Bioactive components in ethanol extract of *Citrullus lanatus* rind using Gas Chromatography- Mass Spectroscopy.

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ABSTRACT

The knowledge of bioactive compounds present in a plant can be helpful in utilizing it for a medicinal purpose in life. The Gas Chromatography-Mass Spectrometry (GCMS) analysis of ethanol extract of Citrullus lanatus rind (CLR) was done to identify bioactive compounds and selectivity or abundance percentage in ethanol extract of CLR. The extract was obtained by fractionating crude ethanol CLR extract in order of increasing polarity, n-Hexane, chloroform, ethylacetate and ethanol. The GC-MS analysis was carried out on GC system comprising Gas Chromatograph interfaced to Mass Spectrometer (GC-MS) instrument. The bioactive compounds obtained were compared with database of spectrum of known components stored in the gas chromatography-mass spectrometry library. The GC-MS analysis of ethanol extract of CLR revealed the presence of androst-4-en-9-thiocyanomethyl-11-ol-3,17-dione a steroid which is a precusor for androgen and estrogen production. As well as cyclooctasiloxane hexadecamethyl and octasiloxane hexadecamethyl in which both of them contain silicon an essential trace element for collagen and bone formation .The presence of these compounds validates the use of ethanol extract of CLR as a good antioxidants, antibacterial agents and stimulant in precursor of male reproductive hormone production. The ethanol extract of CLR can be of great value in ethno-veterinary practice.

Keywords: Bioactive, Ethanol, *Citrullus lanatus* rind, GC-MS

INTRODUCTION

Plants are now occupying significant positions in herbal medicine, homoeopathy, allopathic medicine and aromatherapy[1]. Many of these indigenous medicinal plants are used as spices and food plants they are also sometimes added to foods meant for curing various diseases for medicinal purposes due to the prescence of various bioactive compounds found in there phytochemical composition[2]. Many plants are cheaper and more accessible to most people especially in developing countries than orthodox medicine, and there is lower incidence of adverse effects after use[2].The awareness of medicinal plants usage is a result of the many years of struggles against illnesses due to which man learned to pursue drugs in barks, seeds, fruit bodies, leaves and other parts of the plants[3]. The use of medicinal plants had been established to be the most useful in the treatment of diseases and had provided important source of materials to pharmaceuticals industries worldwide through the knowledge of their bioactive components[4]. The phytochemicals found in medicinal plants example of which are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides are primarily make up of bioactive compounds [5]These compounds vary widely in chemical structures, functions and are grouped accordingly. Some examples of these bioactive compounds are carotenoids, flavonoids, carnitine, choline, coenzyme Q, carbohydrates, esters, dithiolthiones, phytosterols, phytoestrogens, glucosinolates, polyphenols, taurine also including vitamins and minerals while they stimulate there pharmacological effects. Most of the bioactive compounds have antioxidant, anti-inflammatory, antimicrobial and anti-carcinogenic properties[6]. Thus, they can exhibit there hepatoprotective, protective effects on cardiovascular diseases and treatment of different diseases. The use of spectrophotometric analysis had been explored for the quantitative analysis of phytochemicals found in plants[7] while Gas Chromatography-Mass Spectrometer (GC-MS) had been used to confirm the bioactive compounds found in plants[8]. Also, Fourier Transform Infrared (FTIR) spectroscopy analysis was used to analyze the bioactive components of Watermelon rind aqueous extract[4].

Watermelon, also known as *Citrullus lanatus* is a warm season crop from the cucurbit family[9]. Watermelon biomass can be categorized as three main components which are the flesh, seed, and rind. Watermelon constitutes approximately 68% flesh, the rind 30%, and the seeds 2% of the total weight[10]. *Citrullus lanatus* has nutritive values which are beneficial to human health. The plant has pharmacological activities and therapeutic potentials such as antibacterial, antifungal, antimicrobial, antiulcer, antioxidant, anti-inflammatory, gastro-protective, analgesic, laxative, hepato-protective, against prosthetic hyperplasia and atherosclerosis[11]. The

