

GC–MS Analysis of Ethanol Leaves Extract of *Solanum Americana*

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Abstract – GC-MS analysis was carried out in order to investigate the phytoconstituents present in the ethanolic extract of *Solanum americana* leaves. The structural formula of the phytochemicals of *S. americana* leaves were determined by analysing of the spectra of GC-MS using the reference standard of National Institute of Standard and Technology (NIST). GC-MS analysis of *S. americana* leaves showed the presence of eighteen bioactive compounds. The compounds are 4-(5-(2-Naphthoxy)pentylthio)-5-chloro-uracil, propanamide, 2-(dimethylamino)-N-[2-methyl-1-[[3,3a,12,13,14,15,16,16a-octahydro-8-methoxy-13,16-dioxo-14, 4-Amino-1-methyl-5-nitropyrzazole, 1,2-Benzenediol, o-octanoyl-o'-(thiophen-2-acetyl), 7H-1,4-Dioxino[2,3-c]xanthen-7-one, 2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-5-methoxy, benzoic acid, p-(cyclohexyloxy)-, 3-(2-methylpiperidino)propyl ester, quinoline, 6,6'-[2,6-naphthalenediyl]di-2,1-ethenediyl]bis[1-ethyl-1,2,3,4-tetrahydro, 2,5,8,9,11-Pentaacetoxy-2,3-dihydrospiro(4H)benzofuro[2,3-g]-1-benzopyran-4,2'(5'H)-furan]-5'-one, 4-(d-Xylosyloxy)-6,7-dimethoxy-9-[3,4-(methylenedioxy)phenyl]naphtho[2,3-c]furan-1(3H)-one, benzenamine, N,N-didodecyl-4-nitro, 1-Hydroxy-3-(hydroxymethyl)anthraquinone, O,O'-bis(trimethylsilyl), d-Glucopyranoside, 1,4a,5,7a-tetrahydro-7-(methoxymethyl)cyclopenta[c]pyran-1,5-diyl]bis-, 2,2',3,3',4,4',6,6', 5,12-Naphthacenedione, 6,8,10,11-tetrakis(acetyloxy)-8-[(acetyloxy)acetyl]-7,8,9,10-tetrahydro-1-methoxy, salicylic acid, monoanhydride with 1-butaneboronic acid, cyclic ester, tetrabenzo[e,i,o,s][1,4,7,11,14,18]dithiatetraazacycloicosine, 11,12,13,14,26,27-hexahydro, 6-Chloro-2-(3,5-diphenyl-pyrazol-2-yl)-benzothiazole, (23S,24S,25S)-26,26-dichloro-23-ethyl-6-methoxy-3,5:24,26-dicyclocholestane, 9H-Carbazole-9-carboxaldehyde, 1-hydroxy-3-methyl-2-(3-methyl-2-butenyl) and 2,4-cyclopentadiene-1,2,3,4-tetracarboxylic acid, 5-(4-bromo-2-quinoliny)-, tetramethyl ester. It was concluded that the phytocompounds support the use of *S. americana* leaves for the management of pancreatic cancer, prostate cancer, asthma, allergic rhinitis, and osteoarthritis.

Keywords – Ethanol, Gas Chromatography, Mass Spectra, Plant, *Solanum Americana*.

I. INTRODUCTION

The genus *Solanum* is regarded to be one of the widest and most complicated genera among the Angiosperms [1], and the most popular and widest genus of the family Solanaceae [1]. It consist of about 2000 species disperse

across subtropical and tropical territories of Asia, Africa, Americas, Australia and India [2]. The genus is well dispersed in Brazil with about 350 species broadly distributed from north to south in diverse phytogeographic territories [3].

Ripe fruit from *S. americana* have been used in making jams and preservative. *S. americana* has been reported to possess anticancer [4], antiviral, antimicrobial [4] and antidiabetic properties [5]. It has also been established that extracts of *S. americana* can be used in the management of joint pains, protozoa infections, cooling, bladder spasm, vermifuge, cough, gastric ulcer,[5] and asthma [6]. Steroidal saponins are outstanding components in *Solanum* species. Spirostanic saponins isolated from *S. chrysotrichum* leaves showed significant inhibitory activity against dermatophytes and yeasts. Nuatigenoside isolated from *S. sisymbriifolium* roots displayed anti-hypertensive effect in experimental hypertensive rats [7]. Indioside, borassoside and yamoscine displayed cytotoxic activity against several human cancer cell lines [8]. The isolation of torvosides from the leaves of *S. torvum* has been reported [9]. The anticonvulsant activity of *S. torvum* in zebrafish seizure assays have also been reported [9]. Antifungal activity *S. torvum* extract against *Aspergillus flavus* and *Fusarium verticillioides* have been established [10].

Many authors have reported the GC-MS analysis of natural products [11-13]. To the best of our knowledge, the GC-MS analysis of *S. americana* ethanol leaf extract have not been reported in literature. Biological active phytochemicals present in the leaves of *S. americana* is hereby studied for future reference studies. This study is aimed at the determination of compounds present in *S. americana* ethanol leaves extract by GC–MS analysis.

II. METHODOLOGY

2.1 Plant sample

Fresh *S. americana* leaves were harvested from Ohafia, Abia State Nigeria on 25th June, 2020. The leaves were identified by a Botanist at the Department of Plant Science and Biotechnology, MOUAU, Nigeria.

2.2 Extraction of crude extracts

The *S. americana* leaves were air dried at room temperature for 3 days. The dried roots were grounded using electric grinder. The powdered *S. americana* leaves were subjected to extraction using ethanol. The extract was then subjected to evaporation by using rotary evaporator.

