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Phytochemical profiling, oral glucose tolerance, body weight changes and insulin response of methanol fraction of *Hensia crinita* extract on Wistar Rats

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ABSTRACT

Phytochemical profiling, oral glucose tolerance, body weight changes and insulin response fraction of *Hensia crinita* (H.C) leave extract was investigated in this study. Using Gas Chromatography –mass spectrometry (GC-MS) analytical method for phytochemicals analysis, the methanol fraction of H.C showed the presence of fourteen compounds and revealing with respect to percentage abundance, high levels of Oleic acid (27.13%), Hexadecanoic acid (11.5%), ethylbenzene(9.92%), nonane (9.85%), 7-oxalic{4.1.0}heptane (9.83%), 1,2,3-trimethylbenzene (8.05%), Oxalic acid (7.42%) as well as other compounds. Body weight showed significant increase ($P<0.05$) treated groups when compared to the control. Oral glucose tolerance level was also increased in the extract treated groups. A significant increase ($P<0.05$) in serum insulin level was observed in extract treated compared to diabetic control. Both ethanol crude extract and methanol fraction showed positive responses to oral glucose test, body weight changes and insulin levels activity and therefore may be incorporated in herbal decoctions in the management of diabetes.

Keyword: Phytochemical, antidiabetic, Oleic acid, *Hensia crinita*

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Introduction

Active ingredients present in natural products have been the source of nutrients and medicines for years and they may still be the leads for new drugs (Newman, 2008). Medicinal plants are used by 80% of the world population as the most prevalent or available medicines especially in developing countries (Hashim *et al*., 2010) and are considered to be less toxic, safer and more affordable when compared to synthetic drugs and may be used with little or no adverse effects.

Diabetes mellitus, a major public health concern is a metabolic disease whose cause may be due to dysfunctional insulin receptors or insufficient production of insulin by the beta cells of the pancreas (Adeyi et al. 2012) with multifaceted complications (Kathleen, 1996; Sharma et al. 2008).

Heinsia crinita (Rubiaceae), a well-known medicinal plant has gross leafy calyx-lobes, which produces sweet esculent yellow or reddish fruits (Ajibesin, 2008) is spread across the tropical region including Cameroon, Guinea, Congo Basin and Nigeria). It is a useful tropical plant commonly found in the South-eastern part of Nigeria with the local name “Atama” which is of the Efik origin and “Tonoposho” which is of Yoruba origin. The plant is reported to be rich with phytochemical which possess antihypertensive, antimicrobial, antidiabetic, antiplasmodial, nephroprotective, hepatoprotective properties (Abo, 2000; Ajibesin 2003; Mahesh, 2008; Ebong *et al*., 2014). With this medicinal attributes, this study seeks to evaluate the phytochemical profile using GC-MS analysis, oral glucose tolerance, body weight changes and insulin response of extracts of *H. crinita* Stz-induced albino Wistar rats

Materials and Methods

Plant material

Fresh and mature leaves of *Heinsia crinita* were collected from Calabar metropolis, Cross river state, Nigeria. It was authenticated by Dr. Michael Eko a botanist in the Department of Botany, University of Calabar. A voucher specimen (EUDB/S01/13) was deposited in the Herbarium unit of the Department of Biochemistry, University of Calabar, Nigeria The leaves were washed under running tap water, dried under room temperature (25 ± 15˚C), homogenized to get a coarse powder with a manual blender and macerated in 99.7 percent ethanol solution for 48 h before filtering with a cheese cloth and subsequently with whatman No.1 filter paper. The solvent was distilled off in the rotary evaporator to obtain a solid residue of 200g.

Portions (150g) of the ethanolic crude plant extract were successively subjected to serial solvent fractionation using n-hexane and methanol solvents respectively. The crude extract was chromatographed on silica gel (60-120 mesh size) packed in a glass chromatographic column and eluted in succession using the aforementioned solvents in order of increasing polarity. Fractions of each solvent systems were collected in beakers and evaporated to dryness and the menthol fraction used for the present study.

