Free Radical Scavenging Activity and GC-MS Analysis of Solvent Extracts of *Lamium flexuosum* (TEN)

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Abstract— *Lamium flexuosum* has a potential of medicinal property and it is used in the treatment of malaria by rural dwellers. The aim of this research is to evaluates the free radical scavenging activity of a plant extract, a few simple steps using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. The antioxidant activity of hexane, ethyl acetate, methanol and water extracts of *Lamium flexuosum* was measured using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. The highest antioxidant activity was found to be in hexane extract (HCE) followed by methanol extract (MCE) then the ethyl acetate extract (EaCE) with percent radical scavenging activity of 72%, 56%, 60%, respectively. The GC-MS analysis of the MCE and EaCE fractions revealed the presence of Hexadecanoic acid methyl ester and Octadecanoic acid methyl ester (methyl stearate) and respectively. These compounds might be responsible for its free radical scavenging activities as well as the anti-malarial activity of the plant.

Keywords: GC-MS, Lamium Flexuosum (TEN)

I. INTRODUCTION

Free radicals are highly reactive and unstable compounds produced in the body during normal metabolic functions or introduced from the external environment such as pollution and cigarette smoke. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in cells by different means [15]. Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides. ROS and RNS may also cause DNA damage that could lead to mutation. All aerobic organisms, including humans, have antioxidant defenses that protect against oxidative damage [15]. However, these natural antioxidant mechanisms can be inefficient, and hence dietary intake of antioxidant compounds becomes important. Herbal therapy has been widely used to treat a large variety of various ailments and symptoms, and there is a recently growing interest on the herbal therapy in the world. It has also been believed that medicinal plants are an important source of alternative chemical reagents with potential therapeutic effects [3, 31]. In this context, plant-derived antioxidant compounds are getting as more importance for prevention of free radical formation, DNA mutation and lipid peroxidation. Moreover the plant-derived compounds such as phenolic acids, phenolic diterpenes, flavonoids and other important secondary metabolites are known to have strong antioxidant properties which can be used as alternative synthetic antioxidant products for these free radicals mediated pathogenesis [3, 34] Human bodies are protected from oxidative damage of free radicals through some complex defense systems which are called antioxidants. Antioxidant works to maintain the oxidant at optimum level and to reduce free radical, stopping it from forming before it can disrupt living cells in our body. However, excessive oxidants or free radicals can cause cell damage and lead to chronic diseases.

Antioxidant properties of plants due to the presence of phytochemicals have gained much interest in pharmaceutical research [21]. One of the potential uses of plant-derived compounds is to be used as antioxidant agents [15, 2, 12]. It is well known that antioxidants may be useful in protecting from cancer and other mutation-related diseases. Antioxidants radicalize themselves to stabilize free radicals. These then stabilize the charge by delocalization of an electron in their aromatic ring [30]. An array of secondary metabolites like phenolics [18], flavonoids, carotenoids [11], steroids and thiol compounds [23] act as antioxidants. Antioxidants have found a promising place in food industry [13], cosmetics, antiaging products [10], healthcare [26] and pharmaceutical industry [22].The hypothesis is that the use of antioxidants that scavenge reactive oxygen species (ROS) provides biological resistance to free radicals, retards the process of aging, and decreases the risk of age-associated degenerative diseases, such as cancer, cardiovascular diseases, immune system decline, and brain dysfunction [31]. Significant antioxidant properties have been recorded with phytochemicals that are necessary for the reduction in the occurrence of many diseases. Many plants often contain substantial amounts of antioxidants such as ascorbic acid, β-carotene and α-tocophenol [7], carotenoids, flavonoids and tannins, etc., and thus can be utilized to scavenge the excess free radicals from human body (Rachh *et al*. 2011). Antioxidant vitamin may improve immune system functions and may even delay some of the effects of aging [5]. Although there are some synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are commonly used in processed foods, it has been reported that these compounds can have some side effects [24]. Free radicals generated in the body can be removed by body's own natural antioxidant defenses e.g. Glutathione, catalase, etc. However, endogenous antioxidant defense are not completely efficient. Therefore, dietary antioxidants are required to lessen the overall effect of antioxidant stress due to excessive free radicals occurring in our system [33]. More researchers have begun to formally study the health benefits of herbs and spices. In general, fresh herbs and spices are healthier and contain higher antioxidant levels compared to their processed counterparts [5]. Natural antioxidants tend to be safer and they also possess antiviral, anti-inflammatory, anticancer, antitumor and hepatoprotective properties [9,16]. Therefore, the evaluation of antioxidant activity of various plant extracts is considered as an important step in the identification of their ability to scavenge the free radicals [14]. The potent antioxidant nature of the active extracts makes them potential sources of chemo-preventive compounds or

