

Chemical Information from GCMS of Ethanol Extract of *Solanum melongena* (Aubergine) Leaf

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Abstract *Aubergine is often regarded as a local vegetable that is rarely consumed in Nigeria and other countries of the world. However, based on some nutritional data reported for this plant, the present study is aimed at investigating the phytochemical constituent of ethanol extract of this vegetable using GCMS. The results obtained indicated that ethanol extract of aubergine contains 1,1,3-trimethyl-cyclopentane (1.66%), alpha-undecane(2.21%),3,5-di-t-butylphenol(4.42%),6-methyl-1-heptanol (1.38%), dimethylmalononitrile (0.28%), (5Z)-9-methyl-5-undecene (1.10%),metholene 2216 (11.60%), n-hexadecanoic acid (4.97%), ethyl stearate (2.21%), methyl linolelaidate (17.96%), methyl ester 9-octadecanoic acid (18.78%), 13Z)-13-octadecenal (8.29%), trans-3-oxabicyclo[4,4,0] decane -(6.63%), 5-methyl-1,2,3,6-tetrahydropyridazine (1.66%), oleamide (2.49%), E-9-tetradecenal (5.52%) and 2,6-dimethyl-1,5-heptadiene (8.84%). Most of the detected phytochemicals have several biological activities such as antimicrobial, anticancer, antiepileptic and other activities. Some were found to have some industrial roles indicating that much can be derived from this vegetable than it is currently known.*

Key Words: *Solanum melongena leaf ethanol extract, phytochemicals, biological activity, application*

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1.0 Introduction

Edible plants are known for their nutritional value (which can be assessed based on detail proximate, vitamins, mineral and toxicant contents (Eddy and

Udoh, 2005). However, much research attention is paid on proximate, mineral and vitamin based nutritional requirement than on the usefulness of phytochemicals on the health and maintenance of the human system and other applications (Eddy *et al.*, 2004). Phytochemicals are products of primary and secondary metabolism in plants. Most of them have biological activities and are useful for several pharmaceutical or medicinal formulations (Aikoye, 2020; Abdulazeez *et al.*, 2020; Ikpeazu *et al.*, 2020). Literature is scanty on phytochemicals content of *Solanum melongena L.* However, Sohani *et al.* (2019) stated that the major phytochemicals in the plant are flavonoids, tropane, glycoalkaloids, arginine, lanosterol, gramisterol and aspartic acid and has also link their presence to several biological activity including spasmogenic activity, lowering of intraocular pressure, antiplatelet and calcium blocking activities, hypolipidemic action, hepatoactivity, cardiac activity, antipyretic activity and possess analgesic, antidiabetic activity and is useful in some lung problems. Similar phytochemical constituents were highlighted by Eddy *et al.* (2010) and he attributed corrosion inhibition potential of the plant to the presence of this phytochemicals. Fidrianny *et al.* (2017) found that the different organs of aubergine plants have different biological activity due to variation in concentration of phytochemical and solvent choice. They reported that the lowest IC50 of DPPH scavenging activity 1.14 µg/ml and the lowest EC50 of FRAP capacity 49.80 µg/ml was given by ethanolic leaves extract of eggplant. Ethanolic leaves extract of egg plant also presented the highest total phenolic content (TPC) (8.87 g gallic acid equivalent/100 g), while the highest total flavonoid content was shown by ethyl acetate leaves extract (24.50 g quercetin equivalent/100 g). Anbuseivi *et al.* (2019) used GCMS to analyse nasunin extracted from coloured egg plant peels.

GCMS instrument is a useful instrument for investigating phytochemicals in plants (Eddy *et al.*, 2009, 2011). However, the use of this instrument for analysis of phytochemicals in Aubergine leaf has not been extensively reported. However, Macleod * De Toconis (1983) use GC to analyse volatile aroma in the leaf of aubergine and found the presence of hydrocarbon (which constituted 70% of the samples and included 20 acyclic alkanes) and low concentration of total volatiles (*ca* 2.4 µg/kg). Vanitha *et al.* (2016) used GCMS to evaluate component of leaf and salt stress callus of eggplant and obtained several phytochemicals constituent. The present study is aimed at applying GCMS to analysed identified phytochemicals in ethanol extract of *Solanum melongena* leaf.