Animals

Thirty albino *Wistar* rats weighing between 100-150g were obtained from the Animal House of the Department of Biochemistry, University of Calabar. The rats were housed in modern cages which were well ventilated and allowed a week for acclimatization. The temperature and relative humidity were standard with approximately 12h light and dark cycle. The animals were allowed access to food and water *ad libitum* throughout the period of the experiment. The protocol was in accordance with the guidelines of the National institute of Health (NIH) publication (1985) for laboratory Animal Care and Use and approved by the Collage of Medical Sciences Animal Ethics Committee University of Calabar, Nigeria (Atangwho *et al*., 2013)..

Induction of diabetes mellitus

Diabetes was induced intraperitoneally in overnight fasted animals with freshly prepared solution of 50mg/kg streptozotocin (Sigma Aldrich, St. Louis, USA) in 0.1M sodium citrate buffer. After five (5) days, the animals with fasting blood glucose >7.8mmol/l or >180mg/dl were considered diabetic and used for the study.

Treatment procedure

Thirty adult albino Wistar rats weighing 100-150g were grouped into five (5) consisting of six animals as follows:

Group I: Normal healthy rats given 0.2ml of

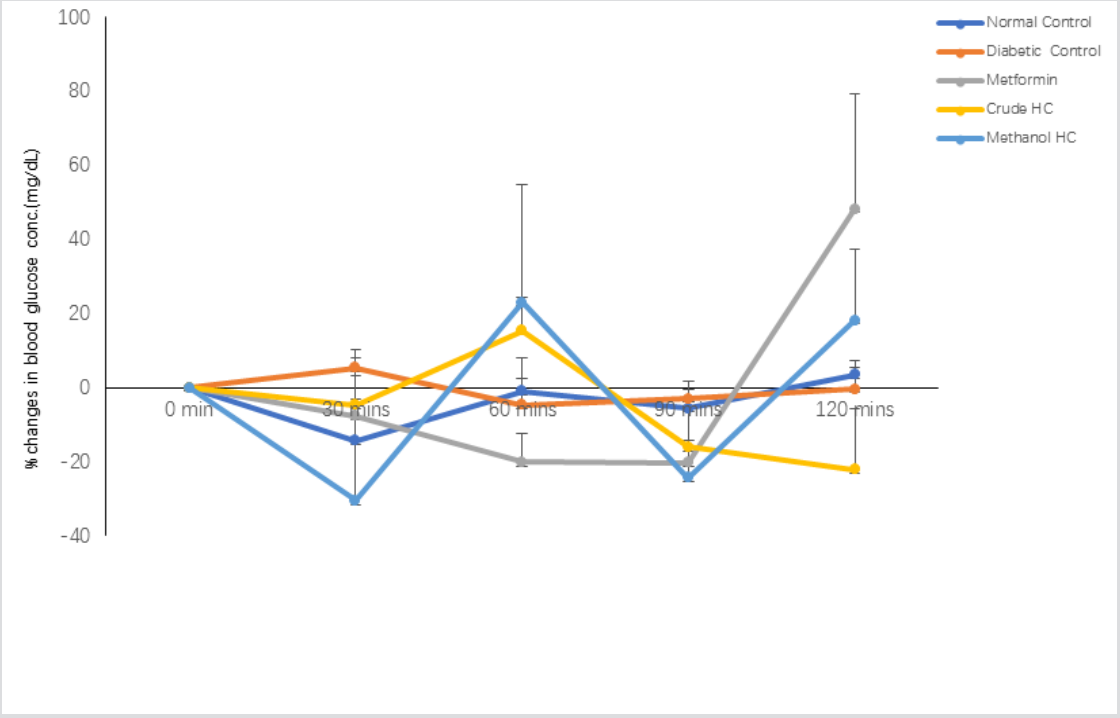

Fig 2: Effect of ethanolic crude extract and methanol fraction of *H. crinita* on oral glucose tolerance in stz induced diabetic rats

