AFRICA ENVIRONMENTAL REVIEW Journal
ISSN: 1727-8341

AER Journal Volume 5, Issue 1, pp. 162-174, June, 2022

RESEARCH ARTICLE
Available Online at *http://ojs.uoeld.ac.ke/*

Chironomid Diversity in the River Nzoia Basin in Kenya

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Abstract

Chironomids belong to the dipteran family Chironomidae, one of the most widely distributed insect groups in the world. Chironomid larvae are found in freshwaters and can withstand polluted waters which makes them suitable for use in evaluation of aquatic ecosystem health. However, there is incomplete taxonomic knowledge of larvae and scarcity of ecological data on local species in Tropical and Southern Hemisphere regions. The study set out to identify the chironomid genera in the River Nzoia Basin in order to contribute to knowledge on the chironomid diversity in Kenya. Larvae were collected from different sections of River Nzoia and River Sosiani, a subwatershed of River Nzoia, between December 2008 and June 2009. The genera of chironomid larvae were identified using morphological characteristics. Results showed a total of five genera of larvae in the basin with Chironomus, being the most abundant. Simpson's Reciprocal index in the Nzoia basin was 2.13 with the subfamily Chironominae making 96.54 % of all larvae identified. The diversity of chironomids was not high in the study area but the occurrence and abundance of the Chironomus genus at all the sampled areas makes it possible for their use in water quality studies in the region.

Keywords: Chironomus, Bioindicator, Water Quality

INTRODUCTION

Chironomids

These are a group of insects belonging to the dipteran family Chironomidae, found on all continents in the world, from 81°N to 68°S, and from 5600 m above sea level to a depth of 1000 m below sea level; making them one of the most widely distributed in the world (McGavin, 1992). Being holometabolous, the first three stages i.e. egg, larval, pupal, are aquatic stages; while the adult is an aerial stage (Péry et al., 2005). There are some terrestrial larvae (Delettre, 2005) but the aquatic ones are the majority, and are found in all types of freshwater habitats worldwide (Francis, 2004; Mousavi et al., 2003). These larvae occur in large numbers and live in all types and conditions of rivers and lakes (Wilson & McGill, 1977). There are four phases, or instars in the larval stage, with complete molting between each instar. These larvae have a head capsule that is nonretracting and consists of sclerotized chitin, which bears opposing mandibles, eyespots, antennae, and various other sensory structures (Francis, 2004). Adults do not bite and survive for one to two weeks after emergence (McGavin, 1992). Most species feed on decayed organic material, algae and minute plants or aquatic animals (McGavin, 1992).

Because they represent a predominant part of benthic communities in freshwater habitats, chironomids are of great interest in ecology. They have an important role in detritus processing and are important preys for other animals in these ecosystems (Delettre, 2005; Faria et al., 2006; Garcia & Suarez, 2007; Jeffries & Mills, 1990). Chironomid larvae

can also tolerate polluted waters because of the hemoglobin that gleans most of the limited oxygen from the water. They can survive periods of anoxia hence their association with pollution (Jeffries & Mills, 1990; Weber & Vinogradov, 2001). Chironomids are therefore can be used for assessment of the toxicity of contaminated sediments as well as various water conditions (Mousavi et al., 2003; Park & Kwak, 2010). Presence of chemicals in aquatic systems may alter the development of their morphological structures such as the mandibles, mentum, antennae, and epipharyngeal pecten and this makes chironomids especially suitable as biological indicators in these systems (Hudson & Ciborowski, 1996; Nazarova et al., 2004). Chironomids have been used by researchers as a tool of environmental impact assessment, toxicity testing and evaluation of aquatic ecosystem health (Carew et al., 2003; Leal et al., 2004).

The family Chironomidae, belongs to one of the most ecologically important classes of invertebrates (Faria et al., 2006; Marziali et al., 2010; Park & Kwak, 2010; Siqueira et al., 2008; Weber & Vinogradov, 2001). It is also the most speciose and abundant groups in any aquatic environment in the tropical regions. (Siqueira et al., 2008). There are over 10,000 species in this family (Weber & Vinogradov, 2001). The Chironomidae family consists of six subfamilies, 148 genera and over 700 species recorded from America north of Mexico (Foote, 1991).

Different types of chironomids can be identified using their morphological differences (Epler, 2001; Foote, 1991).. The mentum is often one of the most noticeable structures of the head capsule. It is a toothed plate on the anterior ventral margin of the head capsule. The number of teeth as well as their shape can play an important role in identification (Epler, 2001). Identification to species level is at times time consuming and difficult (Marziali et al., 2010; Sinclair & Gresens, 2008). This is due to the use of minute structures and probability of wear and damage confusing the identification (Sinclair & Gresens, 2008). There are keys and diagnoses that are used in the identification to species level. (Epler, 2001). The identification also requires considerable and uncommon expertise. Misidentification rates at 7.5-9% have been shown to occur at species level through quality control, even when experienced taxonomists perform the identifications. (Carew et al., 2003). Use of DNA in identification is beginning to gain popularity in order to take care of the above limitations.

Studies have been carried out on species diversity of chironomids in different aquatic habitats (Al-Shami et al., 2006; Fesl, 2002; Marziali et al., 2010). The sub-family Chironominae was the most common and abundant in the studies. According to Epler (2001), there are seven subfamilies namely Chironominae, Orthocladiinae, Prodiamesinae, Diamesinae, Podominae, Telmatogetoninae and Tanypodinae. In tropical and Southern Hemisphere regions, however, there is incomplete taxonomic knowledge of larval Chironomidae and scarcity of ecological data on local species (Verschuren & Eggermont, 2006). Through qualitative studies, pH has been shown to be a significant variable in midge distributions (Francis, 2004). This is because the pH directly affects the physiology of aquatic organisms by influencing enzyme function and ionic balance. Chironomid taxa that are tolerant of low pH levels are able to maintain internal pH-balance and possess, hemoglobin, which provides greater buffering capacity. They also tend to be large-bodied. (Francis, 2004).

In tropical lowland African lakes, the following genera are widely distributed: *Polypedilum Dicrotendipes, Nilodorum Kiefferulus, Chironomus, Cladotanytarsus Procladius,* (Verschuren & Eggermont, 2006). Others that were found in Kenyan Lakes were *Ablabesmyia, Pentaneurini indet, Tanypoinae indet. Cricotopus* (Eggermont et al., 2005). The species found in Africa are widespread in the continent as

70.2 % of the diversity found in West Africa using subfossil collections, was also found in East Africa. Most of West African chironomid species are also found in the drier regions of East, North and Southern Africa (Eggermont et al., 2005).

There is very minimal literature available on composition and species diversity of chironomids in rivers in Kenya. Information on the diversity of the chironomids will help in establishing the types available and whether they are suitable for studying ecosystem health in the region. The study therefore sought to identify the chironomid genera in the Nzoia Basin in order to reduce this knowledge gap. The specific objectives of the study were to identify the genera of chironomid larvae found in the River Nzoia Basin and calculate the species diversity at the different stations.

The Study Area

River Nzoia, with a length of 252 km, runs from Mount Elgon to Lake Victoria (Fig 1). It lies between 2700-1134 m above sea level therefore having an average fall of 4m per 1000m (Osano, 2002). The river receives effluent from major towns such as Kitale, Eldoret, Webuye, Kakamega and Mumias as well as agricultural run-off from the river basin. The effluent consists of both domestic and industrial wastewaters. Eldoret contributes industrial wastewater from textile and food processing industries. (Osano, 2002). The larvae were sampled form four stations. Station 1 at Mount Elgon lies between latitude 00º 43' N and longitude 34º 45' E, 4313 m above sea level.

Figure 1: Position of River Sosiani (No.5); a subwatershed of River Nzoia in relation to other stations (Nos 1-4) on River Nzoia from where Chironomid larvae were collected for identification during the study.

Temperatures vary between 10.0ºC and 24.0 ºC while rainfall is bimodal ranging from 1400 and 1600 mm (Republic of Kenya, 2002c). Station 2 at Moi's Bridge lies between latitude 00º 55' N and longitude 35º 00' E, 1800 m above sea level. Temperatures vary between 18.0ºC and 26.1ºC while rainfall is bimodal with a mean of 960 mm. Intensive agriculture is carried in this area (Republic of Kenya, 2002d). Station 3 is at Webuye and lies between latitude 00º 30' N and longitude 34º 40' E, approximately 1600 m above sea level. Temperatures vary between 21.0ºC and 25.0ºC and the bimodal rainfall mean is 1440 mm (Republic of Kenya, 2002a). Station 4 at Mumias lies between latitude 00º 20' N and longitude 34º 54' E, 1130 m above sea level. Temperatures vary between 26.0ºC and 30.0ºC while rainfall is bimodal ranging from 760 and 1015 mm (Republic of Kenya, 2002b). Station 1 is a pristine locations high up Mount Elgon, Station 2, Moi's Bridge is in an agricultural area that receives run-off consisting of compounds resulting from agricultural activities such as pesticides. Station 3 in Webuye was contaminated with effluent from pulp and paper mill factory, Panpaper Mills in Webuye, which was still operational at the time of sampling of the larvae from River Nzoia. Station 4 in Mumias has its waters polluted from effluent from Mumias Sugar Factory as well as agricultural run-off from surrounding sugar-cane growing farms. River Sosiani is the only river that crosses through Eldoret town and is the recipient of the sewage effluent from the two working sewage treatment works as well as industries liquid effluent. The river enters the town limits at an elevation of 2140 m East of Eldoret and leaves at an elevation of 2070 m, flowing in a south east-north west direction. This river is a major tributary of River Nzoia, which flows into Lake Victoria (Fig 1). The chironomids were sampled from three sites along River Sosiani; Site 1, a point upstream before the river enters town; Site 2, a point after the river has passed through the Central business district (CBD) and Site 3, a point just after the Sewage Treatment Works' effluent discharge point, downstream of the river. The sites were chosen to represent sections that are taken to have varying amounts of pollution with level of pollution increasing from site 1 to site 3 which is expected to have increased pollution from the wastewater effluent that is discharged into the river.

MATERIALS AND METHODS

Sampling of Chironomids

The chironomids were sampled from River Nzoia and its tributary River Sosiani between December 2008 and June 2009, to evaluate the diversity of the chironomid species in the River Nzoia Basin. A sampler made of nylon net basket of mesh size 0.5 mm (D-frame net) (William & Feltmate, 1992) was used to get the larvae from the sediment as described in (Khazenzi, Osano, Wakhisi, & Raburu (2011). The larvae were transferred into 70% ethanol and then transported to the laboratory for identification.

Identification Process

In the laboratory, the larvae heads were cut and digested in 10% Potassium hydroxide solution overnight to eliminate muscle tissue. Chironomids tend to lie laterally so a needle was used to remove the head capsules. These were washed in distilled water, rinsed with 90% ethanol and mounted on microscope slides, ventral side up, using Euparal mountant (Epler, 2001). The larvae were identified by observing their menta under a compound microscope at between 100 - 400X magnification. The menta were identified using keys from (Epler, 2001) and (Harrison, 2003) as well as diagrams from (Al-Shami et al., 2006; Foote, 1991; Özkan, 2006) and (Watts et al., 2003).