2.0 Materials and Methods

Aubergine leaves were obtained from a garden in Ikot Ekpene and were washed with distilled water and allowed to dry to constant weight under the sun. The dried leaves reduced to a powder form and soaked in ethanol (Eddy *et al.*, 2011b; Eddy and Odiongenyi, 2010). The mixed solvent was recovered using cold extractor, leaving behind, acetone/ethanol extract of *Piper guineense* leaves.

The produced extract was used for GCMS analysis using spectroscopically pure acetone solvent (Eddy *et al.*, 2011b). The GCMS-QP2010 PLUS Shimadzu (made in Japan) instrument was used for the analysis. The analytical steps taken were plunger speed (high), syringe injection speed (high), viscosity/compression time (0.2 second), injection mode (normal), pumping time (5), injection port dwell time (0.3 second), terminated air cap (No), plunger washing speed (high), washing volume (8µl), syringe suction position (0), syringe injection position (0) and used three solvent vial (3). The operational setting of the GCMS instrument were column oven temperature (60°C), injection temperature (200°C), injection mode (split), flow control mode (linear velocity), pressure (100.2 kPa), total flow (6.2 ml/minute), linear velocity (46.3 cm/sec), purge flow (3.0ml/min) and split ratio (1.0). The high-pressure injection, carrier gas server and splitter hold functions were switch off. The initial rate of oven temperature program was 5 °C/min and was gradually increased to 140°C after which the temperature was increased to 280 °C at a rate of 10 °C/minute. Some heat unit and detector functions were checked in order to ensure

consistency. These included column oven, SPL2, MS, SPL2 carrier, SPL2 purge and were ensured to be on. However, the APC setting was turned off.

Other setting functions of the machine were ion source temperature (200 °C), interface temperature (250 °C), solvent cut time ((2.50 minutes), detector gain mode (relative), detector gain (0.00kV), threshold (1000). The analytical start time was 3 minutes and the machine run for 45 minutes using ACQ scan mode at a scan speed of 769. However, mass/charge started at 50 and ended with 400 units. Gas chromatogram and mass spectrum were automatically plotted and suggested chemical structures were obtained using the National Science Technology library installed in the machine. Percentage concentrations of each identified component was calculated using area normalization

3.0 Results and Discussion

Fig. 1 shows the GCMS spectrum of ethanol extract of aubergine. The identity of phytochemicals in ethanol extract of aubergine are recorded in Table 1. 1, 1, 3-trimethylcyclohexane (CAS:4516-69-2) was identified in peak 1 under retention time of 14 minutes and base peak of 56 while the molecular ion has m/z value of 97. The compound produces carbon (IV) oxide and water after complete combustion but cannot be hydrolysed because it does not contain a hydrolysable functional group. It is a useful intermediate for organic synthesis and is a major component of gasoline (Montgomery, 2007). Ahmad *et al.* (2018) also identified 1,1,3-trimethylcyclopentane in the hexane extract of *Garcinia antroviridis* root. It is constituent of typical crude oil hydrocarbon mixture (Auria *et al.*, 2008; Purewal, 2012). Zubair *et al.* (2013) also found this hydrocarbon in hexane extract of *Bambusa arundinaceae* leaves which exhibited antimicrobial and haemolytic functions. Mata *et al.* (2018) detected the compound in essential oil of *Anacardium occidentale* L. Fermentation products of *Eichhornia crassipes*, *Pistia stratiotes* and *Salvinia molest* weeds were also found to contain 1, 1, 3-trimethylcyclopentane. The plant seems to have hope for biofuel production.