compounds that can significantly prevent carcinogenesis and other diseases [1]. Ravishankara *et al*., (2002) investigated evaluation of antioxidant properties of root bark of Hemidesmus indicus R. Br. (Anantmul). Above investigation carried out using several *in vitro* and *ex vivo* models, methanolic extract of *H. indicus* root bark was found to scavenge superoxide radical, nitric oxide radical, hydroxyl radical and inhibited the lipid peroxidation. The antioxidant activity of the extract can be attributed to the presence of tannins and other phenolic compounds. The free radical scavenging property may be one of the mechanisms by which this compound is effective in traditional medicine. [19]

II. MATERIALS AND METHOD

A. Collection of Plant Material

The plant (*Lamium flexuosum*) was collected from Kasuwan Kaji Azare and it was identified by a Botanist at Faculty of Science, ATBU Bauchi. The whole of the plant sample (excluding the root) was washed to remove dust, cut in to small pieces and it was dried under shade for two weeks in controlled condition to avoid contaminants. The dried sample was pulverized to powder using mortar and pestle. The pulverized sample was then stored in well cleaned container for further usage.

B. Preparation of Samples

The powdered sample (100 g) was added to 800 ml of solvent (hexane). The mixture was made in a sterile flask and wrapped in aluminum foil to avoid evaporation and exposure to light for three days at room temperature. The flask was shaken three times in a day. After three days of soaking in a solvent, the extract was filtered through Whatman No. 1 filter paper and allowed to evaporate at room temperature. The liquid extract was further evaporated to dryness. The residue was dried, weighed and the process was repeated for ethyl acetate, methanol and distilled water respectively.

C. Antioxidant Evaluation

The DPPH scavenging activity of different extracts of *Lamium flexuosum* was measured according to the procedure described [8]. Radical scavenging activity of plant extracts against stable DPPHradical was determined spectrophotometrically. About 1 mL of each solution added to 1 mL of 0.004 % methanolic DPPH free radical solution. After 30 minutes, the absorbances were taken at 517 nm by a UV spectrophotometer (Hitachi-U-20) which was compared with the corresponding absorbance of standard ascorbic acid concentrations (100 µg/mL). Methanol was used to zero the spectrophotometer absorbance of the DPPH radical without antioxidants. The control was measured and special care was taken to minimize the loss of free radical activities of DPPH.

Radical scavenging activity was expressed as percentage inhibition and calculated using:

% Radical scavenging activity =

Blank absorbance – sample absorbance x100

Blank absorbance

D. GC-MS Analysis

GC-MS analysis of the extract was performed using a Thermo GC - Trace Ultra VER: 5.0, Thermo MS DSQ II employing the following conditions : column Elite -DB 35 - MS Capillary standard non - polar column , Helium (He) was used as a carrier gas at a constant flow of 1.0 ml/min and an injection volume of 1 µL was employed, the oven temperature was programmed from 700C raised to 2600C at 60C/min. Total running time was 37.49 Min. GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