Some of the preserved larvae from River Sosiani were mounted whole on slides using DPX mountant after dipping them in xylene to remove the alcohol. Apart from the menta, both the head and tail parts were also used in the identification of the larvae. Differences in their morphology were used to put them into different genera. Larval heads of the

remaining larvae were cut and prepared as above before mounting on slides using DPX mountant. The slides were left to dry for one week before observing them under a microscope at between 100-400X magnification and identifying them as mentioned above.

Species Diversity

This takes into account the species richness (the number of species at a particular site) and the evenness with which individuals in the community are distributed among the species (Karmondy, 1996). Species evenness refers to the relative abundance with which each species is represented in a community. Species diversity is commonly measured using Shannon's diversity index or Simpson's diversity indices (Gorelick, 2006; Karmondy, 1996).

Simpsons Reciprocal index was used in this study.

Simpson's Reciprocal Index, $1/D = (\Sigma \rho i^2)^{-1}$.

Where ρi is the proportion of the total number of specimens i expressed as a proportion of the total number of species in the ecosystem (Gorelick, 2006; Karmondy, 1996).

The value of the index begins from 1 and the higher the figure the greater the diversity. The maximum value is the number of species in the sample (Gorelick, 2006)

Determination of Physical Characteristics of Water

Physical characteristics of River Sosiani water were taken each time sampling of the larvae was done. The characteristics recorded were Temperature, Dissolved Oxygen, and pH. Dissolved Oxygen and Temperature were measured using an Auto calibrated Hannah Instrument, H1 9143 microprocessor. The pH was measured using a pH 90 WTW meter. All the measurements were done in situ. These characteristics were important for giving an idea about the nature of the river water.

Data Analysis and Presentation

Data for identification of chironomid genera in the River Nzoia basin was presented in terms of percentage abundance for the different genera in the different stations. Means of the water characteristics together with their standard deviations were also calculated for the different sites on River Sosiani.

RESULTS

Diversity of the Chironomids in River Nzoia Basin

Observation of the mentum of different larvae obtained from River Nzoia, yielded four types as shown in the plates below. Plate 1 shows larval head mentum of a *Chironomus*. It has one large tooth flanked on either side by single smaller teeth and two larger and four smaller outer teeth on either side. (Epler, 2001; Özkan, 2006; Watts et al., 2003) and resembles one identified in (Al-Shami et al., 2006) (Plate 2).

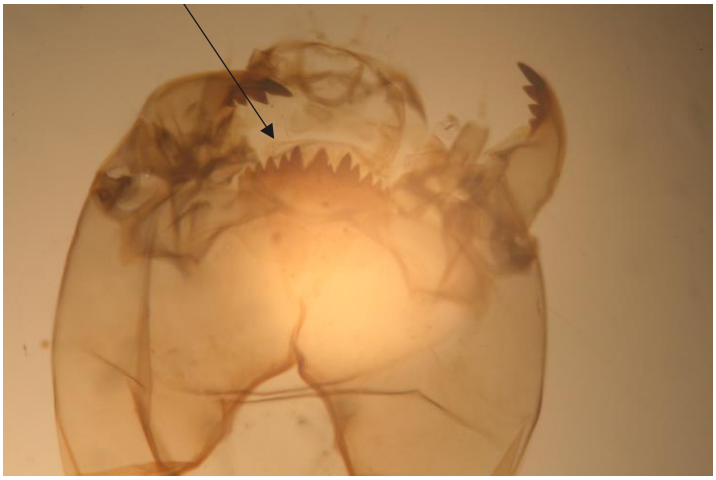

Plate 1: Chironomid larval head whose mentum was identified as that of *Chironomus* obtained from all the sampled areas in the study.

Plate 2: *Chironomus kiiensis* Tokunaga, 4th instar. Source: (Al-Shami et al., 2006).

Plate 3: shows larval head identified as *Polypedilum* after comparison with larval key from (Eggermont et al., 2005; Epler, 2001; Özkan, 2006) showing mentum with median and second lateral teeth longer than first lateral teeth, which distinguishes *Polypedilum* from most members (Plate 4).

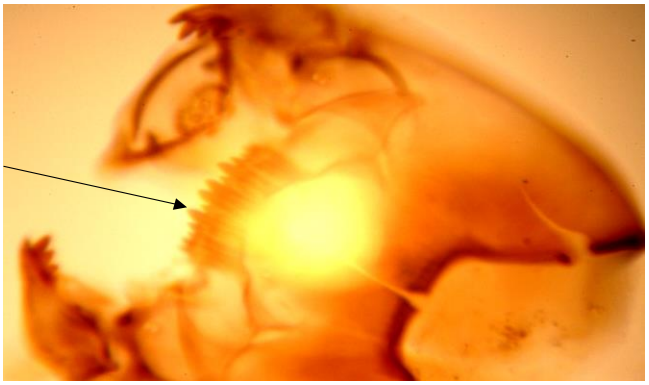

Plate 3: Chironomid larval head whose mentum was identified as that of *Polypedilum* obtained from stations 2 and 4 on River Nzoia during the study period.

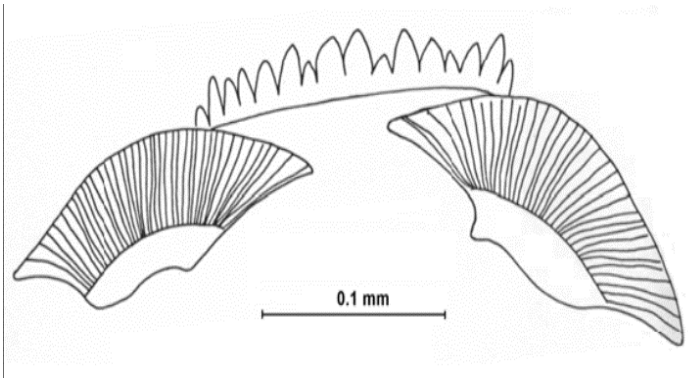

Plate 4: *Polypedilun convictum* (Walk.) (Mentum). Source (ÖZKAN, 2006).

Plate 5 shows larval head whose mentum has an odd number of teeth with a single wide median tooth. This was identified as *Cricotopus* from what was identified by Foote (1991) - Plate 6.

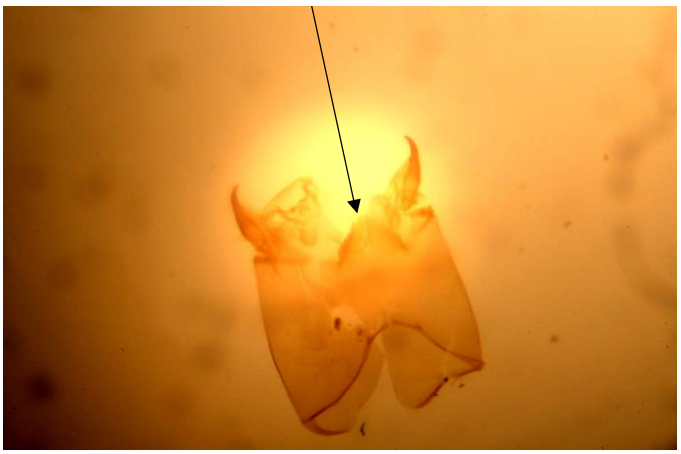

Plate 5: Chironomid larval head whose mentum was identified as that of *Cricotopus* obtained from station 4 along River Nzoia during the study.

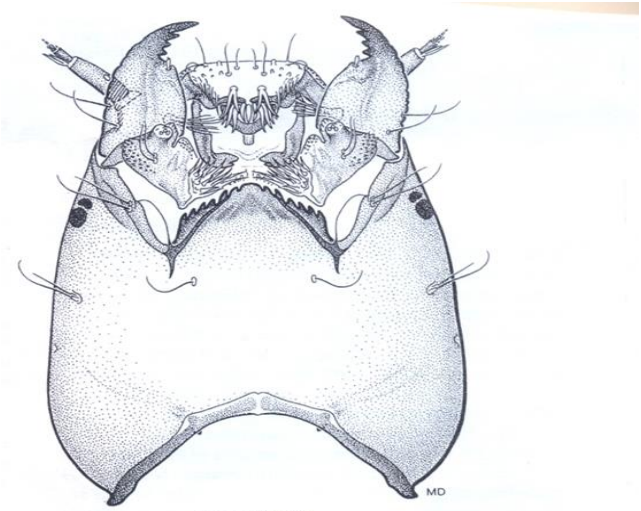

Figure 37.166

Figure 37.165. Chironomidae. Head capsule of *Micropsectra* sp., ventral. Found on bottom sediments and detritus (from Manual of Nearctic Diptera, Vol. 1).

Figure 37.166. Chironomidae. Head capsule of *Cricotopus* sp., ventral. Found on vegetation, detritus, and in sediments (from Manual of Nearctic Diptera, Vol. 1).

Plate 6: Head capsule of Chironomidae *Cricotopus sp*. Source: (Foote, 1991).

Ablabesmyia was identified from the anteriorly narrowed, elongate-oval head capsule (Epler, 2001; Foote, 1991). The identification of the larvae from the River Nzoia Basin revealed the composition shown in Table 1.

Table 1: Percentage distribution of genera found at different sites along River Nzoia and River Sosiani during the study period

Sites/stations Temperature Range	*Chironomus* %	*Ablabesmyia* %	*Micropsectra* %	*Polypedilum* %	*Cricotopus* %
Sosiani site 1 16-23.6 ºC (n=3).	100	-	-	-	-
Sosiani Site 2 16-23.6 ºC (n=43).	95.3	4.7	-	-	-
Sosiani Site 3 16 - 23.6 ºC (n=8).	71	-	29	-	-
Station 1 Mt Elgon 10.0 - 24.0 ºC (n=352).	100	-	-	-	-
Station 2, Moi's Bridge 18.0 - 26.1 ºC (n=352)	92.3	-	-	7.1	-
Station 3, Webuye 21.0 - 25.0 ºC (n=352).	100	-	-	-	-
Station 4, Mumias 26.0 - 30.0 ºC (n=363).	1.7	-	-	90.9	7.4

From Table 1, it is seen that Larvae from Station 1 and Station 3 on River Nzoia and Site 1 on River Sosiani, were all *Chironomus*. The Simpson's Reciprocal index for River Sosiani was 1.15. Simpson's Reciprocal index for Site 2 on the river was 1.10 and that for Site 3 was 1.92. Simpson's Reciprocal index for Station 2 on River Nzoia was found to be 1.15 while that of Station 4 was 1.19.