1-Undecene (CAS Number: 821-95-4) was found in peak 2 with characteristics retention time and base peak values of 19.433 minutes and 56 respectively. The molecular ion was found at m/z value of 111. The compound is unsaturated aliphatic hydrocarbon and is an example of hydrocarbon lipid molecule



that is hydrophobic, insoluble in water and relatively neutral. Alpha undecene is a known plant metabolite. Hunziker *et al.* (2015) reported that 1-undecene is produced by strains inducing volatile-mediated *P. infestans* growth inhibition and that when it was supplied to *P. infestans* it significantly reduced mycelial growth, sporangium formation, germination, and zoospore release in a dose-dependent manner. Hunziker *et al.* (2015) and Guevara-Avenidaño *et al.* (2019) observed that 1-undecene has strong antifungal activities and is the main active compound released by *Pseudomonas fluorescens*. Kong *et al.* (2020) also observed that 1-undecene is the most abundant volatile in strain ST-

TJ4. The antifungal activity of 1-undecene against *R. solani* AG-1(IA), has also been reported by Tagele *et al.* (2019). Zhou *et al.* (2014) also found that 1-undecene has some antibacterial and antifungal activity. The presence of 1-undecene in the flower, leaf and stem of *Senecio pandurifolus* and associated microbial activity have been confirmed by Kahrman *et al.* (2011). It is also a major volatile compound in *Ballota nigra subsp uncinata* (Rigano *et al.* (2020). 1-undecene is a known biomarker for identification of *Pseudomonas aeruginosa* strain.