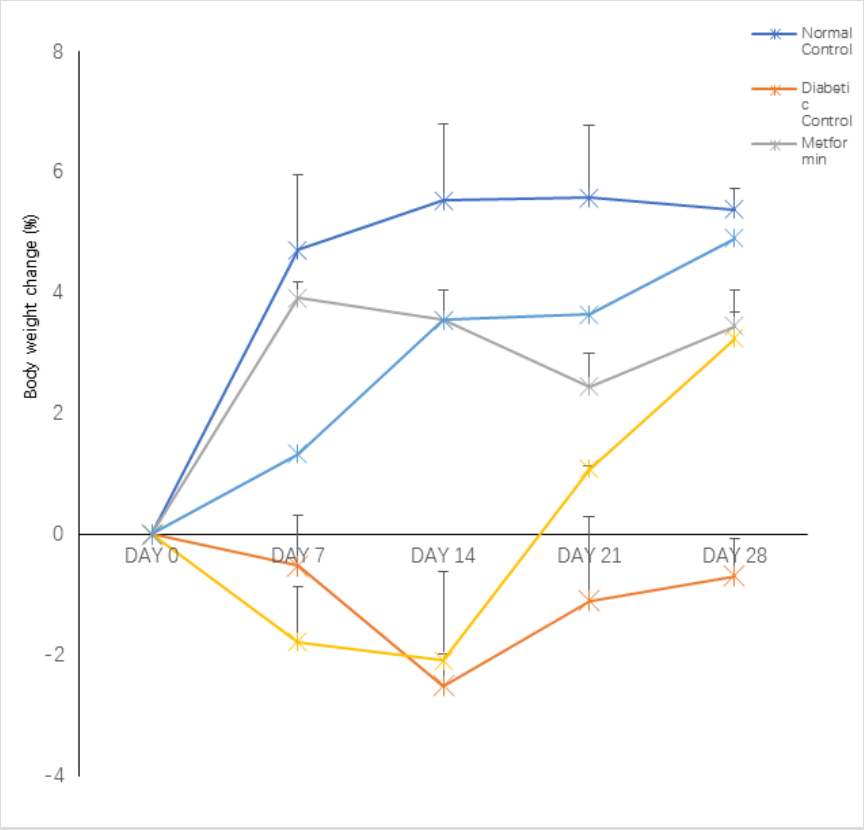

Fig 4: Effect of ethanolic crude extract and methanol fraction of H. crinita on body weight in stz induced diabetic rats

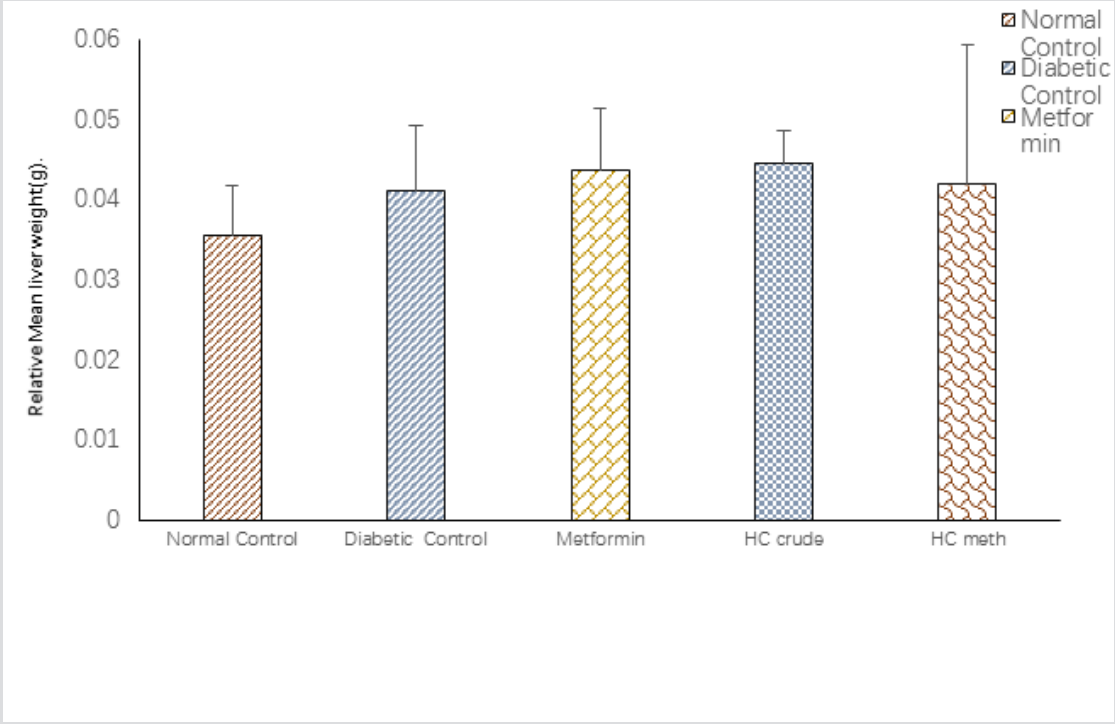

Fig 5: Effect of ethanolic crude extract and methanol fraction of H. crinita on relative liver organ weight in stz induced diabetic rats