Citrullus lanatus rind seems to be receiving much attention recently, maybe due to efforts to reduce the environmental toxicants that it may cause since it is regularly discarded when the *Citrullus lanatus* fruit is consumed. It was noted that Kumar *et al.,*[8] considered the use of GC-MS to study the sugars found in aqueous extract of *Citrullus lanatus* rind. While Bichi et al.,[4] explored the use of GC-MS and FITR to study the bioactive components found in aqueous extract of CLR, but this is in exhaustive because the aqueous extract of CLR cannot be stored or preserved for a long period of time. With the great awareness on the phytochemicals and pharmaceutical importance of CLR recently this will create needs for the CLR stored or preserved for a long period of time by preparing the ethanol extract of CLR. All through extraction, the choice of solvent is preferable to select two solvents with low polarity (n-hexane, chloroform) containing bioactive compounds in highly organic medium, two with medium polarity (dichloromethane, ethanol) containing bioactive compounds in both organic and inorganic media, and one with the highest polarity (water).[12] Meanwhile ethanol had been shown to be used as a preservative because it is effective in knocking out organisms that could pose danger to consumers[13].

This study now considered the use of GC-MS to analyze the bioactive components of ethanol extract of CLR and compared with the study on the aqueous extract of CLR that was done by Bichi *et.al.,*[4] . This will provide more information on active pharmaceutical ingredients (API) present in CLR when it is stored or preserved in ethanol solvent for long time before consumption by animal or use in livestock since ethanol is a good preservative solvent.

MATERIALS AND METHODS

Plant Specimen

The *Citrullus lanatus* fruit was collected in Abeokuta, Ogun state Nigeria. The rind was carefully removed from the fruit using a sharp knife and cut into small pieces. It was identified and authenticated in Botany department University of Ibadan Oyo state and voucher number UIH 22872 was assigned. The *Citrullus lanatus* rinds were chopped into small pieces and air-dried under room temperature. This was done until it attains a constant dry weight. The dried rind was grinded to powdery form using a grinder (Euro premium blender^R Ultima made in China). The powdered CLR was soaked/macerated in 95% ethanol for 72 hours. The mixture was filtered twice using muslin bag. The obtained filterate was concentrated using a rotary evaporator (Heidolph laboaratory efficient model 517-01002-002 Germany) at 40^0C[14].

Fractionation process of crude ethanol extracts of CLR.

The crude ethanol CLR extract was fractionated using the following solvents (n-hexane, chloroform, ethylacetae, ethanol and water) according to their polarity index[12].The fractionation was initiated by pre-absorption process which was done by mixing silica gel to the crude ethanol CLR extract and air-dried overnight. The silica gel was poured into Vacuum liquid chromatograph (VLC) chamber or distillation tube under pressure using pump to closely packed the silica gel very well. A whatman filter paper was placed in between while n-hexane with lowest polarity index was added. This brought out a colored filtrate initially but later became colorless as the volume of the n– hexane increases. It was later stopped after clear n-hexane filtrate which is colorless was obtained. This gave the n-hexane filtrate labelled CLRH, the procedure continued with chloroform based on polarity index[12]. This procedure continued with chloroform, ethyl acetate followed by ethanol to obtain ethanol fraction of CLR. These procedures were carried out at room temperature. Each of the filtrates was concentrated with rotary vacuum evaporator at 40^0C[13]. The resulting extracts were weighed and labelled as CLRH, CLRC, CLREA and CLREF for n-hexane, chloroform, ethyl acetate and ethanol fractions respectively.

Identification of bioactive components in ethanol extract of CLR using GC-MS.

The ethanol extract of CLR was sent to Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology Kumasi Ghana for gas chromatography-mass spectrometer (GC-MS).

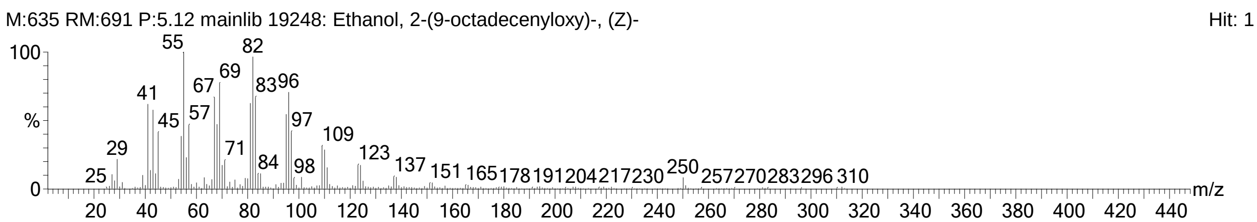
Gas Chromatography Mass Spectrometer Procedure.

