Section: Research Paper

Analysis and characterization of molecular components of *Aloe vera* using GCMS

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Abstract:

In the present study, we focused on one of the most expressive herbal plants, *Aloe vera,* which is unique for applications in general use and in treating various diseases. The evaluation of phytochemical components was based on molecular formula, molecular weight, retention time, and peak area. The obtained peaks were compared with the NIST library. The major phytochemical components revealed by GC-MS analysis of *Aloe vera* in the present study are 9-Octadecenoic acid, methyl ester (also known as Oleic Acid) (36.80%), Propanoic acid (8.16%), Ammonium acetate (7.20%), and 2, 3-Btanediol [S-(R^*,R^*)]- (4.19%); 6-Octadecenoic acid, methyl ester, (Z)- (3.39%); Phthalic acid, decyl isobutyl ester (3.30%); Methyl 9-cis,11-trans-octadecadienoate (2.89%); Heptadecanoic acid, 16-methyl-, methyl ester (2.63%); Hexadecanoic acid, methyl ester (2.21%); Ethanol,2,2'-oxybis- (1.97%); Acetic acid, methyl ester (1.86%); 2(5H)-Furanone, -methyl (1.62%); Glycerin (1.61%); Octanoic acid (n-Caprylic acid) (1.34%); Ethyl iso-allocholate (1.16%); 1,25-Dihydroxyvitamin D3, TMS derivative (0.67%); 9,12,15-Octadecatrienoic acid, 2,3-dihydroxy propyl ester, (Z, Z, Z)- (0.53%); Octaethylene glycol monododecyl ether (0.30%); Pterin-6-

carboxylic acid (0.23%); Methyl 3-(acetyloxy)-20-hydroxyurs-12-en-28-oate (0.15%); Tetraacetyl-d-xylonic nitrile (0.05%) and Nitro-L-arginine (0.04%). The presence of the identified components suggests the anti-bacterial, anti-fungal, anti-microbial, anti-cancer, anti-tumor, anti-diabetic activities, anti-oxidant, analgesic, anti-inflammatory, wound healing potential, reproduction booster, and neuroprotective potential of the *Aloe vera* leaf gel.
Keywords: *Aloe vera*, phytochemical compounds, anti-oxidant, neuroprotective, GC-MS.

INTRODUCTION:

Around the world, there have been great ancient histories of preferred herbal medicine to cure many diseases (Brantner & Grein, 1994). Our ancestors credibly experimented by testing the whole plant, leaves or only roots, and the whole plant extract, but the modern era endorsed individual chemical entities (Habtemariam, 2017). *Aloe vera,* one of the most promising herbal medicinal plants, has been used in wound healing and cosmetic products since ancient times. This therapeutic plant species belongs to the genus Aloe, where "*aloe*" is the ancient Arabic name of the plant and "*vera,"* which indicates authenticity and genuineness (Reynolds, 2004). *Aloe barbadensis Miller,* the botanical name for Aloe vera, is a kind of green shrub and a member of the Asphodelaceae (Liliaceae) family. *Aloe vera* radiance has excellent potential and impact over the past 2000 years in health, beauty, medicine, and skincare properties. The Egyptians named Aloe the "plant of immortality." One of the oldest plants is aloe vera which has medicinal properties that include anti-cancer (Unlu *et al.*, 2016), anti-bacterial (Chen *et al.*, 2004), anti-microbial (Cock, 2008), antiviral (Li *et al.*, 2014), anti-inflammatory, antineoplastic (Chen *et al.*, 2014), etc. There are some antagonistic approaches to *Aloe vera's* medicinal properties, but the deep knowledge of the active compounds present in the *Aloe vera* plant makes it unique. The *Aloe vera* leaf can be divided into three parts: *Aloe vera* latex, *Aloe vera* leaf gel, and green rind or plant cuticle. Many theories, evidence, and studies exist about Aloe vera and its pharmacological activity. *Aloe vera* has many valuable, researchable, and interesting compounds, making it more interesting with medicinal properties. Interestingly, based on its GC-MS analysis, *Aloe vera* has some compounds that directly work on anti-cancer, anti-bacterial, and neurological disorders. Besides all these qualities, *Aloe vera* also contains some biological (metabolic) pathway intermediates that activate or control metabolic activities. All the compounds like enzymes, amino acids, vitamins, minerals, proteins, and organic

acids, have shown their therapeutic potential during in-vivo studies. *Aloe vera* contains major components like anthraquinones (aloe-emodin, aloetic acid, aloin, ethanol, ester of cinnamic acid), saccharides (cellulose, glucose, mannose, acetylated mannan), vitamins (B1, B2, B6, C, β-carotene, choline, folic acid, α-tocopherol), enzymes (amylase, oxidase, lipase, cyclooxydase, carboxypeptidase), low-molecular-weight substances (cholesterol, steroids, triglycerides, lignins, salicylic acid), etc. (Choi & Chung, 2003). Besides these biological components, *Aloe vera* contains natural anti-oxidant like SOD and catalase (Suzuki *et al.*, 1979; Sabeh et al., 1996). The present study discusses the biological activities of functional components isolated from *Aloe vera* gel and their relative significance in medicinal and therapeutic uses.

MATERIALS AND METHOD

Preparation of plant extract:

Fresh leaves of *Aloe barbadensis Miller* (*Aloe vera*) are commonly collected from a nearby Rohtak, Haryana, India, garden. The leaf surfaces were extensively washed with ethanol to eliminate all signs of soil, grime, and other debris before being de-skinned with a surgical blade sterilized in ethanol. Crude pulp material was extracted in pure form by applying pressure (in a sterilized pestle and mortar) to the clear pulp. The isolated material was spun on a tabletop centrifuge for 10 minutes at 3750 rpm to get its semi-solid state. Thereafter, it was filtered with Whatman filter paper. The extracted *Aloe vera* pulp was stored at -20 °C in a deep freezer until use.

Gas Chromatography-Mass Spectroscopy:

Aloe vera gel was analyzed by GC-MS using a thermoscientific TSQ column quantum XLS with a DBJ-MS capillary column that was 30 mm ×0.25 mm ×0.25 m in size. Helium (99.9%) was utilized as the cryogenic gas, and 1 mL of a transparent liquid solution was kept in autoinjection in the sample vial before being injected into the GC column with a syringe set to split mode 10:1. The ion source temperature was 280 °C at the time of injection. The system temperature was kept at 250 °C. The column temperature was first programmed to be 110 °C for 2 minutes, then increased by 10 °C/min to 200 °C and 5 °C/min to 280 °C. 17.09 minutes was the whole GC-MS run time. Compounds' spectra were compared to a NIST reference (National Institute of Standards and Technology). Figure 36 depicts an image of a gas chromatography-mass spectroscopy device.

Identification of Components:

The NIST database, which contains more than 62,000 patterns, was used to analyze the mass spectrum from the GC-MS. The NIST collection contained the mass spectra of the components that were known to exist. Standard interpretation approaches were used to ascertain the components of the test materials' names, molecular weights, and structures. The NIST collection contained the mass spectra of the known components. The components of the test materials' names, molecular weights, and structures were ascertained using conventional interpretation approaches.

RESULTS AND DISCUSSION

The utilization of GC-MS was practical and valuable for identifying the bioactive compounds in *Aloe vera*. According to the results, various bioactive phytochemical compounds were identified in the GC-MS analysis of *Aloe vera* gel (**Figure 1**). Retention time (RT), molecular formula, molecular weight (MW), percentage composition, and CAS number are used to identify phytochemical substances (Table 1). The main compounds include 9-Octadecenoic acid, methyl ester (36.80); Propanoic acid (8.16); 6-Octadecenoic acid, methyl ester, (Z)- (3.39); Phthalic acid, decyl isobutyl ester (3.30); Methyl 9-cis,11-trans-octadecadienoate (2.89); Heptadecanoic acid, 16-methyl-, methyl ester (2.63); Hexadecanoic acid, methyl ester (2.21); 2-Propanone, 1-hydroxy- (1.86); Glycerin (1.61); Pterin-6-carboxylic acid (0.77); 1,25-Dihydroxy vitamin D3, TMS derivative (0.67); 9,12,15-Octadecatrienoic acid, 2,3-dihydroxy propyl ester, (Z, Z, Z)- (0.53); Nitro-L-arginine (0.04).