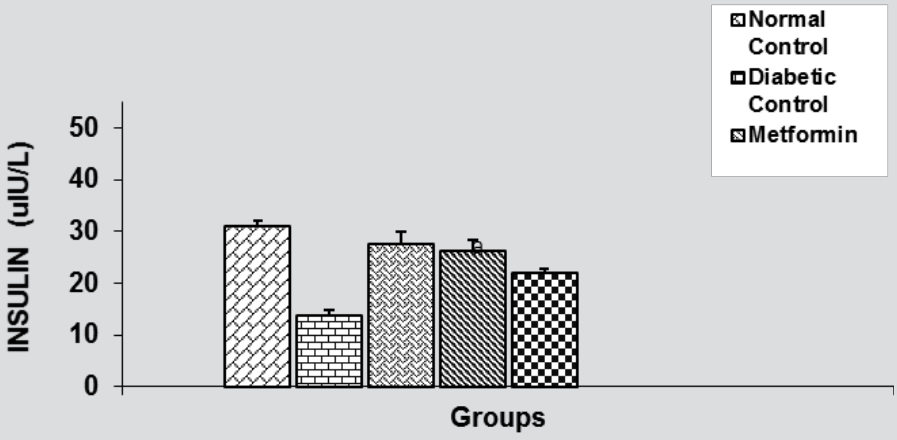

Fig 6: Effect of ethanolic crude extract and methanol fraction of H. crinita on body weight in stz induced diabetic rats. Values are expressed as mean ± SEM, n = 5 * Significantly different from Normal control at $p<0.05$; a significantly different from Diabetic control at $p<0.05$;b significantly different from Metformin at $p<0.05$

10% DMSO

Group II: STZ-induced diabetic rats given 0.2ml of 10% DMSO

Group III: Diabetic rats treated with metformin drug (500mg/kg body weight)

Group IV: Diabetic rats treated with ethanolic extract of H. crinita (400mg/kg body weight)

Group V: Diabetic rats treated with methanol fraction of H. crinita (400mg/kg body weight)

Treatment was administered by oral gavages twice daily for 28 days. The plant crude extract and fraction were reconstituted in 10% dimethylsulphoxide (DMSO) before use. Body weight changes were observed in the experimental animals at 7 days interval throughout the duration of treatment.

Oral glucose tolerance test:

Oral glucose tolerance test (OGTT) in diabetic rats. Twenty-six rats were used for the test and the procedure, dosage of extracts/metformin/saline and animal grouping were as described above. Furthermore, the rats were orally administered glucose (2 g/kg by weight) 30 min after dosing, and blood samples were obtained via the tail puncture at time 0 (prior to glucose dosing) and at 0, 30, 60, 90 and 120 min after glucose administration to measure the glucose level

Inulin test:

ELISA kit (Insulin, monobind Inc Lake Forest, CA 92630, USA) were used to measured serum insulin levels at the end of the 28 days experimental period

Statistical analysis

Results obtained were analyzed using SPSS package version 20 and presented as Mean ± SEM. The statistical significance was measured by One-way Analysis of variance (ANOVA) with a post hoc Donett value at $P<0.05$.

Results and Discussion

DISCUSSION

Due to the high prevalence of diabetes worldwide, extensive research is still being performed to develop new antidiabetic agents and determine their mechanisms of action, consequently, a number of diabetic animal models have been developed and improved over the years (Islam and Loots, 2009). Chemically induced type I diabetes is the most commonly used animal model of diabetes utilizing streptozotocin as the principal agent to produce experimental diabetes (Tan et al., 2005).

In this study, ethanolic crude extract and methanol fraction of H. crinita in STZ induced diabetic rats were assessed for their antidiabetic activity. Oral administration of the crude extract and methanol fraction (400mg/kg) caused a marked reduction in the blood glucose level after the 4th hour when compared with the diabetic untreated group thus producing a significant anti diabetic effect. This is in agreement with reports from previous study which indicated that some plant extracts possess antidiabetic properties, potentiate insulin release and utilize peripheral glucose thereby reversing chemically induced hyperglycemia (Okonkwo and Okoye, 2009; Pulipaka *et al*., 2012).