The total percentages of genera of larvae along River Nzoia obtained in this study were Chironomus, 52. 37%; Polypedilum, 44.26% and Cricotopus, 3.37%. In River Sosiani, Chironomus was the most abundant making 93.4% of the total larvae. Ablabesmyia and Micropsectra were found in equal proportions of 3.3% each in the river. Ablabesmyia belongs to the subfamily Tanypodinae while Cricotopus belongs to the subfamily Orthocladiinae. Chironomus, Micropsectra and Polypedilum belong to the subfamily Chironominae. The most common subfamily in the River Nzoia basin studied was therefore Chironominae, which made 96.54% of the identified larvae with Chironomus genus as the most abundant. The overall percentage of all the larvae in the two rivers studied was *Chironomus*, 54.6%, *Polypedilum*, 41.7% *Cricotopus*, 3.2%, *Ablabesmyia* 0.24% and *Micropsectra* 0.24%. *Ablabesmyia* belongs to the subfamily Tanypodinae while *Cricotopus* belongs to the subfamily Orthocladiinae. *Chironomus*, *Micropsectra* and *Polypedilum* belong to the subfamily Chironominae. Simpson's Reciprocal index was found to be 2.13 with the subfamily Chironominae making 96.54% of the total larvae identified in the River Nzoia basin.

Physical Water Characteristics of River Sosiani water

These were recorded for the river water only at the three sites where sampling was done. The mean levels obtained for the characteristics are shown in the Table 2 below.

Table 2: Physical characteristics of River Sosiani water together with mean nitrate concentration from different sites along the river during the study period

Characteristic (unit)	**KCC**	**Kipkaren**	**Huruma**
	Site 1	Site 2	Site 3
Dissolved Oxygen (%±SD)	81.73±0.37	80.35±2.35	78.93±1.99
Temperature (^{o}C±SD)	21.93±0.81	22.10±0.50	21.23±1.17
pH (±SD)	7.25±0.20	7.26±0.24	7.50±0.16
Nitrate (mgL^{-1} NO_3-N)	0.33±0.32	0.80±0.39	1.42±0.18

From Table 2, it is observed that the dissolved oxygen decreased from Site 1 to Site 3 while the pH gets more alkaline. The temperature does not follow any trend.

DISCUSSION

Identification of different types of chironomids has been carried out using their morphological differences in several studies (Al-Shami et al., 2006; Eggermont et al., 2005; Epler, 2001; Foote, 1991; Marziali et al., 2010). This study used the mentum in the identification of the larvae to genus level. This is because identification to species level is at times time consuming and difficult (Marziali et al., 2010; Sinclair & Gresens, 2008) due to the use of minute structures and probability of wear and damage confusing the identification (Sinclair & Gresens, 2008). Identification to species level also requires considerable and uncommon expertise (Carew et al., 2003).

The results on the most abundant genera agree with past studies that find the subfamily Chironominae in large numbers and C*hironomus* as most common in different parts of the world (Al-Shami et al., 2006; Fesl, 2002; Garcia & Suarez, 2007; Marziali et al., 2010). C*hironomus* occurred at all the stations along River Nzoia as well

as all the sites along River Sosiani as shown in Table1.

Chironomus is commonly associated with presence of decomposing aquatic macrophytes as well as organic matter (Silva et al., 2008). The *Chironomus* genus is also ecologically versatile, being found in flowing waters or standing as well as clean or polluted water (Epler, 2001; Silva et al., 2008). This may explain why it is found at all the sites, both in River Sosiani and River Nzoia. Along River Sosiani, Site 1 is located upstream as the river is about to enter the town. This area is relatively less polluted as compared to the two sites downstream. *Chironomus* was the only type of chironomid larvae that was found at this place. Site 2 is a point after the river has passed the Central business district (CBD). The water has been polluted by runoff from the town and other activities such as car washing and wastewater discharge from industries. Site 3 is the most polluted site because of the sewage effluent discharged into the river upstream, close to this point. This information is reflected in some of the parameters shown in Table 2.

Along River Nzoia the *Chironomus* was found in both the pristine locations (Station 1) as well as the parts of the river affected by anthropogenic activities, Station 2, and Station 3. Station 4 in Mumias had a higher percentage of *Polypedilum* which belongs to the same family as *Chironomus.* The area has its waters polluted with effluent from Mumias Sugar Factory as well as agricultural run-off from surrounding sugar-cane growing farms. Chironomus has been used as an indicator for pollution in several studies. (Mousavi et al., 2003; Nyakeya et al., 2018; Park & Kwak, 2010)

The difference in the composition of larvae at the different sites of River Nzoia may be due to the difference in climate that results in the change of moisture content as well as environmental differences including the quality of the water arising from anthropogenic activities. Species richness is related to a number of factors, which include geographical factors such as latitude, altitude and depth in aquatic environs; other factors include the environment e.g., age of the environment, productivity of environment, harshness of the environment, climatic variability; and biological attributes such as predation, competition and successional status of the community (Begon et al., 1990). The diversity of the chironomid community is also influenced by the plant community which is directly affected by changes in the moisture regime (Driver, 1977). Chironomids have been found to be indicators of the overall water quality status of sites, with different taxa assemblages according to ecological gradients linked to anthropogenic impact (Garcia & Suarez, 2007; Marziali et al., 2010). The genus *Cricotopus* is tolerant to changing environmental conditions (Epler, 2001). *Cricotopus* was found at only one site in this study, station 4, on River Nzoia as it gets closer to Lake Victoria. This was unlike the study by (Garcia & Suarez, 2007) where it was found in all the sections of a stream that had differences in some physicochemical features. Station 4 was located in an area that receives effluent from the sugar factory and which may also have been polluted with effluent from a paper mill that is located several kilometers upstream. *Cricotopus* larvae are often associated with plants and are found in a variety of aquatic habitats. The climate of station 4 is different from the rest of the stations. This implies a difference in the type of plants growing in the area as is evidenced by the growing of sugar cane as the main cash crop unlike the other stations where maize is the main cash crop.

Polypedilum and *Chironomus* are mainly found in fine sediment with high content of organic matter of anthropogenic or natural origin (Leal et al., 2004). *Polypedilum* larvae are also found in a wide range of habitats under a variety of environmental conditions, ranging from heavily degraded to pristine (Epler, 2001; Silva et al., 2008). *Polypedilum nubifer* can become extremely abundant in

shallow, warm, eutrophic waters subject to seasonal drying; it is a common midge in tropical and subtropical waters. (Jacobsen & Perry, 2007). Station 4 is the warmest of all the stations with temperatures varying between 26.0ºC and 30.0ºC (Republic of Kenya, 2002b). Station 2, although at times cool, experiences a high maximum temperature of 26.1ºC (Republic of Kenya, 2002d), which may explain the presence of a few *Polypedilum* at the area. Station 1 and Station 3 have temperature ranges of 10.0ºC to 24.0ºC (Republic of Kenya, 2002c) and 21.0ºC to 25.0ºC (Republic of Kenya, 2002a) respectively.

During the period from January to July 2009, Site 3 along River Sosiani was found to have many larvae that were also big in size, up to 13mm long. This was unlike Site 1 that had a considerable amount and Site 2 that had very few except in November 2008 when there were more larvae than in the other sites. The large number and big size of the larvae at Site 3 may have been because of the organic material from the sewage wastewater that is discharged into the river at a nearby point upstream. The main food source for most diptera are sewage fungi and bacteria (Arimoro et al., 2007). The water level was relatively low during this period hence decreasing the dilution factor of the sewage effluent. Chironomids are good indicators of organic enrichment (Leal et al., 2004).

Differences in environmental aspects of habitats, producing microhabitats, contribute to the spatial distribution of taxa in water bodies (Bazzanti et al., 2010). The differences in genera found at the different sites in River Sosiani and the different stations along River Nzoia may be explained along this line. At all the sites from River Sosiani as well as along River Nzoia, the species richness was not high; about three different genera were found in each of the rivers

The species diversity is not high in both rivers as seen from the Simpson's Reciprocal index. According to the ''River Continuum theory'' species richness should reach a maximum in rivers with an order around five (Garcia & Suarez, 2007). The rivers in this study are of lower order; River Sosiani is of second order while River Nzoia is third order. At the same time the species richness of the rivers is likely to have been underestimated to a certain degree since the identification was only at the genus level. A similar case was observed in a study by (Garcia & Suarez, 2007). The species diversity index range of 1.10 – 2.13, covering three subfamilies, is lower than that found by (Drake, 1982) which was in the range 1.5–3.0 covering five subfamilies. These numbers are also lower than those found in the study by (Marziali et al., 2010) which were 51 genera obtained from six rivers. Identification of the larvae to species level may have resulted in a higher index because studies by (Verschuren & Eggermont, 2006) found genera in East Africa, most notably *Ablabesmyia*, *Chironomus*, and *Polypedilum* to contain many species in nature.

Ablabesmyia and *Micropsectra* were found in minute quantities in River Sosiani probably due differences in the microhabitats and water quality. According to (Epler, 2001), *Ablabesmyia* larvae prefer softer, less alkaline water. *Ablabesmyia* was found at Site 2 whose pH of the river water 7.26 (Table 2). *Micropsectra* was found at Site 3, which was the most polluted.

CONCLUSION

In conclusion, the River Nzoia basin does not have a high diversity of chironomids but the chirominae subfamily family is most abundant. The *Chironomus* genus is spread out in most of the basin in both the pristine areas and those affected by anthropogenic activities. The occurrence of the *Chironomus* genus at all the sampled areas makes it possible to use these insects for water quality studies in the region. It is recommended that the identification of the larvae can be done using DNA in order to get a better species diversity index.

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AFRICA ENVIRONMENTAL REVIEW Journal
ISSN: 1727-8341

AER Journal Volume 5, Issue 1, pp. 175-193, June, 2022

RESEARCH ARTICLE
Available Online at *http://www.aer-journal.info*

Investigation of *Pavonia urens* as Potential Biosorbent in Phytoremediation of Metal Pollutants through Complexation

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Abstract

Environmental pollution involving metal ions represents a potential threat to life. This is due to different chemical wastes discharged to the environment with no or minimal treatment to reduce and decrease the harmful compounds. Several herbs have been reported for use in heavy metals removal in wastewater. This study aimed at investigating the phytochemicals present and demonstrating the possible use of Pavonia urens leaves as adsorbent material through formation of a complex with selected metals zinc, copper and nickel from aquatic environment. The plant was collected from Uasin Gishu County and air dried before crushing. The grounded powder was soaked in three organic solvents (hexane, ethyl acetate, and acetone) of increasing polarity each for 48 hours followed by filtration and drying it. The extracts were placed on silica gel columns for thin layer chromatography (TLC) separation, with visibility reagents to monitor the fractions. Five fractions obtained were labelled 1A, 2A, 3A, 4B and 5 A. The GC-Mass spectrometer (MS) gave the functional groups hydroxyl (OH), amine (-NH), and (-COOH). The ability of Pavonia urens to complex with bimetallic ions in aqueous solution was also investigated using UV-VIS spectrometry which characterized absorption frequencies. The interaction of these ions with various functional groups of the bio-sorbent surfaces was revealed by UV-VIS analysis which showed that copper ions were complexed while zinc was the least giving the order $Cu^{2+} > Ni^{2+} > Zn^{2}$.The results from this study showed that P. urens is a promising alternative as an eco-friendly, low-cost bio-sorbent that can effectively complex with heavy metals in aqueous solution. The phytochemical screening showed the plant is rich in bioactive compounds such as steroids, flavonoids and terpenoids. Therefore, P urens is worth consideration for investigation in the pursuit for main compounds in drug discovery and as adsorbent.