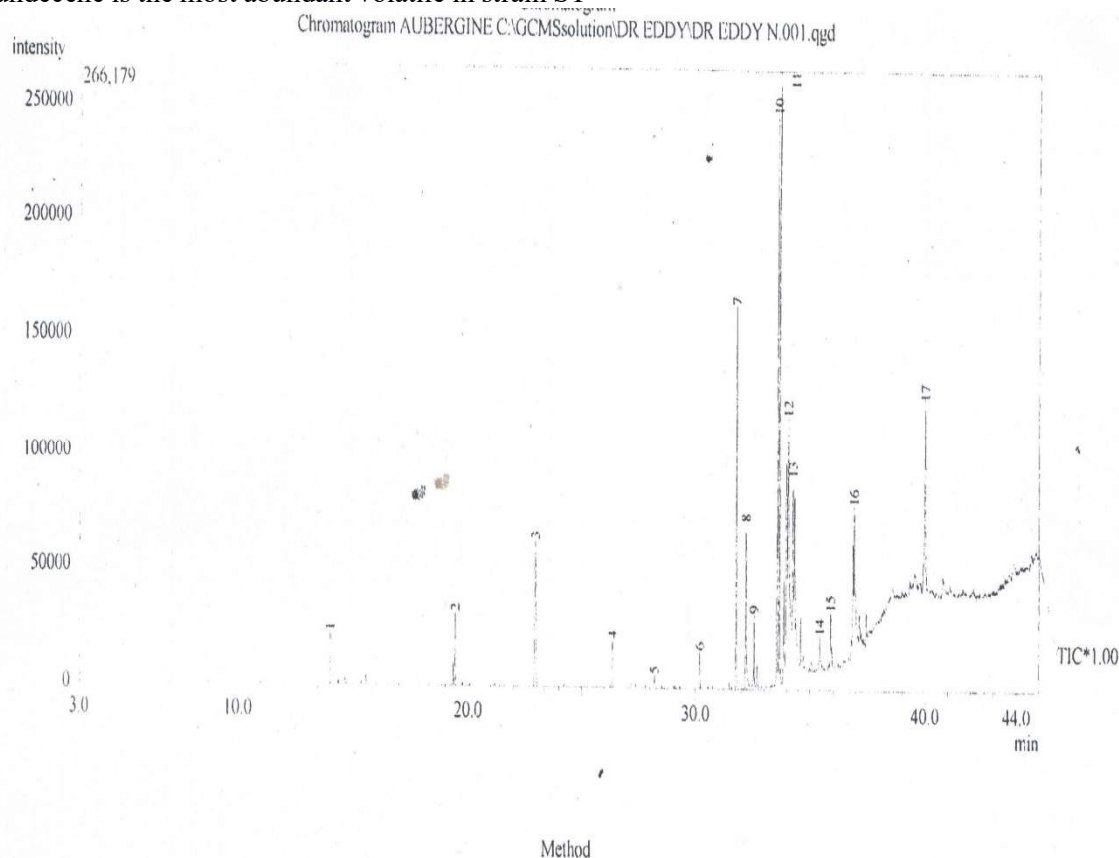


Fig. 1: GCMS spectrum of *Solanum melongena* leaf

Table 1: Phytochemicals in ethanol extract of *Solanum melongena* fruit

| Peak | Name of Compound | Retention time (min) | Base peak | Molar mass | Molecular ion peak | %C |
|------|-----------------------------|----------------------|-----------|------------|--------------------|------|
| 1 | 1,1,3-trimethylcyclopentane | 14.00 | 56.00 | 112.21 | 97 | 1.66 |
| 2 | Alpha undecene | 19.433 | 55.00 | 154 | 111 | 2.21 |
| 3 | 3,5-di-t-butylphenol | 22.942 | 57.05 | 206 | 206 | 4.42 |
| 4 | 6-methyl-1-heptanol | 26.350 | 55.00 | 112 | 97 | 1.38 |
| 5 | Dimethylmalononitrile | 28.200 | 93.10 | 94.11 | 93 | 0.28 |



| | | | | | | |
|----|---|--------|-------|--------|-----|-------|
| 6 | (5Z)-9-methyl-5-undecene | 30.183 | 57.05 | 168.32 | 97 | 1.10 |
| 7 | Metholene 2216 (methyl ester hexadecenoic acid) | 31.775 | 74.05 | 270 | 270 | 11.60 |
| 8 | n-hexadecanoic acid | 32.208 | 60.00 | 256 | 256 | 4.97 |
| 9 | Radia 7185 (ethyl stearate/ethyl ester octadecanoic acid) | 32.567 | 88.10 | 312 | 101 | 2.21 |
| 10 | Methyl linolelaidate | 33.575 | 67.05 | 294 | 294 | 17.96 |
| 11 | Methyl ester 9-octadecanoic acid (methyl ester elaidic acid) | 33.658 | 55.00 | 296 | 264 | 18.78 |
| 12 | (13Z)-13-octadecenal | 34.04 | 55.00 | 266.50 | 97 | 8.29 |
| 13 | Trans-3-oxabicyclo[4,4,0]decane | 34.242 | 67.00 | 140.22 | 109 | 6.63 |
| 14 | 5-methyl-1,2,3,6-tetrahydropyrazine | 35.417 | 98.15 | 98.15 | 98 | 1.66 |
| 15 | Crodamide O (armoslip CP/oleyl amide/adogen 77/oleic acid amide/9-octadecamide) | 35.892 | 59.00 | 281.10 | 97 | 2.49 |
| 16 | E-9-tetradecenal | 36.908 | 55.00 | 210 | 129 | 5.52 |
| 17 | 2,6-dimethyl-1,5-heptadiene (skvalen/spinacene/supraene) | 39.98 | 60.05 | 124.22 | 121 | 8.84 |

3,5-di-*t*-butylphenol (CAS Number: 1138-52-9) was identified in peak 3 at retention time, base peak and molecular ion values of 22.942 minutes, 57.05 and 206 respectively. This compound has also been identified by Aikoye (2020) in ethanol extract of *Chromolaena odorata* leaf. 3,5-DTBP has also been detected in flowers of *Aesculus chinensis* (Gao *et al.*, 2018), fungal *Coriolus versicolor* (Yuan *et al.*, 2019), *Aquilaria sinensis* (Lour.) Gilg (Meiw *et al.*, 2007), whole plants of *Hedyotis lancea* Thunb. Pan *et al.*, 2012), and seeds of *Plukenetia volubilis* L. (Chen *et al.*, (2018). Rathma *et al.* (2016) reported that 3, 5-di-*t*-butylphenol exhibited anti-biofilm and conventional fungicidal activity against *Candida* species and elucidate the underlying mechanisms. *Tert*-butylphenol compounds produced from the culture of *Paenibacillus odorifer*, a bacterial strain associated with the crustose lichen, *Rhizocarpon geographicum*, were reported to exhibit significant cytotoxicity against B16 murine melanoma and HaCaT human keratinocyte cell lines with micromolar half maximal inhibitory concentration (IC₅₀) values. Generally, it has been found that most heterocyclic compounds containing di-*tert*-butyl phenol display various types of biological activity in addition to their antioxidant ability (Ziaka *et al.*, 2006; Yehye *et al.*, 2012).