The initial temperature was 80°C for 0 minutes, at ramp 10°C/min to 250°C the hold period was minutes and the ramp rate of 5^0c/min to 280°C. Later the hold time was 15 minutes injected at 250°C the volume was 0µl split at 10-1 the carrier gas was helium while the solvent delay was 4min and transfer temperature was 280°C the source temperature was 220°C scanned at 50 to 450Da and the column was maintained at 30.0m by 250µm. The spectra of different compounds found in ethanol CLR extract were obtained through GC-MS analysis. The spectra were nomenclated according to their respective peak spectrum with mass, M; relative mass RM, while selectivity % was calculated as M/RM X 100 %. The selectivity % indicates the quantity, availability, or amount of a specific compound found in the ethanol CLR extract (CLRCE). But the classification, molecular formula, molecular weight, and biological uses of each compound were sourced in Pubcem[15].

RESULTS AND DISCUSSION

GC-MS analysis on ethanol extract of CLR revealed the following bioactive compounds with the following different mass spectra showing different peaks.

Number 1



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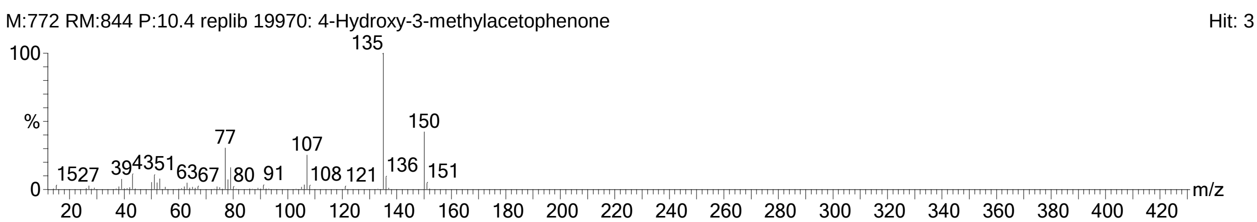

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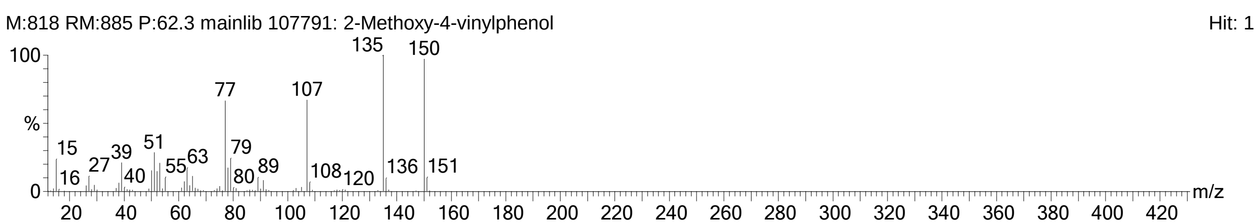
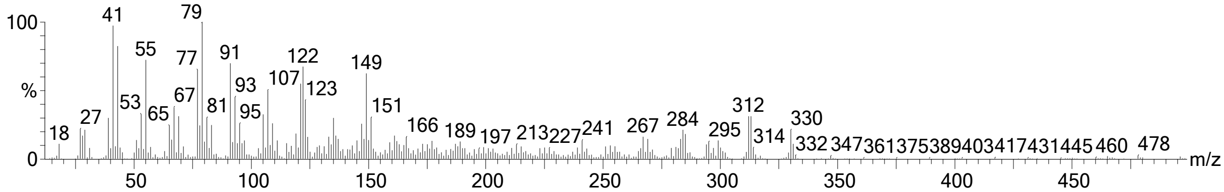


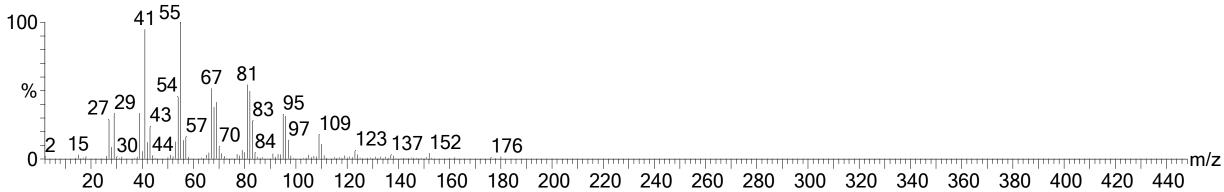
Number 4

M:511 RM:511 P:24.7 mainlib 44123: 2,4,6-Decatrienoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-t... Hit: 1



