



Spectroscopic Elucidation of the Biodeterioration Potentials of *Aspergillus niger* on *Lagenaria siceraria* Seed Oil

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Authors' contributions

This work was carried out in collaboration between all authors. Author PMD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DK and BM managed the analyses of the study. Author AA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Vegetable oil was extracted from *Lagenaria siceraria* seeds and some physico-chemical analysis determined. The refractive index of 1.47, saponification value of 465.9 mgKOH/g, iodine value of 15.86 I₂/100 g, Acid value mgKOH/g of 2.52 and density of 0.93 were obtained. GC-MS of both the un-inoculated and inoculated oil was measured before and after four weeks of incubation with *Aspergillus niger*. The disappearance and or appearance of some absorption peaks which correspond to hydroxyl, carbonyl, methyl, and carboxylic acid functional groups were identified. MS provided the mass fragments of the possible components formed during the degradation of the oil to include 12-methyl-methyl ester, tetradecanoic acid, hexadecanoic acid, heptadecanoic acid, oleic acid, 2, 6, 10-Dodecatrien-1-ol, 3, 7, 11-trimethyl (E,E) and pentadecanoic acid as biodegradation

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by-products. Despite, low acid value obtained which may have suggested stability of the oil to oxidation, its high iodine value indicated a rather high level of unsaturation i.e. presence of double bonds. The position of double bonds was viewed as possible sites for the initial attack by the microorganisms.

These absorption bands disappeared after inoculation and incubation with *Aspergillus niger* after four weeks with prominent peaks noticed to be of fatty acids as a result of the action of *Aspergillus niger* on the *Lagenaria siceraria* oil. The fragmentation of the triglycerides suggested that the unsaturation is not on an even number of carbon atom along the chain length resulting in the propyl – like breakage with molecular weight of 42 (C₃H₇) whereas, fragmentation on even carbon atom resulting in ethyl-like breakage with molecular weight of 28 (C₂H₂). The GC-MS showed that acidic metabolites were mostly produced. This is similar to the hydrolysis of vegetable oil by other agents where sterol and fatty acids are usually produced in the ratio 1:3. Therefore, it is recommended that the storage of these oils in the presence of common mold such as *Aspergillus niger* at temperature above 37°C would lead to its deterioration. However, *Lagenaria siceraria* seed oil can be used as lubricants, for cooking and paint production.

Keywords: Vegetable oil; seed; *Aspergillus niger*; biodegradation; biodeterioration; inoculation; incubation.

1. INTRODUCTION

Recently, there has been an increase in the demand for a renewable source of raw materials in the fuel industry as biodiesel and non-renewal natural resources like petroleum. Vegetable oils have provided this opportunity with seeds, oil and cake used in a range of foods either as beverages or source of protein. Some are used as ingredient in a range of body-care products and nutritional supplements [1] used for industrial oils, cosmetics and pharmaceuticals, among other composites [2]. The high demand of vegetable oil has necessitated research in seeds yielding high quantity and quality of oil [3,4] so that the need for nonedible plants seed oils to supplement the vast areas of applications remains a challenge [5]. The biodegradation of oil is viewed as either the modification of a substance by a microorganism which may cause changes in some specific measurable properties of the substance [6] or any alternation in the physical characteristics of a compound. Intermediate metabolites may be produced, detected and or isolated [7]. The behaviour of vegetable oil towards biodegradation has been viewed to depend on the amount of unsaturated and saturated fatty acids [8]. Degradation of vegetable oil is essentially caused by the oxidation of unsaturated fatty acid which is converted to hydroperoxides and subsequent break down into smaller molecules usually hydrocarbons, aldehydes, alcohols and ketones [7]. The stability of or freedom from the development of rancidity of any vegetable oil is an important property and define its quality [9]. Spectroscopic measurements have been

employed for either the detection of contaminants and or additives of degradation by products of some vegetable oils [10]. GC- MS has been used to detect adulteration of palm oils and other edible oils which provided quick and unambiguous results. The ability of different molecules to absorb radiation at specific wavelengths enable their identification [11]. Also, the measurement of size of the absorption peak which is directly proportional to the amount of the specific molecules present provided additional insight into the composition of oils. However, spectroscopic measurements are complimentary to other physical property tests when proper information on the degradation of oil is required [12]. The research work is aimed at using GC-MS technique to identify metabolites produced when *Lagenaria siceraria* seed oil is degraded by *Aspergillus niger*.

