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Identification of some Organic Compounds in Sugarcane vinasse by Gas Chromatography- Mass Spectrometry and Prediction of their Toxicity Using TEST Method

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ABSTRACT

The varied characteristics of vinasse compounds make the control of vinasse pollution very challenging. To meet this challenge, it is necessary to detect and predict the organic substances that cease toxicity and pollution as well. GC-MS was used to identify the organic compounds and Toxicity Estimation Software Tool (TEST) method was used to estimate the toxicity of the substances.

In this work we measured toxicity by TEST method in term of (LC_{50}) 48 hr. mg/l and LC_{50} (48 hr) -Log_{10} (mol/L). In general about 15 compounds were identified. Most compounds were phenolic and carboxylic acids. The highest toxicity compounds were 1,2,3- triethoxy-5-methyl benzene and 3,4,5- trimethoxy phenol which is 0.9 and 1.09 mg/l respectively, while 2-phenyl ethanol and 4,4-dimethyl- 3-(3- oxobutyl)cyclohex-2-enone with the smallest toxicity, 13.14 and 11.7 mg/l respectively.

KEY WORDS: Pollution, Ecosystem, BOD, COD, LC_{50}.

1. INTRODUCTION

Pollution control is one of the major worries of humanity today. Disposed untreated industrial wastewater directly into natural ecosystem cause a serious of environmental problems. Among the industrial wastewater, vinasse has a high content of organic and inorganic loads with acidity nature. Vinasse is considered as the most hazards industrial waste water, due to the dark brown color, high biological oxygen demand (BOD) and chemical oxygen demand (COD) 45-100g/l and 90-210g/l respectively (Satyawali and Balakrishnan, 2008). Vinasse considered as one of the main sources of soil, rivers, surface and ground water pollution (Mohan et al, 2009).The recalcitrant nature and various characteristics of vinasse (Espana-Gamboa, 2011) make the control of vinasse pollution is very challenging. Moreover the colored compound in vinasse has antioxidant properties and become toxic to all living systems including microorganisms (Soni Tiwari, 2012), therefore the quality and the predicting the toxicity compounds of vinasses needs more attention to estimate and reduce their environmental impacts.

Substantial efforts has been expended to determine the composition of various types of vinasse (Dowd, 1994). Many researchers reported that the most important organic components of sugar cane vinasse are ethanol, acetic acid, glycerol, and lactic acid (Dowd, 1994; Decloux and Bories, 2002), In addition to these low molecular weight compounds, vinasses may contain melanoidins ,humic acid and phenolic compounds (Fitz Gibbon, 1998). On the other hand many studies focus in the vinasse organic matter quality and mineralization potential using ionic chromatography (Parnaudeau, 2008), while Benke (1997) and Emmanuel (2009) studied the characterizations of organic matter of vinasse using spectroscopic techniques. Hyphenated techniques such as gas chromatography-mass spectrometry GC/MS can provide a detailed chromatographic profile of the sample and consequent measurements of the relative or absolute amounts of the components. The number of components measured will depend on the resolution of the chromatographic system and the specificity of the detection technique. (John et al., 2004), therefore GC/MS is suitable technique for vinasse analysis.

Software tools computer programs has been extensively used to predict the toxicity and drug discovery (Manal, 2013). Such programs can provide baseline data for environmental hazard, risk assessment of chemicals. Moreover these programs are easier, cheaper, effective and less time consuming compared with laboratory procedures. Quantitative structure activity relationship (QSAR), ecological structure activity relationship (ECOSAR) and structure activity relationship (SAR) models, are being increasingly used to fill the data gaps in chemical, Pharmaceutical, agrochemical, food additives and other industrial toxicity databases. These tools can offer a means of assessing toxicity of chemicals that lack appropriate experimental test (Patricia, 2011; 2012). Such programs were used Fish, rats and daphnia magna as indicators of toxicity.