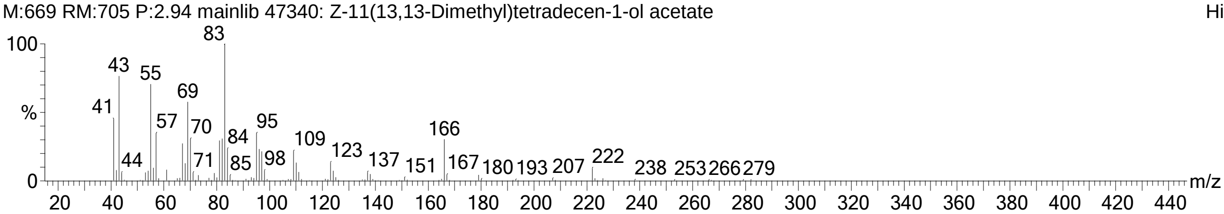
Number 5

M:629 RM:772 P:3.99 mainlib 18131: 1,12-Tridecadiene Hit: 3



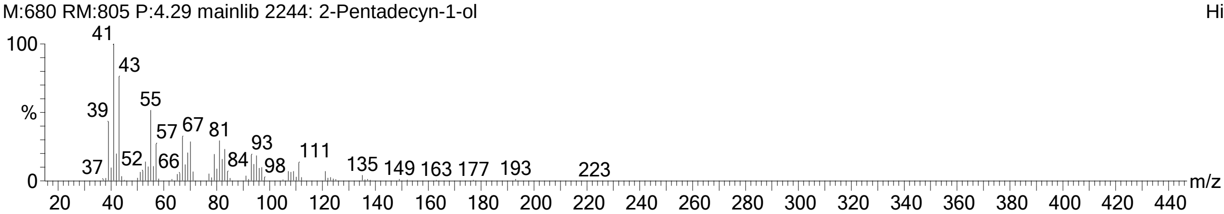
Number 6

M:669 RM:705 P:2.94 mainlib 47340: Z-11(13,13-Dimethyl)tetradecen-1-ol acetate Hit: 3



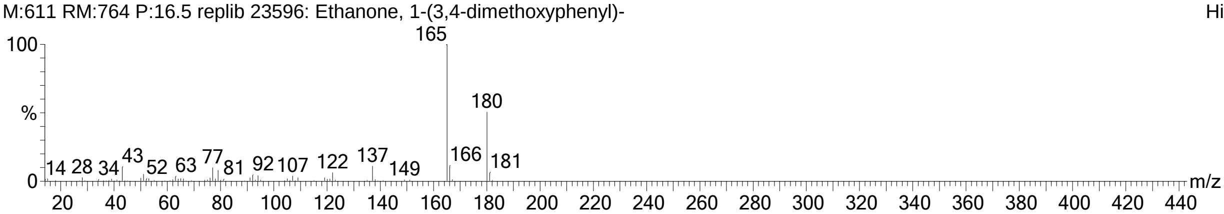
Number 7

M:680 RM:805 P:4.29 mainlib 2244: 2-Pentadecyn-1-ol Hit: 1



Number 8

M:611 RM:764 P:16.5 replib 23596: Ethanone, 1-(3,4-dimethoxyphenyl)- Hit: 1



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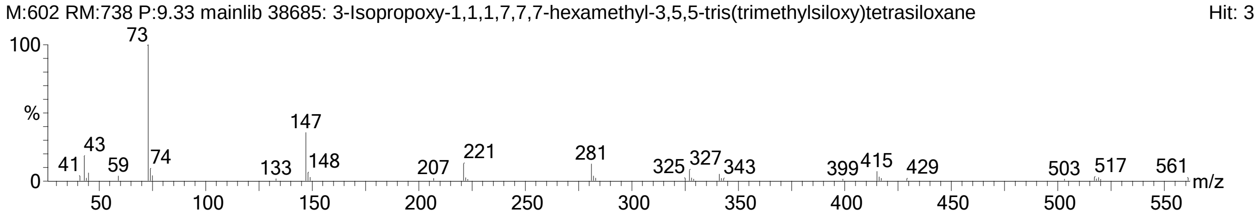