2. EXPERIMENTAL

2.1 Collection of Materials

Mature wild *Lagenaria siceraria* fruits (Fig. 1) were collected in Sangere, Gerei Local Government Area of Adamawa State, Nigeria. The fruits were broken and their seeds were collected and washed with water (Fig. 2a). The pericarps were shelled out manually and seeds (Fig. 2b) dried under the sun for 4 days then grind and stored for further analysis.

2.2 Extraction of Oil from Seeds

Using Kyari's [13] method of extraction, 300 ml of n-hexane was poured into a round bottom flask

and 100 g of the sample was placed in a filter paper thimble and inserted in the centre of the Soxhlet extractor. The Soxhlet extractor was heated at 60°C with solvent boiling point vapour rose through the vertical tube into the condenser at the top. The liquid condensate dripped into the filter paper thimble in the centre which contains the solid sample to be extracted. The extract seeped through the pores of the thimble and filled the siphon tube where it flowed back down into the round bottom flask. This was allowed to continue for 3 hours and the condenser was removed at the end of the extraction. The flask containing the remaining mixture was connected to Liebig condenser and heated to a temperature of 70°C during which the n- hexane evaporated off and was recovered in a conical flask. This procedure was repeated until complete extraction of oil was achieved.

2.3 Determination of Saponification, Acid, Iodine Value and Refractive Index

Physico-chemical analysis of oil were done according to the AOACS [14] standard procedures and further calculations were carried out to obtained relevant values.

2.4 Biodegradation of Seed Oil

The biodegradation of seed oil was carried out according to the ASTM: D 5864: Standard practice for the evaluation of the action of microorganism in oil. Visible fungal growth and the production zones of cleaning around the cells were regarded as evidence of ability to degrade oil. Degradation studies were done by dispensing

1.0 g of PDA broth-grown culture of each fungal isolate into sample and incubating them at 37°C for 4 weeks at the Department of Microbiology, Modibbo Adama University of Technology Yola, Adamawa State, Nigeria. Growth of microorganisms was observed in all the oils and the change in colour of oils were noted.

2.5 GC-MS Measurement

The GC-MS analysis was done at American University of Nigeria using Model –GC-MS-7890A, Agilent Technologist Inert MSD-597CM. Carrier gas –Helium, ⁶³Ni electron capture detector, low polar HP 5Ms column, Column dimension -30 cm x 0.34 mm, column oven temp-60°C, detector temperature 300°C, injection temperature 250°C, flow rate of carrier gas 1.61 ml/min, pressure of 100.2 kpa, linear velocity of 46.3 cm² and injection mode- split.



Fig. 1. Matured *Lagenaria siceraria* (Bottle gourd) fruit



a



b

Fig. 2. Seeds (a =with; and b =without shells) from matured *Lagenaria siceraria* (Bottle gourd) fruit

