

Determination of the Chemical Composition of Banana Leaves Wax

Kaana Asemave^{a*}, Stephen Anthony Ujah^b

^{a,b}Dept. Chemistry, Benue State University, Makurdi - Nigeria

^aEmail: kasemave@gmail.com

Abstract

Banana cultivation generates an enormous number of agricultural residues, such as pseudo-stems and leaves. These residues are regarded as an unutilized sustainable biomass resource. Hence the research considers extraction of banana leaves wax and its characterization. Soxhlet apparatus was used for the extraction and a yield of 0.97wt% was found. Thereafter, solubility, FTIR, GC-MS, and UV/visible analyses were performed on the wax. The wax was soluble in hexane and ethyl acetate but insoluble in methanol and ethanol. The FTIR absorption peaks of 3373.2-3384.4, 2922.2-2851.4, 1738.9-1666.1 cm^{-1} implied the presence of alcohol, saturated organic compounds, and then ester/aldehyde/ketones/carboxylic acid, respectively. The λ_{max} for the wax was found at 360 nm. The second absorption was found at 400 nm. These results indicate the presence of unsaturated compounds. Thus, the wax contains long chain alkanes, alcohols, esters, aldehydes and carboxylic acids. However, the predominant groups of chemicals present in the wax are palmitic acid (C_{16}), linoleic acid (C_{18}); lesser amounts of esters, alcohols, aldehydes, and alkanes. The banana wax can be used in making cosmetics, inks, candles, packaging materials and so on.

Keywords: Agricultural residues; Banana Wax; Palmitic acid; Linoleic acid.

1. Introduction

Banana is an economically important tropical crop cultivated worldwide in 4.54×10^6 ha yielding 6.93×10^7 t annually [1]. This cultivation generates a large amount of residue since each plant produces only one bunch of bananas. After its harvesting, the pseudostems and leaves are cut and usually left in the field or incinerated [2]. Furthermore, the banana harvesting residues are regarded as waste and a recognized source of environmental pollution as the case may be [3]. Shah and his colleagues reported each hectare of banana crop generates nearly 220 t of plant residual waste [4]. The putative amount of the annual global banana waste is 9.99×10^8 t.

* Corresponding author.

This huge amount of the waste has some advantages, such as its potential use in its conversion into industrial products, like wax recovery from the leaves; because its leaves are covered with wax [5,6,7]. These can be recovered and possibly applied for many purposes. Plant waxes are used for a number of purposes; including automobile wax, food coating, confections, cosmetics, medicines, and chemical bases [8,9,10]. In 2003, Japan imported 3081 t of carnauba wax, 259 t of other natural plant waxes, and 712 t of bees' wax [9]. Carnauba wax is extracted from palm tree leaves and is the most popular commercial wax because of its high melting point, hardness, toughness and luster [4]. If the properties of the banana leaves wax are similar to natural commercial wax such as carnauba, then banana wax may become an alternative material. Interestingly, using banana wax byproducts would greatly benefit tropical developing countries by creating a high value product from unutilized biomass resources of banana cultivation. Also, it will be to the best of interest to use plant waxes in addition to their conventional counterparts for commercial purposes in order to meet the ever-increasing demand of man. Furthermore, waxes are used commercially in cosmetics for making varnish, lipsticks, lotions and creams [11]. In dentistry, applications of waxes include baseplate wax, bite registration wax, indicator wax, sticky wax, utility wax and diagnostic wax-up. As for commercial applications and automobile industry, waxes are applied in the production of candles, car polish, lubricants, and dyes. Waxes are also used in paper, textile and match impregnants, fruit and food preservatives, insulators for electrical wires, manufacture of polishes and pharmaceuticals. Besides the commercial industries, waxes are used as starting materials for chemical conversions such as chlorination, oxidation, cracking and polymerization [11]. Plant-based waxes have been given attention for these purposes because of increased in consumers awareness about biobased products and also due the sustainability of such materials [8]. This is also in line with the principles of Green Chemistry that emphasis the use of renewable materials [12,13,14,15,16]. More so, there is emphasis on edible films and coatings from natural products, unlike synthetic and nonedible films. Their use contributes to reducing environmental pollution. Plant based waxes are also readily decomposed than the polymer material. The market of wax industry is also expanding and growing at an encouraging rate. It is also noted that natural waxes are increasing in demand, especially plant-based waxes [8]. In general, plant waxes are made up of long-chain hydrocarbon compounds such as alkanes, primary alcohols, aldehydes, secondary alcohols, ketones, esters and other derived compounds[11,17,18]. More so, plant leaves waxes may also contain hydroxy- β -diketones, oxo- β -diketones, alkenes, branched alkanes, acids, esters, acetates and benzoates of aliphatic alcohols, methyl, phenylethyl and triterpenoid esters. This leaves surface wax serves as a mechanical barrier which protects the plant tissues against UV radiation and bacterial or fungal attacks and also reduces water loss in summer time[19,20]. Currently, the four recognized classes of wax are animal waxes, plant waxes, mineral waxes, and synthetic waxes [11,21]. However, there are paucity of studies on the chemical composition of plant waxes, especially banana leaves wax [22]. Therefore, the study is aimed at finding the chemical composition of banana wax as alternative to popular waxes.