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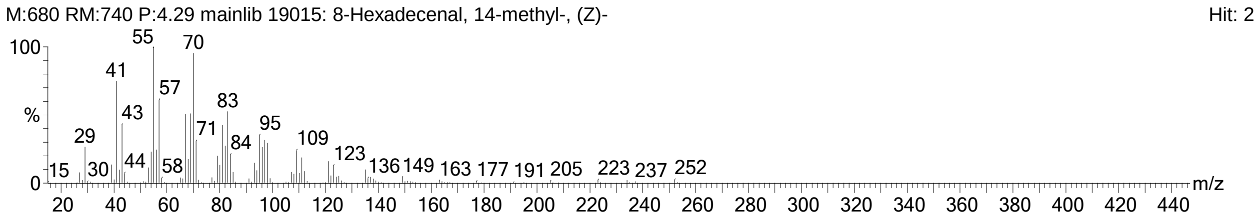

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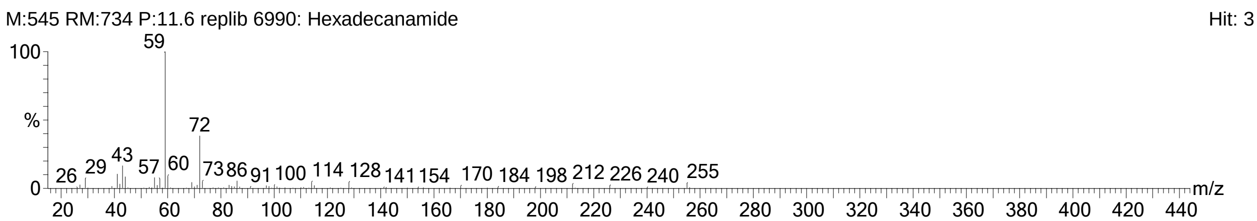

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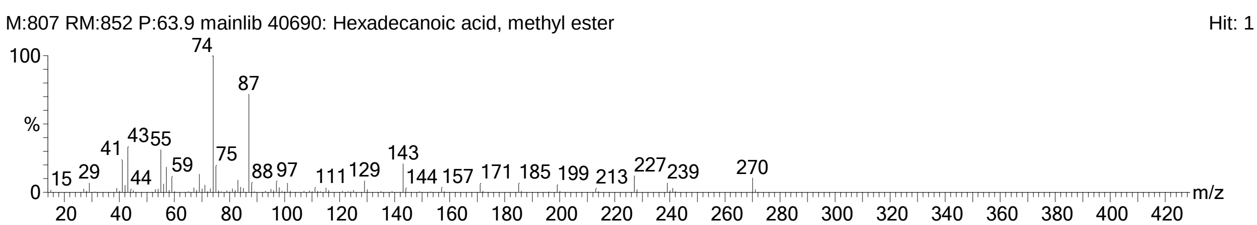

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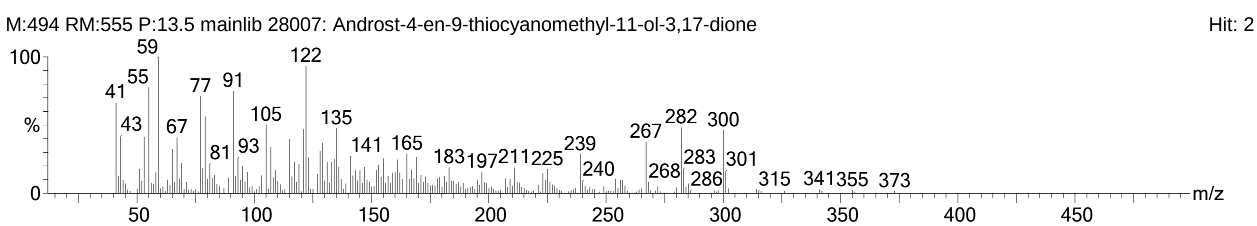