The experiments carried out in this study were designed to characterize vinasse and to identify some of organic compounds using GC-MS technique. TEST version (4.1 US Environmental Protection Agency, 2012) method was used to estimate the toxicity of identified compounds. Daphnia magna was used to express the toxicity in term of the lethal concentration that kills fifty percent of test population (LC50) -48 hr. mg/L and LC50 (48 hr) -Log10 (mol/L), which indicates to lethal concentration that kills fifty percent of test population (daphnia magna) (Ruiz et al., 2012) To our best knowledge there has never been any report regarding estimation of vinasse toxicity using TEST method.

2. MATERIAL AND METHODS

2.1. Chemicals: Hexane, Sodium sulfate and sulfuric acid (98%) were purchased from (Merck, Germany), Dichloromethane (DCM) obtained from (Suprasolv, Germany). N,O-bis trimethylsiyl trifluoroacetamide (BSTFA), Trimethylchlorosilane (TMCS) were obtained from (supelco, USA). Sodium hydroxide was purchased from (Fluka, Germany). Ultrapure water (Elga, USA) was used for the preparation all aqueous solutions.

2.2. Vinasse: The vinasse used in this work was obtained from Kenana Sugar Company, an ethanol distillery located at White Nile State, Sudan. Sample were collected directly after the distillation. The main chemical characteristics of the vinasse were determined according to the Standard Methods for Examination of Water and Wastewater table (1). (Clesceri, 1998).while total organic carbon (TOC), total carbon (TC) and total nitrogen (TN) were determined by using total organic carbon analyzer (Shimadzu TOC-L).

Table.1.Vinasse characterization

Parameters	Concentration	Parameters	Concentration
pH	3.5	TN	6.669
BOD	68.978 mg/l	TS	111.46 mg/l
COD	125.777 mg/l	TDS	63.8 mg/l
TOC	489.60 mg/l	TSS	13.0 mg/l
TC	490.00 mg/l	K	121.3 mg/l
IC	0.40 mg/l	Na	120.55 mg/l

2.3. Sample Preparation: The solvent extraction method were used, sample of raw vinasse were placed in separation funnels. Hexane and dichloromethane (DCM) were added in ratio 1:4 for each and shook manually for approximately 5 min. Sodium sulphate was added to the separated organic phase, then filtered and collected in pre-weighted a glass flask. The solvents were removed from organic layer by rotary evaporation (37 °C). Hexane organic layer extracted was reduced under a gentle nitrogen stream (approximately 50 min). The concentrated extracts were stored in a refrigerator at 4°C until analysis. DCM organic layer extract was transferred to vial and TMCS was added, heated to 70°C for 4 hr and dried under nitrogen stream (approximately 50 min), stored in fridge until GCMS analysis.

2.4. GC-MS Analysis: The analyses of the extract were performed by gas chromatography mass spectrometry. These analyses were carried out on a Hewlett- Packard Model 6890 gas chromatograph with splitless injector and a VB-5 5% phenylmethylepolysiloxane column (30 m Length, 0.25 mm I.D., 0.25 μm film thickness) equipped with a Hewlett- Packard Model 6890 mass selective detector provide with a HP ChemStation data acquisition system. Helium (purity 99.999%) was used as a carrier gas. The chromatographic conditions are present in table (2). The data for analysis was acquired from electron impact (EI) mode 70 (eV), scanning from 50-550 amu at 1.5 sec/scan.

Table.2.GCMS condition

Oven Temperature program	Initial oven temperature 60°C, hold for 2 minutes; then up to 280°C at 6°C/min, then held at 280°C for 20 min
Gas flow rates	1.2 ml/min
Injection port temperature	290°C
Injection mode	Splitless (1 min) (1.0-1.4 μl; hot needle technique)
Column inlet pressure	10.4 pis
Average Velocity	40cm/s
Temperature of transfer line	300°C
Solvent delay	4 min

3. RESULT AND DISSECTION

The GCMS analysis of various compounds from vinasse were extracted by hexane and DCM. The identification of unknown compounds was initially accomplished by comparison with the MS library (NIST) and comforted by using Chemo bio draw program version ultra 11.0, and/or by interpreting the fragmentation pattern of the mass spectra. The comparison of the mass spectrums with the data base on MS library gave about 80% - 95% match as well as confirmatory compound structure match.