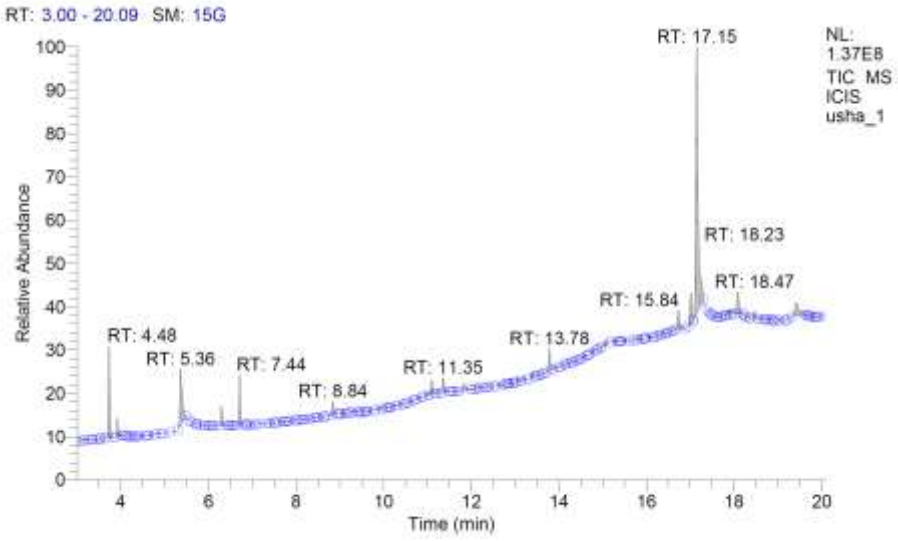

Figure 1: GC-MS Chromatogram of Aloe vera gel

Table 1: Various phytocompounds identified by GC-MC in the ethanolic extract of Aloe vera gel.

Sr. No.	RT	Name of Compound	Molecular Formula	MW	Area (%)	CAS# No.
1	3.73	Propanoic acid	$C_6H_{12}O_3$	132	8.16	63697-00-7
2	3.92	Acetic acid, methyl ester	$C_3H_6O_2$	74	1.86	79-20-9
3	4.48	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	207	0.23	948-60-7
4	5.36	Ammonium acetate	$C_2H_7NO_2$	77	7.20	631-61-8
5	6.12	Nitro-L-arginine	$C_6H_{13}N_5O_4$	219	0.04	2149-70-4
6	6.71	2,3-Butanediol, [S-(R*,R*)]-	$C_4H_{10}O_2$	90	4.19	19132-06-0
7	7.44	Methyl 3-(acetyloxy)-20-hydroxyurs-12-en-28-oate	$C_{33}H_{52}O_5$	528	0.15	14356-56-0
8	8.84	2 (5H)-Furanone, 3-methyl	$C_5H_6O_2$	98	1.62	22122-36-7
9	11.10	Glycerin	$C_3H_8O_3$	92	1.61	56-81-5
10	11.35	Ethanol, 2,2'-oxybis-	$C_4H_{10}O_3$	106	1.97	111-46-6
11	11.83	Octanoic acid (n-Caprylic acid)	$C_8H_{16}O_2$	144	1.34	124-07-2
12	13.78	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.21	112-39-0
13	15.84	Octaethylene glycol monododecyl ether	$C_{28}H_{58}O_9$	538	0.30	3055-98-9
14	16.73	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	298	2.63	5129-61-3
15	17.01	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	296	3.39	2777-58-4
16	17.15	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	36.80	112-62-9
17	17.88	1,25-Dihydroxyvitamin D3, TMS derivative	$C_{30}H_{52}O3Si$	488	0.67	55759-94-9
18	17.96	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)- [Linolenin, 1-mono-]	$C_{21}H_{36}O_4$	352	0.53	18465-99-1
19	18.08	Methyl 9-cis,11-trans-octadecadienoate	$C_{19}H_{34}O_2$	294	2.89	NA
20	18.23	Tetraacetyl-d-xylonic nitrile	$C_{14}H_{17}NO_9$	343	0.05	NA
21	18.47	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	1.16	NA
22	19.42	Phthalic acid, decyl isobutyl ester	$C_{22}H_{34}O_4$	362	3.30	NA

9-octadecenoic acid, a methyl ester observed at retention time 17.15 with a 36.80% peak area (**Figure 2**), is recommended as a saturated fatty acid having anti-bacterial, anti-oxidant, and anti-microbial effects by several researchers (Awa *et al.,* 2012; Satpute *et al.,* 2010; Sermakkani & Thangapandian, 2012; Rahman *et al.,* 2014). Propionic acid observed at retention time 3.7 with an

8.16% peak area (**Figure 3**) has been shown to have anti-oxidant, anti-cancer, analgesic, anti-bacterial, and anti-diabetic effects (Dracheva et al., 2009; Avetisyan et al., 2010; Berzosa et al., 2011).