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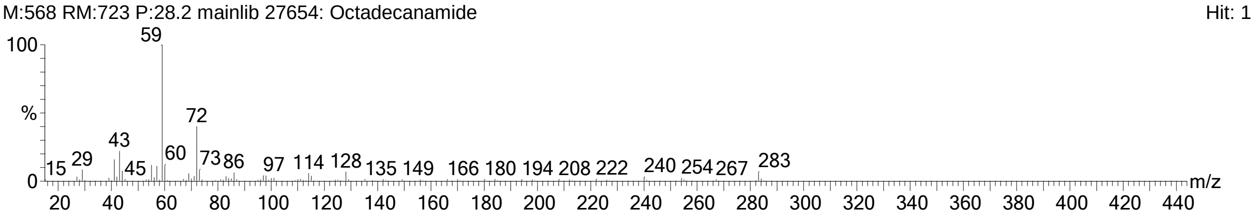

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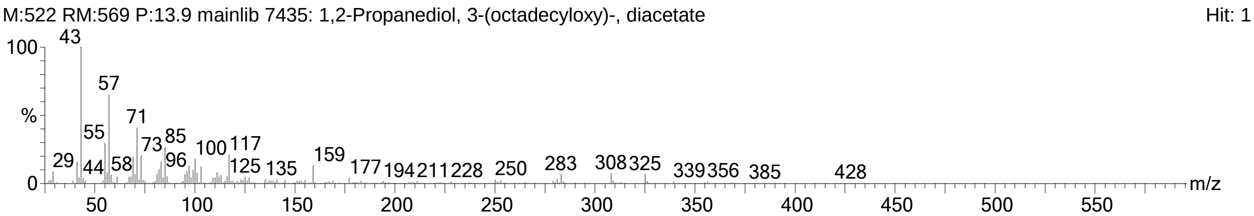

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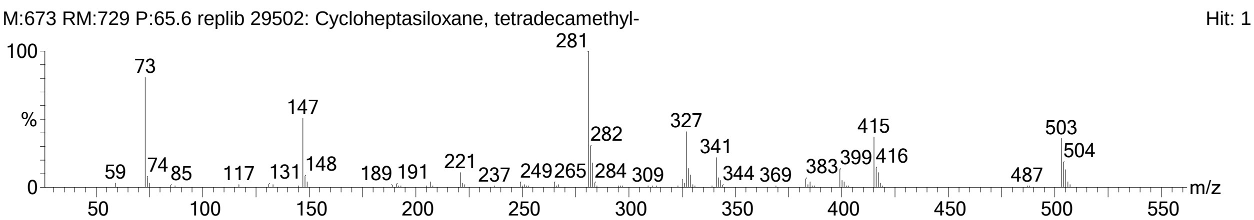

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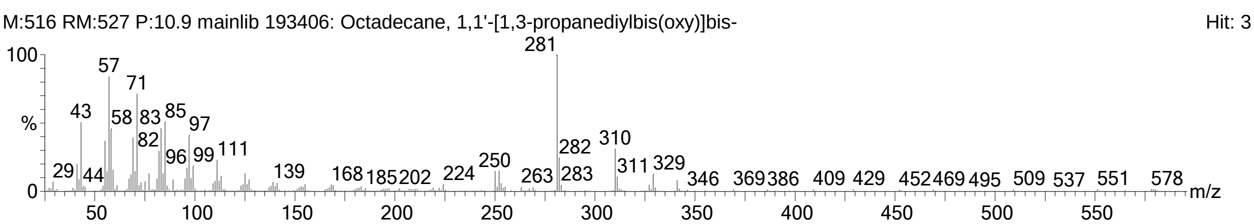

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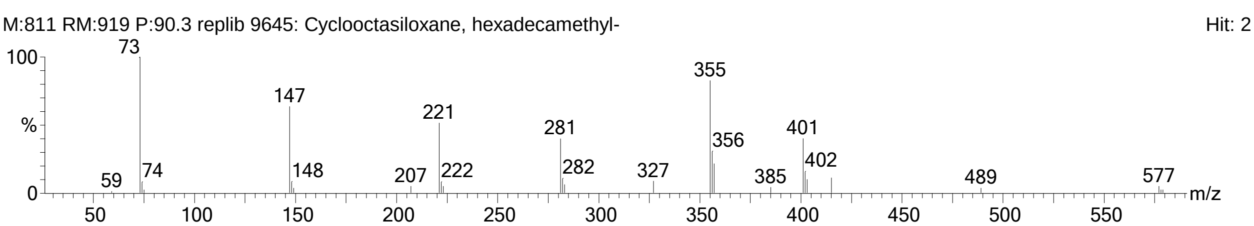