2. Materials and methods

Soxhlet extractor, GC-MS (5977A MSD), FTIR (Agilent Cary 630 FTIR), UV-Spectrometry, beakers, mortar and pestle, measuring cylinders, isomantle, hexane, methanol, ethyl acetate, ethanol, distilled water were used.

2.1. Methods

Sample Preparation: The leaves were shade dried for few days to a constant weight and then crushed directly by grinder without adding any solvent in order to increase the surface area for extraction. The resulting stuffs were kept for subsequent soxhlet extraction. The wax extraction: Using conventional soxhlet extraction method, 14 g of the banana plant material was placed in a thimble-holder (25 × 80 mm thimble) and about 350 mL of normal hexane was measured and placed in the round bottom flask of the extractor [18]. The thimble was placed inside the soxhlet extractor and the side arm lagged with glass wool. The solvent was heated using the isomantle until it began to evaporate, moving through the apparatus to the condenser and the condensate then dripped into the reservoir containing the thimble, when the liquid reaches the overflow level, a siphon aspirates the solution of the thimble-holder and unloads it back into the distillation flask, carrying extracted solutes into the bulk liquid. Solvent and solute in the flask were separated using distillation; solute was left in the flask while, fresh solvent passed back into the plant sample. The cycle of operation was repeated until approximate complete extraction was achieved. The powdered leaves were extracted with the appropriate volume of solvent at 80 °C for about suitable time (4 h) to achieve approximate complete extraction. The solvent was removed from the extract with the aid of rotary evaporator Solubility test: Preliminary test of solubility was carried out with different solvents. Simply, 20 mg of the extracted wax (solute) was mixed with 1 mL of different solvents (methanol, ethyl acetate, ethanol and hexane) to assess the level of solubility. After a while, a gentle shaking was done to confirm the solubility. Gas Chromatography-Mass Spectrometry (GC/MS) analysis: The GC/MS model; 5977A MSD at the multi-user science research laboratory of the Ahmadu Bello University, Zaria - Nigeria was used for the analysis of the sample to ascertain the constituents present. GC analyses of intact cuticular wax extracts or fractions was carried out, with the instrument's working condition; a high polarity column (HP-88) of dimension 60 m×0.25 mm, 0.20 µm. the carrier gas used is Helium at 1.4 mL/min constant flow. The oven temperature of between 125 °C to 220 °C was employed. The detector (FID) temperature used is 260 °C while, the injector temperature is 250 °C. The extracted wax in above was diluted with n-hexane and injected into the GC/MS machine. A chromatogram of the was obtained and each peak was matched to the respective compounds using the instrument's library, via their mass spectra. FTIR analysis: The FTIR model; Agilent Cary 630 FT-IR, at the multi-user science research laboratory of the Ahmadu Bello University, Zaria - Nigeria was used for the analysis of the sample to ascertain the functional composition of the sample. The extracted wax which was in semi-solid form was smeared on the polished salt plate made of KBr. A second plate was then placed upon it to sandwich the sample and was clamped together firmly. The excesses by the edges of the two plates were wiped off and the mounted onto to the sample holder and the analysis was carried out. UV/Visible spectrum of the banana wax: UV analysis for the wax was determined by dissolving 20 mg of the wax into 20 mL hexane. Then the absorbance of the sample was scanned between 200 – 800 nm.

3. Results and discussion

3.1. The extracted wax

The wax extracted from the banana leaves is shown below in Figure 1.