3. RESULTS AND DISCUSSION

3.1 Properties of *Lagenaria siceraria* Seed Oil

Table 1 shows the physicochemical analysis of *Lagenaria siceraria* seeds oil revealing that the seeds contain high amount of oil (52%/100 g). This is similar to the amount of oil obtained from egusi, an edible seed (53.20%), but higher than that obtained from non-edible seeds of pawpaw (40.10%), sweet orange (43.10%), cotton seed 24% and mustard seed 40.0% [15]. The colour of oil changed from pale yellow to brown-yellow. This could be as a result of the heating time during solvent recovery which may have destroyed some pigments. Many vegetable oils are known to change colour i.e. decolourises at higher heating temperature [16]. The refractive index of the oil is 1.47 and within the acceptable range of 1.4677 to 1.4707 for virgin and refined plant oils [17]. The oil is less dense than water with 0.91 specific gravity which is similar to the specific gravity of 0.9261 obtained for *Hyptis spicigera* seed oil [18]. Oils with specific gravity less than 1.0 flow easily and are reported to be useful in cream production [19]. Specific gravity is commonly used in conjunction with other parameters to assess the purity of oil [20]. The saponification value measure the average molecular weight or chain length of all the fatty acids present in oil. The saponification value of 465.9 mgKOH/g was obtained which is higher than the one reported by Kubde et al. [21]. Low saponification value indicates long chain fatty acids with fewer number of carboxylic functional groups per unit mass of the oil whereas higher saponification value is due the presence of short chain fatty acids. The oil has higher iodine value (126.90) than palm kernel oil 15.86 I₂ /100 g, soya beans oil 119.21 I₂ /100 g and 84.4 I₂ /100 g for groundnut seed oil. A higher iodine value indicates high degree of unsaturation in other word, high amount of double bonds in fatty acid of the oil. High iodine value justifies utilization of the oil in paint production while low values are suitable for soap making [22,23]. Acid value of 2.52 is lower than that reported for egusi (4.30), pawpaw (9.46) and sweet orange seeds (7.59) [15]. The acid value indicates the amount of carboxylic acid groups in the oil. Usually, the suitability of vegetable for consumption can be determine for the acid value [17]. Lower acid values have been obtained for most edible vegetable oils. Also, low acid value indicates that the oil is stable to oxidative degradation cause by

either overexposure to oxygen, heat and microorganisms [18].

3.2 Biodegradation of Seed Oil

The acid value of oil accounts for the presence of free fatty acids which are prone to hydrolysis by lipolytic enzymes and oxidation [23]. The low acid value of 2.52 indicates that the oil will be stable over a long period of time against microorganisms otherwise rancidity may occur. The presence of natural antioxidants, flavonoids and other compounds which were not destroyed by overheating of oil during solvent recovery may have enhanced the resistance to oxidation. However, the stunning ability of some fungi to destroy substances known to be resistance to biodegradation provided the ground for the test. The degradation of *Lagenaria siceraria* seed oil by *Aspergillus niger* could have been made possible due to the high degree of unsaturation i.e. double bonds, which may have served as the initial point of attack by the microorganisms as they search for food. The microorganisms may have secreted enzymes which initiated the formation of free radicals. At suitable temperature free radical may have reacted quickly with oxygen to either propagate or caused chain scission. Usually, chain scissions are reported rather than any polymerization reaction. Also, the biodegradation products that were identified served by GC-MS supported chain scissions. The colour, odour and viscosity of the seed oils was observed to have changed after inoculation with the microorganisms and incubated for four weeks [24] however, the physicochemical analysis of the oil was not done. The structural properties such as the lower molecular weight, high degree of unsaturation and the nature of functional groups in the oil could have been responsible for the relative ease to degradation by *Aspergillus niger* [25].

Table 1. Physicochemical analysis of *Lagenaria siceraria* seed oil

| Parameters | Properties |
|------------------------------------|-----------------|
| Oil yield (%) | 52 |
| Colour | Brownish yellow |
| pH | 6.25 |
| Density | 0.93 |
| Specific gravity | 0.91 |
| Refractive index at 38°C | 1.47 |
| Iodine value I ₂ /100 g | 126.90 |
| Acid value mgKOH/g | 2.52 |
| Saponification value mgKOH/g | 465.91 |