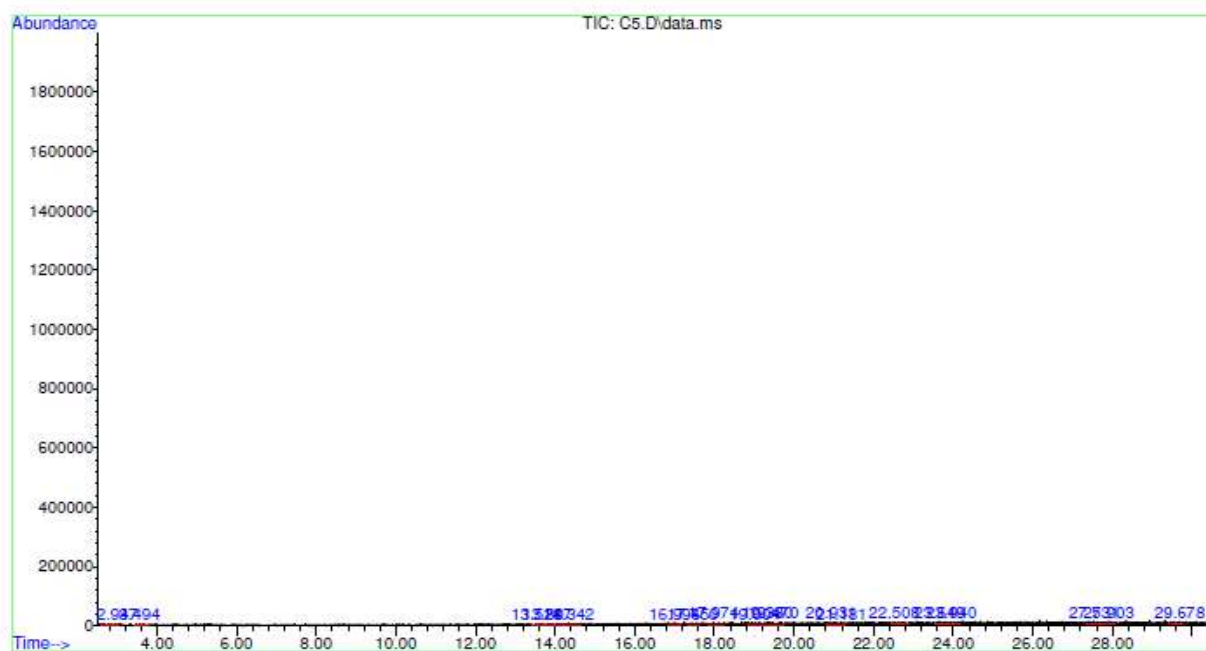
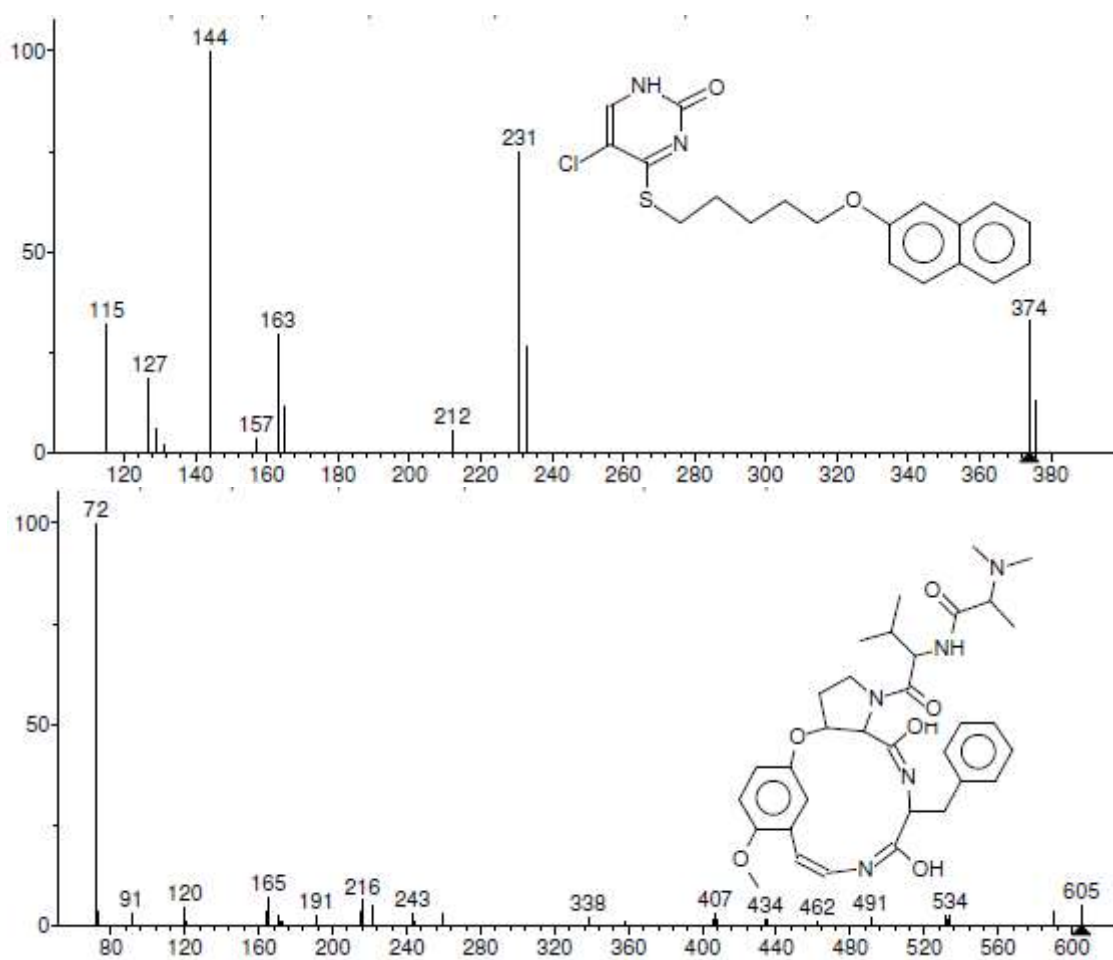
2.3 GC-MS Analysis