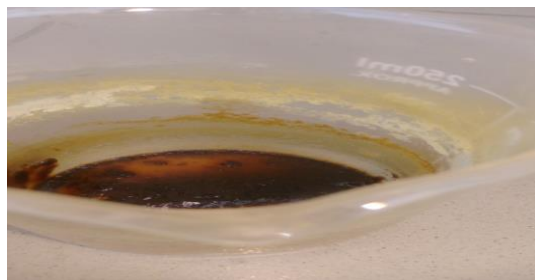


Figure 1: The banana wax

The yield of the wax determined was 0.97wt%. The amount of the wax extracted is similar the previous report [21]. In addition, banana leaf wax was extracted using hexane reflux. *Musa liukuensis M. acuminata*, and *M. chiliocarpa* yielded 0.58%, 1.05%, and 1.41% (dry basis), respectively [22]. The wax was found to be soluble in hexane and ethyl acetate but partially soluble in methanol and ethanol. This indeed is expected of waxes since they are made of long chain alkanes, alcohols, esters, aldehydes, and carboxylic acids. As lipids they are mostly soluble in hexane and much less polar solvents such as ethyl acetate. Meanwhile, very high polar solvents like methanol and water will not dissolve waxes very well.

3.2. FTIR of the wax

The FTIR spectrum of the wax is given below in Figure 2. Therefore, the characteristic peaks for common functional groups identified from the spectrum are as described in the Table 1.

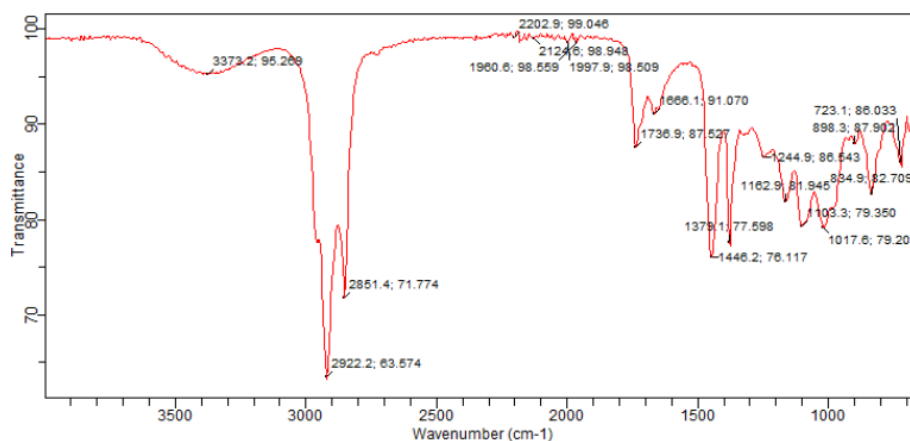


Figure 2: FTIR spectrum of the wax

Table 1: Characteristics FTIR absorption peaks of the banana wax

Peaks (cm ⁻¹)	Function groups	Possible compounds
3373.2	O -H stretch	Alcohols
2922.2 – 2851.4	C – H stretch	Alkanes
1738.9 – 1666.1	C = O stretch	Aldehyde, Ketone, Carboxylic acids, Ester

The absorption peaks of 3373.2-3384.4, 2922.2-2851.4, and 1738.9-1666.1 cm^{-1} implied the presence of alcohol, saturated organic compounds, and then ester/aldehyde/ketones/carboxylic acid. Asemave [23,24] and Asemave and his colleagues [25,26,27,28,29,30,31] reported similar evaluations of FTIR spectra. Furthermore, Reddy and his colleagues [32] also confirmed the presence of C=O group at similar absorption values to the one reported in this work. Similarly, wheat straw waxes were extracted and were found to contain high value wax compounds including free fatty acids, fatty alcohols, alkanes, wax esters, sterols, aldehydes, and β -diketones. The solvent properties did not affect the composition of the extracts but changed the relative abundance of the different compounds [21].