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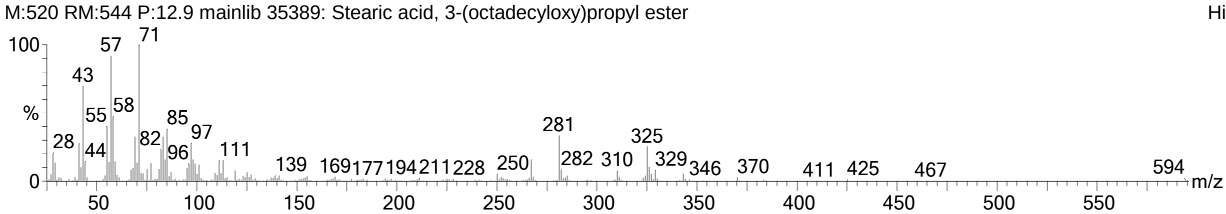

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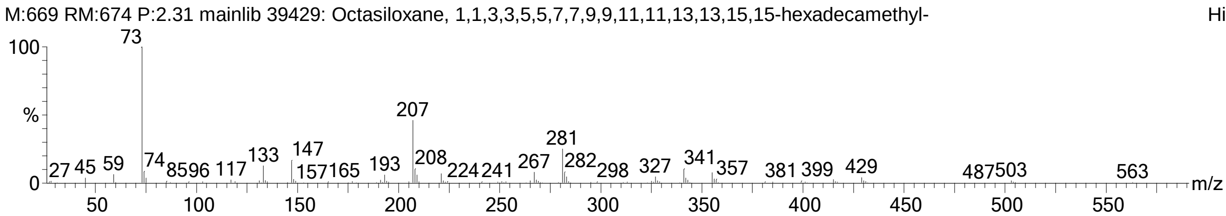

The serial number in the table below follows same trend as seen in the numbering of spectra shown above.

Table 1: Different bioactive compounds found in ethanol *Citrullus lanatus* rind extract using Gas Chromatography-Mass Spectrometry analysis showing there chemical classifications and some of their uses.

S/NO	M	RM	Selectivity %	Compound name	Structure	Molecular weight	Classification	Natural Uses
1	631	690	91.44 92.8	Ethanol,-2-(9,12-Octadecadienyloxy)-	$C_{20}H_{42}O_2$	314	Alcohol	Useful in medicine as cough syrups, tonic as a good solvent, color additives, enchanced flavour, cosmetics and beauty lotions.
2	7	8	91.82	4-Hdroxy-2-	$C_9H_{10}O_2$	150.2	Ketone	Antimicrobial

	86	56	243	methyacetophenone.				and antioxidant effects
3	818	885	92.42938	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.2	Carbohydrate	Calorific or Energy
4	511	511	100	Decatrienoic acid,1a,2,5,5a,6,9,10,10a Octahydro-5,5a-dihydroxy-4-(hydroxymethyl 1,1,7,9.	$C_{10}H_{14}O_2$	166.2	Carboxylic acids	Antioxidants
5	629	772	81.47668	1,12-Tridecadiene	$CH_3(CH_2)_{11}CH_3$	184.4	Alkene	Useful in research laboratory as distillation chaser, it can cause skin irritation.
6	669	705	94.89362	11(13,13-Dimethyltetradecen-1-ol acetate	$C_{12}H_{24}O_2$	200.3	Ketone	
7	680	805	84.47205	2-Pentadecyn-1-ol	$C_{15}H_{32}O$	228.4	Ketone	Antibacterial effects against E.Coli/Antidiarrheal against Clostridium butyricum.
8	611	764	79.97382	Ethanone,-1-(3,4-dimethoxyphenyl	$C_{16}H_{26}O$	234.4	Ketone	Odor agent/Unmask unpleasent

								odor
9	602	738	81.57182	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy(tetrasiloxane	$C_{18}H_{52}O_7Si_7$	577.2	Organosilicon	Collagen formation. Pubcem[15].
10	680	740	91.89189	8-Hexadecanal, 4-methyl	$C_{16}H_{32}O$	240.4	Aldehyde	Flavouring agent of Food additives. Action on cellular location at endoplasmic reticulum,extra cellular and membrane
11	545	734	74.25068	Hexadecanamide	$C_{16}H_{33}NO$	255.4	Fatty amide	Found in aging mouse brain. Also use in skin conditioning[15]
12	747	850	87.88235	Hexadecanoic acid ,methyl ester.	$C_{16}H_{32}O_2$	256.4	Palmitic acid/Organic acid	Therapeutic effect on multiple meloma cells, no effects on normal peripheral blood mononuclear cells (PBMN).