6-methyl-1-heptanol (CAS number, 26952-21-6) also called isooctyl alcohol was detected in peak 4 with retention time of 26.350 minutes, base peak of 55.00 and molecular ion of 97. This compound was

also identified by Aikoye (2020) in ethanol extract of *Chromolaena odorata*. 6-methyl-1-heptanol is a primary alcohol and a volatile organic compound. It is a preferred solvent in the making of cutting and lubricating oils, in hydraulic fluids, and in the production of other chemicals. 6-methylheptan-1-ol is a primary alcohol in which the heptane is substituted by a *methyl* group at position 6 and a hydroxy group at position 1. It has a role as a mammalian metabolite. Okwu and Ighodaro (2009) has also identified this compound in the stem bark of *Dacryodes edulis* G. and reported its effectiveness against antibacterial activity. Antimicrobial activity of 6-methyl-1-heptanol has been identified (McDonnell *et al.* 1999; Tanner and Wilson, 1943). Isooctyl alcohol is useful as plasticizers, intermediate for nonionic detergents and surfactants, hydraulic fluid, resin, solvents, emulsifier, antifoam, in coating, intermediate to introduce isooctyl group, froth flotation foam modifier, and as cosmetic ingredients (Ash, 2004). Peak 5 showed evidence for the presence of dimethylmalononitrile (CAS Number: 7321-55-3) with retention time, mass peak and molecular ion *m/z* at 28.200 minutes, 93.10 and 93 respectively. Other names for the compound are DMMN; 2,2-dimethylmalononitrile; dimethylpropanedinitrile; 2,2-dimethylpropanedinitrile; propanedinitrile; 2,2-dicyanopropane; dimethyldicyanomethane. It is a useful reagent for transnitration with aryl nucleophiles and for the synthesis of heterocycles



(Luescher, 2019). It is also a useful intermediate for synthesis of aryl nitrile. According to Reeves et al. (2015), transnitration with DMMN is unique because it avoids the use of toxic reagents and transition metals and occurs under mild reaction conditions, even for extremely sterically hindered substrates. Li et al. (2020) have reported the use of DMMN as a cyanating reagent for the Rh(I)-catalyzed aromatic C-H cyanation with dimethylmalononitrile. Mills *et al.* (2016) also stated that malononitriles are valuable synthetic intermediates for many applications, including the synthesis of herbicides and other biologically active molecules, and the synthesis of chiral ligands for asymmetric catalysis. Line 6 indicated the presence of (5Z)-9-methyl-5-undecene (also known as 5-Undecene, 9-methyl-, (Z)-, (Z)-9-Methyl-5-undecene, (5Z)-9-Methyl-5-undecene) with CAS number, 74630-65-2. The compound was identified with GC retention time of 30.183 minutes, mass peak and molecular ion peak of 57.05 and 97 respectively. Literature is scanty on bioactivity and uses of (Z)-9-Methyl-5-undecene. However it has been reported to be an active and hydrocarbon constituent of sesame and olive oils (Cheseto *et al.*, 2020). Aikoye (2020) also reported the presence of (5Z)-9-methyl-5-undecene in ethanol extract of *Chromolaena odorata* leaf. Also some of its isomers including 6-methyl-2-undecene and 7-methyl-3-undecene have been found in plants that exhibit good antimicrobial activity (Bhardwaj, 2018). Therefore, these components may also exhibit biological activities against some microorganism. Metholene was observed at peak 7 at retention time of 31.775 minutes, base and molecular ion peaks of 74.05 and 270 respectively. Aikoye (2020) isolated this compound in ethanol extract of *Chromolaena odorata* leaf at a retention time of 31.758 minutes, which is very close to the present value. In the leaves of *Sesuvium portulacastrum L.*, Its present accounted for strong antibacterial and antifungal properties (Chandrasekaran *et al.*, 2011, 2008). Lima *et al.* (2011) and Canales *et al.* (2011) also stated that this compound has significant antibacterial and antifungal capacities. Suresh *et al.* (2014) also confirmed the antibacterial activity of this compound through a study on target algal species. Hexadecanoic acid was detected in peak 8 at retention time, base peak and molecular ion peak of 32.208 minutes, 60 and 256 respectively. Similar compound has been detected by Chibuzo and Okop