Keywords: Toxic, Urban, Industrial Effluents, Aquatic Ecosystem, Accumulate, Bioremediation

INTRODUCTION

A number of toxic substances, mainly metals are found in the environment, such as in water, soils, and rocks, and are also discharged into the surrounding from anthropogenic resources, largely commercial and industrial (Masindi & Muedi, 2018; Jaishankar *et al*., 2014; Bhagure & Mirgane, 2011). These anthropogenic activities include; agriculture, urbanization, industrial effluents and release of poorly treated sewage to aquatic ecosystems (Kahlon *et al*., 2018).

Heavy metals are elements of high atomic weight greater than 4g/cm^3 and toxic or poisonous even at low concentrations (Jarup 2003). These metals have several uses due to their physical and chemical features, such as multi-valency, reactivity, capacity to

generate coloured solutions, strength, and complex formation (Madasamy *et al.*, 2019).

Heavy metal accumulation in soil, water, and air is a global environmental hazard caused by industrialization, urbanization, and mining (Ukaogo *et al*., 2020; Islam *et al*., 2018). Heavy metals have been reported to cause physical and mental disabilities, reduced IQ, organ dysfunction, neurological diseases, respiratory infections, gastrointestinal issues, maternal health problems, malignancies, and even death (Tchounwou *et al*., 2012; Jaishankar *et al*., 2014; Vigneri *et al*., 2017; Iannitti *et al*., 2010). These contaminants damage the immune system, making the body more prone to infections. The majority of research in Kenya has only focused on heavy metal levels in water, soil, air, and food (Lalah *et al*., 2008; Dsikowitzky *et al*., 2013; Akenga *et al*., 2020; Kinuthia *et al*., 2020; Akenga *et al*., 2016; Githaiga, *et al*., 2021; Nyambura *et al*., 2020; Inoti *et al*., 2012). Few research however, have focused on the removal of heavy metals from contaminated water, soil, and air.

Conventional physical or chemical repair procedures aren't cost-effective or eco-friendly, necessitating new alternatives (Aransiola *et al*., 2019; Yan *et al*., 2020). Phytoremediation is a plant-based technique that uses plants to absorb and remove contaminants from soil or reduce their bioavailability (Petruzzelli *et al.*, 2013; Muthusaravanan *et al.*, 2018; Parmar & Singh, 2015). Plants can absorb ionic substances from the soil at low quantities via their roots (Arao *et al*., 2010). Several plant materials have strong coordination bonding capabilities for heavy metal ions (Gardea-Torresdey *et al*., 2000). These plants' cell walls contain proteins and lipids with strong affinity for heavy metal ions, such as hydroxyl, carboxylate, and amino groups (Kwon *et al*., 2007; Park *et al*., 2005). These groups have the ability to bind heavy metals using an electron pair to complex the metal ions in solution. These methods are cheaper and more efficient than chemical or physical methods, and they reduce chemical and biological sludge. Complex formation and metal recovery is also possible (Gopalakrishnan et al., 2010).

Plants such as *Helianthus annuus* (Zhao et al., 2019), *Cannabis sativa (*Ćaćić*, et al., 2019), Nicotiana tabacum (*Evangelou *et al*., 2007), and *Zea mays (*Xiaomei *et al*., 2005), have been reported to effectively remove heavy metals from contaminated soil through phytoextraction. In Kenya, a few plants have been used to remove heavy metals such as bamboo (Bosire, 2014), Eichhornia crassipes (Ndeda & Manohar, 2014), *Eichhornia crassipes* (Matindi *et al*., 2022).

The Sustainable Development Goals (SDG) of the United Nations place a heavy emphasis on eliminating environmental pollution (Leal- Filho *et al*., 2019). SDG 3.9 aims to "significantly reduce the number of fatalities and diseases caused by hazardous chemicals in air, water, and soil pollution and contamination" by 2030. The Kenya Vision 2030 is to provide its citizens with a clean, secure, and sustainable environment by the year 2030. These goals can be achieved through reclaiming of polluted areas. This study utilized *Pavonia urens* plant as it is used by communities' especially in North Rift region treating diabetes and for washing utensils. Therefore, this work carried out the phytochemical study on this plant to determine its bioactive components and evaluated the potential of those bioactive components to adsorb heavy metals by complexation. Some of the plant materials possess binding capacities of heavy metal ions through coordinate bond hence cleaning the environment.

METHODOLOGY

Materials and Instrumentation

Reagents/Chemicals

The reagents used were of analytical grade and were; sulphuric acid, acetic acid, ethanol, distilled water, n-hexane, concentrated hydrochloric acid, chloroform, acetone, ethyl acetate and methanol.

Instrumentation

The following equipment was used to analyse the samples: GC-MS (Turbo mass type 20141128) and UV-VIS spectrophotometer (Shimadzu, UK).

Sample Processing

The leaves of the plant were washed and weighed before air drying for 2 weeks to remove water completely. Its weight was taken again after grinding into fine powder so as to increase the surface area of the sample and enhance the contact between the solvent and the sample.

Phytochemical Screening

According to (Uddin & Rauf, 2012) phytochemical screening of crude extracts and fractions was done for quality control. To test for tannins, each 0.2 g crude extract was cooked on a water bath and filtered. Adding three drops of ferric chloride ($FeCl_3$) solution to the filtrate gave a positive tannin test result. Anthraquinones were detected by boiling 1 g of each crude extract in 10 percent HCl for a few minutes, then it was filtered and cooled. The 5 mL $CHCl_3$ was added to the filtrate, which was then boiled with 4 drops 10% ammonia and showed a rose-pink colour indicating the presence of anthraquinones. To test for flavonoids, 0.5 g of each crude extract was dissolved in 5 mL 10% NaOH and 3 mL 2 M HCl, flavonoids were detected by decolorizing a yellow solution. As part of the Liebermann burchard reaction, 1 g of each crude extract was treated with 2 mL acetic anhydride, followed by 2 mL concentrated H_2SO_4. Blue, green, or red colour change indicated steroid presence. To test for terpenoids, 0.5 g of each crude extract was mixed with 2 mL chloroform ($CHCl_3$) and 3 mL conc. H_2SO_4 to form a layer, a reddish interface showed terpenoids. Three drops of copper acetate (Cu $(CH_3COO)_2$) solution were added to 2 g of each crude extract to test for diterpenes. Diterpenes were detected by a shift in colour from blue to emerald green. 2 g of each crude extract was heated for 2-3 minutes with 5 mL of 2 percent H_2SO_4. With 2 drops of Dragendrof's reagent. Orange precipitate indicated alkaloids (Arya *et al*., 2012).

RESULTS AND DISCUSSION

Phytochemical Investigation of the crude extracts of *Pavonia urens*

The preliminary crude extract investigation involved phytochemical analysis which carried out to identify the main constituents of *P. urens* plant, and the findings are presented in Table 1.

Table 1: Phytochemical screening of hexane and ethyl acetate crude extracts

Chemicals	Hexane extract	Ethyl acetate extract
Diterpenes	+	+
Steroids	-	+
Terpenoids	+	+
Alkaloids	-	-
Anthraquinones	-	-
Tannins	-	+
Flavonoids	+	+

As shown in Table 1, hexane extracts contained flavonoids, terpenoids and diterpenes while ethyl acetate extract had tannins, flavonoids, steroids, terpenoids and diterpenes. Flavonoids, terpenoids and diterpenes were present in both hexane and ethyl acetate extract. These findings are in line with many other findings that have reported these secondary metabolites that are found in medicinal plants and have biological functions such as antidiabetic, complexation, antioxidant, and anti-carcinogenic (Ginwala *et al.,* 2019).The presence of enormous O-H, C=O, C= C, C - H and C-N groups in phytochemicals of heterocyclic compounds in crude extracts

indicates the higher potential of the extracts binding with metal ions since these functional groups have lone pair of electrons on oxygen and nitrogen atoms (acting as donors) and the metals (acting as electron acceptors).

Fractionation

Gradient mixture of n-hexane gave three semi pure fractions (1, 2 and 3) which were further fractionated. Fraction 1 gave two fractions on column chromatography. The two were labelled 1A (white crystals in colour) and 3A (yellow in colour). Semi-pure fraction 2 produced one fraction, this spot was labelled 2A (yellowish brown in colour). The semi- pure fraction 3 had two fractions on column chromatography. The first one was labelled 4B (cream in colour) and 5A (white solid in colour), respectively.

Spectroscopic Analysis of fractionated fractions

GC- MS

Fractions; 1A – 5A were characterized by GC-MS. The results presented in GC-MS chromatogram reveals the presence of 15 peaks in Fraction 1A, Figure 1. NIST library match characterized them into several different phytochemical constituents. Elution occurred between retention times (RT) ranging from 7.866 to 29.187. The details of each compound were tabulated in Table 4.2. n-tetracosanol-1(40.73%) and hen eicosane (10.13%) predominantly occurred among the compounds characterized. Other compounds present in very less quantity in the fraction as presented in Table 2. The structures are shown in Figure 2 below.

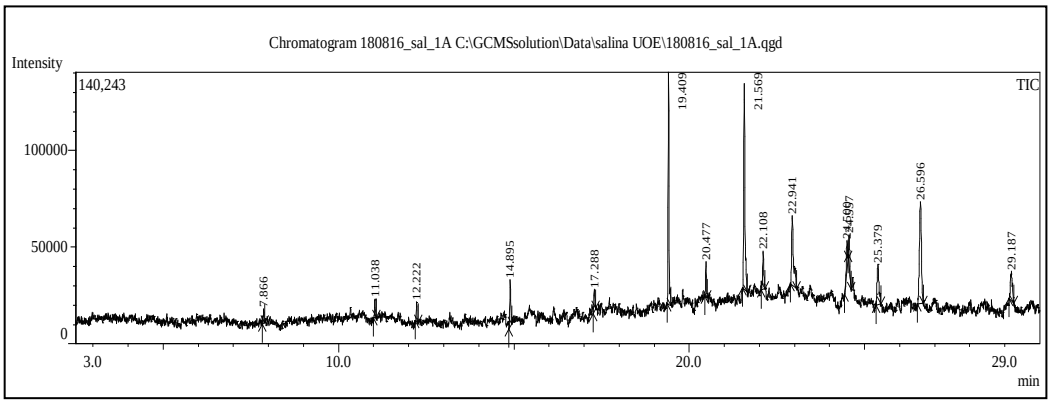


Figure 1: Fraction 1A chromatogram

Table 2: Peak list for Fraction 1 A

S/N	Retention time	Area	Area %	Height	Height %	Name
1	7.866	10616	0.9	7358	1.53	Nonanal
2	11.038	15385	1.31	9683	2.01	Undecanal
3	12.222	15351	1.31	9975	2.08	Tetradecane
4	14.895	41096	3.5	24155	5.03	Hexadecane
5	17.288	19972	1.7	11104	2.31	Heptadecane
6	19.409	221635	18.85	119837	24.93	n-Tetracosanol-1
7	20.477	31953	2.72	18255	3.8	Tetracosane
8	21.569	257304	21.88	106582	22.17	n-Tetracosanol-1
9	22.108	43364	3.69	19583	4.07	Henicosanal
10	22.941	119050	10.13	36041	7.5	Heneicosane
11	24.5	31076	2.64	14707	3.06	Bacteriochlorophyll-c-stearyl
12	24.557	25710	2.19	14154	2.94	Heneicosane
13	25.379	77508	6.59	20940	4.36	Docosanal
14	26.596	209209	17.79	52828	10.99	Tetracosane
15	29.187	56469	4.8	15458	3.22	Tetracosane