III. RESULT AND DISCUSSION

A. The Anti-Oxidant Activity Assay

The results of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity or antioxidant activity of HCE, EaCE, MCE and WCE of the whole plant (*Lamium flexuosum*) excluding the root was carried out and the results are shown in the figure 1 below . The positive standard, Ascorbic acid was used. The highest total antioxidant activity was recorded with n-Hexane extract followed by methanol extract and the malarial pathogenesis was reported to be associated with free radicals formation and decrease of antioxidant level [32], this suggests that an increase in the body antioxidant can initiate the decrease in parasite number and severe infection at the long run. and ethyl acetate as it's in the study conducted by Jamuna *et al.* 2014 "the extracts of *H. radicata* had significant scavenging effects on the DPPH radical which was increase with increase in the concentration of the sample from 50-250 µg/mL. The lowest antioxidant activity was found with the water extract. Sumathy *et al.* 2013 reported that "A freshly prepared DPPH solution is of deep purple colour with absorption maxima at 517 nm and in the presence of antioxidant, this colour disappears due to quenching of DPPH free radicals and converting them into a colourless product 2,2-diphenyl-1-picryl hydrazine" Petroleum ether, chloroform and methanolic extracts of *M. oleifera* and standard ascorbic acid were found to be scavenger of DPPH radical with an IC_{50} of 124.75, 112.08, 54.34 and 13.86 µg/mL, respectively [29]. Methanolic extract was found to be good scavenger of DPPH radical. Kumbhare *et al.* 2012 reported that Petroleum ether, chloroform, ethyl acetate soluble fraction of methanolic extracts of *M. oleifera* and ascorbic acid were found to be scavenger of nitric oxide radical with an IC_{50} of 93.32, 65.12, 54.83 and 12.59 µg/mL, respectively. Ethyl acetate soluble fraction was found to be good scavenger of nitric oxide radical.

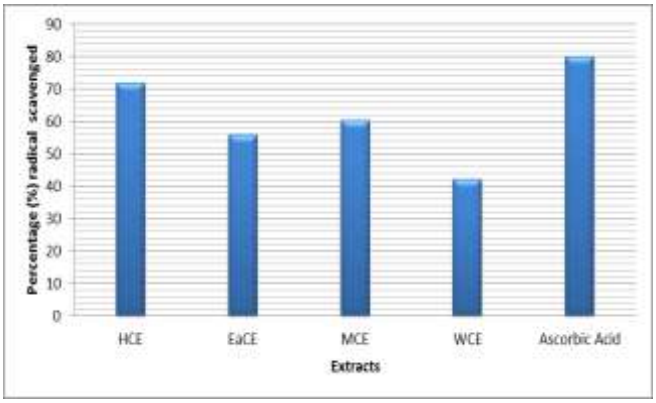

Fig. 1: The anti-oxidant Activity of the crude extract

B. GC- MS analysis

The main components identified by the GC-MS analysis of the fractions of EaCE and MCE of *Lamium flexuosum* are listed in Tables 1-2. The constituents were identified by comparison with the NIST database. The mass spectral data for the compounds whose molecular weight was observed at 270 M/Z (Table 1) and 298 M/Z (Table 2). The identified constituents showed 97% similarities to those in the database. Hexadecanoic acid methyl ester and Octadecanoic acid methyl ester (Methyl Stearate) has been identified as the components of MCE of *Lamium flexuosum.* The fragmentation patterns of the major constituents identified in the MCE of *Lamium flexuosum* are shown in plate 1 and 2.

Retention Time	Molecular Weight	Proposed Identity	Mass of Fragment Ions And Percentage Abundance
14.517	270	Hexadecanoic acid methyl ester	270 (10%), 239 (12%), 211 (2%), 183 (2%), 169 (2%), 155 (2%), 141 (7%), 127 (3%), 113 (2%), 99 (2%), 74 (100%), 55 (42%), 43 (85%)

Table 1: Mass Spectral Data of Hexadecanoic acid, methyl ester

Retention Time	Molecular Weight	Proposed Identity	Masses of Fragment Ions and Percentage Abundance
16.373	298	Octadecanoic acid methyl ester (methyl stearate)	298 (10%), 267 (4%), 255 (12%), 241 (2%), 237 (2%), 227 (2%), 199 (7%), 185 (5%), 157 (2%), 143 (10%), 115 (5%), 87 (69%), 74 (100%), 55 (51%), 43 (69%)

Table 2: Mass Spectral Data of Octadecanoic acid methyl ester (methyl stearate)