In the Glucose tolerance test, an important diagnostic tool for diabetes, blood sugar lowering effect was observed between 30-90 min in the crude extract and methanol fraction treated groups. Also both crude extract and methanol fraction of *H. crinita* decreased fasting blood glucose level significantly ($P<0.05$) throughout the duration of the experiment and compared favorably with the result of metformin treated diabetic rats. This observed features may be attributed to the presence of secondary bioactive principles which has been reported to be present in this plant in this study and also those earlier reported by Abo *et al*. (2011) such as flavonoids, polyphenols, saponins and tannins in the crude extract and methanol fraction of this plant. This study corroborates previous study by Mgbeje *et. al*., (2013) and Edet *et al*., (2013) that plant extracts containing polyphenols, flavonoids, saponins and other secondary compounds of plant metabolism actually have antidiabetic properties.

Diabetes has been associated with a characteristic loss of body weight, which is due to continuous excretion of glucose, decreased peripheral uptake of glucose, glycogen synthesis, increased muscle wasting and loss of tissue proteins (De-

Table1: Phytochemicals identified in methanol fraction of *Hensia crinita* GC-MS analysis

S/N	RT	NAME	MOL. FOR.	MOL. WT	% ABUDANCE
1	3.52	Ethylbenzene	C_8H_{10}	106	9.92
2.	4.65	1-ethyl-2- methyl benzene	C_9H_{12}	120	4.39
3	4.91	nonane	C_9H_{20}	128	9.85
4	5.14	1,2 ,3 trimethyl benezene	C_9H_{12}	120	8.05
5	6.39	2, 7-dimethyl octane	$C_{10}H_{22}$	142	4.13
6	9.04	2, 4-dimethyl benezene	C_8H_{18}	114	1.11
7	11.94	3, 3,-diimethyl hexane	C_9H_{18}	114	0.99
8	14.49	2,8-dimethyl undecane	$C_{13}H_{28}$	184	1.17
9	19.23	n-hexadecanoic Acid	$C_{16}H_{32}O_2$	256	11.5
10	20.62	cyclopro-panentanoic acid	$C_{20}H_{38}O_2$	310	1.99
11	20.87	13-Docosenoic acid	$C_{23}H_{44}O_2$	352	2.98
12	21.61	Oleic acid	$C_{18}H_{34}O_2$	282	27.13
13	25.04	7-oxalic{4.1.0} heptane	$C_{10}H_{16}O_2$	168	9.82
14	26.61	Oxalic acid	$C_{26}H_{48}O_4$	412	7.42
					100%

RT= Retention time.

fronzo *et al*., 1992). This condition was ameliorated and a significant improvement in the body weight of the crude extract and methanol fraction treated groups was observed when compared with the diabetic untreated rats. Serum insulin levels was significantly decreased in the diabetic untreated group when compared to the normal control group, this is due to the selective destruction of the β-cells of the pancreas thereby resulting in insulin deficiency or inability of peripheral tissues to utilize insulin. A significant response to glucose level was observed in groups treated with crude extract and methanol fraction of *H. crinita* and comparing favorably with the effect in the metformin treated group. This may be due to the ability of the extracts to influence the β-cells to stimulate insulin secretion and restored insulin sensitivity.

The GC–MS analysis of methanol fraction of H.C was carried out, which revealed approximately 14 phytochemical compounds (Table 1), Oleic acid (27.13%-Linoleic acid), Hexadecanoic acid (11.5%-Palmitic acid), Ethylbenzene (9.92%), n-Nonane (9.85%), alpha- Limonene (9.83%-Terpene), 1,2,3-trimethylbenzene (8.05%) and Oxalic acid (7.42%-Dicarboxilic acid) where all found to be present in significant concentrations. The identified fatty acids present in this plant have been documented by (Erasto *et al*.,2007), showing an underlining nutritional and pharmacological importance of this plant, and also playing significant roles as metabolic precursors of physiologically active mediators of inflammatory, immunological, blood clotting mechanisms, hypocholesterolemic, anti-cancer, anti-anxiety and anti- diabetic activity in experimental models (Suresh and Das 2007).

CONCLUSION

The results indicate that the ethanolic crude extract and methanol fraction of *H.crinita* possesses potent bioactive phytochemicals with antidiabetic actitiy.