3.1. Identification of hexane extractable: The typical total ion chromatograms (TIC) of hexane extractable were given in figure (1). While Table 3 represented the compounds extracted by hexane.

3.2. Identification of 2-phenyl ethanol: The EI mass spectrum of 2-phenyl ethanol. MW 122 is given in fig (2) the base peak is found at m/z 91 corresponding to $M[C_7H_7]^{+\cdot}$ result from the loss of $[CH_3O]^{\cdot}$ the 2-phenyl ethanol appeared at R.T of 5.647 in total ion chromatogram.

3.3. Identification of 4-ethyl-3-methoxy phenol: The EI mass spectrum of 4-ethyl-3-methoxy phenol MW 152 is given in fig (3). The base peak is found at m/z 137 corresponding to M[$C_8H_9O_2$] $^{+\cdot}$ Due to the loss of [CH_3]·. The 4-ethyl-3-methoxy phenol appeared at R.T 6.873 in total ion chromatogram.

3.4. Identification of 2,6- dimethoxy phenol: The EI mass spectrum of 2,6- dimethoxy phenol MW 154 is given in fig (4) the base peak is found at m/z 139 corresponding to M[$C_7H_7O_3$]$^{+\cdot}$. due to the loss of [CH_3]· . 2,6- dimethoxy phenol appeared at R.T 7.575 in total ion chromatogram.

3.5. Identification of 1,2,3- triethoxy-5-methyl benzene: The EI mass spectrum of 1,2,3- triethoxy-5-methyl benzene MW 182 is given in fig(5). The MW of 183 probably due to the isotope of C^{13}. The base peak is found at m/z 167 corresponding to M[$C_9H_{11}O_3$] $^{+\cdot}$ Due to the loss of [CH_3]·. 1,2,3- triethoxy-5-methyl benzene appeared at R.T 9.742 in total ion chromatogram.

3.6. Identification of Dodecanoic acid: The EI mass spectrum of Dodecanoic acid MW 200 is given in fig (6) the loss of [C_2H_5]· results in M[$C_{10}H_{19}O_2$] $^{+\cdot}$ at m/z 171 .While the base peak is found at m/z 73, Dodecanoic acid appeared at R.T 10.225 in total ion chromatogram.

3.7. Identification of 3,4,5- trimethoxy phenol: The EI mass spectrum of 3,4,5- trimethoxy phenol MW 184 is given in fig(7). The base peak is found at m/z 169 corresponding to M[$C_8H_9O_4$] $^{+\cdot}$ Due to the loss of [CH_3]·. The 3,4,5- trimethoxy phenol appeared at R.T 10.675 in total ion chromatogram.

3.8. Identification of 4-allyl-2, 6-dimethoxy phenol: The EI mass spectrum of 4-allyl-2, 6-dimethoxy phenol MW 194 is given in fig (8) the loss of [CH_3]· results in M [$C_{10}H_{11}O_3$] $^{+\cdot}$ at m/z 179, while the base peak is found at m/z 91, 4-allyl-2,6-dimethoxy phenol appeared at R.T 10.967 in total ion chromatogram.

3.9. Identification of (E) -1-(3-hydroxy-2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-one: The EI mass spectrum of (E) -1-(3-hydroxy-2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-one MW 208 is given in fig (9) the loss of [CH_3]· results in M[$C_{12}H_{17}O_2$] $^{+\cdot}$ at m/z 193, while the base peak is found at m/z 69, (E) -1-(3-hydroxy-2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-one appeared at R.T 11.300 in total ion chromatogram.

3.10. Identification of of 1,1,4,4-tetramethyl-2,5-dimethylenecyclohexane: The EI mass spectrum of of 1,1,4,4-tetramethyl-2,5-dimethylenecyclohexane MW 164 is given in fig (10) the base peak is found at m/z 149 corresponding to M[$C_{11}H_{17}$] $^{+\cdot}$ Due to the loss of [CH_3]·. The 1,1,4,4-tetramethyl-2,5-dimethylenecyclohexane appeared at R.T 13.150 in total ion chromatogram.