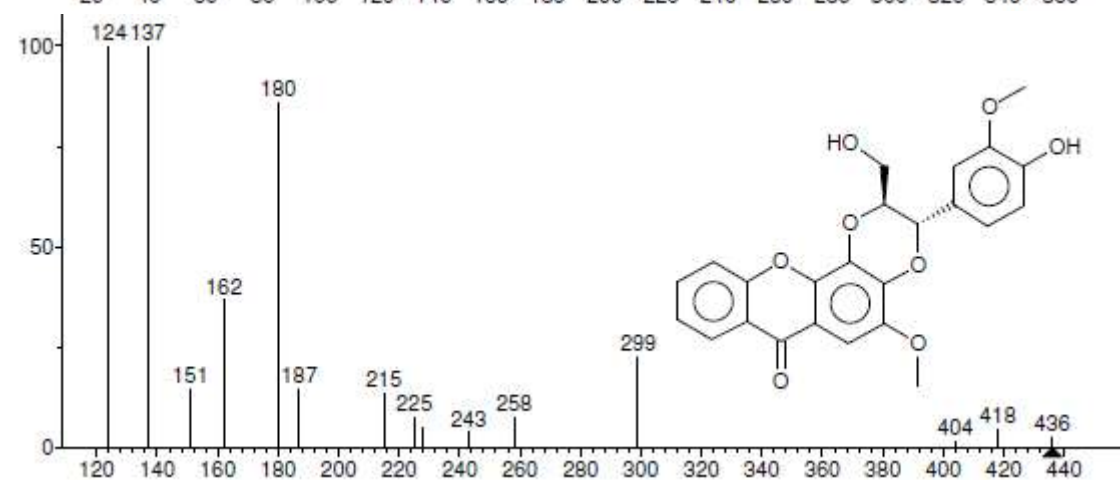
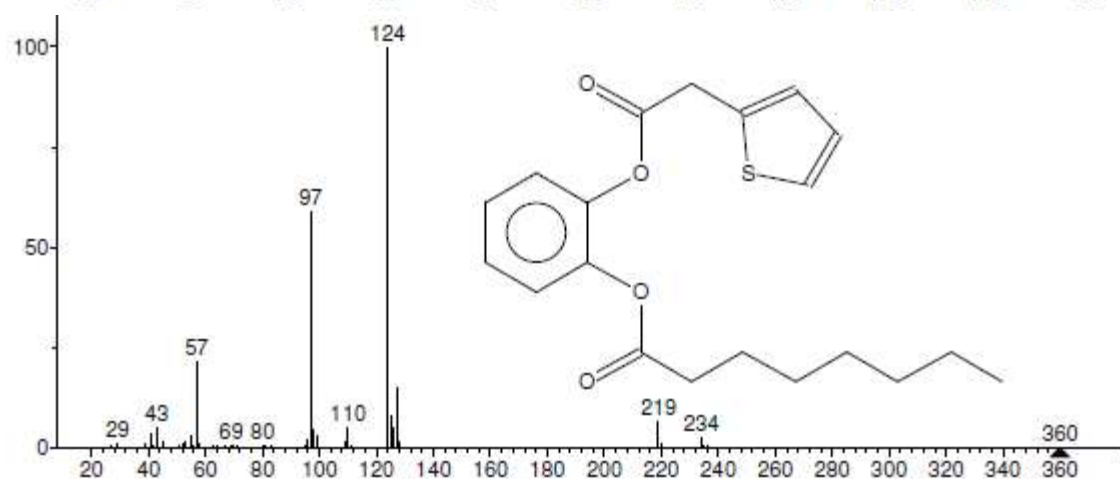
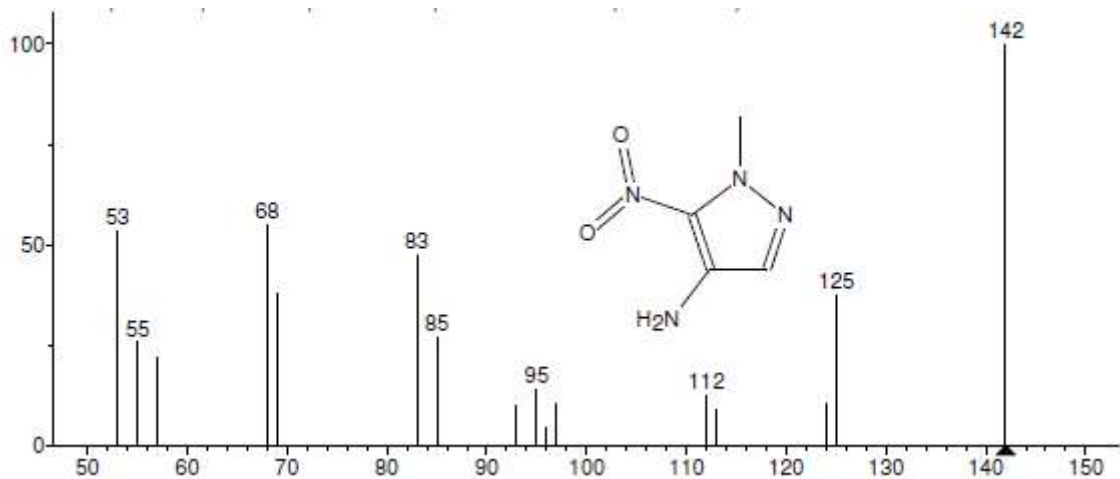
The GC–MS spectra of bioactive compounds of *S. americana* leaves extracts were analysed using agilent 6890N gas chromatography equipped with an auto sampler connected to an agilent Mass Spectrophotometric Detector . A micro-litre of sample was injected in the pulsed splitless mode onto a 30m x 0.25 mm ID DB 5MS coated fused silica column with a film thickness of 0.15 micrometer. Helium gas was used as a carrier gas and the pressure of the column head was maintained at 20 psi to give a constant of 1ml/min. The temperature of the column was held initially at 55 °C for 0.4 min and then raised to 200 °C at a rate of 25 °C/mins,. It was increased to 280 °C at a rate of 8 °C/mins and to a final temperature of 300 °C at a rate of 25 °C/mins for 2 mins . The identification time was based on retention time. Components with lower retention time eluted first before the ones of higher retention time.