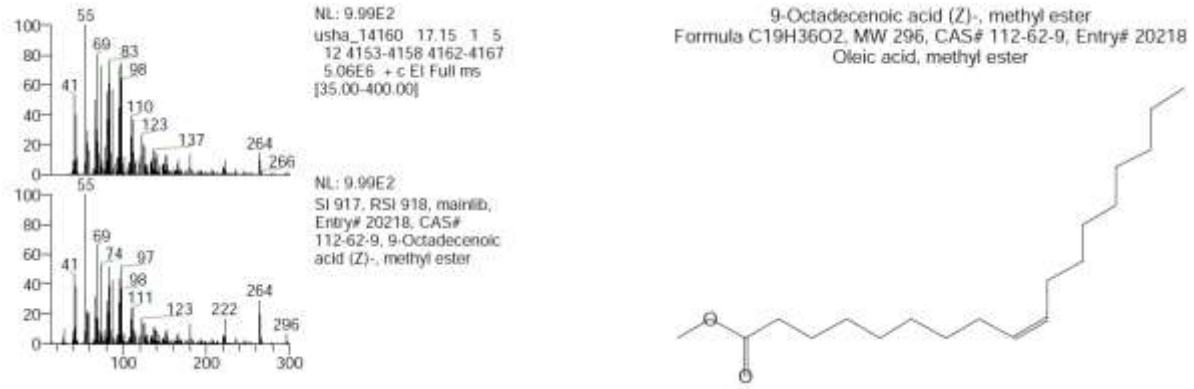

Figure 2: 9-Octadecenoic acid, methyl ester

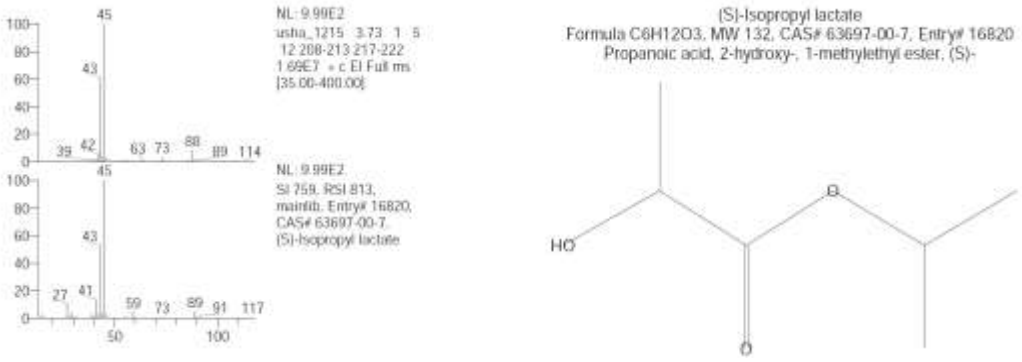

Figure 3: Propanoic acid

Ammonium acetate was observed at a retention time of 5.36 with a 7.20% peak area (**Figure 4**), and 2-butanediol [S-(R*, R*)] was observed at a retention time of 6.71 with a 4.19% peak area (**Figure 5**). Tarric acid, also known as 6-octadecanoic acid, observed at a retention time of 17.01 with a 3.39% peak area (**Figure 6**), is a fatty acid that is often studied because of the triple bond in position 6. Biological activity studies have indicated anti-fungal characteristics and the potential to inhibit hepatic fibrosis in vitro. Phthalic acid derivatives observed at retention time 19.42 with a 3.62% peak area (**Figure 7**) had anti-tumor, anti-inflammatory, and anti-bacterial characteristics. They may have been used to treat chronic cardiovascular and cerebrovascular diseases (Shengbo et al., 2015). Saranya et al. (2013) reported that phthalates have anti-bacterial and pharmacological properties. The anti-microbial properties were assumed to be caused by a phthalic acid derivative (Nakalembe

& Kabasa, 2012). The ester methyl 9-cis, 11-trans-octadecadienoate observed at retention time 18.08 with a 2.89% peak area (**Figure 8**) has anti-oxidant and anti-cancer effects (Berdeaux et al., 1998). Heptadecanoic acid, 16-methyl-, methyl ester observed at retention time 16.73 with 2.63% peak area (**Figure 9**) has been reported to have anti-oxidant properties (Ponnamma & Manjunath, 2012).

