine triphosphate synthase molecule, effectively increasing the energy products obtained (Meyrat, 2019).

Conclusion

The study found out several similarities in physical chemical parameters, composition and ionic stability of the biocatalytic extracts analyzed. The pH, conductivity and solid content of the biocatalysts were similar except for *Osyris lanceolata* which had relatively lower values. *Acanthaceae spp*. extracts had lower oxygen concentrations compared to the other samples. Only *Santaraseae spp*. extracts had more than 35mg/L of volatile fatty acids. All the other biocatalysts had volatile acids in the range of 29-32mg/L. Similar FT-IR patterns were observed in the spectra of all the biocatalysts. All samples had characteristic peaks at 2250cm^{-1}, 2850cm^{-1} and two close peaks at around 1500cm^{-1}.

Analogous UV VIS spectra were also observed with a broad peak around 400nm wavelength. *Terminalia b.* and *Osyris lanceolata* extracts showed more multiplicities in the spectra citing more conjugation. The biocatalysts recorded high level uniformity in bio-metal concentration and speciation. *Acanthaceae spp.* extracts were found to have the most bio-metal abundance while *Terminalia b.* samples had the least. The ions of $Cu(OH)_2$, $Fe(OH)^{2+}$, $ZnOH^{+}$, $Cr(OH)^{2+}$ and Co^{2+} were found to be dominant in all biocatalyst extracts. *Terminalia b.* and *Osyris lanceolata* were found to have relatively higher sulfate, phosphate levels while nitrate levels were similar in all the samples except for *Terminalia b.* biocatalysts.

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