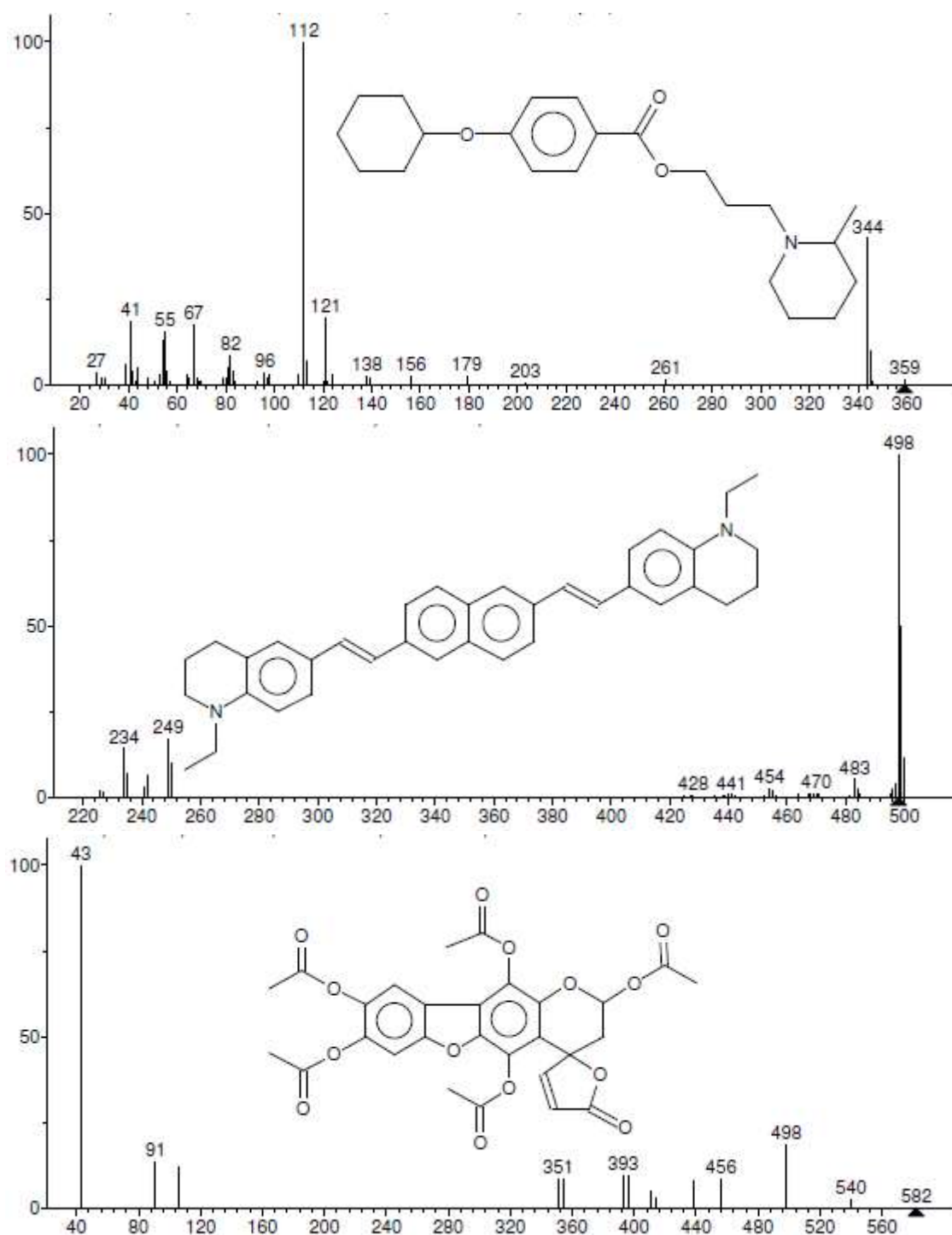
2.4 Identification of chemical constituents

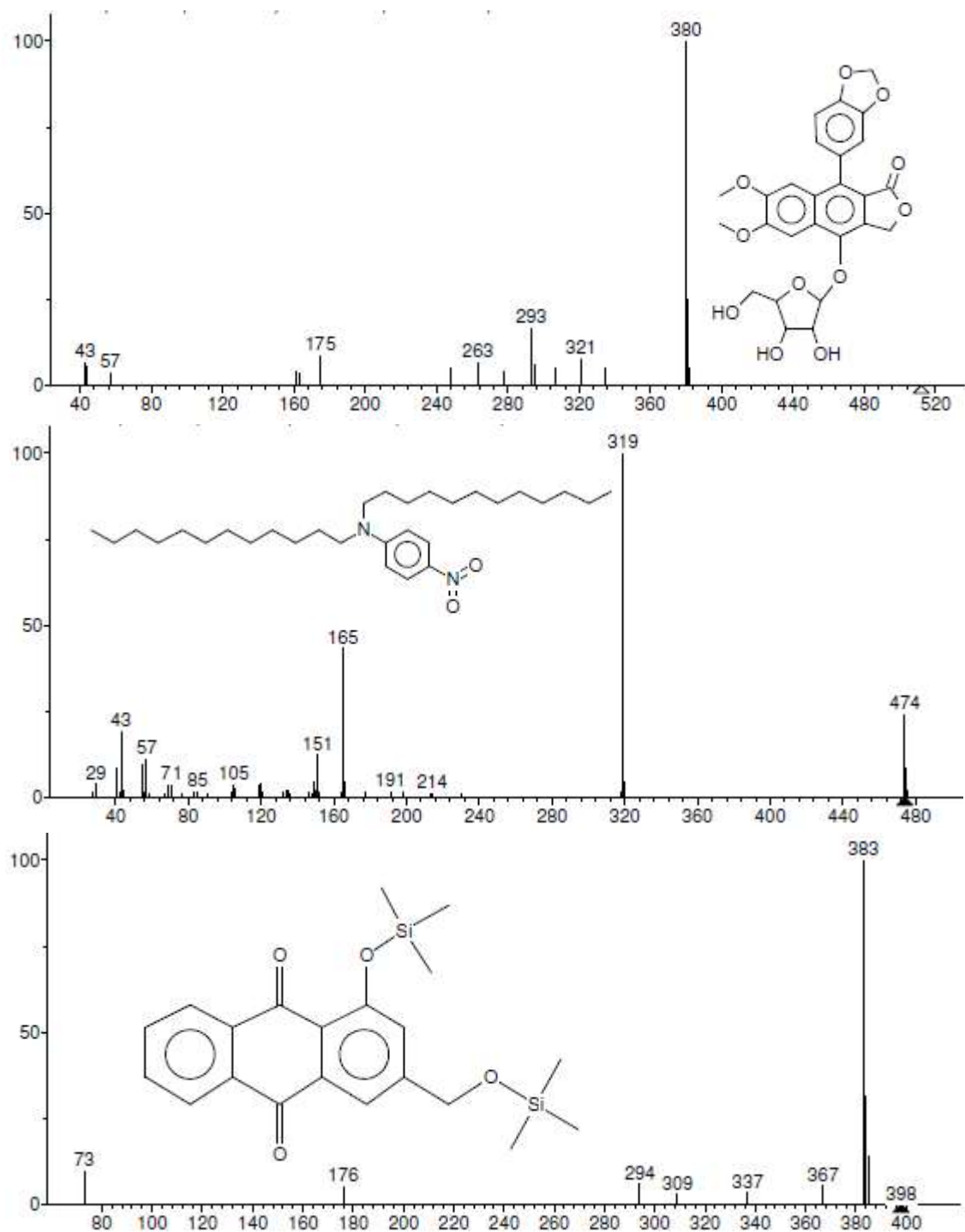
The structural formula of the compounds present in ethanol extract of *S. americana* leaves were ascertained by the interpretation of mass spectrum of GC-MS using the reference standard of NIST library. The mass spectra of the unknown compounds were cross-matched with the spectra of the known compounds stored in the NIST library.