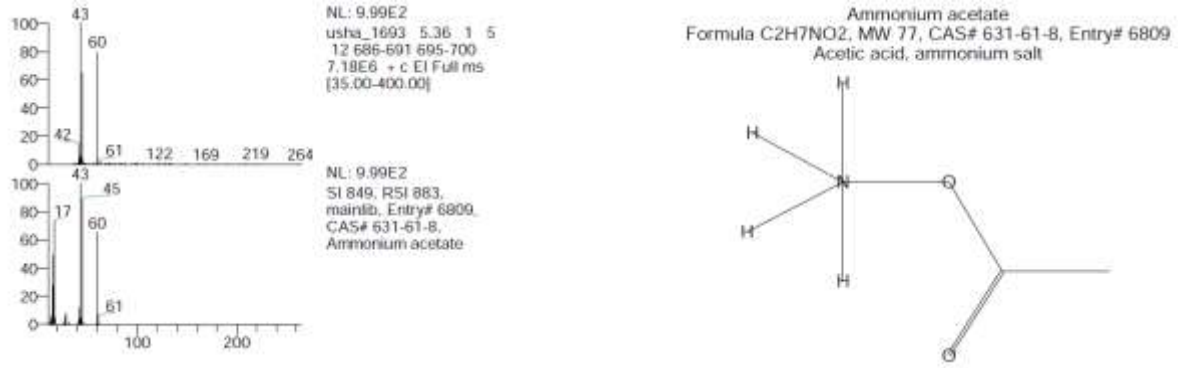

Figure 4: Ammonium acetate

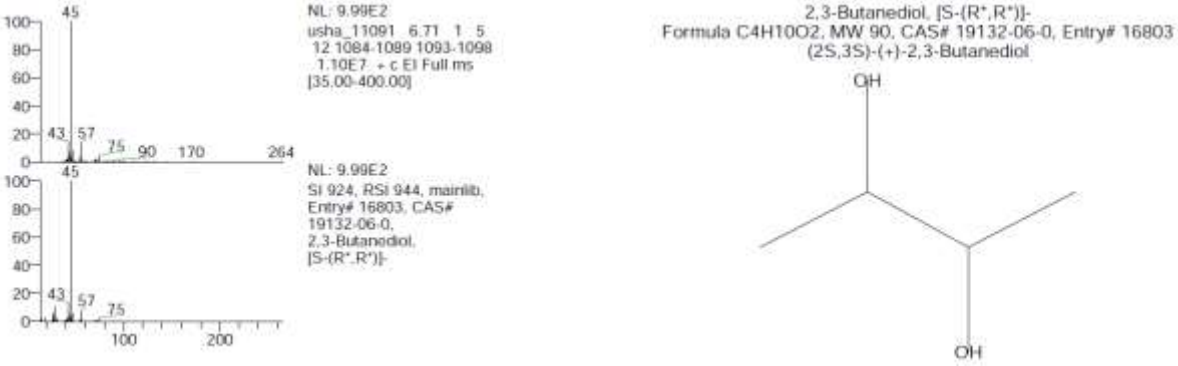

Figure 5: 2,3-Butanediol, [S-(R*,R*)]-

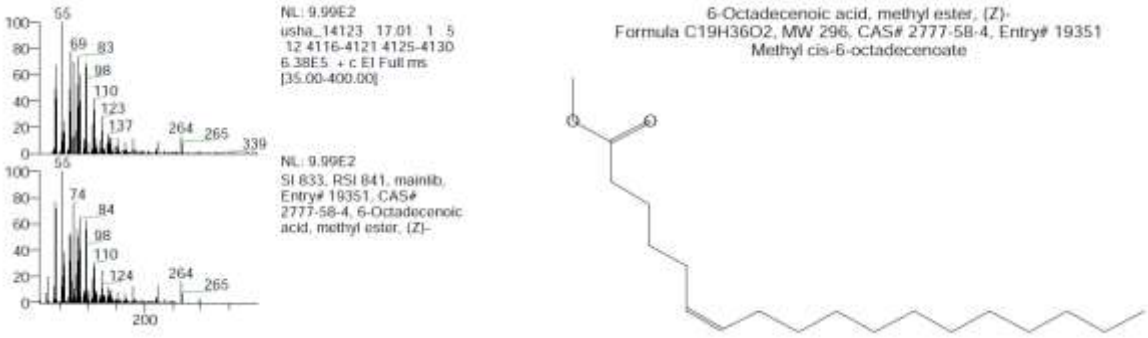

Figure 6: 6-Octadecenoic acid, methyl ester, (Z)-

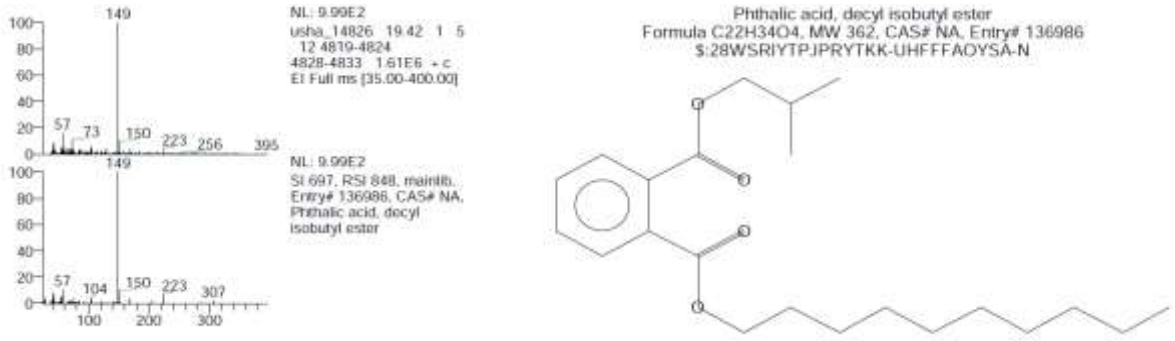

Figure 7: Phthalic acid, decyl isobutyl ester

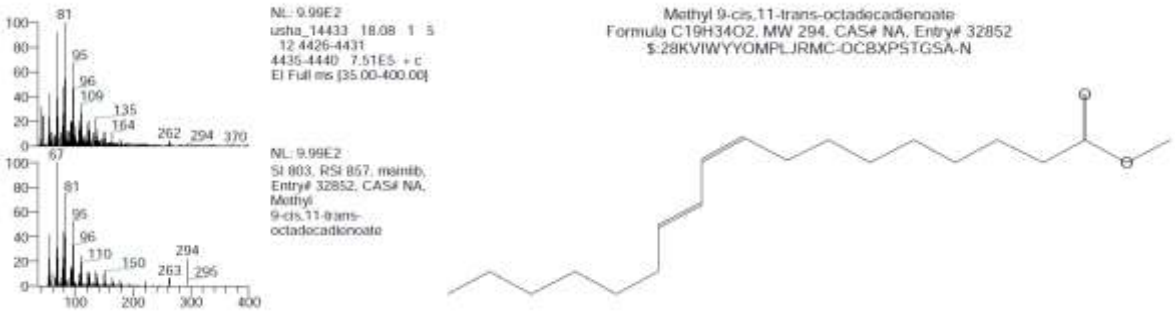

Figure 8: Methyl 9-cis,11-trans-octadecadienoate

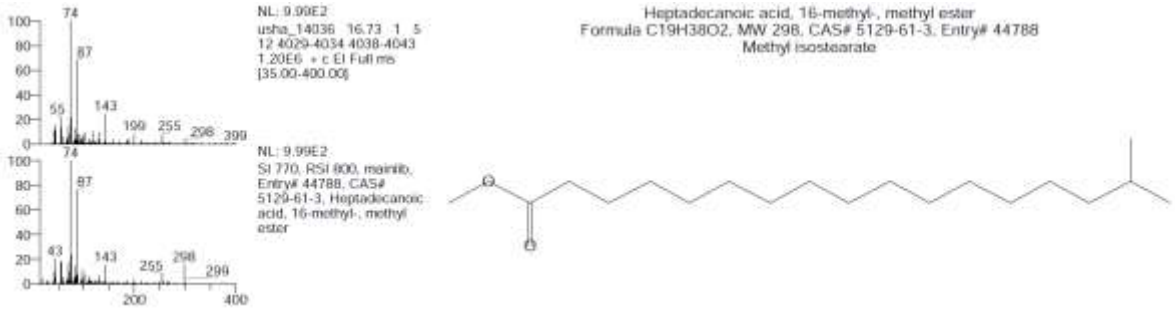

Figure 9: Heptadecanoic acid, 16-methyl-, methyl ester

According to studies, hexadecanoic acid, methyl ester (palmitic acid methyl ester), observed at retention time 13.78 with 2.211% peak area (**Figure 10**), has potent anti-bacterial, anti-oxidant, anti-fungal, nematicide, pesticide, anti-androgenic flavor, hemolytic, and 5-Alpha reductase inhibitory properties (Akpuaka *et al.,* 2013; Sermakkani & Thangapandian, 2012; Pinto *et al.,* 2017; Chandrasekaran *et al.,* 2011). It has also been demonstrated as a novel neuroprotective compound in cerebral ischemia (Lee et al., 2019). Ethanol, 2,2'-oxybis-, was observed at a retention time of 11.35 with a 1.97% peak area (**Figure 11**), and acetic acid, methyl ester, was observed at a retention time of 3.92 with a 1.86% peak area (**Figure 12**). 2(5H)-Furanone, 3-methyl, was observed at a retention

time 8.84 with a 1.62% peak area (**Figure 13**). Glycerin, observed at a retention time of 11.10 with a 1.61 % peak area (**Figure 14**), is an odorless, colorless, viscous liquid with a sweet, non-toxic flavor. Glycerides are lipids that include the glycerin backbone. Glycerin is commonly used in wound and burn treatments due to its anti-bacterial and antiviral characteristics. It is also used as an accurate indicator of liver illness. Glycerin is widely used in the food sector as a sweetener and a humectant in pharmaceutical formulations. Glycerin is a denaturant, fragrance ingredient, hair conditioner, humectant, oral care agent, oral health-care medicine, skin protectant, skin conditioning agent—humectants, and viscosity-reducing agent. Octanoic acid (n-Caprylic acid) was observed at a retention time of 11.83 with 1.34 % peak areas (**Figure 15**).