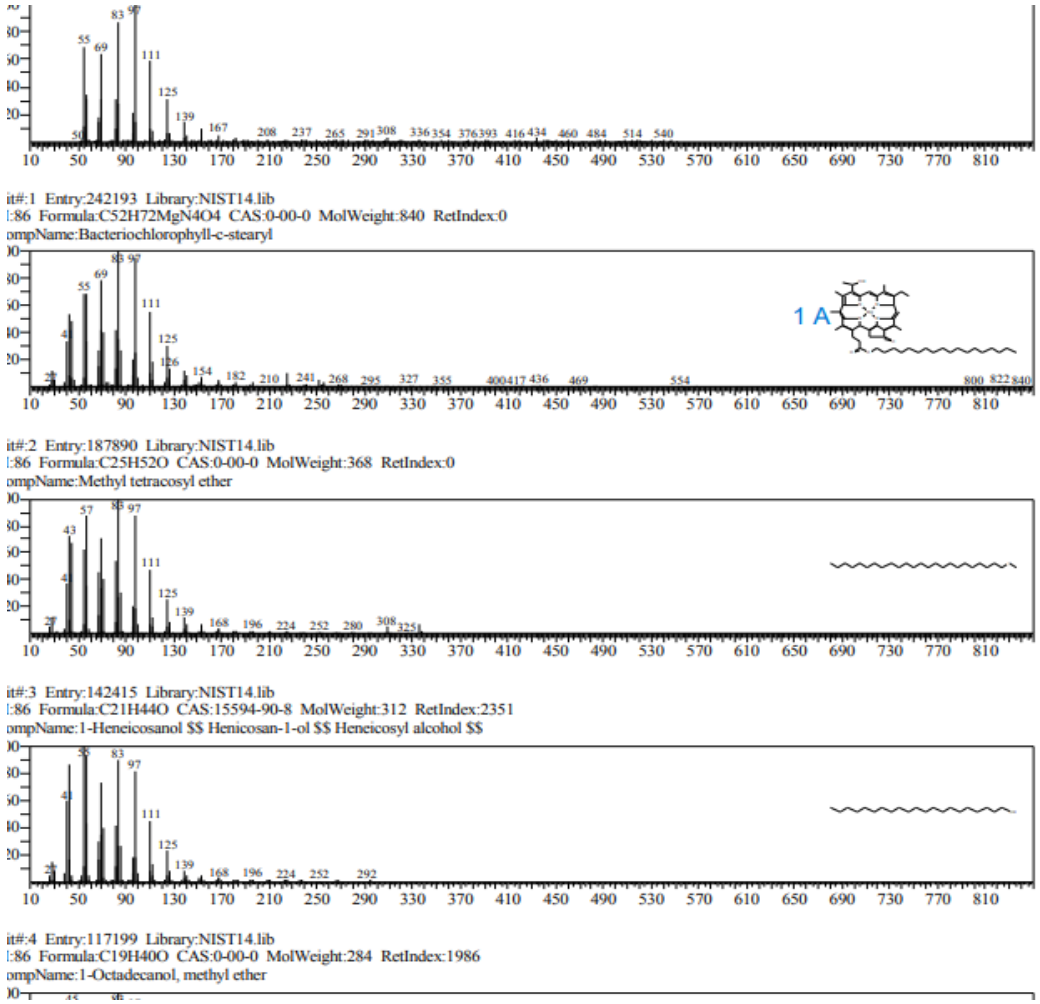


Figure 2: Structures of fraction 1A

The results presented for GC-MS chromatogram revealed the presence of 11 peaks in Fraction 2A and are shown in Figure 3. NIST library match characterized them into several different phytochemical constituents. Elution occurred between retention times (RT) ranging from 14.596 to 28.375. The details of each compound are tabulated in Table 3. Squalene (70.01 %) and tetratriacontane (13.27 %), predominantly occurred among the compounds characterized. Other compounds were present in low fractions. The structures are in Figure 4 below.

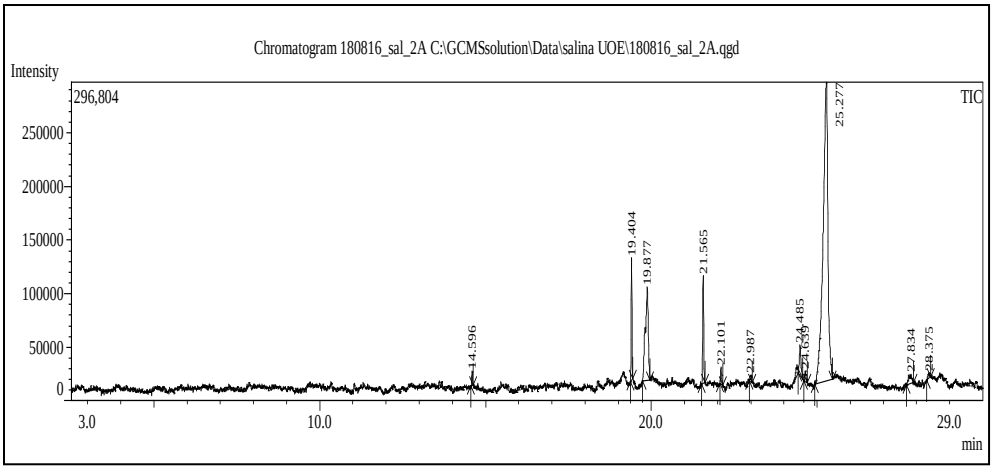


Figure 3: Fraction 2A chromatogram

Table 3: Peak list for fraction 2A

S/N	Retention time	Area	Area %	Height	Height %	Name
1	14.596	25597	0.63	13566	2.03	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-
2	19.404	199887	4.93	114316	17.14	n-Tetracosanol-1
3	19.877	538468	13.27	87300	13.09	Tetratriacontane
4	21.565	218815	5.39	101071	15.15	n-Tetracosanol-1
5	22.101	35323	0.87	15888	2.38	Eicosanal-
6	22.987	12595	0.31	5398	0.81	1,1'-Biphenyl, 2-(phenylmethyl)-
7	24.485	93703	2.31	27674	4.15	n-Tetracosanol-1
8	24.639	25340	0.62	9638	1.45	n-Tetracosanol-1
9	25.277	2841078	70.01	278236	41.72	Squalene
10	27.834	50623	1.25	8650	1.3	2-Bromotetradecane
11	28.375	16666	0.41	5190	0.78	Nonadecyl heptafluorobutyrate

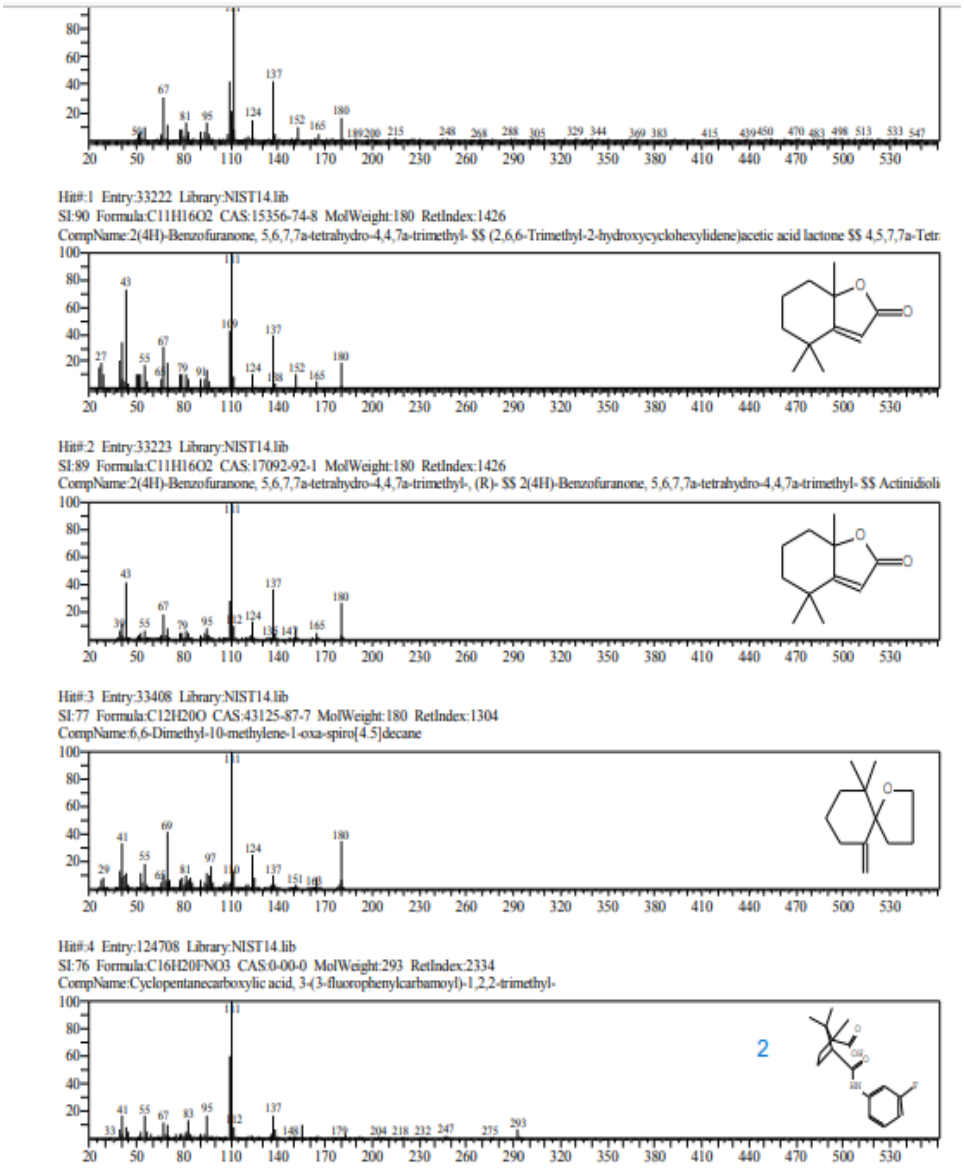


Figure 4: Structures of fraction 2A

The results presented in GC-MS chromatogram revealed the presence of 13 peaks in Fraction 4B and shown in Figure 5. NIST library match characterized them into several different phytochemical constituents. Elution occurred between retention times (RT) ranging from 12.454 to 19. 789. The details of each compound are tabulated in Table 4. Hexadecenoic acid (37.04 hexadecenoic acid methyl ester (18.14 %), and hexacontane (9.03 %), predominantly occurred among the compounds characterized. Other compounds were present in very low quantity in the fractions as presented in Table 4. The structures are as shown in Figure 6 below.