13	494	555	89.00901	Androst-4-en-9-thiocyanomethyl-11-ol3,17-dione.	$C_{21}H_{27}NO_3S$	373.5	Steroid	Metabolite in androgen and estrogen production[15]
14	549	622	88.26367	9-Octadecenamide	$C_{18}H_{35}NO$	281.5	Amide or Stearylamide	Useful in food additives, Hypnotics and sedatives[15]
15	522	569	91.73989	1,2-Propanediol ,3-(Octadecycloxy)-dio-acetate	$C_{25}H_{48}O_5$	428.6	Ester	Therapeutic methods and pharmaceutical compositions for treating warts with tellurium compounds[15]
16	673	729	92.31824	Cycloheptasiloxane Tetradecamethyl	$C_{14}H_{42}O_7Si_7$	519.6	Alkane	
17	516	527	97.91271	Octadecane1,1,-(1,3-propanediybis(oxy)bis.	$C_{39}H_{80}O_2$	581	Lipid	Nucleic acid transfer carrier, compound for manufacturing nucleic acid
18	811	919	88.2481	Cyclooctasiloxane,hexadecamethyl	$C_{16}H_{48}O_8Si_8$	593.2	Organosiloxane and Macrocycle.	Collagen formation
19	520	544	95.58824	Steric acid,3-(Octadecycloxy)propyl ester	$C_{39}H_{78}O_3$	595	Ester	

20	669	674	99.25816	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,13,13,15-hexadecamethyl	$C_{16}H_{50}O_7Si_8$	607.3	Heterocyclic Organic Compound	Natural active pharmaceuical ingredients for oxidative acivities

Discussion

This study revealed that Octahydro-5,5a-dihydroxy-4-(hydroxymethyl decatrienoic acid has the highest selectivity % of 100% which is a carboxylic acid with molecular formular $C_{10}H_{14}O_2$ and molecular mass of 166 followed by hexadecamethyloctasiloxane has the next selectivity % of 99.25% which is an heterocyclic acid with the with molecular formular $C_{18}H_{54}O_7Si_8$ molecular mass of 607. It was noted that these two bioactive compounds are good natural active pharmaceutical ingredients as antioxidants. This suggests that ethanol CLR will possibly be a good antioxidant agent. The least bioactive compounds found in the ethanol extract of CLR were 1-(3,4-dimethoxyphenylethanone a ketone with a selectivity % of 79.9% while Hexadecanamide a fatty amide with selectivity % of 74.3%, both have anticancer effects. This indicates that ethanol CLR has a slight effect against cancer growth and diseases.

It was noted in this study that the following bioactive compounds and groups ethanol-2-(9,12-octadecadienyloxy)-**;** ethanone-1-(3,4-dimethoxyphenyl; 8-hexadecanal 4-methyl; methyl ester hexadecanoic acid ; hexadecamethyl octasiloxane in alcohol, carbonyl, aldehyde, palmitic acid, heterocyclic organic acid respectively were seen in this study and earlier study[4] This shows that ethanol extract of CLR possess good antioxidant and antimicrobial potentials. In addition to all other bioactive compounds found in ethanol CLR seen in Table 1 in this study and in aqueous CLR[4]. The ethanol CLR extract it is also found to contain androst-4-en-9-thiocyanomethyl-11-ol-3,17-dione which is a precursor for androgen and estrogen production male animals. As well as cyclooctasiloxane hexadecamethyl and Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,13,13, 15- hexadecamethyl in which both contain silicon an essential trace element for collagen and bone formation[15] .

CONCLUSION

Conclusively, in this study it can be established that the ethanol extract of CLR will better preserve CLR for livestock consumption as well as improving the bioactive compounds to make it suitable for male reproductive hormone production, collagen and bone formation. It can also be useful in ethnoveterinary practice.

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