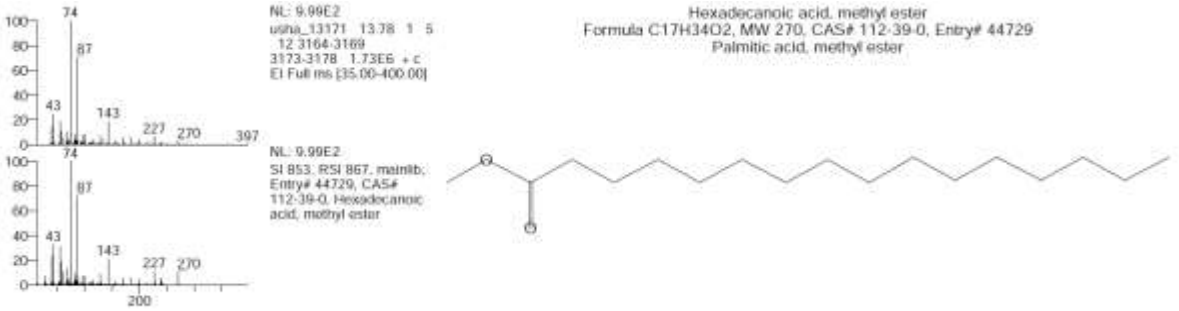

Figure 10: Hexadecanoic acid, methyl ester

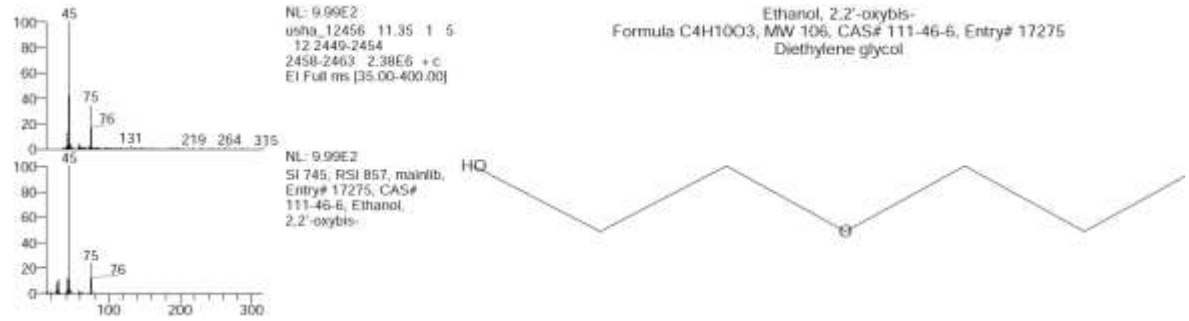

Figure 11: Ethanol, 2,2’-oxybis-

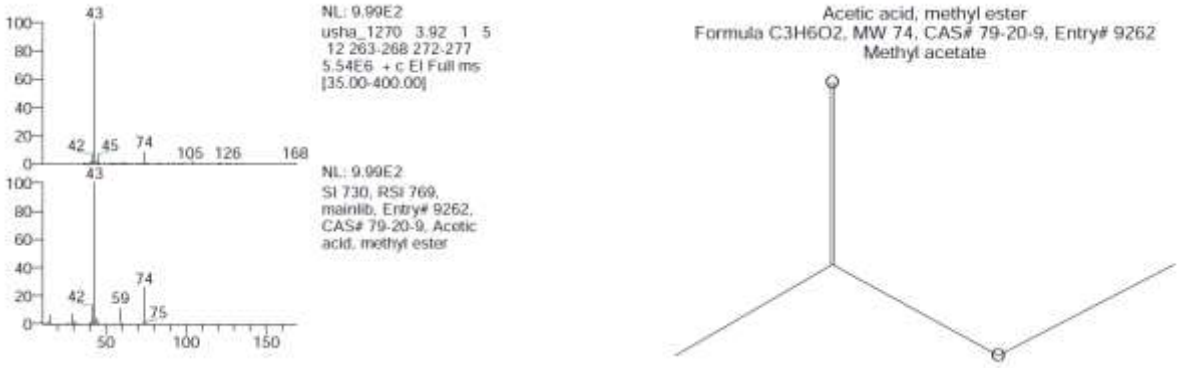

Figure 12: Acetic acid, methyl ester

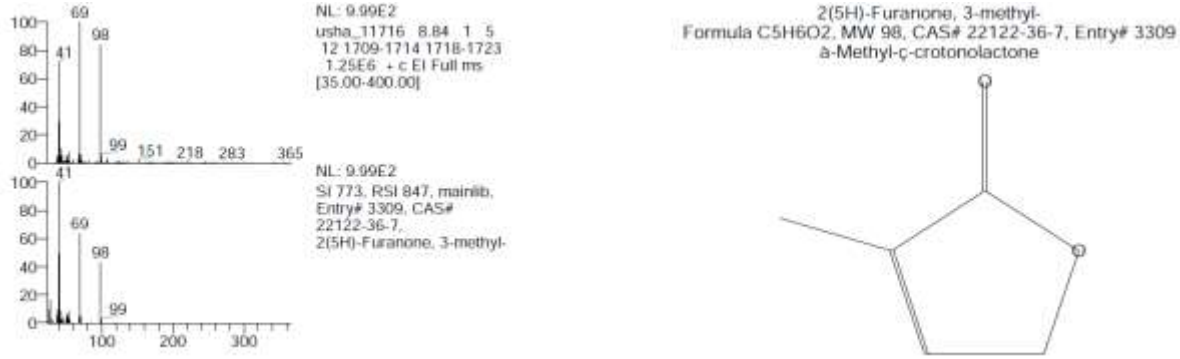

Figure 13: 2 (5H)-Furanone, 3-methyl

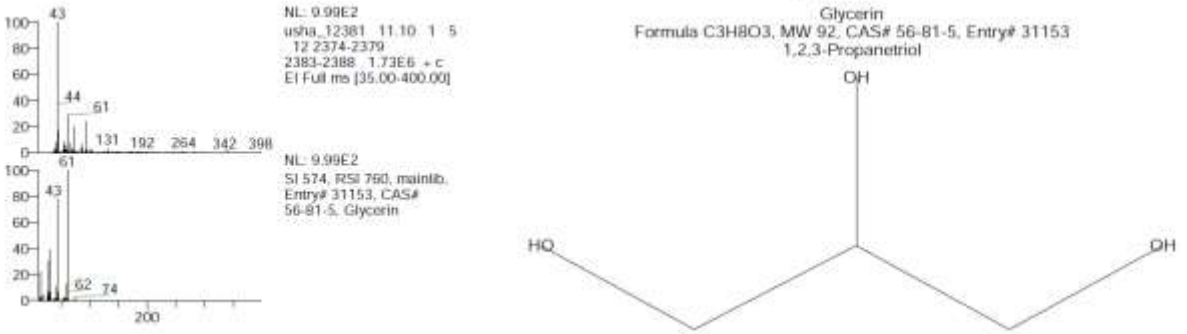

Figure 14: Glycerin

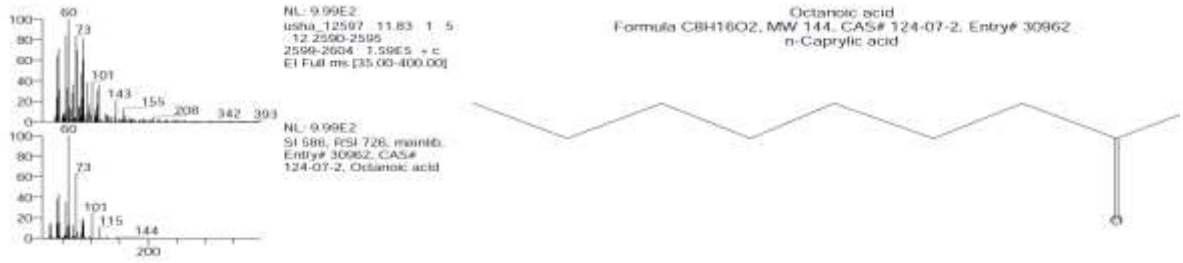

Figure 15: Octanoic acid (n-Caprylic acid)

Ethyl iso-allocholate observed at retention time 18.47 with 1.16 % peak area (**Figure 16**) has been reported to be a sterol compound, and it may be used as an anti-bacterial, anti-oxidant, anti-tumor, cancer preventive, and chemopreventive and pesticide. Ethyl isoallocholate has been reported as a sterol compound, and it may be used as an anti-bacterial, anti-oxidant, anti-tumor, cancer preventive, pesticide, and chemopreventive agent. 1, 25-Dihydroxyvitamin D3, TMS derivative was observed at retention time 17.88 with 0.67% peak area (**Figure 17**). In vertebrates, vitamin D is primarily used to maintain calcium homeostasis; however, lack of vitamin D has been related to an increased risk of hypertension, autoimmune diseases, diabetes, and cancer (Holick, 2004; Lappe et al., 2007; Hyppönen et al., 2001; Pittas et al., 2007; Kendrick et al., 2009; Cantorna & Mahon, 2004). Photochemical conversion of 7-dehydrocholesterol produces vitamin D3 in the skin. However, dietary vitamin D intake is critical due to a shortage of sunlight throughout the winter, especially in northern nations. The methyl ester of 9, 12, and 15-octadecatrienoic acid observed at a retention time of 17.96 with 0.53% peak area (**Figure 18**) is also known as α-linolenic acid. Linolenic acids are essential in humans to maintain normal conditions, cellular membranes, brain activities, nerve impulse transmission, anti-inflammation, anti-oxidation, and neuroplasticity (Li et al., 2010). These acids are building blocks for arachidonic and docosahexaenoic acids, which are required for brain and retina growth and function. It is considered a crucial and necessary consumable since it aids in delivering oxygen from the atmosphere to the blood plasma, synthesizing hemoglobin, and cell division. It has also been reported to exhibit psychotropic effects by modulating neurotransmission, anti-inflammation, anti-oxidation, and neuroplasticity (Lin et al., 2010). Octaethylene glycol monododecyl ether was observed at a retention time 15.84 with a 0.30% peak area (**Figure 19**). Pterin-6-carboxylic acid was observed at a retention time 4.48 with a 0.23% peak area (**Figure 20**). Pteridines are a broad and structurally diverse category of natural chemicals involved in cofactor and vitamin biosynthesis pathways. The term "pterins" refers to their derivatives, and various pterin derivatives have been isolated from nearly all living organisms. They are a complex collection of biological molecules with a distinctive ring structure and a nitrogen heterocyclic molecule with a 2-amino-4-hydroxypteridine structure in their moieties. They are classified into two types: conjugated pterins, which have highly complicated chains (folic acid and derivatives), and unconjugated pterins, which have short chains. Xanthopterin, isoxanthopterin, 6, 7-dimethylpterin, 6-biopterin, 6-xydroxymethylpterin, pterin, and pterin-6-carboxylic acid are among these chemicals (Koslinski et

al., 2011; Basu, 2011). Although the molecular basis for this assertion is not fully understood, the available literature data point to this group of chemicals as potential biomarkers for cancer detection (Koslinski et al., 2011; Basu, 2011; Gamagedara et al., 2011). Methyl 3-(acetyloxy)-20-hydroxyurs-12-en-28-oate was observed at a retention time of 7.44 with a 0.15% peak area (**Figure 21**). Tetraacetyl-d-xylonic nitrile was observed at a retention time of 18.23 with a 0.05% peak area (**Figure 22**). Nitro-L-arginine was observed at a retention time of 6.12 with a 0.04% peak area (**Figure 23**). An essential amino acid, l-arginine, is a nitric oxide (NO) precursor with significant vascular relaxant characteristics. Intravenous arginine (Morikawa et al., 1992, 1994) or NO donors (Zhang & Iadecola, 1994) have been proven to diminish ischemia infarction. However, NO synthase (NOS) inhibitors have been demonstrated to aggravate brain injury (Kuluz et al., 1993). The ability of nitro-arginine to protect against ammonia toxicity and ammonia-caused alterations in brain energy and ammonia metabolites has been experimentally examined (Kosenko et al., 1995). The results of the present study are consistent with the previous investigations that observed the phyto-constituents of Aloe vera's ethanolic extract, like hexadecanoic acid and 9-octadecenoic acid, methyl ester (oleic acid), 9,12,15-octadecatrienoic acid, and 2,3-dihydroxy propyl ester (Z, Z, Z) (Arunkumar & Muthuselvam, 2009; Lakshmi & Rajalakshmi, 2011; Hadizadeh et al., 2013; Ghosh et al., 2015).