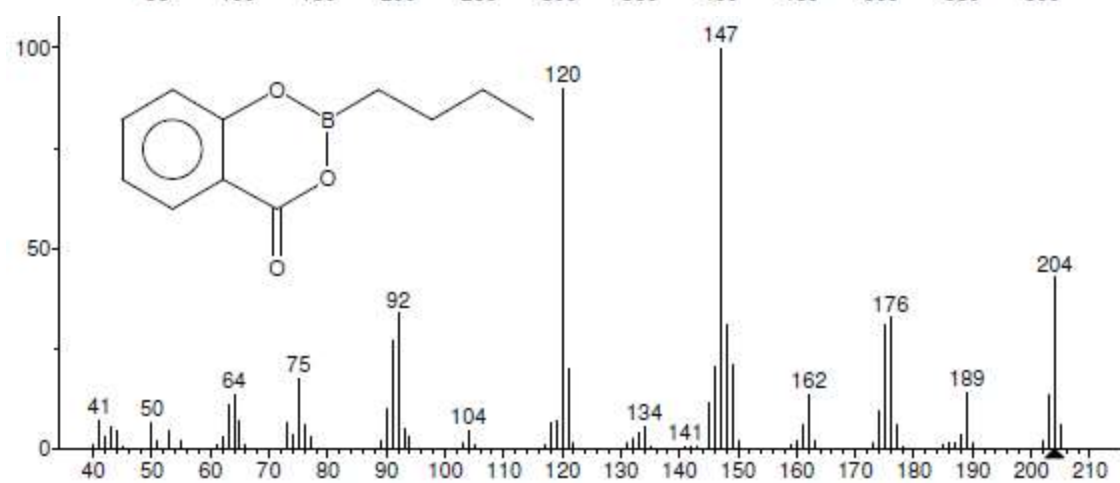
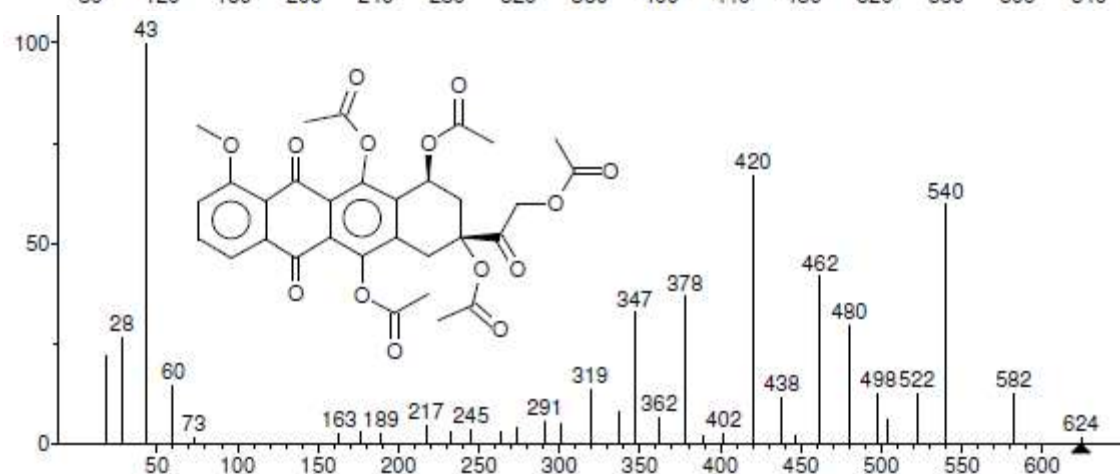
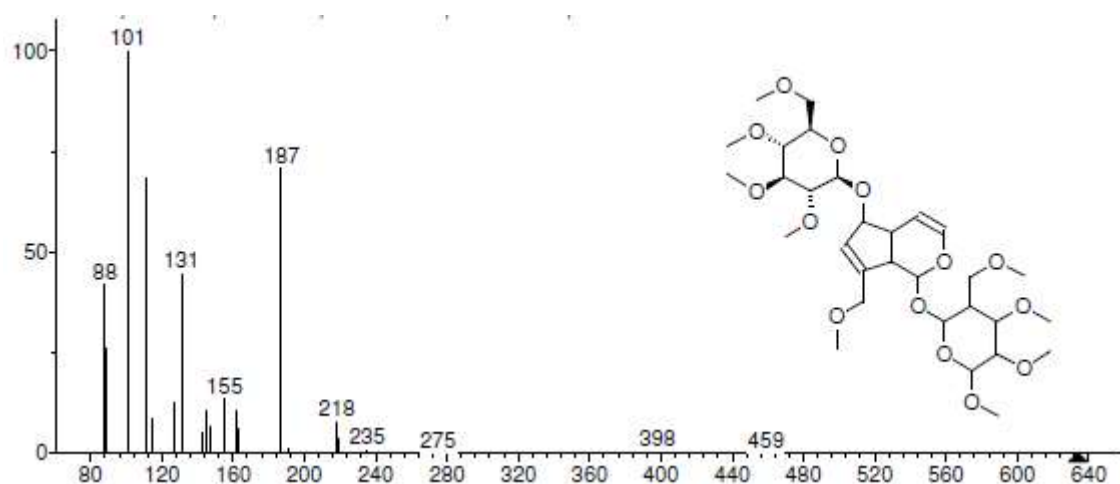
III. RESULTS AND DISCUSSION