ACKNOWLEDGEMENT

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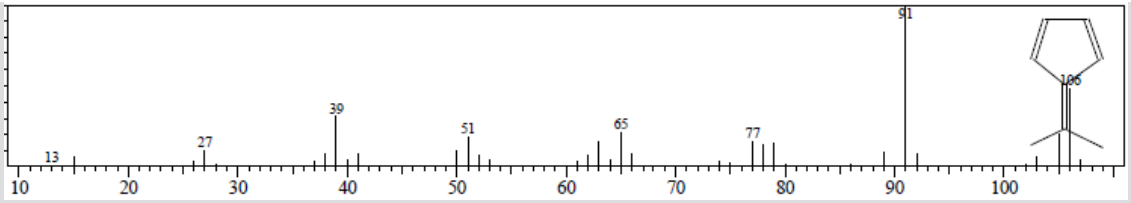

Plate1; Ethylbenzene

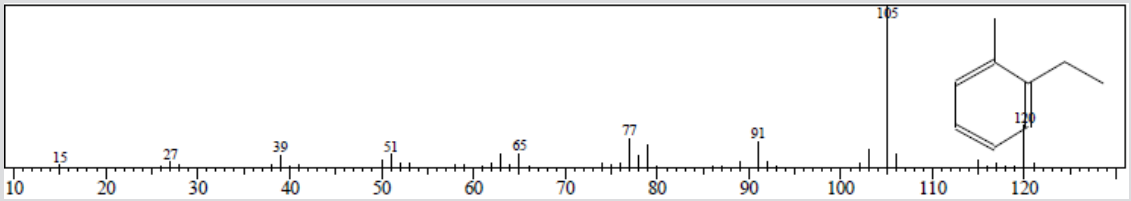

Plate2: 1-Ethyl-2-methylbenzene

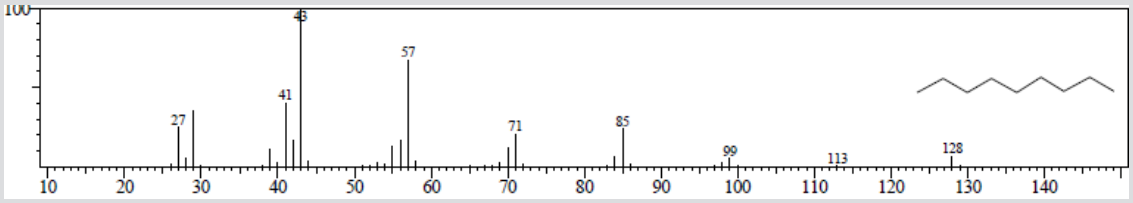

Plate 3: Nonane

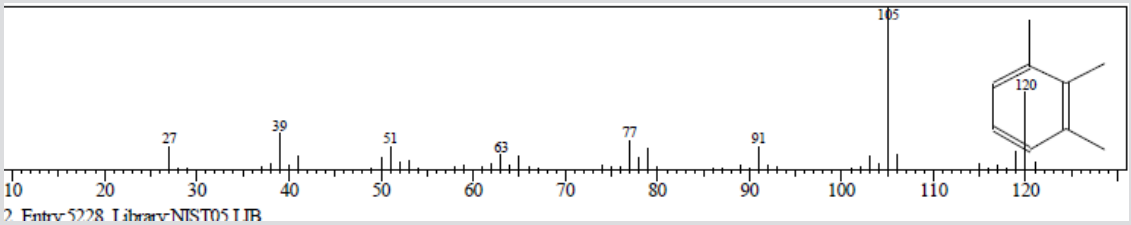

Plate 4: 1,2,3-trimethyl benzene

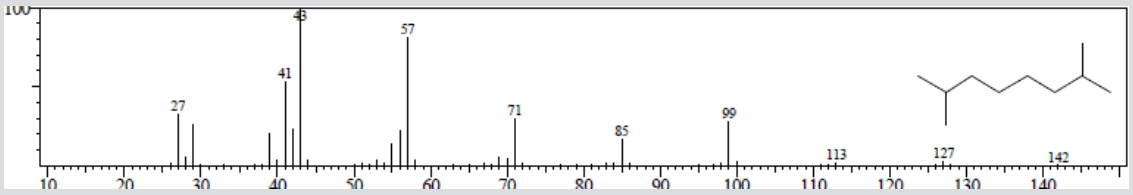