3.11. Identification of Methyl palmitate: The EI mass spectrum of Methyl palmitate. MW 270 is given in fig (11). The loss of [C_3H_7]· results in [$C_{14}H_{27}0_2$] $^{+\cdot}$ At m/z 227.The base peak is found at m/z 74, the Methyl palmitate appeared at R.T 17.608 in total ion chromatogram.

3.12. Identification of Palmitic acid: The EI mass spectrum of Palmitic acid .MW 256 is given in fig (12). The loss of [C_2H_5]· results in M[$C_{14}H_{27}0_2$] $^{+\cdot}$ At m/z 227.While the base peak is found at m/z 73, palmitic acid appeared at R.T 18.367 in total ion chromatogram.

3.13. Identification of methyl stearate: The EI mass spectrum of methyl stearate MW 298 is given in fig (13) the loss of [C_3H_7]· results in M [$C_{16}H_{31}0_2$] $^{+\cdot}$ At m/z 255.While the base peak is found at m/z 74, methyl stearate appeared at R.T 22.050 in total ion chromatogram.

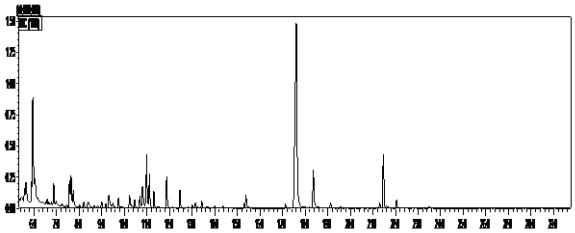


Fig.1.Total ion chromatogram of vinasse extracted by hexane