3.3. GC-MS results

About 13 peaks were found from the GC-MS chromatogram of the wax. These peaks correspond to peculiar compounds as suggested by the library. It was found from the % area that the most abundant chemical in the banana leaves wax were palmitic acid, C_{16} (60% by peak area); linoleic acid, C_{18} (25% by peak area); and stearic acid, C_{18} (6% by peak area). In addition to these chemicals, alkanes, esters, aldehydes, and alcohol in combination formed the remaining 9% by peak area of the wax extracted. Similar chemical composition of plant waxes have been previously reported by Sin [21]. Therefore, these waxes can find use in commercial and industrial purpose for manufacturing of cosmetics, ink, varnish, polish, candles, crayons, and also in food (especially) packaging materials [8,9,10]. Such recommendations have also been made in the literature. Thus, the banana leaves can indeed provide alternative plant waxes for different industrial applications. Furthermore, Attard reported that the dominant hydrocarbon in the sugar rind and bagasse waxes was found to be hentriacontane, $33.6 \pm 1.7 \text{ ug/g}$ of dry plant; while triatriacontane (C_{33}) was the most abundant hydrocarbon in the sugarcane leaves ($303.6 \pm 13 \text{ ug/g}$ of dry plant). The leaves had the highest amount of hydrocarbons with $594.3 \pm 20.7 \text{ }\mu\text{g/g}$ of dry plant followed by the rind ($483.3 \pm 36 \text{ }\mu\text{g/g}$ of dry plant) [18]. In addition, the rind gave only even-chained saturated fatty acids with chain lengths ranging from C_6 to C_{24} . However, a much wider distribution of chain lengths were identified ranging from C_6 to C_{30} and a large variety of odd-chain fatty acids (C_7 to C_{25}) albeit in smaller quantities were detected in the leaf and bagasse waxes [18]. Palmitic acid (C_{16}) was the dominant fatty acid in all the extracts, where a high quantity was found in the leaf wax ($866.8 \pm 4.2 \text{ }\mu\text{g}$ of plant). The leaf wax demonstrated the highest quantity of saturated fatty acids studied, with a total concentration of $1515 \pm 11.7 \text{ }\mu\text{g/g}$ of dry plant, while the rind had the smallest amount ($212.9 \pm 24.2 \text{ }\mu\text{g/g}$ of dry plant) [18]. Saturated fatty acids have the potential to be used in a wide range of applications including soaps, detergents, cleaning polishes, and lubricating oils [18]. Furthermore, similar to our findings, it has been previously reported that banana wax is known to contain myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, eicosapentaenoic acid, behenic acid, and lignoceric acid; where palmitic acid (C_{16}), linolenic acid (C_{18}), and behenic acid (C_{22}) are the major constituents [5]. The fatty acid composition and the physicochemical properties of banana wax are similar to those of carnauba wax. Thus banana wax could be used as a replacement for carnauba wax for polishing leathers, glasses, wooden furniture, and automobiles [5]. More so, banana leaf wax was extracted using hexane reflux was studied. Subsequently, the *M. chiliocarpa* leaf wax was analyzed after saponification using gas chromatography. The predominant acid was C_{22} , and the major alcohols were C_{28} and C_{30} . The results demonstrated that the banana leaf wax is a potential source of natural wax for industrial material and is expected to be used extensively [22].

3.4. Results of UV-Visible spectrum of the wax

The UV/Visible spectrum of banana leaves wax is given below in Figure 3.

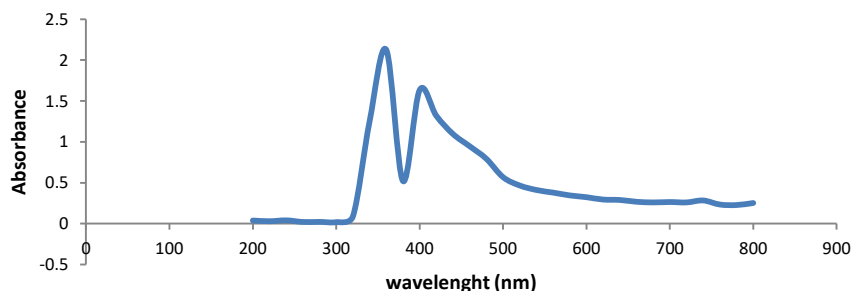


Figure 3: UV/Visible spectrum of banana leaves wax

The UV/Visible analysis was carried between 200 – 800 nm. The λ_{\max} for the wax was found at 360 nm and 400 nm as may be seen in the Figure 3 above. These results indicate the presence of unsaturated compounds. Similarly, Inarkar and Lele [18] reported two major peaks from UV/Visible spectrum of sugar cane peel wax at 230 and 270 nm. They also observed that beyond 400 nm there was no peak found which is similar to the spectrum obtained from this banana leaves wax.