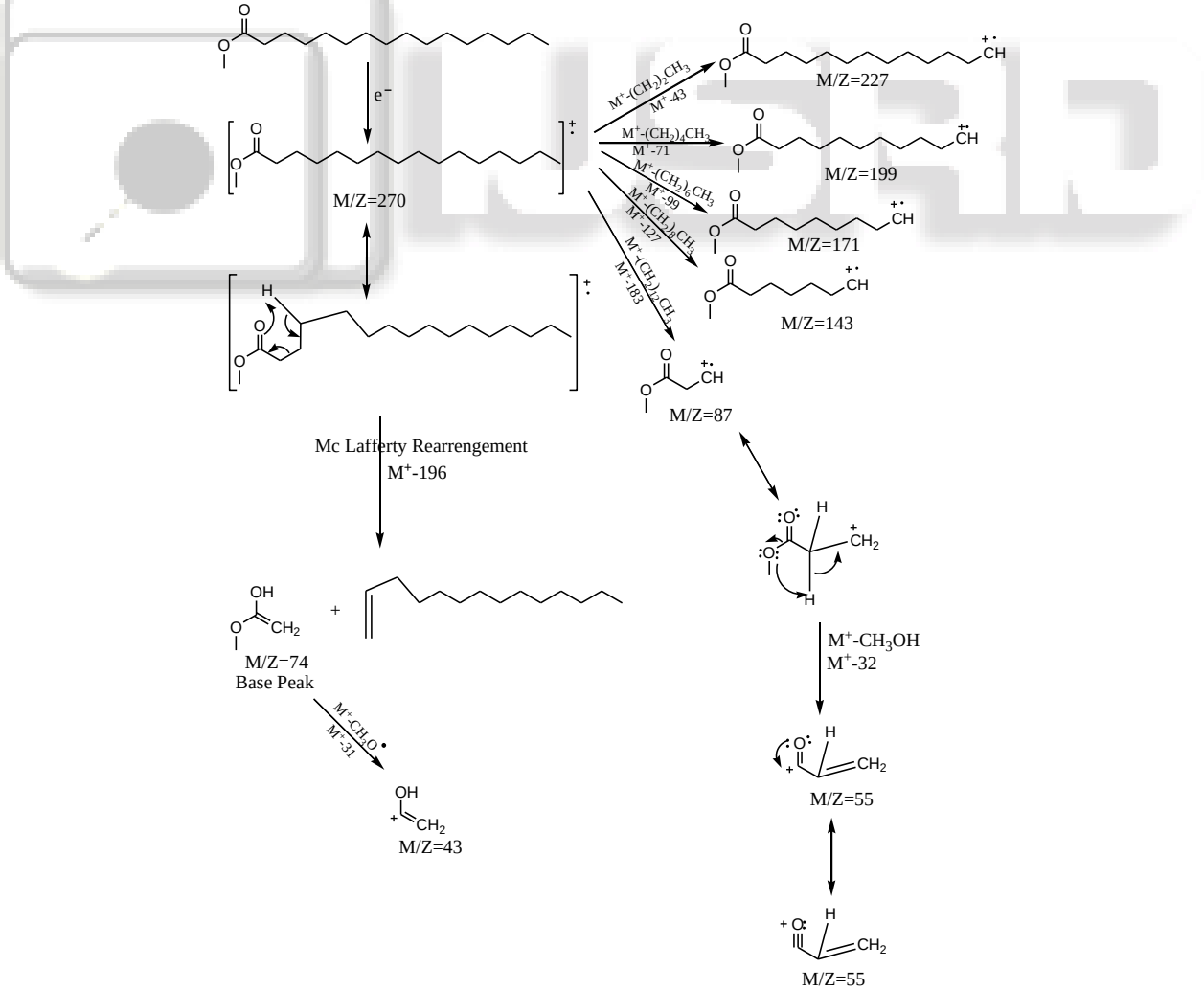

Plate 1 : Mass Fragmentation of Hexadecanoic Acid methyl ester