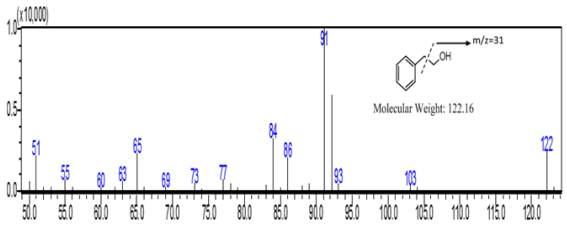


Fig.2.The mass spectrum analysis of 2-phenyl ethanol

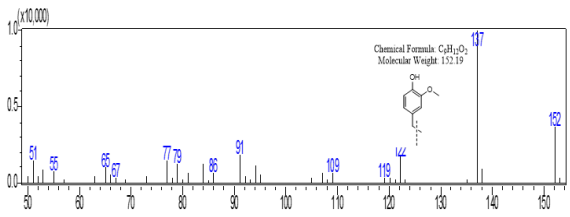


Fig.3.The mass spectrum analysis of 4-ethyl-3-methoxy phenol

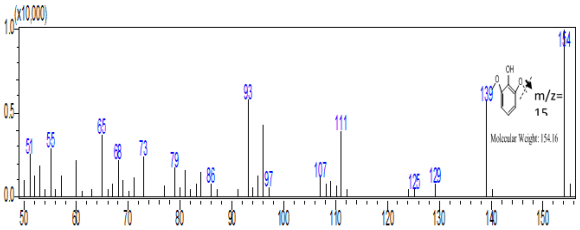


Fig.4.The mass spectrum analysis of 2,6-dimethoxy phenol

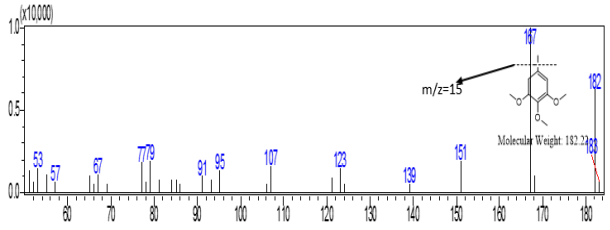


Fig.5.The mass spectrum analysis of 1,2,3-triethoxy-5-methyl benzene

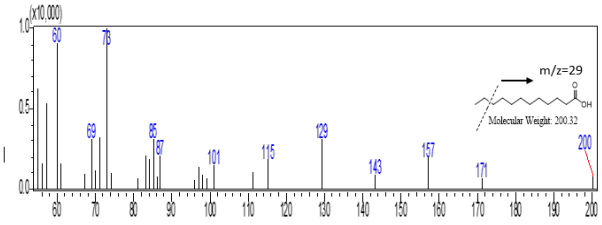


Fig.6.The mass spectrum analysis of Dodecanoic acid

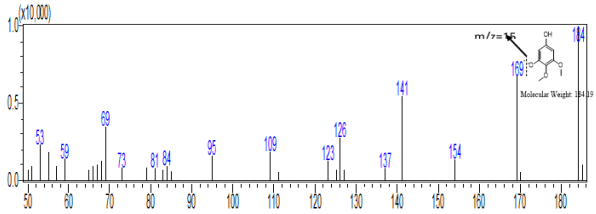


Fig.7.The mass spectrum analysis of 3,4,5-trimethoxy phenol

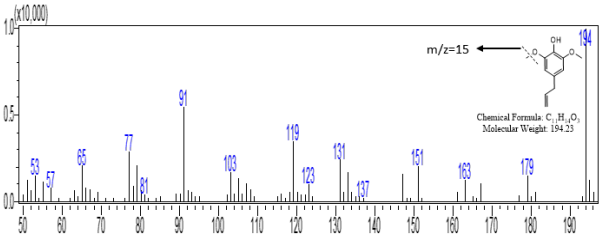


Fig.8.The mass spectrum analysis of 4-allyl-2,6-dimethoxy phenol

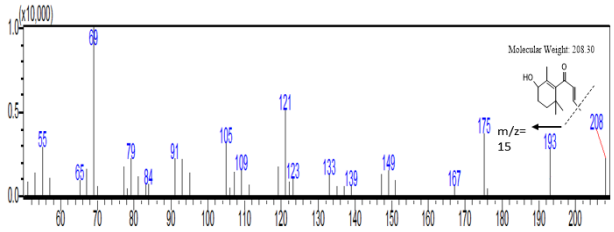


Fig.9.The mass spectrum analysis of (E) -1-(3-hydroxy-2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-one

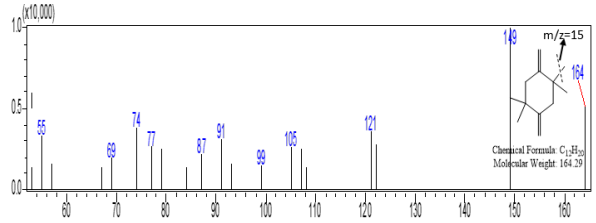


Fig.10.The mass spectrum analysis of 1,1,4,4-tetramethyl-2,5-dimethylenecyclohexane