4. Conclusion

About 0.97wt% of the banana leaves wax was extracted using soxhlet extraction process. As expected, the wax was found to be soluble in hexane and ethyl acetate but insoluble in methanol and ethanol. The FTIR absorption peaks of 3373.2-3384.4, 2922.2-2851.4, 1738.9-1666.1 cm^{-1} implied the presence of alcohol, saturated organic compounds, and then ester/aldehyde/ketones/carboxylic acid, respectively. The λ_{\max} for the wax was found at 360 nm and 400 nm. The GC-MS analysis showed that the % area of the most abundant chemical in the banana leaves wax were palmitic acid, C_{16} (60% by peak area); linoleic acid, C_{18} (25% by peak area); and stearic acid, C_{18} (6% by peak area). In addition to these chemicals, alkanes, esters, aldehydes, and alcohol in combination formed the remaining 9% by peak area of the wax extracted. Since the chemical composition of the banana wax is similar to other waxes it can find use in commercial and industrial purpose for manufacturing of cosmetics, ink, varnish, polish, candles, crayons, and also in food (especially) packaging materials.

Acknowledgements

We thank the Dept. Chemistry Benue State University, Makurdi – Nigeria for supporting the research with their facility.

References

- [1]. N. V. Phirke, R. P. Patil, S. B. Chincholkar, and R. M. Kothari, “Recycling of banana pseudostem

- waste for economical production of quality banana,” *Resour. Conserv. Recycl.*, vol. 31, pp. 347–353, 2001.
- [2]. N. Cordeiro, M. N. Belgacem, I. C. Torres, and J. C. V. P. Moura, “Chemical composition and pulping of banana pseudo-stems,” *Ind. Crop. Prod.*, vol. 19, pp. 147–154, 2004.
- [3]. J. B. Ulloa, J. H. van Weerd, E. A. Huisman, and J. A. J. Verreth, “Tropical agricultural residues and their potential uses in fish feeds: the Costa Rican situation,” *Waste Manag.*, vol. 24, pp. 87–97, 2004.
- [4]. T. YANAGIDA, N. SHIMIZU, and T. KIMURA, “Extraction of Wax from Banana Leaves as an Alternative Way of Utilizing Agricultural Residues,” *Japan J. Food Eng.*, vol. 61, pp. 29–35, 2005.
- [5]. S. Charumane, S. Yotsawimonwat, P. Sirisa-ard, and K. Pholsongkram, “Characterization and chemical composition of epicuticular wax from banana leaves grown in Northern Thailand,” *Songklanakarin J. Sci. Technol.*, vol. 39, no. 4, pp. 509–516, 2017.
- [6]. P. Kolattukudy, “Cutin, suberin and waxes,” in *Lipids: structure and function*, P. K. Stumpf, Ed. New York, Academic Press, 1980, pp. 571–646.
- [7]. L. Kunst and A. Samuels, “Biosynthesis and Secretion of Plant Cuticular Wax,” *Prog. Lipid Res.*, vol. 42, pp. 51–80, 2003.
- [8]. S. Pashova, “Plant waxes – nature, types and application. Excellence In Business, Commodity Science and Tourism, Bucharest Academy Of Economic Studies, Faculty of Commerce,” *Forum Ware Int. Spec. Issue*, pp. 166–170, 2001.
- [9]. M. Chughtai and S. Khan, “No Title,” *Pakistan J. Sci.*, vol. 23, p. 61, 1971.
- [10]. W. Christie, *Lipid Analysis*, (3rd edition). 2003.
- [11]. J. W. T. Huat, “Extraction of Wax from Plants for Possible Commercial Application,” *Universiti Malaysia Saraw Ak*, 2013.
- [12]. J. R. Dodson et al., “Bio-derived materials as a green route for precious & critical metal recovery and re-use,” *Green Chem. (RSC)*, pp. 1–15, 2015.
- [13]. K. Asemave and T. A. Asemave, “African Shea Butter as a Staple and Renewable Bioproduct,” *Int. J. Sci. Res.*, vol. 4, no. 12, pp. 2133–2135, 2015.
- [14]. K. Asemave, F. P. Byrne, J. H. Clark, T. J. Farmer, and A. J. Hunt, “Modification of bio-based β -diketone from wheat straw wax: synthesis of polydentate lipophilic super-chelators for enhanced metal recovery,” *RSC Adv.*, vol. 9, no. 7, 2019.
- [15]. K. Asemave and T. T. Anure, “The bioactivities of the neem (seeds, leaves and their extracts) against *Callosobruchus maculatus* on *Vigna Subterranean L.*,” *Prog. Chem. Biochem. Res.*, vol. 2, no. 3, pp. 92–98, 2019.
- [16]. K. Asemave, “Bioactivity of *Arachis Hypogaea* Shell Extracts against *Staphylococcus aureus* and *Pseudomonas aeruginosa*,” *Prog. Chem. Biochem. Res.*, vol. 4, no. 3, pp. 331–336, 2021.
- [17]. T. Yanagida, N. Shimizu, and T. Kimura, “Extraction of wax and functional compounds from fresh and dry banana leaves,” *Japan J. Food Eng.*, vol. 6, no. 1, pp. 79–87, 2005.
- [18]. T. Attard, “Supercritical CO₂ extraction of waxes as part of a holistic biorefinery,” *University of York*, 2015.
- [19]. M. Riederer and L. Schreiber, “Protecting against water loss: Analysis of the barrier properties of plant cuticles,” *J. Exp. Bot.*, vol. 52, no. 363, pp. 2023–2032, 2001.