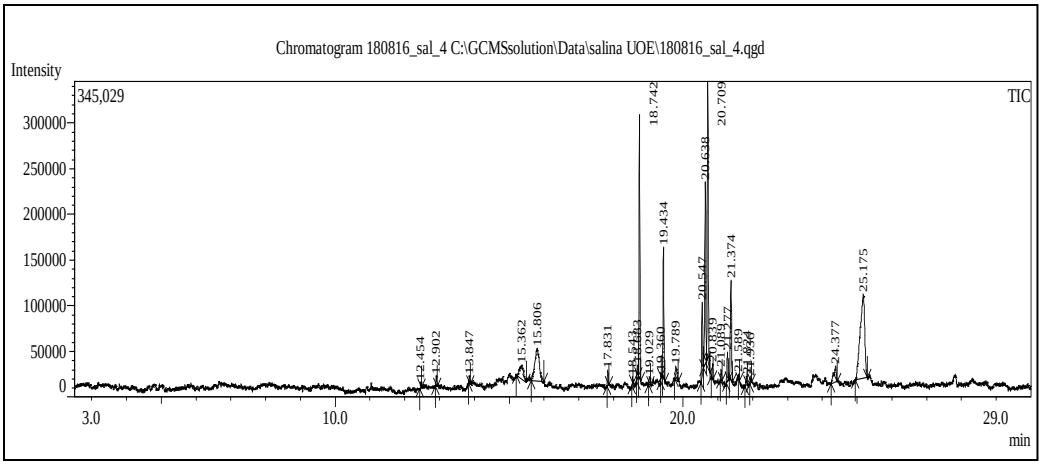


Figure 5: Fraction 4B chromatogram

Table 4: Peak list for fraction 4B

S/N	Retention time	Area	Area %	Height	Height %	Name
1	12.454	10815	0.81	6115	1.05	Benzene, 1,2-dimethoxy-4-propenyl-, (Z)-
2	12.902	20253	1.51	11412	1.97	Nonanoic acid, 9-oxo-, methyl ester
3	13.847	11952	0.89	8229	1.42	Nonanoic acid, 9-oxo-, ethyl ester
4	15.362	121195	9.03	15856	2.73	Tetratriacontane
5	15.806	314112	23.4	35954	6.2	Tetratriacontane
6	17.831	28147	2.1	16466	2.84	2-Pentadecanone, 6,10,14-trimethyl-
7	18.543	8484	0.63	8448	1.46	7-Hexadecenoic acid, methyl ester, (Z)-
8	18.683	22294	1.66	14393	2.48	1,6,10,14,18,22-hexacosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-(.+/-.)-
9	18.742	497413	37.04	288780	49.81	Hexadecanoic acid, methyl ester
10	19.029	17286	1.29	8893	1.53	Undec-10-ynoic acid, tridec-2-yn-1-yl ester
11	19.36	6136	0.46	6954	1.2	Ethyl 9-hexadecenoate
12	19.434	243517	18.14	143494	24.74	Hexadecanoic acid, ethyl ester
13	19.789	40742	3.04	14920	2.57	Tetratetracontane

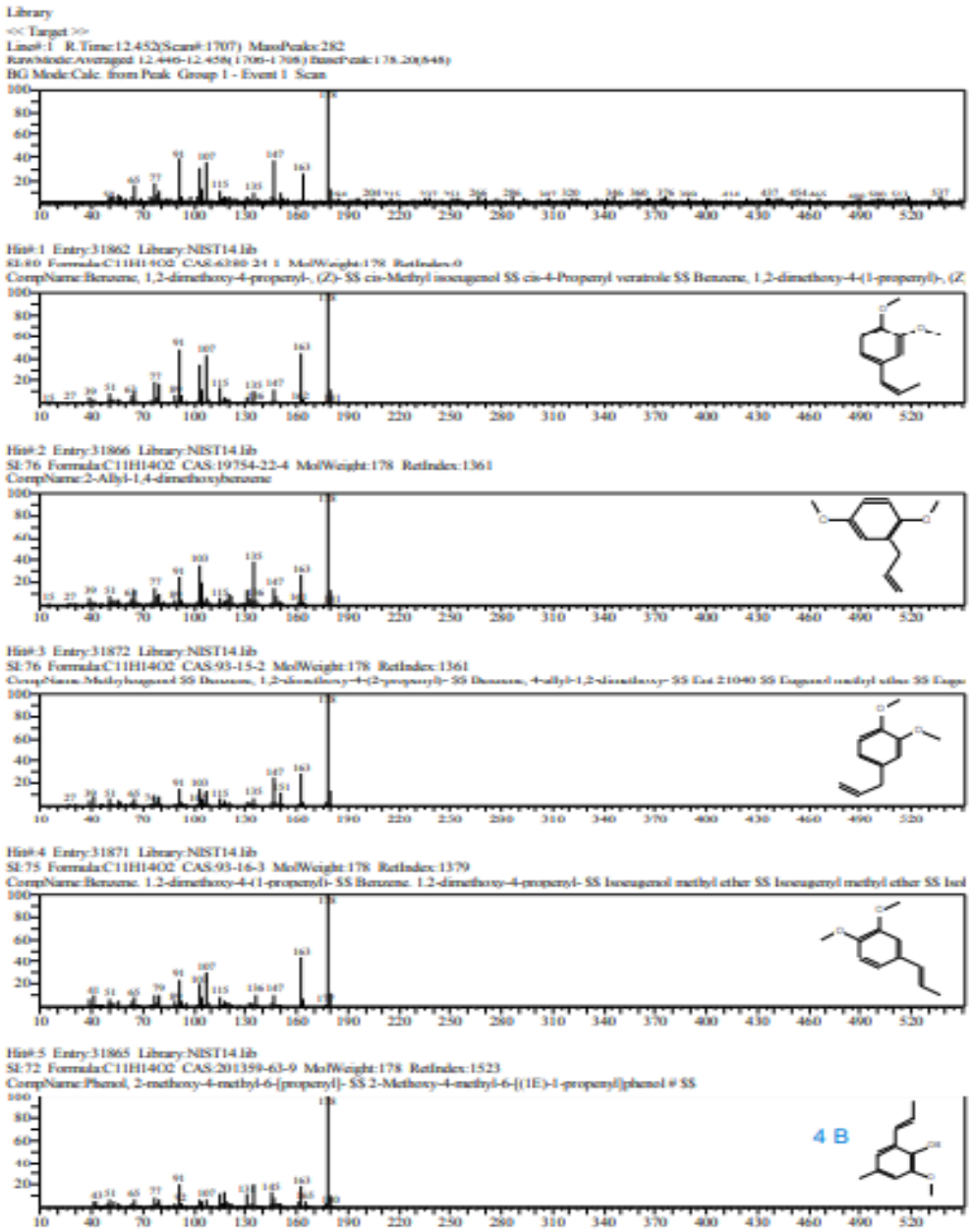


Figure 6: Structures of fraction 4B

The results of fraction 5A shown in GC-MS chromatogram revealed the presence of 9 peaks shown in Fraction 5 A and shown in Figure 7. NIST library match characterized them into several different phytochemical constituents. Elution occurred between retention times (RT) ranging from 14.955to 24. 818. The details of each compound are tabulated in Table 5. Phytol (29.1 %) tetratriacontane (15.59 %), and

hexatriacontane (10.81 %), predominantly occurred among the compounds characterized. Other compounds were present in very low quantities in the fraction as presented in Table 5. The structures are in figure 8.

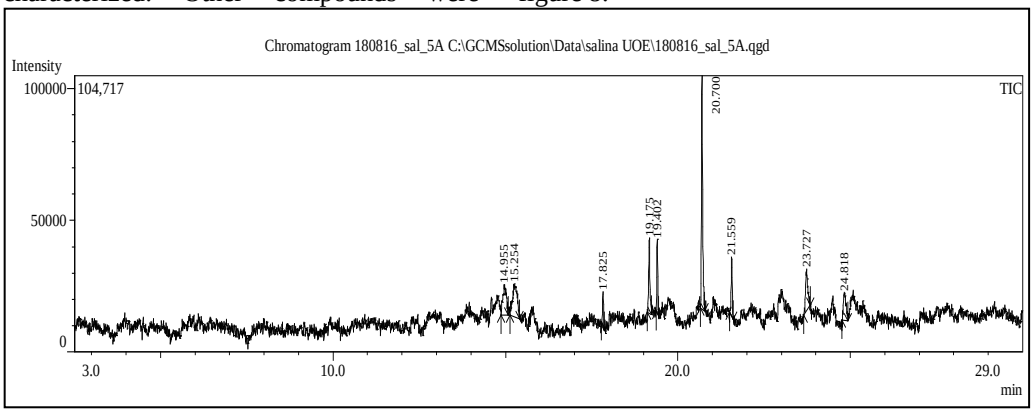


Figure 7: Fraction 5A chromatogram

Table 5: Peak list of fractions 5A

S/N	Retention time	Area	Area %	Height	Height %	Name
1	14.955	79971	10.89	11658	5.05	Hexatriacontane
2	15.254	114467	15.59	12459	5.39	Tetratriacontane
3	17.825	23760	3.24	13297	5.75	2-Pentadecanone,6,10,14-trimethyl-
4	19.175	73313	9.99	28466	12.32	l-(+)-Ascorbic acid 2,6-dihexadecanoate
5	19.402	52572	7.16	27975	12.11	9-Tricosene, (Z)-
6	20.7	213681	29.1	88332	38.22	Phytol
7	21.559	49245	6.71	22394	9.69	n-Tetracosanol-1
8	23.727	71790	9.78	16008	6.93	10-12-Pentacosadiynoic acid
9	24.818	55364	7.54	10479	4.54	10-12-Pentacosadiynoic acid