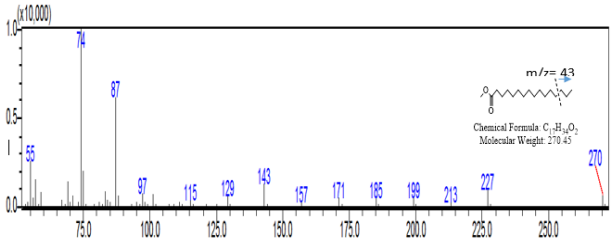


Fig.11.The mass spectrum analysis of Methyl palmitate

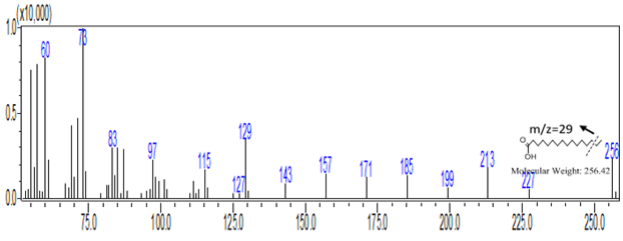


Fig.12.The mass spectrum analysis of Palmitic acid

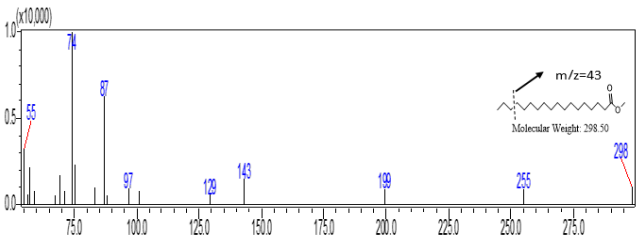


Fig.13.The mass spectrum analysis of methyl stearate

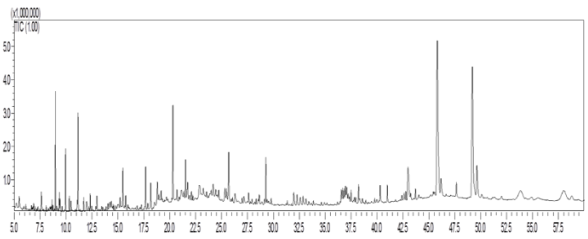
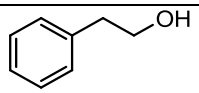
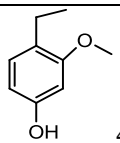
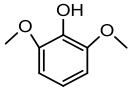
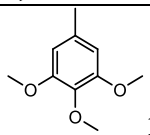
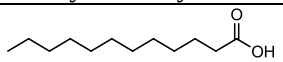
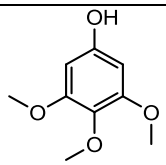
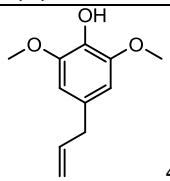
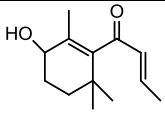
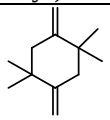
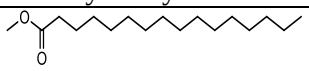
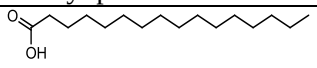
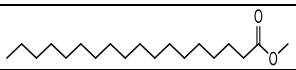


Fig.14.Total ion chromatogram of vinasse extracted by DCM

Table.3.Identification and toxicity prediction of compounds extracted by hexane

R.T.	Structure and Name	Mol. Wt.	Area %	Class	Daphnia magna LC50(48hr)Log 10 (mol/L)	Daphnia magna LC50(48hr)mg/L
5.647	 2-phenyl ethanol	122.16	2.23	alcohol	3.97	13.14
6.873	 4-ethyl-3-methoxy phenol.	152.19	1.42	phenol	4.99	1.55
7.575	 2,6- dimethoxy phenol	154.16	2.47	phenol	4.33	7.22
9.742	 1,2,3- tri ethoxy-5-methyl benzene	182.22	0.64	Aromatic (benzene ring)	5.30	0.91
10.225	 Dodecanoic acid	200.32	1.16	Carboxylic acid	4.32	9.49
10.675	 3,4,5- trimethoxy phenol.	184.19	1.12	phenol	5.23	1.09
10.983	 4-allyl-2,6-dimethoxy phenol.	194.23	5.59	phenol	5.11	1.51
11.300	 (E) -1-(3-hydroxy-2,6,6-trimethyl cyclohex-1-enyl)but-2-en-1-one.	208.30	1.43	ketone	5.04	1.89
13.150	 1,1,4,4-tetramethyl-2,5-dimethylenecyclohexane	164.29	0.70	Cyclo alkane	4.53	4.88
17.608	 Methyl palmitate	270.45	37.23	ester	5.34	1.37
18.367	 Palmitic acid.	256.42	4.12	Carboxylic acid	4.75	4.58
22.050		298.5	0.73	ester	5.42	1.18

As observed in table (3) methyl palmitate extracted by hexane was 37.23 % which indicates that it was the highest concentration in vinasse, while the 1,2,3- triethoxy-5-methyl benzene was the lowest concentration 0.64%.