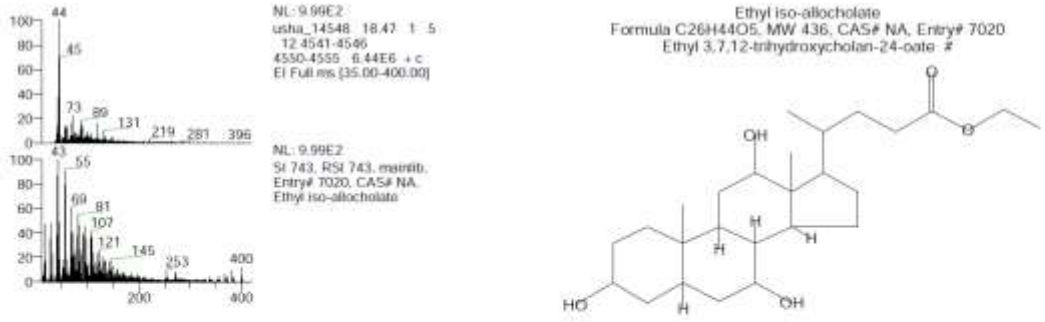

Figure 16: Ethyl iso-allocholate

Figure 17: 1, 25-Dihydroxyvitamin D3, TMS derivative

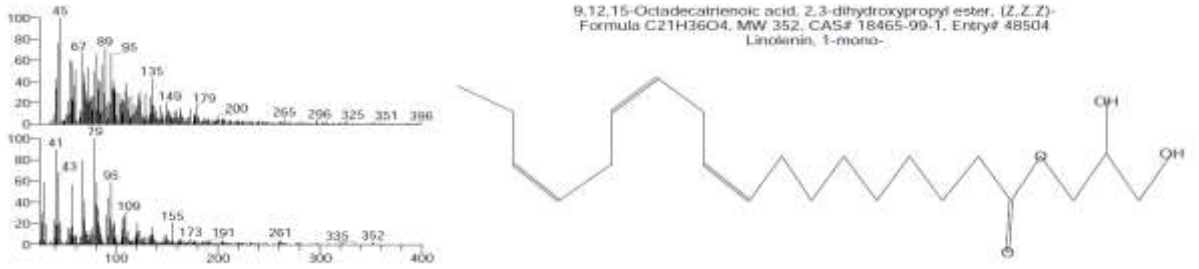

Figure 18: 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-

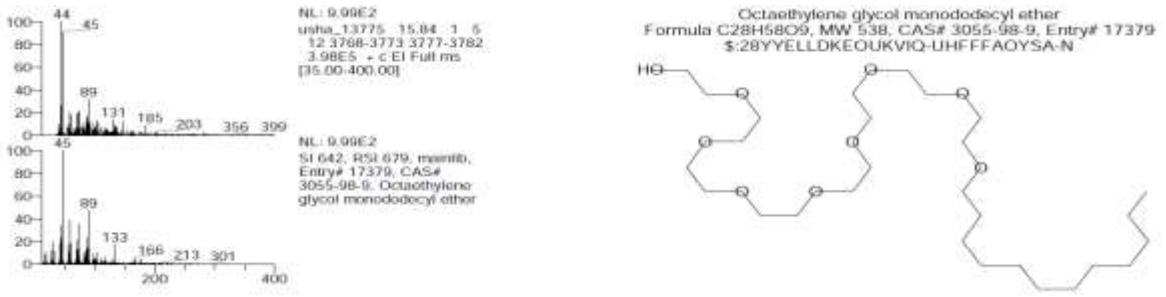

Figure 19: Octaethylene glycol monododecyl ether

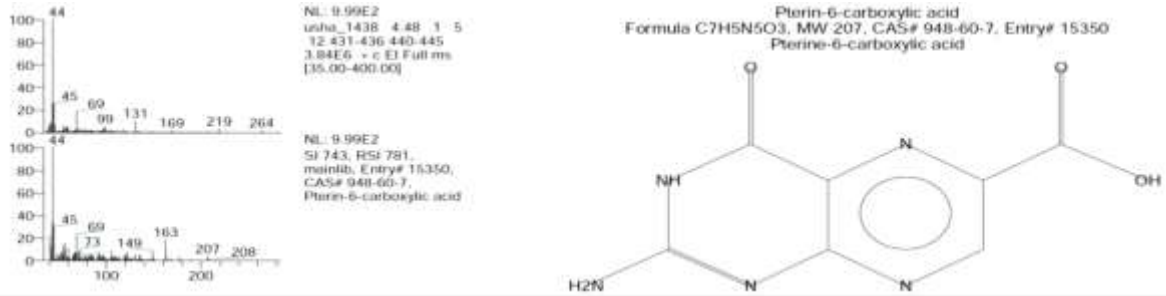

Figure 20: Pterin-6-carboxylic acid

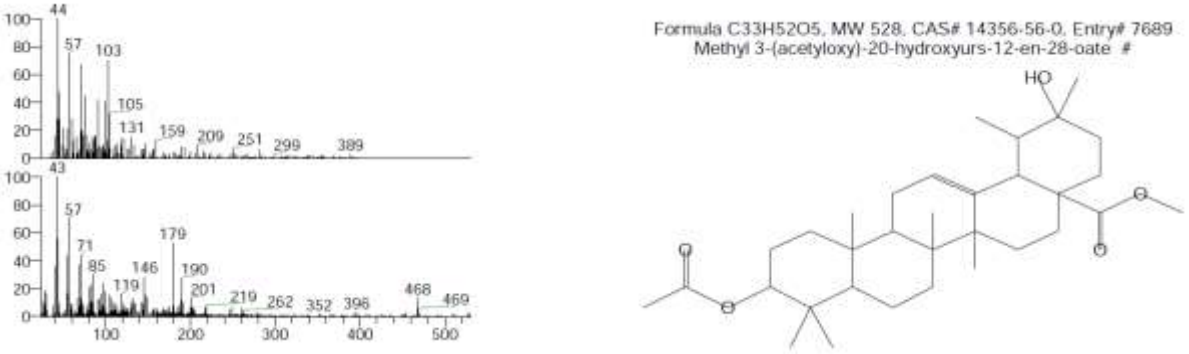

Figure 21: Methyl 3-(acetyloxy)-20-hydroxyurs-12-en-28-oate

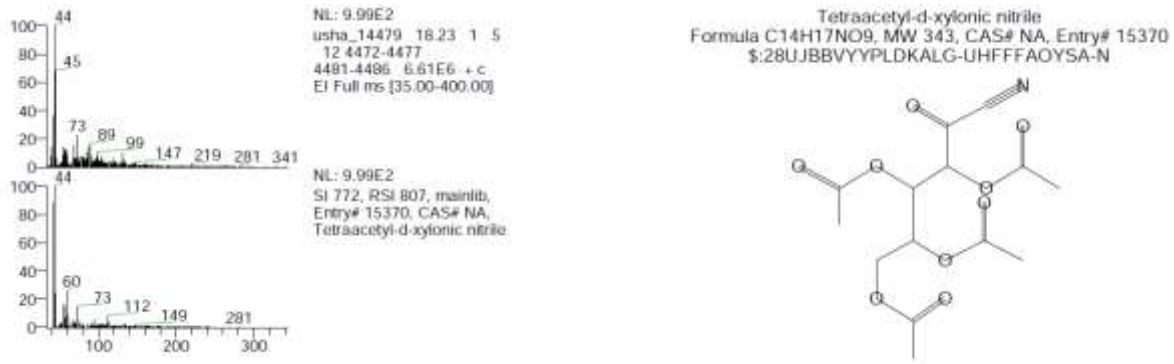

Figure 22: Tetraacetyl-d-xylonic nitrile

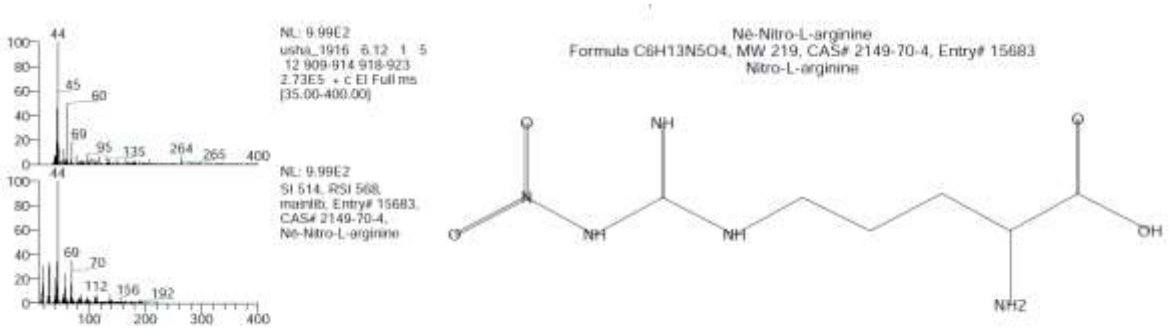

Figure 23: Nitro-L-arginine

The present study findings suggest that the significant anti-bacterial, anti-fungal, anti-microbial, anti-cancer, anti-tumor, anti-diabetic activities, anti-oxidant, analgesic, anti-inflammatory, wound healing potential, reproduction booster and neuroprotective potential of the *Aloe vera* leaf gel can be attributed to its 9-Octadecenoic acid, methyl ester. Propanoic acid; Ammonium acetate; 2, 3-Btanediol, [S-(R^*, R^*)]6-Octadecenoic acid, methyl ester, (Z)-; Phthalic acid, decyl isobutyl ester; Methyl 9-cis, 11-trans-octadecadienoate; Heptadecanoic acid, 16-methyl-, methyl ester; Hexadecanoic acid, methyl ester; Ethanol, 2,2'-oxybis-; Acetic acid, methyl ester; 2(5H)-Furanone, -methyl; Glycerin; Octanoic acid (n-Caprylic acid); Ethyl iso-allocholate; 1,25-Dihydroxyvitamin D3, TMS derivative; 9,12,15-Octadecatrienoic acid, 2,3-dihydroxy propyl ester, (Z, Z, Z)-; Octaethylene glycol monododecyl ether; Pterin-6-carboxylic acid; Methyl 3-(acetyloxy)-20-hydroxyurs-12-en-28-oate; Tetraacetyl-d-xylonic nitrile; and Nitro-L-arginine compounds identified by GC-MS analysis.

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CONFLICT OF INTEREST:

There is no conflict of interest with the writers.

References:

Akpuaka, A., Ekwenchi, M. M., Dashak, D.A., Dildar, A. (2013). Biological Activities of Characterized Isolates of n-Hexane Extract of Azadirachta indica A. Juss (Neem). New York Science Journal; 6(6): 119-124. (ISSN: 1554-0200). http://www.sciencepub.net/newyork [accessed Dec. 6, 2015].

Arunkumar, S. & Muthuselvam, M. (2009). Analysis of Phytochemical Constituents and Anti-microbial Activities of Aloe vera L. against Clinical Pathogens. World Journal of Agricultural Sciences, 5, 572-576.