- [20]. R. Hamilton, *Waxes: Chemistry, Molecular Biology and Functions*. Dundee: The Oily Press, 1995.
- [21]. E. H. K. Sin, "The extraction and fractionation of waxes from biomass," University of York, 2012.
- [22]. T. Yanagida, N. Shimizu, and T. Kimura, "Extraction of wax from banana as an alternative way of utilizing agricultural residues," *Japan J. Food Eng.*, vol. 6, no. 1, pp. 29–35, 2005.
- [23]. K. Asemave, "Biobased Lipophilic Chelating Agents and their Applications in Metals Recovery," University of York, UK, 2016.
- [24]. K. Asemave, *Supplement of Inorganic Kinetics: Substitution Reaction of Cobalt (III) with Amino acids*, First. Saarbrücken, Germany: LAP LAMBERT Academic Publishing, 2012.
- [25]. K. Asemave, S. G. Yiase, and S. O. Adejo, "Kinetics and Mechanism of Substitution Reaction of Trans-Dichloro-bis-(Ethylenediammine) Cobalt (III) Chloride with Cysteine, Aspartic acid and Phenylalanine," *Int. J. Sci. Technol.*, vol. 2, no. 5, pp. 242–247, 2012.
- [26]. K. Asemave, S. G. Yiase, S. O. Adejo, and B. A. Anhwange, "Substitution Reaction of trans-dichloro-bis-(ethylenediammine) Cobalt (III) Chloride and Phenylalanine-A Kinetics and Mechanism Study," *Int. J. Mod. Chem.*, vol. 1, no. 2, pp. 93–101, 2012.
- [27]. K. ASEMAVE, S. G. YIASE, S. O. ADEJO, and B. A. ANHWANGE, "KINETICS AND MECHANISM OF SUBSTITUTION REACTION OF trans-DICHLOROBIS (ETHYLENEDIAMMINE) COBALT (III) CHLORIDE WITH ASPARTIC ACID," *Int. J. Inorg. Bioinorg. Chem.*, vol. 2, no. 1, pp. 11–14, 2011.
- [28]. K. Asemave, S. Yiase, and S. O. Adejo, "Kinetics and Mechanism of Substitution Reaction of Trans-Dichloro-bis-(Ethylenediammine) Cobalt (III) Chloride with Cysteine, Aspartic acid and Phenylalanine," *Int. J. Sci. Technol.*, vol. 2, no. 5, pp. 242–247, 2012.
- [29]. K. Asemave, S. G. Yiase, and S. O. Adejo, "Kinetics and Mechanism of Substitution Reaction of trans-Dichlorobis (ethylenediammine) cobalt (III) chloride with Cysteine," *Int. J. Mod. Org. Chem.*, vol. 1, no. 1, pp. 1–9, 2012.
- [30]. K. Asemave, S. G. Yiase, S. O. Adejo, and B. A. Anhwange, "Kinetics and Mechanism of Substitution Reaction of trans-dichloro-bis-(ethylenediammine) cobalt (III) chloride with Aspartic acid," *Int. J. Inorg. Bioinorg. Chem.*, vol. 2, no. 1, pp. 11–14, 2011.
- [31]. K. Asemave, B. Anhwange, and U. J. Ahile, "Hydrolysis of Fatty Esters in Dichloromethane/ Methanol," *FUW Trends Sci. Technol. J.*, vol. 2, no. 1B, pp. 521–524, 2017.
- [32]. P. R. REDDY, M. RADHIKA, and P. MANJULA, "Synthesis and characterization of mixed ligand complexes of Zn(II) and Co(II) with amino acids: Relevance to zinc binding sites in zinc fingers," *J. Chem. Sci.*, vol. 117, no. 3, pp. 239–246, 2005.