3.14. Identification of DCM extractable: The typical total ion chromatograms (TIC) of DCM extract were given in figure (14). TMCS were used as derivatiztion method. Most of compounds detected not reported because they contain Si which is come from silylation such as (3,4-dimethoxy phenoxy) trimethylsilane and the toxicity not reported as well (table 3). As shown in table (3) only three compounds were identified, this might be due to the method of silylation, which is resulting of poor chromatographic peaks.

3.15. Identification of 2,6 dimethoxy phenol: The EI mass spectrum of 2,6 dimethoxy phenol MW 154 is given in fig (15) the base peak is found at m/z 154. The loss of $[CH_3]\cdot$ results in M $[C_7H_7O_3]^{+\cdot}$ at m/z 139. The 2,6 dimethoxy phenol appeared at R.T 7.608 in total ion chromatogram.

3.16. Identification of Methyl palmitate: The EI mass spectrum of methyl palmitate MW 270 is given in fig (16) the loss of $[CH_3O]\cdot$ results in M $[C_{16}H_{31}O]^{+}$**.** At m/z 239, while the base peak is found at m/z 74, methyl palmitate appeared at R.T 17.66 in total ion chromatogram.

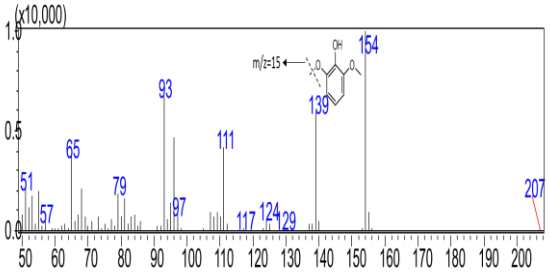


Fig.15.The mass spectrum analysis of 2,6 dimethoxyphenol

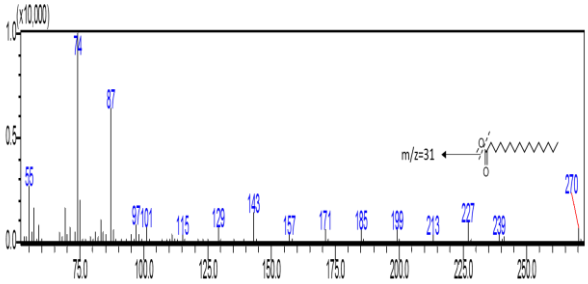
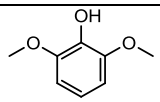
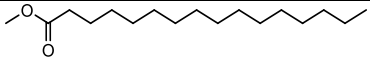
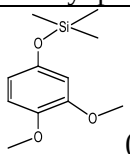


Fig.16.The mass spectrum analysis of methyl palmitate

Table.4.Identification and toxicity prediction of compounds extracted by DCM

R.T.	Structure and Name	Mol.Wt.	Class	**Daphnia magna LC_{50} (48 hr) -Log10 (mol/L)**	**Daphnia magna LC_{50} (48 hr) mg/L**
7.608	 2,6 dimethoxy phenol	154.16	phenol	4.33	7.22
17.667	 Methyl palmitate	270.45	ester	5.34	1.37
8.95	 (3,4-dimethoxy phenoxy)trimethylsilane	226.34	phenol		

In general, the smaller the LC_{50} value, the more toxic the chemical, and the larger the LC_{50} value, the lower the toxicity (Patricia et al., 2012).Hence phenols are the most compounds that influence on human and natural environment (Urszula et al., 2012), and as showed in table 3 and 4, the most compounds detected were phenolic, therefor vinasse can considered as harmful substances to environment. The most toxic compounds detected in vinasse were 1,2,3- triethoxy-5-methyl benzene and 3,4,5- trimethoxy phenol with concentrations of 0.9 mg/l and 1.09 mg/l respectively.

4. CONCLUSION

The study indicates that vinasse has high COD, TOC and highly colored with low pH, these characteristics affect negatively to the environment. About 15 of organic substances were identified by using GC-MS, and the toxicity were estimated by TEST method. 0.9 mg/l of 1,2,3- triethoxy-5-methyl benzene is capable to kill Daphnia magna in 48 hr.

5. ACKNOWLEDGEMENT

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