http://www.idosi.org/wjas/wjas5(5)/9

Avetisyan, A. A., Aleksanyan, I. L. & Durgaryan, V. G. (2010). Synthesis of (2-methylquinolin-4-ylsulfanyl)-substituted acetic and propionic acids and propionitriles. *Russ J Org Chem*, 46, 571–576. https://doi.org/10.1134/S1070428010040226

Awa, E.P., S. Ibrahim and D. A. Ameh (2012). GC/MS analysis and anti-microbial activity of diethyl ether fraction of methanolic extract from the stem bark of ANNONA SENEGALENSIS PERS. *IJPSR*, 3(11): 4213-4218.

Basu, P.; Burgmayer, S.J. (2011). Pterin chemistry and its relationship to the molybdenum cofactor. Coord. Chem. Rev., 255, 1016–1038.

Berdeaux, O., Voinot, L., Angioni, E. et al. (1998). A simple method of preparation of methyl trans-10,cis-12- and cis-9,trans-11-octadecadienoates from methyl linoleate. *J Amer Oil Chem Soc* 75: 1749–1755. https://doi.org/10.1007/s11746-998-0327-x

Berzosa, X., Pettersson, S., Teixidó, J. et al. (2011). A diversity-oriented synthesis of 3-(2-amino-1,6-dihydro-6-oxo-pyrimidin-5-yl) propanoic esters. Mol Divers 15, 595–601. https://doi.org/10.1007/s11030-010-9287-9

Brantner, A and E. Grein (1994). Anti-bacterial activity of plant extract used externally in traditional medicine.J.Ethnopharmacol., 44(1):35-40.Doi:10.1016/0378-8741(94)90096-5. PMID: 7990502.

Cantorna, M.T., Mahon, B.D. (2004). Mounting evidence for vitamin D as an environmental factor affecting autoimmune disease prevalence. Exp. Biol. Med. 229, 1136-1142.

Chandrasekaran, M., Senthilkumar, A., & Venkatesalu, V. (2011). Anti-bacterial and anti-fungal efficacy of fatty acid methyl esters from the leaves of Sesuvium portulacastrum L. European review for medical and pharmacological sciences, 15(7), 775–780.

Chen, J. (2004). Study on isolation, purification, and anti-tumor activity of *Aloe vera* polysaccrahides. Fuzhou: Fujian Medical University.

Chen, R., J. Zhang, Y. Hu, S. Wang, M. Chen, and Y. Wang. (2014). Potential antineoplastic effects of aloe-emodin: A comprehensive review. *The American Journal of Chinese Medicine* 42 (02): 275-88. Doi:10.1142/s0192415X14500189.

Choi, S., & M.H. Chung (2003). A review on the relationship between *Aloe vera* components and their biological effects. Seminars in Integrative Medicine 1(1):53-62.

Cock, I. E. (2008). Anti-microbial activity of Aloe barbadensis miller leaf gel components. *The Internet Journal of Microbiology* 4(2): 17-25.