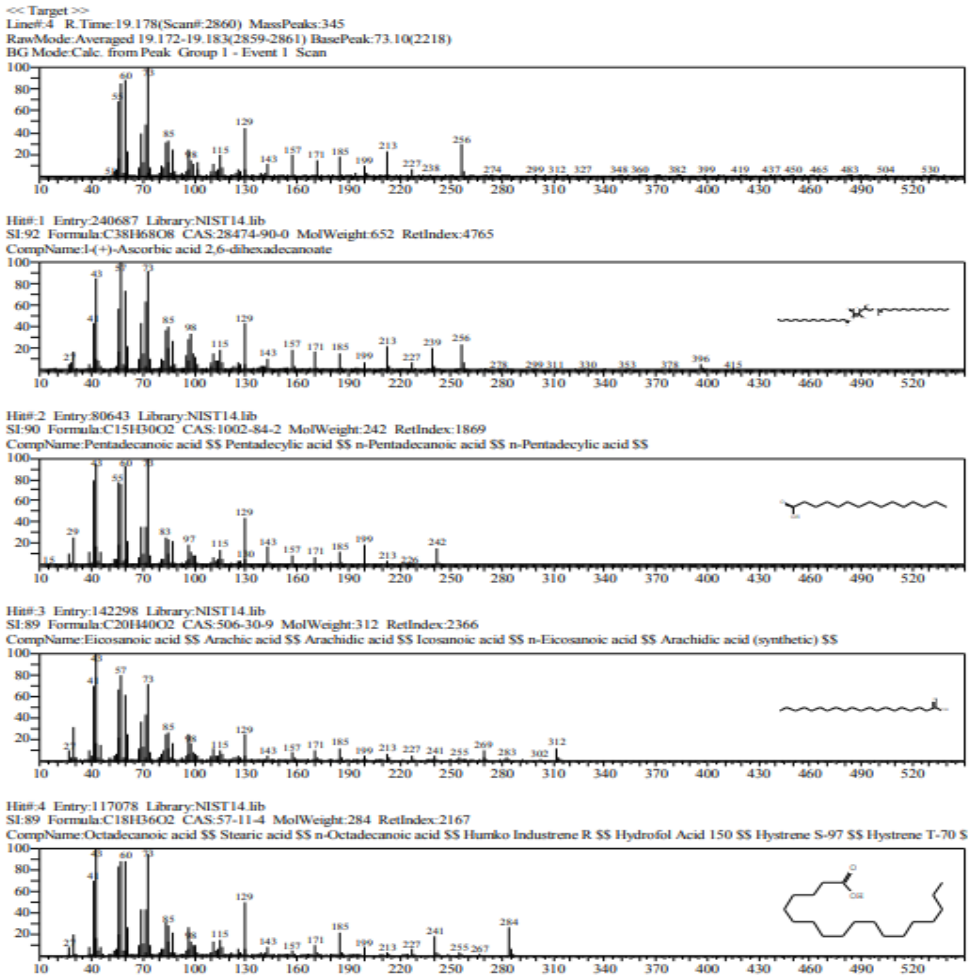
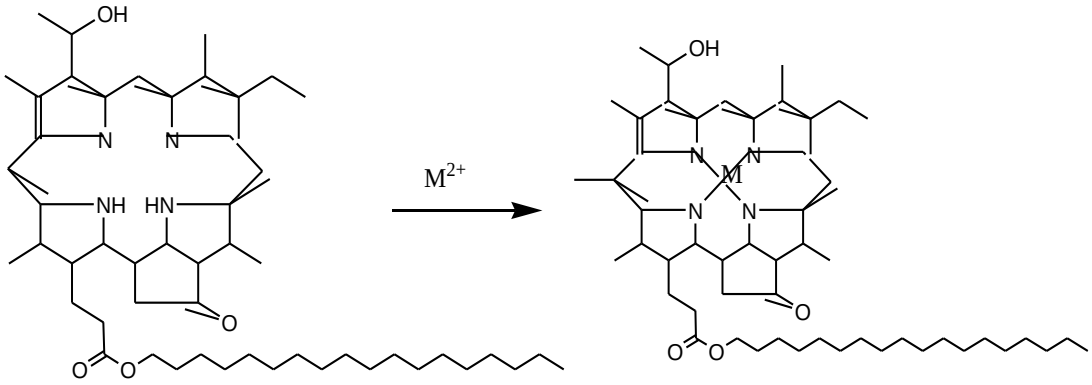


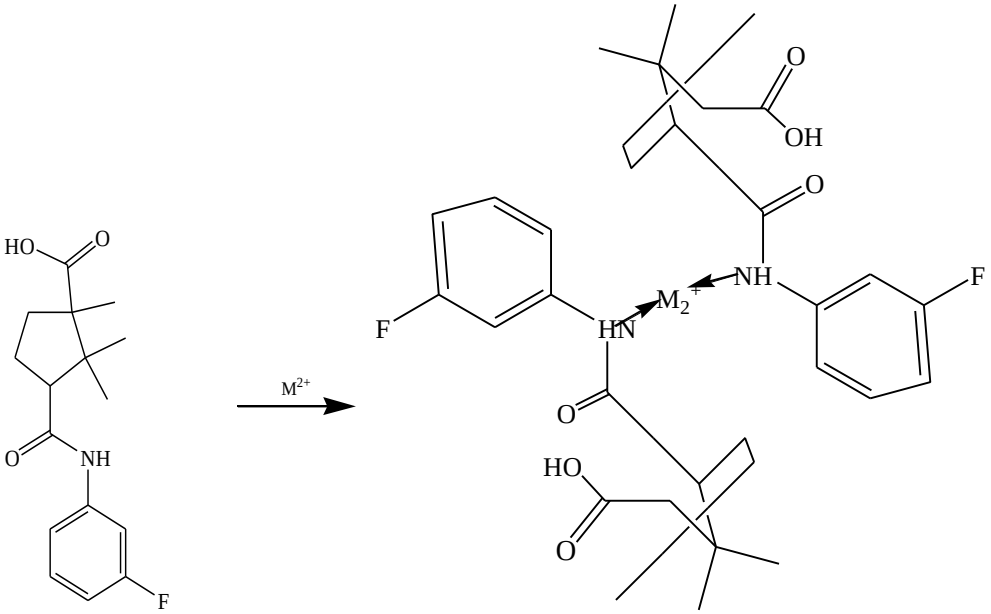
Figure 8: Structures of fraction 5A

Mass fragmentation of fractions 1A – 5A revealed most of these compounds identified contained functional groups with O-H and C=O and a few others with C - N and N-H having lone pairs and thus having the potential to form complexes with metals (coordinate bond formation).

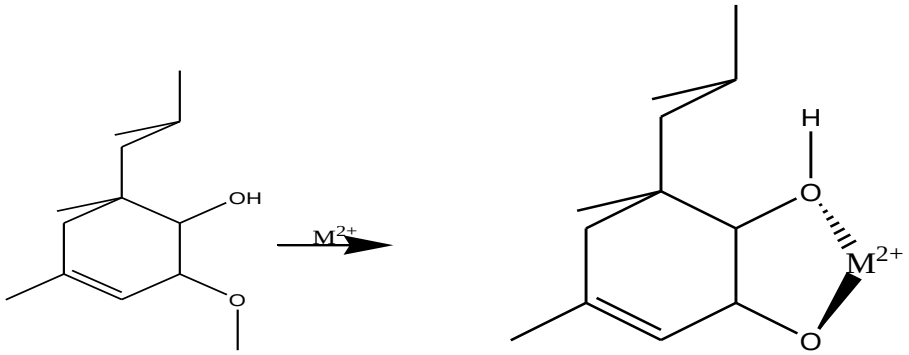
From the chemical, physical, and spectral evidences compared with the ones in literature, possible structures of fraction 1A – 5A with lone pairs can bind with metal ions. Fractions 1A to 5A, is schematic view of proposed binding of metal ions with some structures of some compounds of *Pavonia urens* as shown below.



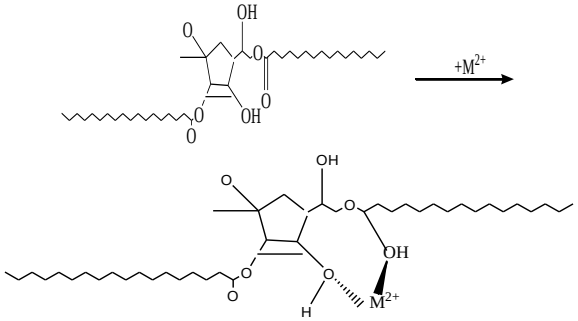
Fraction 1 A: Bacteriochlorophyll-C-stearyl and proposed possible metal complex structure



Fraction 2 A: Cyclopentane carboxylic acid, 3-(-fluorophylcarbonyl)-1, 2, 2-trimethyl and possible metal complex structure



Fraction 4 B: Phenol, 2-methoxy-4-methyl-6-(propenyl) and possible metal complex structure



Fraction 5 A: Ascorbic acid 2,6-dihexadecanoate and possible metal complex structure M^{2+}=Cu^{2+} and Ni^{2+}ions

Scheme 1: The proposed compounds present (fractions (1A, 2A ,4B and 5A) in *Pavonia urens* and possible metal complex structures

Substituents in the benzene ring that remove or donate electrons, the steric effects and inter-molecular, intra-molecular H-bonding affects the absorption spectrum of phenols (Mathiyalagan & Mandal, 2020). In fraction 1A in the scheme, a complex formation of the metal ion with the amino group takes place, other sites like the OH and C=O could not form due to steric hindrance. The epoxy also plays a role of steric hindrance as the group is bulky. This may slow or prevent reactions (Grzelczak *et al.*,2012). This is evident in fractions 2A and 5 A in which, steric hindrance is responsible for the observed shape and geometry of the compound. The steric barrier caused the complex compound change the conformation and geometry of the functional group with the metal ion (Bickelhaupt & Barends,2003).

In fraction 2A from the scheme, the amine group formed a complex with the metal ion while in fraction 5A formation of a complex with C=O and OH occurred. The nature of binding nitrogen and metal ion connection allowed for better ion-functional group interactions (Qureshi *et al*., 2009) The epoxide repelled each other because of molecular interactions and steric hindrance (Mathiyalagan & Mandal, 2020). For Fraction 4B, the complex was formed between the methoxy and hydroxyl group with the metal ion. The oxygen atom has two lone pairs of electrons while methyl groups are bulky but the methyl groups are hindered because of its size.

UV- VIS analysis of complex formation between heavy metals with *Pavonia urens* compounds

Spectroscopy confirmed the production of complex chemicals in metal ion systems. From the UV-VIS spectra, it was observed that there was a shift in wavelength of the plant material when metal ions were added as shown in figure 9.

When copper ions and nickel (II) ions were added separately, a blue-shift of wavelength from original 311 nm to 302 nm and 305 nm, respectively occurred. In addition, there was decrease in absorption frequencies in both cases. This bathometric shift suggests coordination. However, for zinc (II) ions, there was almost no shift observed from original plant material spectrum. The change in shift of wavelength demonstrated coordinate bond formed through the lone pair from the functional groups in the plant cell with the metal ion. For instance, for the -OH, a bond formed can be illustrated as M^{2+}……. O ------- H, here the M^{2+}…. O shows interactions of different groups in plant material having O-atom with different metal ions. The O–H bond of the plant cell was affected by shifts of wavelength involving lowering or increasing absorption bands. For instance, for strong coordinate bonds, M^{2+}…. O will be strengthened while the O-H is

weakened experiencing a shift to longer wavelength that is lowering of absorption and vibrational energies. For weak coordinate bonds, M^{2+}…. O is weakened, while the O-H is strengthened, causing a shift to shorter wavelength hence increasing absorption and vibrational frequencies of O-H bond (Shoaib *et al*., 2011).