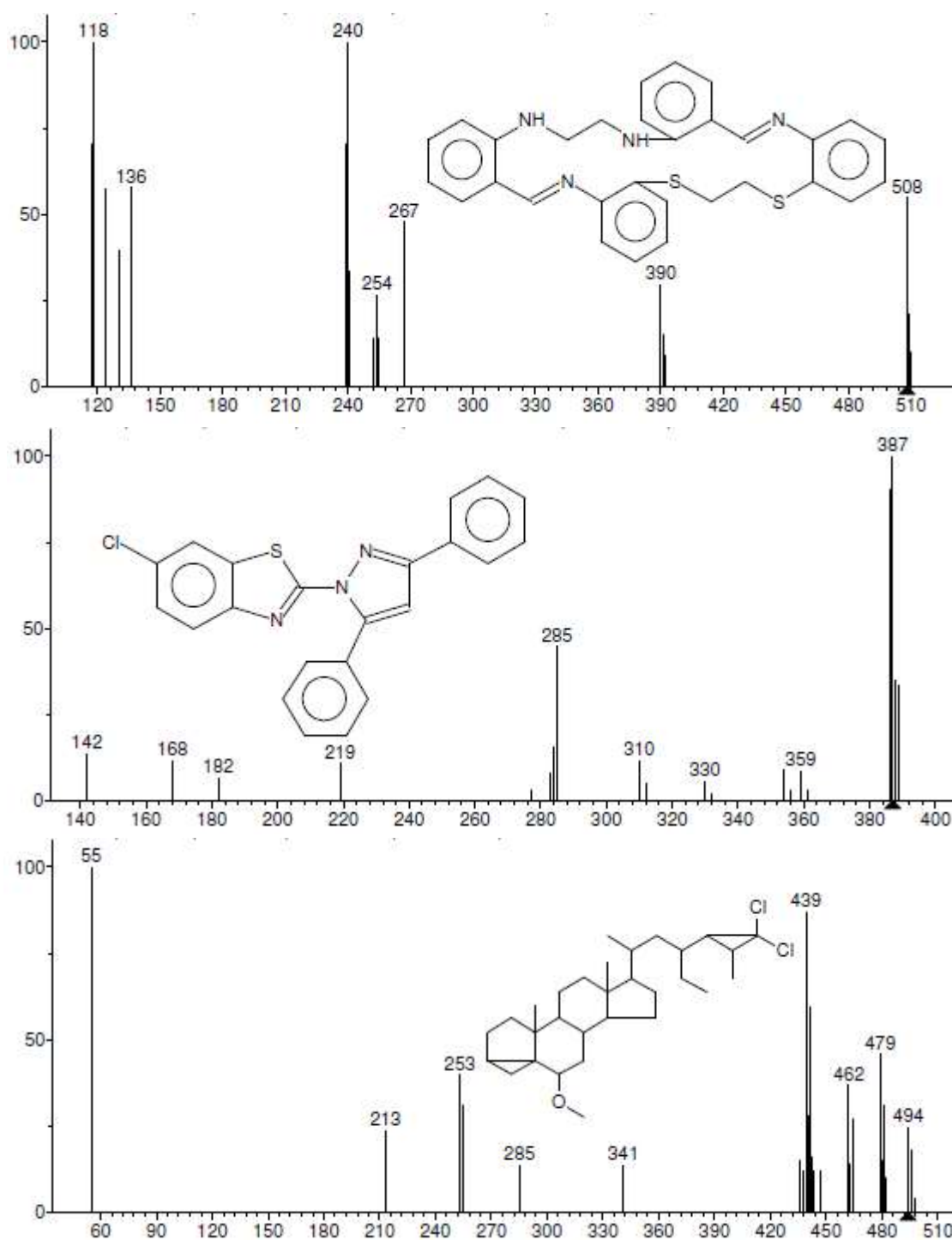
Figure 1: Gas chromatogram of ethanol leave extract of *S. americana*

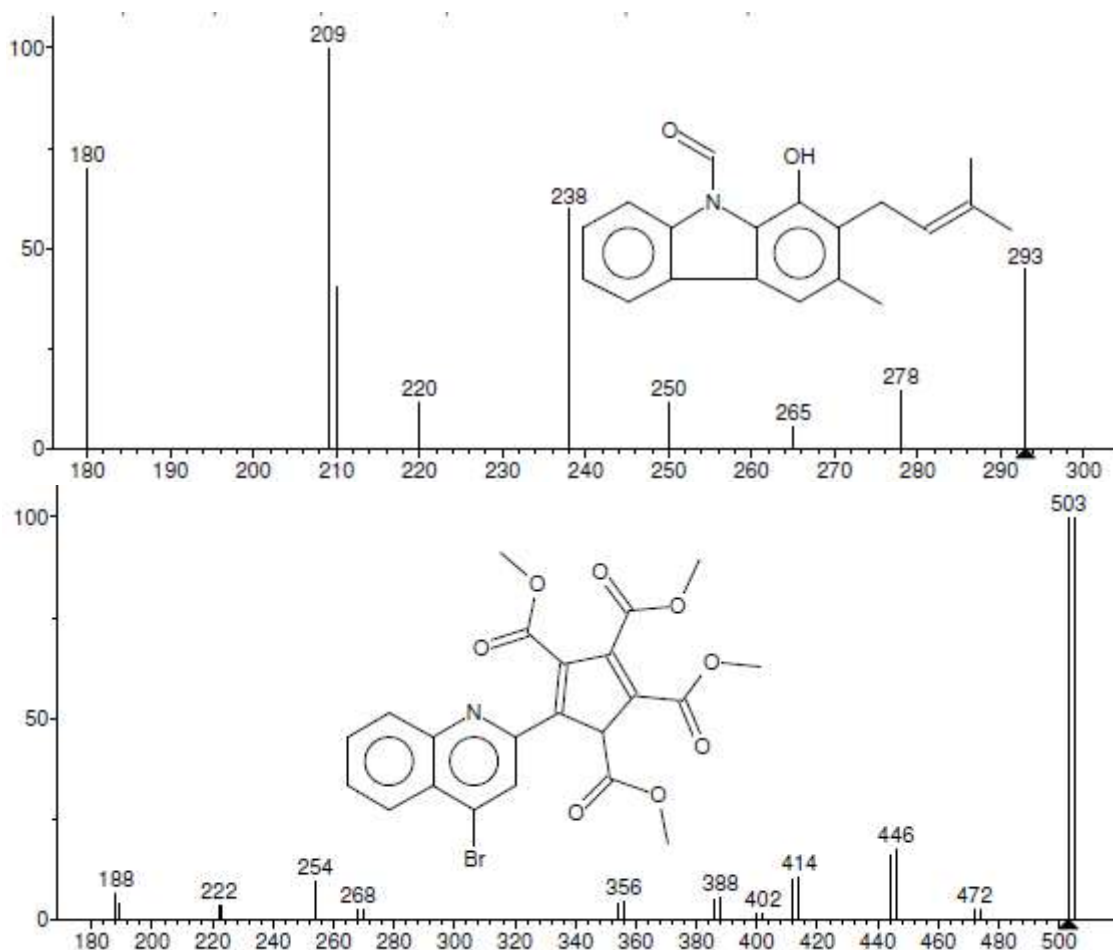










Figure 2: Mass spectra of ethanol leaf extract of *S. americana*Table 1: Bioactive compounds present in ethanol extract of *S. americana* leave