Dracheva, L.V., Dorozhko, E.V., Avramchuk, O.A. et al. (2009). Voltammetric study of the anti-oxidant activity of propionic acid bacteria in liquid cultures. Moscow Univ. Biol.Sci. Bull. 64, 157–160. https://doi.org/10.3103/S0096392509040051

Gamagedara, S., Gibbons, S., & Ma, Y. (2011). Investigation of urinary pteridine levels as potential biomarkers for noninvasive diagnosis of cancer. Clinica chimica acta; international journal of clinical chemistry, 412(1-2), 120–128. https://doi.org/10.1016/j.cca.2010.09.015

Ghosh, G., Panda, P., Rath, M., Pal, A., Sharma, T., & Das, D. (2015). GC-MS analysis of bioactive compounds in the methanol extract of Clerodendrum viscosum leaves. Pharmacognosy research, 7(1), 110–113. https://doi.org/10.4103/0974-8490.147223

Habtemariam, S. (2017). Going back to the good old days: the merit of crude plant drug mixture in 21st century. International Journal of Complementary and Alternative Medicine, Vol. 6, no. 2, p.00182.

Hadizadeh, F., Rahimi, B., Taghiabadi, E., Razavi, M., & Karimi, G. (2013). Evaluation of anticonvulsant effect of two novels 4-[1-(4-fluorobenzyl)- 5-imidazolyl] dihydropyridine derivatives in mice. Research in pharmaceutical sciences, 8(2), 91–95.

Holick, M.F., (2004). Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am. J. Clin. Nutr. 80, 1678S-1688S.

Hyppönen, E., Läärä, E., Reunanen, A., Järvelin, M.R., Virtanen, S.M., (2001). Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. The Lancet 358, 1500-1503.

Kendrick, J., Targher, G., Smits, G., Chonchol, M., (2009). 25-Hydroxyvitamin D deficiency is independently associated with cardiovascular disease in the Third National Health and Nutrition Examination Survey. Atherosclerosis 205, 255–260.

Kosenko, E., Kaminsky, Y., Grau, E. et al. (1995). Nitroarginine, an inhibitor of nitric oxide synthetase, attenuates ammonia toxicity and ammonia-induced alterations in brain metabolism. Neurochem Res 20, 451–456. https://doi.org/10.1007/BF00973101

Koslinski, P.; Bujak, R.; Daghir, E.; Markuszewski, M.J. (2011). Metabolic profiling of pteridines for determination of potential biomarkers in cancer diseases. Electrophoresis, 32 (15): 2044–2054.

Kuluz, J. W., Prado, R. J., Dietrich, W. D., Schleien, C. L., and Watson, B. D. (1993). The effect of nitric oxide synthase inhibition on infarct volume after reversible focal cerebral ischemia in conscious rats. *Stroke* 24, 2023–2029.

Lakshmi PTV, Rajalakshmi P (2011). Identification of Phyto-Components and Biological Activities of *Aloe vera* Through the Gas Chromatography-Mass Spectrometry. Inter. Res. J. Pharm. 2(5):247-249.

Lappe, J.M., Travers-Gustafson, D., Davies, K.M., Recker, R.R., Heaney, R.P., (2007). Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. Am. J. Clin. Nutr. 85, 1586–1591.

Lee, R. H., Couto E Silva, A., Possoit, H. E., Lerner, F. M., Chen, P. Y., Azizbayeva, R., Citadin, C. T., Wu, C. Y., Neumann, J. T., & Lin, H. W. (2019). Palmitic acid methyl ester is a novel neuroprotective agent against cardiac arrest. Prostaglandins, leukotrienes, and essential fatty acids, 147, 6–14. https://doi.org/10.1016/j.plefa.2018.11.011

Li, S. W., T. C. Yang, C.C. Lai, S. H. Huang, J. M. Liao, L. Wan, Y. J. Lin, and C. W. Lin (2014). Antiviral activity of aloe-emodin against influenza a avirus via gelectin-3 up-regulation. *European Journal of Pharmacology* 738: 125-132.Doi:10.1016/j.ejphar.2014.05.028.

Lin, P. Y., Huang, S. Y., & Su, K. P. (2010). A meta-analytic review of polyunsaturated fatty acid compositions in patients with depression. *Biological psychiatry*, 68(2), 140–147. https://doi.org/10.1016/j.biopsych.2010.03.018

Morikawa, E., Moskowitz, M. A., Huang, Z., Yoshida, T., Irikura, K., and Dalkara, T. (1994). l-Arginine infusion promotes nitric oxide-dependent vasodilation, increases rCBF and reduces infarction volume in the rat. *Stroke* 25, 429–435.

Morikawa, E., Rosenblatt, S., and Moskowitz, M. A. (1992). l-Arginine dilates rat pial arterioles by nitric oxide-dependent mechanisms and increases blood flow during focal cerebral ischaemia. *Br. J. Pharmacol.* 107, 905–907.

Nakalembe I., Kabasa J. D. (2012). Anti-microbial activity and biochemical constituents of two edible and medicinal mushrooms of Mid-Western, Uganda. *Res J. Pharmacol.* 6 4–11. 10.3923/rjpharm.2012.4.11

Pinto, M.E.A, Araujo, S.G., Morais M.I., Sa N.P., Lima, C.M., Rosa, C.A., Siqeira E.P., Johann, S., and Lima L. (2017). Anti-fungal and anti-oxidant activity of fatty acid methyl esters from vegetable oils. *Annals of the Brazilian Academy of Sciences*. 1-11.

Pittas, A.G., Lau, J., Hu, F.B., Dawson-Hughes, B., (2007). The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J. Clin. Endocrinol. Metab. 92, 2017-2029.

Ponnamma, S.U., & Manjunath, K. (2012). GC-MS analysis of phytocomponents in the methanolic extract of *Justicia wynaadensis* (Nees) T. Anders. *International journal of pharma and bio sciences*, 3(3): P570-P576

Rahman, M. M., Ahmad, S. H., Mohamed, M. T., & Ab Rahman, M. Z. (2014). Anti-microbial compounds from leaf extracts of *Jatropha curcas*, *Psidium guajava*, and *Andrographis paniculata*. *The Scientific World Journal*, 635240. https://doi.org/10.1155/2014/635240

Reynolds, T. (2004). Aloes: The genus aloe. Boca Raton: CRC press.

Sabeh, F., T. Wright, & S. J. Norton. (1996). Isozymes of superoxide dismutase from *Aloe vera*. *Enzyme and Protein* 49 (4): 212–221. Doi:10.1159/ooo468631.

Saranya, D. K., Sruthy, P. B., Anjana, J. C., Rathinamala, J., Jayashree, S. (2013). GC-MS Analysis of Phytocomponents in Resin of Araucaria Columnaris (Cook Pine) and its Medicinal Uses. International Journal of Applied Biology and Pharmaceutical Technology, 4(3): 272-276. Available online at www.ijabpt.com.

Satpute, S.K., Banat, I.M., Dhakephalkar, P.K., Banpurkar, A.G., and Chopade, B.A. (2010). Biosurfactants, bioemulsifiers and exopolysaccharides from marine microorganisms. Biotechnol Adv 28: 436–450.

Sermakkani, M. and Thangapandian, V. (2012). GC-MS analysis of *Cassia italica* leaf methanol extract. *Asian Journal of Pharmaceutical and Clinical Research,* 5(2):90-94.

Shengbo, G., Wanxi, P., Dongli, L. Bo, M., Minglong, Z., Daochun, Q. (2015). Study on Anti-bacterial Molecular Drugs in Eucalyptus granlla Wood Extractives by GC-MS. Pak. J. Pharm. Sci., 28(4): 1445-1448.

Suzuki, H., Kishida, F. & Miyamoto, J. (1979). Studies on Mutagenicity of S5602 in Ames Test in the Presence of Hepatic S9 Fractions from Several Laboratory Animal Species. Unpublished report from Sumitomo Chemical Co.

Unlu, A., E. Nayir, H. Ay, O. Kirca, and M. Ozdogen. (2016). *Aloe vera* and cancer. *Turk Onkoloji Dergisi-Turkish Journal of Oncology* 31(2): 68-72. Doi:10.5505/tjo.2016.143.

Zhang, F., and Iadecola, C. (1994). Reduction of cerebral ischemic damage by delayed treatment with nitric oxide donors. *J. Cereb. Blood Flow Metab.* 14, 574–580.