Plate 5: 2,7-Dimethyloctane

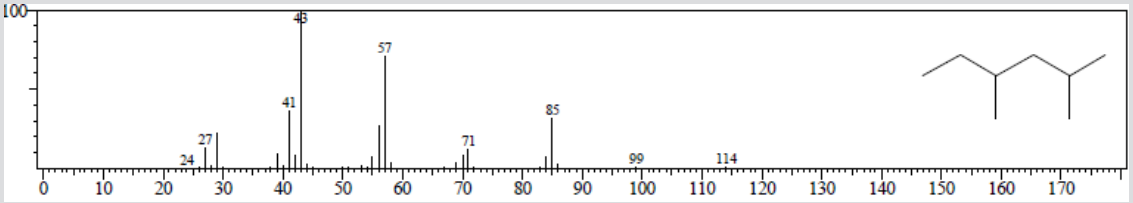

Plate 6: 2, 4 dimethyl hexane

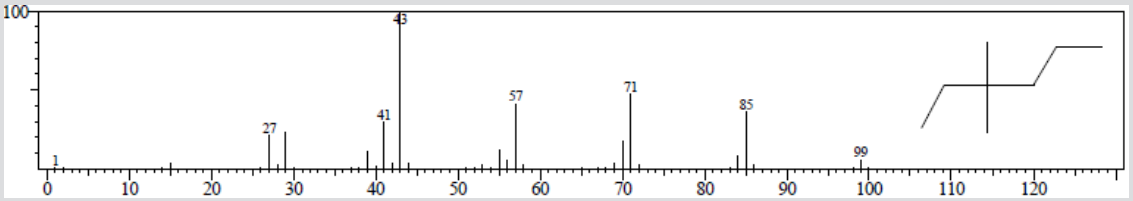

Plate 7: 3,3-Dimethylhexane

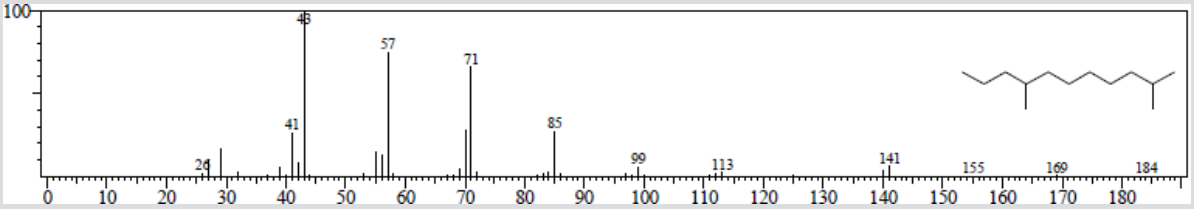

Plate 8: 2,8-Dimethylundecane

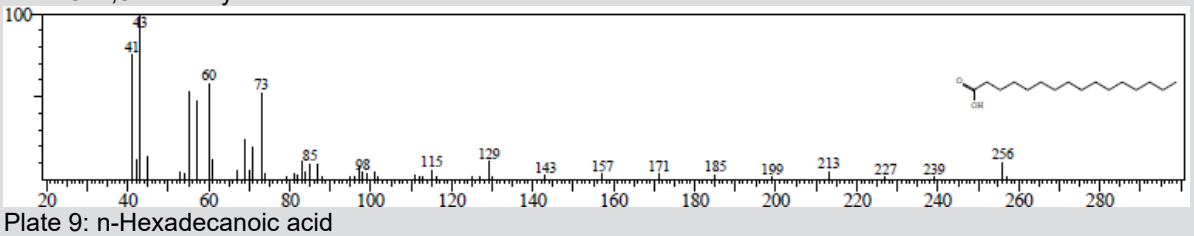

Plate 9: n-Hexadecanoic acid

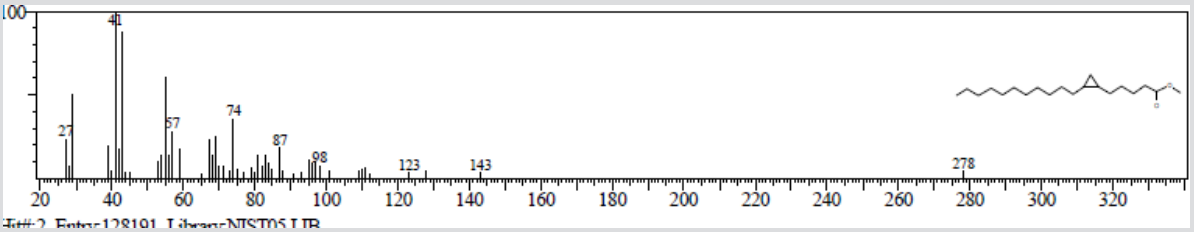