S/No	Compound	Bioactivity
1.	4-(5-(2-Naphthoxy)pentylthio)-5-chloro-uracil	Not found
2	Propanamide, 2-(dimethylamino)-N-[2-methyl-1-[[[3,3a,12,13,14,15,16,16a-octahydro-8-methoxy-13,16-dioxo-14	Not found
3	4-Amino-1-methyl-5-nitropyrazole	Increase aromatic amino Acid Decarboxylase activity, catechol-O-methyltransferase-Inhibitor, methyl-guanidine-inhibitor
4	1,2-Benzenediol, o-octanoyl-o'-(thiophen-2-acetyl)	Acetylcholinergic
5	7H-1,4-Dioxino[2,3-c]xanthen-7-one, 2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-5-methoxy	Not found
6	Benzoic acid, p-(cyclohexyloxy)-, 3-(2-methylpiperidino)propyl ester	Anticancer (pancreas), anticancer (prostate)
7	quinoline, 6,6'-[2,6-naphthalenediyl-di-2,1-ethenediyl]bis[1-ethyl-1,2,3,4-tetrahydro	Not found
8	2,5,8,9,11-Pentaacetoxy-2,3-dihydrospiro(4H)benzofuro[2,3-g]-1-benzopyran-4,2'(5'H)-furan]-5'-one	Not found

9	4-(d-Xylosyloxy)-6,7-dimethoxy-9-[3,4-(methylenedioxy)phenyl]naphtho[2,3-c]furan-1(3H)-one	11B-HSD-inhibitor, 12-Lipoxygenase-inhibitor
10	benzenamine, N,N-didodecyl-4-nitro	NADH-oxidase-inhibitor, NADH-ubiquinone-oxidoreductase-Inhibitor
11	1-Hydroxy-3-(hydroxymethyl)anthraquinone, O,O'-bis(trimethylsilyl)	17-beta-hydroxysteroid dehydrogenase-inhibitor, aryl-hydrocarbon-hydroxylase-inhibitor
12	d-Glucopyranoside, 1,4a,5,7a-tetrahydro-7-(methoxymethyl)cyclopenta[c]pyran-1,5-diyl[bis-, 2,2',3,3',4,4',6,6'	Not found
13	5,12-Naphthacenedione, 6,8,10,11-tetrakis(acetyloxy)-8-[(acetyloxy)acetyl]-7,8,9,10-tetrahydro-1-methoxy-, (8S	Not found
14	Salicylic acid, monoanhydride with 1-butaneboronic acid, cyclic ester	Increase aromatic amino acid decarboxylase activity,
15	Tetrabenzo[e,i,o,s][1,4,7,11,14,18]dithiatetraazacycloicosine, 11,12,13,14,26,27-hexahydro	
16	6-Chloro-2-(3,5-diphenyl-pyrazol-2-yl)-benzothiazole	Not found
17	(23S,24S,25S)-26,26-dichloro-23-ethyl-6_-methoxy-3_,5:24,26-dicyclocholestane	Not found
18	9H-Carbazole-9-carboxaldehyde, 1-hydroxy-3-methyl-2-(3-methyl-2-butenyl)	17-beta-hydroxysteroid dehydrogenase-Inhibitor, aryl-hydrocarbon-hydroxylase-inhibitor, testosterone-hydroxylase-inducer
19	2,4-Cyclopentadiene-1,2,3,4-tetracarboxylic acid, 5-(4-bromo-2-quinoliny)-, tetramethyl ester	Arachidonic-acid-inhibitor

From Table 1, ethanol extract of *S. americana* leave showed acetylcholinergic activity. The neurotransmitter used at the neuromuscular junction is known as acetylcholine. In order to activate muscles, the motor neurons of the nervous system releases acetylcholine. This property means that medications that inhibit cholinergic systems can have disastrous effects like paralysis and convulsions. Acetylcholine, a neurotransmitter in the autonomic nervous system, is an internal transmitter for the sympathetic nervous system and the last product released by the parasympathetic nervous system [14]. Acetylcholine is the dominant neurotransmitter of the parasympathetic nervous system [15]. Ethanol extract of *S. americana* leave showed 12-Lipoxygenase-inhibitory activity. Arachidonate 5-lipoxygenase inhibitors are medications that slow or inhibit the activity of arachidonate 5-lipoxygenase (5-lipoxygenase or 5-LOX) enzyme, which is responsible for the generation of inflammatory leukotrienes. Excessive production of leukotrienes is a primary cause of inflammation in asthma, allergic rhinitis, and osteoarthritis [16]. Aromatic L-amino acid decarboxylase (AADC) was discovered 70 years ago in mammalian tissue [17]. Its specific importance in

pharmacology was established when it was demonstrated that its substrate L- DOPA relieved the clinical symptoms of Parkinson's disease (PD) [18-20] by supplementing lost dopamine from nigrostriatal neurons. 4-Amino-1-methyl-5-nitropyrazole have been reported to increase aromatic amino acid decarboxylase activity [21]. Table 1 shows that ethanol extract of *S. americana* leave has been reported as catechol-O-methyl-transferase inhibitor [21]. Catechol-O-methyltransferase inhibitors are category of medicines that are used along side carbidopa-levodopa therapy in the management of symptoms of Parkinson's disease. Catechol-O-methyltransferase inhibitors can extend the effectiveness of carbidopa-levodopa therapy, and permit for lower doses of carbidopa-levodopa [22]. 2,4-Cyclopentadiene-1,2,3,4-tetracarboxylic acid, 5-(4-bromo-2-quinoliny)-, tetramethyl ester has been reported as arachidonic acid inhibitor [21]. Arachidonic acid and its metabolites have recently generated a heightened interest because of growing evidence of their significant role in cancer biology. Thus, inhibitors of arachidonic acid have originally been of interest in the management of inflammatory disorders and certain types of heart disease. They are now receiving increased attention as

an arsenal against cancer [23]. 4-Amino-1-methyl-5-nitropyrazole is a methylguanidine inhibitors [21]. Methylguanidine is a suspected uraemic toxin that accumulates in kidney failure; however it also exhibits anti-inflammatory effects. Recent evidence suggests that methylguanidine significantly inhibits nitric oxide synthase activity and tumor-necrosis factor (TNF) release [24]. This suggests that methylguanidine can attenuate the degree of inflammation and tissue damage arelated to endotoxic shock.