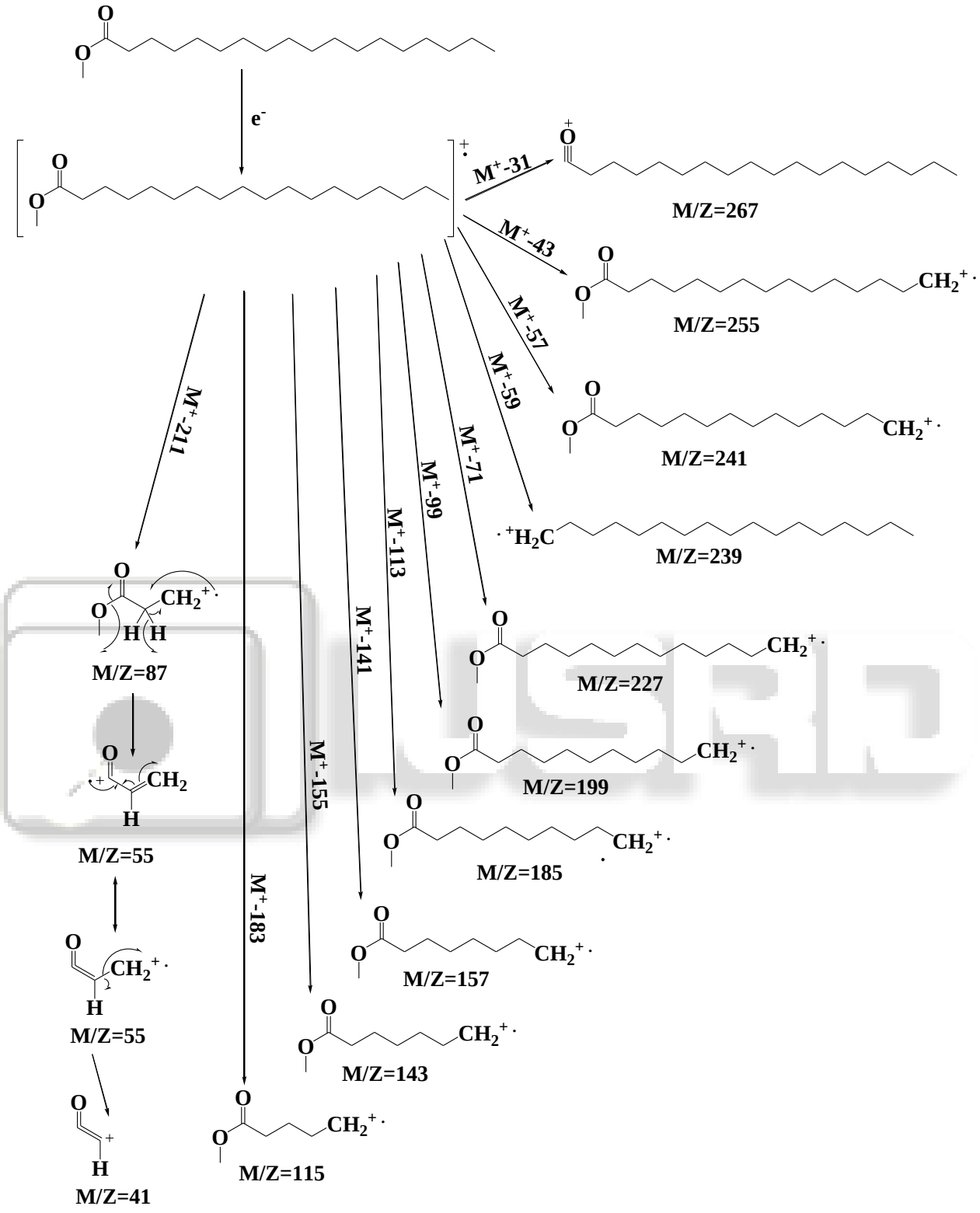

Plate 2: Mass fragmentation of Octadecanoic acid ester (Methl stearate)