Plate 10: Cyclopropanepentanoic acid

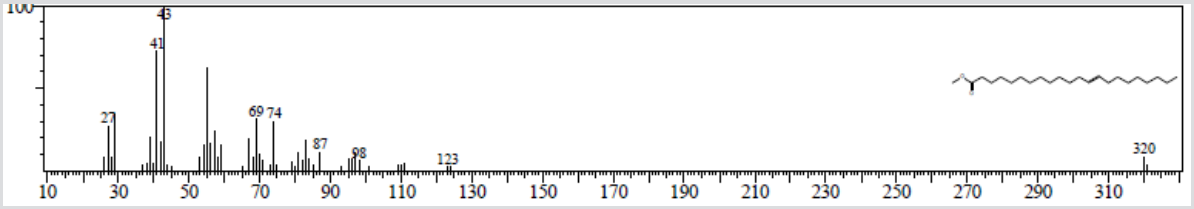

Plate 11: 13-Docosenoic acid

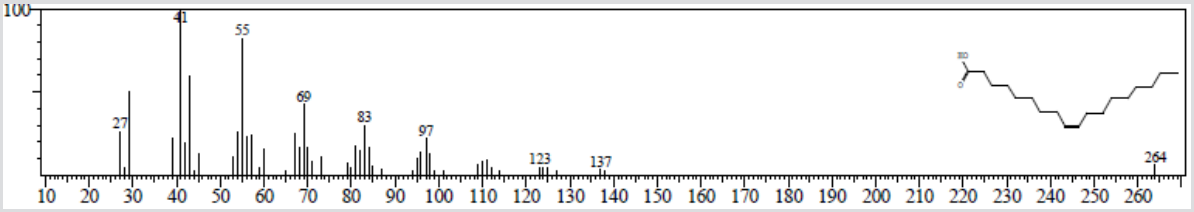

Plate 12: Oleic Acid

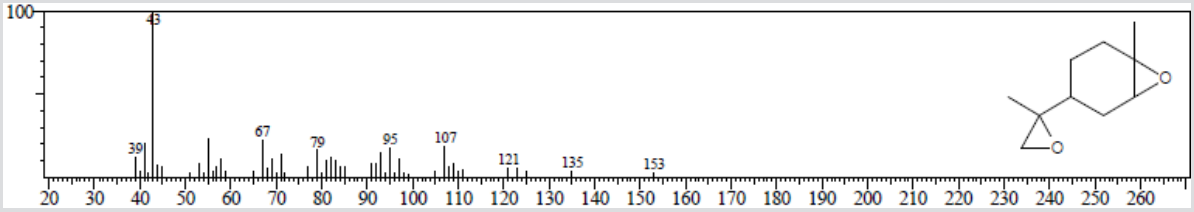

Plate 13: 7-Oxabicyclo[4.1.0]heptane

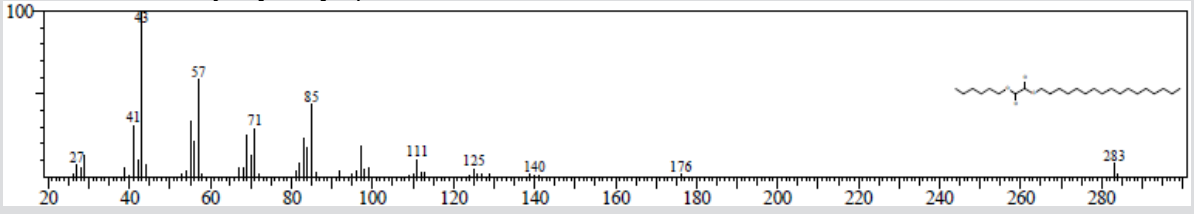

Plate 14: Oxalic acid

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