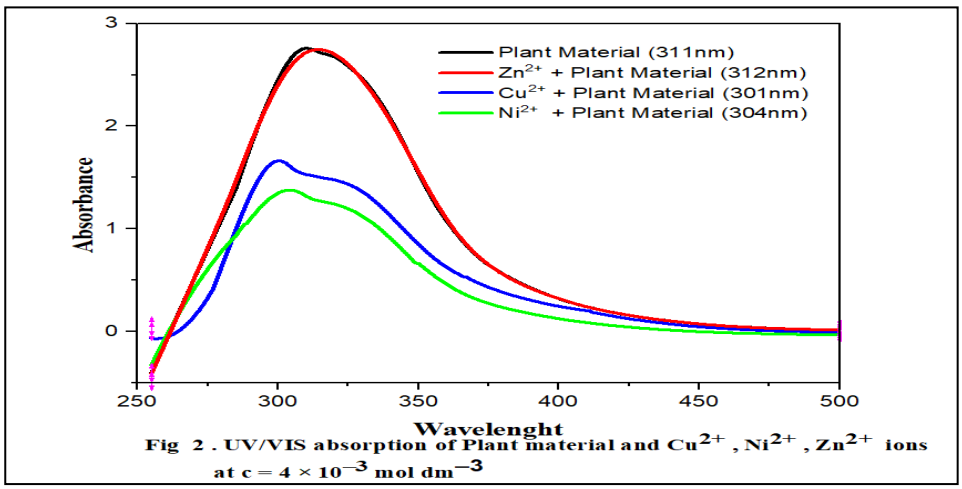


Figure 9: UV/VIS absorption of plant material and Cu^{2+}, Ni^{2+}, Zn^{2+} ions at c=4 x 10^{-3} mol dm^{-3}

Based on the spectra obtained figure 8, copper ions had the largest shift in wavelength range. Overall, the data show that the size of the metal ion's internal coordination sphere changes (Scerri, 2011).

Functional groups can act as coordination centres because they are deprotonated in *Pavonia urens* compounds. These molecules can be partially or entirely deprotonated in biological systems, allowing complexes with metal ions such as copper (II), or nickel (II) (Zabiszak *et al*., 2021) Complexation may be due to ligand-to-metal charge transfer (LMCT). The emergence of new peaks was likely owing to the coordination of deprotonated hydroxyl, carboxyl, and amine groups in plant material with metal ions (Rasheed & Nabeel, 2019). The shift to shorter wavelength of different metals may be related to differences in metal ion size and atomic number (Scerri, 2011). The UV-VIS absorption spectra of zinc metal ions did not change because no complexes were formed and these findings are in agreement with those of Alorabi *et al* (2020).

Zinc is a metallic element with atomic number 30 and electronic configuration is $1s^2 2s^2 2p^6 3d^{10}$, therefore, the 3d orbitals are filled and there are no unpaired electrons for the transition, hence zinc compounds are colourless (Crabtree, 2009) since there is no d-d electronic transition. This confirms why there was no shift. In addition, the coordinate bond between the electron pair acceptor from plant material to 4s orbital of Zn^{2+} is of higher energy because energy then needed to lower these set of orbitals is not achieved (Shoaib *et al.*,2011). However, charge transfer in Zn^{2+}may involve higher energy orbitals such as 4s..The possible binding mode is through the coordination of the nitrogen atoms (N) and the oxygen atoms (O) of the functional groups to form complexes with possible structures as shown in fractions 1A to 5A of Figure 10.

Pavonia urens complexes with metal ions have been established. Monodentate binding to negatively charged oxygen donor atoms (phenolic and carboxylic functional groups) and the related linear free energy connection

for metallic and complexation or bidentate modes were seen in the complexes (Ratié *et al*., 2021). The lone pairs in ligands (OH, C=O and N-H) were donated to the stable and lowest empty orbital of the metal to form coordinate bond.

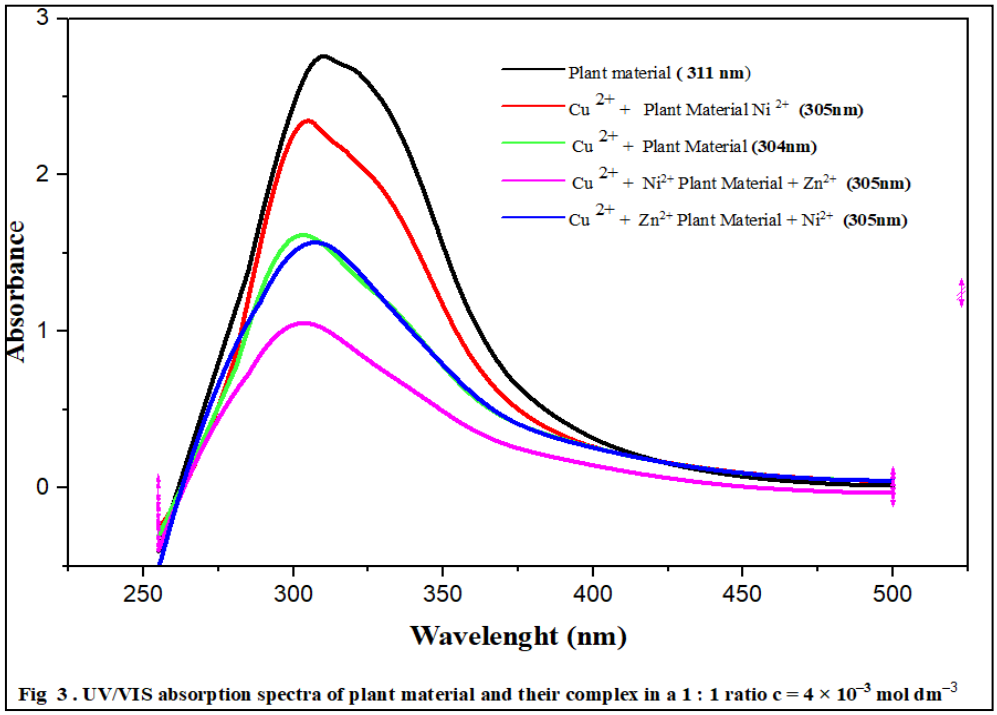


Figure 10: UV/VIS absorption spectra of plant material and their complex in a 1:1 ratio c=4 x 10^{-3} mol dm^{-3}

The atomic radii of the first series transition metals decreased with atomic number. The increased nuclear charge attracts the electron cloud inward, reducing atomic size. Horsefall and Spiff (2005) argued that decreased ionic radius leads to more hydrolysis and less absorption. This confirms that absorption may be linked to the hydration sphere depletion prior to hydrolysis. Ionic size confirms there was high interaction of copper ions with the plant material. Therefore, copper, could have "displaced" zinc and formed bonds with plant material. High energy is needed to break the bond formed between the functional group O-H with copper.

When Cu (II) was added to the plant material, the absorption of metal ions was high because metal ions interact with the active sites of bioactive groups of the plants cell. With the addition of nickel ions, the metal ions compete for binding sites, causing overlapping of absorption bonds due to particle crowding. Due to its smaller ionic radius and higher hydration energy, Zn (II) ions could have easier access to plant pores than Cu (II) and Ni (II) (Shoaib *et al*., 2011).

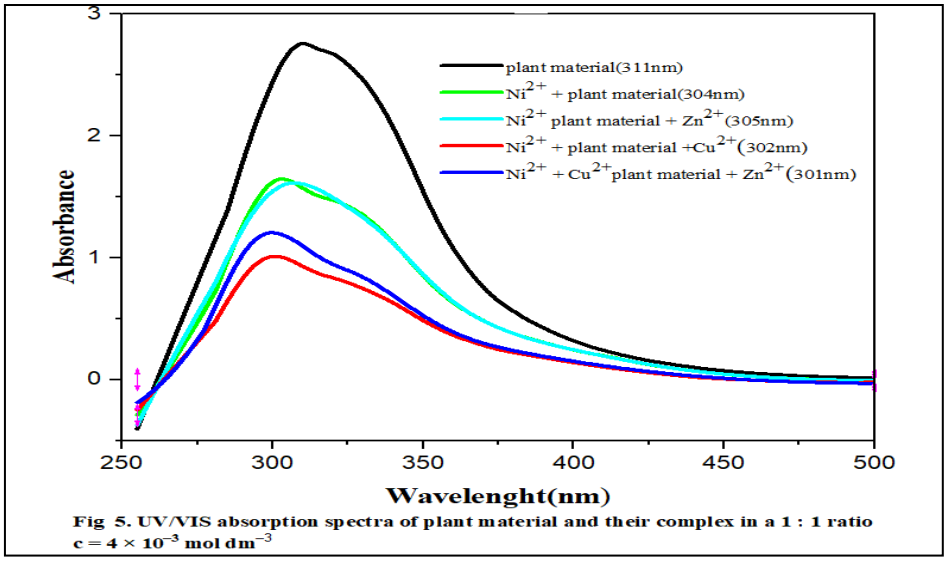


Figure 11: UV/VIS Absorption spectra of plant material and their complex in a 1:1 ratio c=4 x 10^{-3} mol dm^{-3}

In Figure 11, the UV-VIS spectra of nickel with plant material showed shift to shorter wavelength from original 311 nm to 304 nm. The shift to lower wavelength, high frequency forming stronger bond with the functional group. This showed there were high interactions of nickel with the plant material. This was due to greater availability of exchangeable sites or surface area leading to maximum absorbance. When zinc ion was added there was not much change in wavelength although absorption dropped implying the interaction was low or absent. Copper and nickel ions competed for active sites in plant material, which became saturated at a specific metal ion concentration and absorption, however dropped.

Pavonia urens compounds were shown to form complexes with d-electron metal ions. Overall, the data show that metal (II) ions had a higher propensity for coordination with the examined ligands in order of copper (II) > nickel (II) > zinc (II)ions.

CONCLUSIONS

Phytochemical screening showed that *P. urens* contained flavonoids, terpenoids and steroids which account for application of herbal medicine including antidiabetics, anti-carcinogenic activity and metal plant complexation.

Column chromatography of the *pavonia urens* extracts led to fractions 1A, 2A, 4B and 5A using GC-MS. The fractions were identified 1A as bacteriochlorophyll-C-stearyl, 2A as cyclopentane carboxylic acid,3-(3-fluorophylcarbonyl)-1,2,2-trimethyl, 4B as phenol,2-methoxy-4-methyl-6-(propenyl) and 5A as ascorbic acid 2,6-dihexadecanoate. The extracts of *Pavonia urens* had phenolic, alcoholic, aromatic and nitro functional groups.

UV-VIS Spectroscopy confirmed the formation of complexes with metal ion systems. There were shifts in wavelength of the plant material when metal ions were added. When copper and nickel (II) ions were added separately a -shift of shorter wavelength was observed. In addition, there was decrease in absorption in both cases showing bathometric shift through coordination.

Acknowledgement

The authors are indebted to the University of Eldoret for technical support.

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