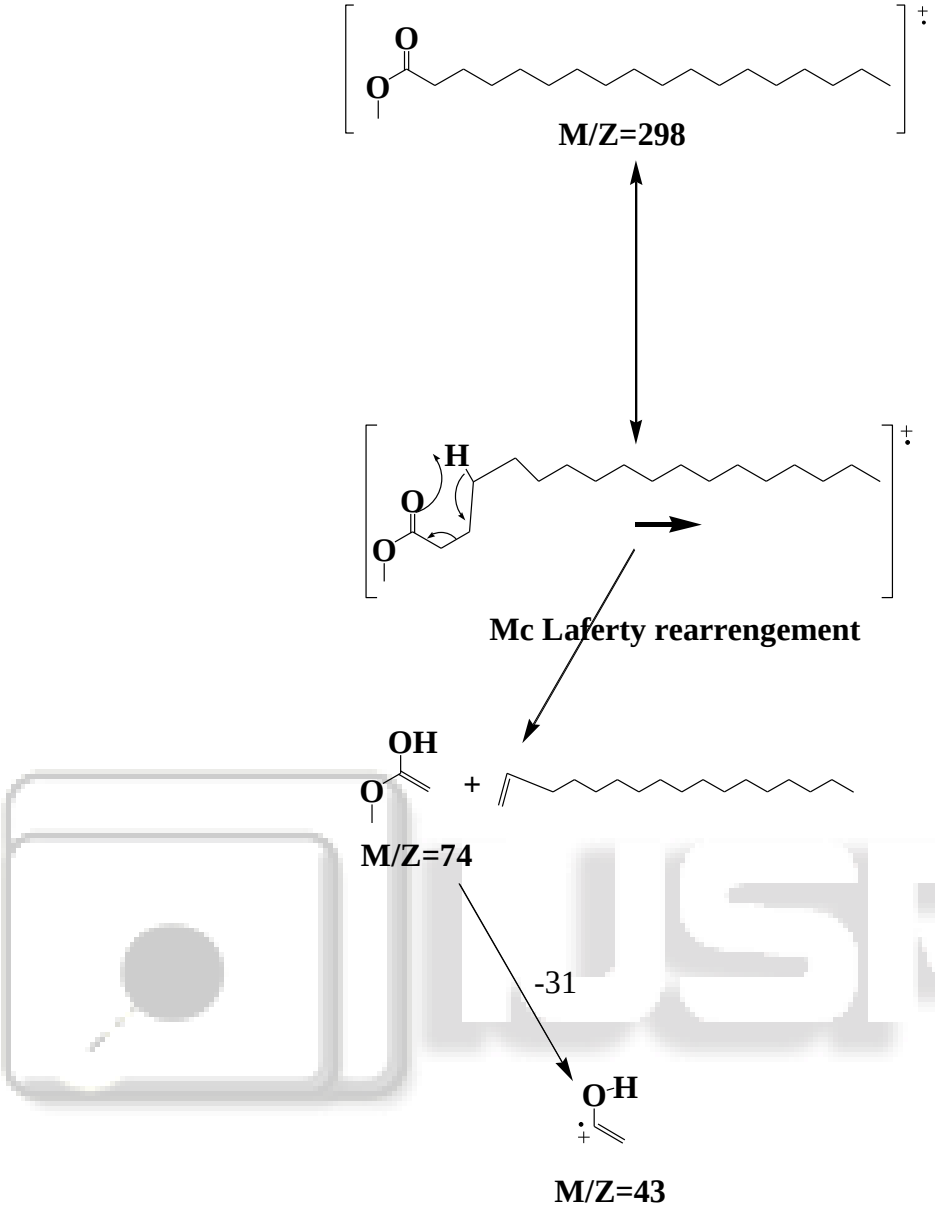

Plate 3: Mass Fragmentation of Octadecanoic acid, methyl ester (methyl stearate) contiune

IV. CONCLUSION

Finally, the present investigation can be concluded that the highest antioxidant activity was observed in hexane extract then methanol extract followed by ethyl acetate extract and the lowest antioxidant activity was observed in water extract. The GC-MS analysis showed that the compound Hexadecanoic acid methyl ester and Octadecanoic acid methyl ester (methyl stearate) were isolated from MCE. It has been suggested that these compounds contribute to the pharmacological activities of *Lamium flexuosum*.

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