(2020) at retention time of 32.283 minutes in acetone extract of *Piper guineense* leaf and in acetone-ethanol extract of the same plant leaf at a retention time of 31.725 minutes (Chibuzo and Aikoye, 2020). Hexadecanoic acid that has been confirmed to have potential antioxidant, antitumor, antiinflammatory, antibacterial and antifungal activities (Vasudevan *et al.*, 2012).

Radia 7185 (also known as stearic acid, ethyl ester; ethyl n-octadecanoate; ethyl octadecanoate; ethyl stearate; ethyl ocatadecanoate; ethyl octadecanoate (ethyl stearate with CAS number: 111-61-5) was identified in peak 9 at retention time of 32.567 minutes. Ethyl stearate has been found to display antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* when isolated from the Indonesian edible oil. It is reported to be a major phytochemical in hexane and chloroform extracts of extracts of *Neilamarcjia cadamba* leaf (Zayed *et al.*, 2014). It was also identified by Kim *et al.* (2020) in volatile organic compounds in *Coreopsis cultivars*. Lazarevic *et al.* (2010) also detected the compound in *Stachys* species and acknowledge its contribution to the antimicrobial and antioxidative activities.

In line 10, methyl linolelaidate (CAS Number: 2566-97-4) was identified. The compound displayed molecular m/z value of 294 and was identified at retention time of 33.575 minutes while the base peak value was 67.05. Khiralla, *et al.* (2020) detected methyl linolelaidate as one of the components of fractions in endophytic fungus *Curvularia papendorfi* and evaluated and found the organism to have strong antiviral, antibacterial and antiproliferative activities. Methyl linolelaidate (25.22 % and RT 18.247) was detected as one of the major metabolites in *Huru crepitans* bark ethanol extract with potential to be a urinary acidifier, inhibitor of uric acid production, antibacterial, prevents inflammation and vasodilatation, displayed anti 5HT (Serotonin), anti HIV integrase activity as well as antidote activity for heavy metals poisoning. Agarwal *et al.* (2017) also detected the compound in methanol extract of *Quisqualis indica* plant.

Methyl ester elaidic acid was the compound detected in peak 11 under base peak of 55 and retention time of 33.658 minutes. The compound is an esterified form of elaidic acid (CAS number. 90250) that has been found in biodiesel produced by the microalga *Botryococcus* (Ashokkumar *et al.*, 2014), in Iranian olive fruit oil (Konoz *et al.*, 2015)



and in certain cultivars of *O. sativa* black rice bran ((Arjinajarn *et al.* 2016). Studies conducted on fatty acids of methyl ester indicated that they generally exhibit strong antimicrobial, and antifungal activities but mild anticandidal activity.

(13Z)-13-octadecenal (CAS Number: 58594-45-9) was the compound of interest in peak 12 of the GCMS spectrum. The retention time, base peak and molecular ion peak that were associated with these peaks were 34.04 minutes, 55 and 97 respectively. Strong biological activities have been reported for aqueous and ethanolic extracts of the leaf and stem bark of *Psorospermum febrifugum* and *Harungana madagascariensis* by Asogwa (2017). Both aqueous and ethanol extracts were rich in (13Z)-13-octadecenal. European Food Safety Authority (2014) classified (13Z)-13-octadecenal as an active compound against pesticides activity.