IV. CONCLUSION

Nineteen compounds have been identified from the GC-MS analysis of ethanol extracts of *S. americana* leaves. The bioactive compounds support the use of *S. americana* leaves in the management of diseases like pancreatic cancer, prostate cancer, asthma, allergic rhinitis, and osteoarthritis

REFERENCES

- [1] Available [Online] at: <https://en.wikipedia.org/wiki/Solanum>. Accessed 22 Aug 2017
- [2] Yokose, T., Katamoto, K., Park, S., Matsuura, H. and Yoshihara, T. (2004). Anti-Fungal Sesquiterpenoid from the Root Exudate of *Solanum abutiloides* Biosci. Biotechnol. Biochem., Vol. 68, 2640–2642.
- [3] Silva, T.M.S., Costa, R.A., Oliveira, E.J. and Barbosa, F.J.M. (2005). 3-Aminofurostane Alkaloids from *Solanum paniculatum* ("Jurubeba Verdadeira") Roots. J. Braz. Chem. Soc. Vol. 16, 1467–1471.
- [4] Available [Online] at: https://en.wikipedia.org/wiki/Solanum_americanum. Accessed 22 Aug 2017
- [5] Silva, E.L., Almeida, L.R.C., Borges, R.M. and Staerk, D. (2017). Dual high-resolution inhibition profiling and HPLC-HRMS-SPE-NMR analysis for identification of α -glucosidase and radical scavenging inhibitors in *Solanum*, Fitoterapia., Vol.118, 42–48.
- [6] Fidrianny, I., Rizkiya, A. and Ruslan, K. J. (2015). Antioxidant activities of various fruit extracts from three *solanum* sp. using DPPH and ABTS method and correlation with phenolic, flavonoid and carotenoid .,Chem. Pharm. Res. Vol. 7, 666–672.
- [7] Ibarrola, D.A., Ibarrola, M.C.H., Montalbetti, Y., Heinichen, O. and Campuzano, M.A.(2011). Cardiovascular Action of Nuatigenosido from *Solanum sisymbriifolium*., Phytomedicine. Vol. 18, 634–640.
- [8] Yen, C.T., Lee, C.L., Chang, F.R., Hwang, T.L., Yen, H.F., Chen, C.J. and Chen, S.L.(2012). Indiosides G–K: Steroidal Glycosides with Cytotoxic and Anti-inflammatory Activities from *Solanum violaceum*, J. Nat. Prod., Vol. 75, pp. 636–643.
- [9] Challal, S., Buenafe, O.E.M., Queiroz, E.F., Maljevic, S., Marcourt, L., Bock, M. and Chem, A.C.S.(2014). Zebrafish Bioassay-Guided Microfractionation Identifies Anticonvulsant Steroid Glycosides from the Philippine Medicinal Plant *Solanum torvum*, Neuroscience. Vol. 5, 993–1004.
- [10] Igwe, K.K., Nwankwo, P.O., Otuokere, I.E., Chika, I. and Amaku, F.J (2016): Studies on the medicinal plant *Acalypha wilkesiana* ethanol extracts phytocomponents by GC-MS analysis, Global Journal of Science Frontier Research, Vol. 16(2), 48–55.
- [11] Ikpeazu, O.V., Otuokere, I.E. and Igwe, K.K. (2020). GC–MS Analysis of Bioactive Compounds Present in Ethanol Extract of *Combretum hispidum* (Laws) (Combretaceae) leaves, International Journal of Trend in Scientific Research and Development, Vol. 4(5), 307 - 313
- [12] Otuokere, I.E., Okorie, D.O., Igwe K.K. and Mathew. U.J. (2016). GCMS Determinatiopn of Bioactive Phytocompounds in *Chromolaena odorata* leaf extract. Int J of Advances in Engineering Tech and Sci. Vol. 2(3), 7-11.
- [13] Abhishek, R.U., Thippeswamy, S., Manjunath, K. and Mohana, D.C.(2015). Antifungal and antimycotoxigenic potency of *Solanum torvum* Swartz. leaf extract: isolation and identification of compound active against mycotoxigenic strains of *Aspergillus*, J. Appl. Microbiol. Vol.119, 1624–1636.
- [14] Tiwari, P., Dwivedi, S., Singh, M.P., Mishra, R. and Chandy, A. (2012). "Basic and modern concepts on cholinergic receptor: A review". *Asian Pacific Journal of Tropical Disease*. Vol. 3 (5), 413–420. doi:10.1016/S2222-1808(13)60094-8.
- [15] David, L. N. and Michael, M. C. (2008). *Lehninger's Principles of Biochemistry*, Fifth Edition. Freeman, W.H. and Co., 359.
- [16] Laufer, S (2003). "Role of eicosanoids in structural degradation in osteoarthritis". *Curr Opin Rheumatol*. Vol.15 (5), 623–627. doi:10.1097/00002281-200309000-00017.
- [17] Holtz, P., Heise, R. and Ludtke, K. (1938). Fermentativer Abbau von L-Dioxyphenylalanin (Dopa) durch Niere. Naunyn Schmiedeberg's Arch exp Path Pharmak. Vol. 191, 87–118.
- [18] Birkmayer, W. and Hornykiewicz, O. (1961). The L-3,4-dioxyphenylalanine (DOPA)-effect in Parkinson-akinesia.. *Wien Klin Wochenschr*, Vol. 73, 787–788.

- [19] Cotzias, G.C., Van Woert, M.H. and Schiffé, L.M. (1967). Aromatic amino acids and modification of Parkinsonism. *N Engl J Med*, Vol. 276, 374–379.
- [20] Yahr, M.D., Duvoisin, R.C., Shear, M.J., Barrett, R.E. and Hoehn, M.M. (1969) Treatment of Parkinsonism with levodopa. *Arch Neurol*, Vol. 21, 343–354.
- [21] Duke, J.A. (1992). Handbook of phytochemical constituents of GRAS herbs and other economic plants. Boca Raton, FL. CRC Press.
- [22] Connolly, B.S. and Lang. A.E. (2014). Pharmacological treatment of Parkinson disease: a Review. *JAMA*. Vol. 311(16) pp. 1670-1683.
- [23] Hyde, C.A.C. and Missailidis, S. (2009). Inhibition of arachidonic acid metabolism and its implication on cell proliferation and tumour-angiogenesis, *International Immunopharmacology*, Vol. 9 (6), 701-715.
- [24] National library of Medicine (2020). National Center for Biotechnology Information. PubChem Database, Methylguanidine. Available at <https://pubchem.ncbi.nlm.nih.gov/compound/methylguanidine>.