Trans-3-oxabicyclo[4,4,0]decane was found in peak 13 of the GCMS spectrum and exhibited retention time of 34.417 minutes, base peak at 67 and molecular ion at 109. Nwafor *et al.* (2015) identified this compound in Hydromethanolic Chloroform Extract of *Xylopia Aethiopica* (Dunal) A. Rich (Annonaceae) Fruits. Ajayi *et al.* (2016) also detected the presence of this compound in essential oil from *Cymbopogon citratus* leaves. Literature is scanty on the bioactivity or applications of trans-3-oxabicyclo[4,4,0]decane indicating that research into the usefulness of this components is widely open for investigation.

5-methyl-1,2,3,6-tetrahydropyrazine was observed in peak 14 at retention time of 35.417. The base ion, molecular ion and molar mass were similar indicating that the ion pass through the detector without fragmentation. Mohan and Krishna (2019) detected 5-methyl-1,2,3,6-tetrahydropyrazine as a phytochemical constituents of *Michelia nilagirica* leaves that exhibited strong anti-inflammatory activity. 2,3,5,6-tetramethylpyrazine (commonly called ligustrazine) is an essential alkaloid that can be derived from 5-methyl-1,2,3,6-tetrahydropyrazine by methylation. The resulting compound has strong antioxidant and, anti-inflammatory activities in addition to neuroprotection ability. Lin (2009) also stated that tetramethyl pyrazine works as antithrombotic agent, antagonist of vasoconstriction and anti-inflammatory compound.

In line 15, crodamide O whose CAS Number is 301-02-0 (also called oleamide, armoslip CP, oleyl amide, adogen 77, oleic acid amide or 9-octadecamide). It is an amide of the oleic acid, a fatty acid and is a known endogenous substance that occurs naturally in the body of animals. It accumulates in the cerebrospinal fluid during sleep deprivation and induces sleep in animals. Researches are ongoing on the use of this compound for medical treatment of mood and sleep disorders, and cannabinoid-regulated depression. The mechanism of action of oleamide in inducing sleep is currently receiving research attention but it is assumed that oleamide interacts with multiple neurotransmitter systems. Oleamide has the ability to bind to the CB1 receptor as a full agonist. Nazeam *et al.* (2018) detected oleamide in active seed extract of *Portulaca oleracea* L and biological activities of the plant's seed was found to possess diuretic properties, antiscorbutic and ability to act as an aperient. Mikautadze *et al.* (2008) isolated oleamide from *Aquilegia vulgaris* and found that oleamide has strong antiepileptic activity.

6E-9-tetradecenal was identified in peak 16 at a retention time of 36.908 minutes, base peak of 55 and molecular ion peak of 129. 9-tetradecenal is a fatty aldehydes because it is a long chain aldehydes with a chain exceeding 12 carbon atoms. It is insoluble in water and a very weak acid. According to Zhang *et al.* (2012), (Z)-9-tetradecenal is a potent sex pheromone in *Helicoverpa armigera*. Boo *et al.* (1995) also reported that the compound is a potent inhibitor of pheromone-mediated communication in the oriental tobacco budworm moth, *Helicoverpa assulta*.

2,6-dimethyl-1,5-heptadiene was observed in peak 17 with associated retention time, base peak and molecular ion peak values of 39.98, 60.05 and 121 respectively. The compound is also known as 2,6-dimethylhepta-1,5-diene, 2,6-dimethyl-1,5-heptadiene or geraniolene. Literature is scanty on the application or biological activities of 2,6-dimethyl-1,5-heptadiene, thus creating a vacuum that should be filled by research.

4.0 Conclusion

Aubergine is a vegetable that has significant nutritional value and also contain phytochemicals that have several pharmaceutical, industrial and medicinal values. It also contain some compounds that have not been deeply researched.



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