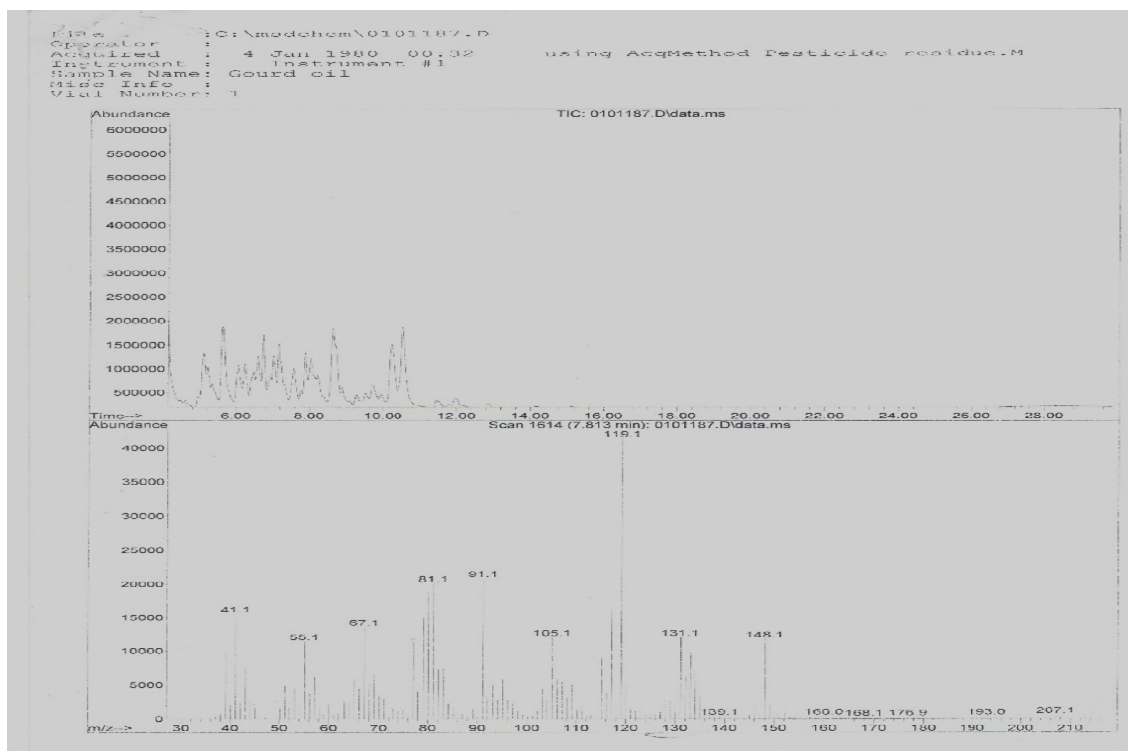


Fig. 3. GC-MS of *Lagenaria siceraria* seed oil Un- inoculated with *Aspergillus niger*

3.3 GC-MS Measurement

Figs. 3 to 11 are the GC-MS spectrograms of both un-inoculated and inoculated *Lagenaria siceraria* (Bottle gourd) seed oil. The composition of the oil before biodegradation show several compounds belonging to aliphatic unsaturated hydrocarbons, organic acids and esters (Fig. 3). This is in agreement with the result of the physicochemical analysis presented in Table 1. Also, vegetable oils have been characterized based on the amount of fatty acids, degree of unsaturation, and size of other smaller molecules presence so that possible areas of applications, quality and quantity could be suggested using GC-MS [24]. The GC-MS revealed the disappearance of some compounds after inoculation and incubation for four weeks earlier shown to be present in the virgin oil. The fatty acids obtained from the biodegraded oil are 12-methyl-methyl ester, tetradecanoic acid, hexadecanoic acid, heptadecanoic acid, Oleic acid, 2, 6, 10-Dodecatrien-1-ol, 3, 7, 11-trimethyl (E.E) and pentadecanoic acid (Figs. 5 to 11). Similar biodegraded by- products consisting of acidic, hydroxyl, ester, saturated hydrocarbons, etc. functional groups have reported [24]. Long-chain

fatty acids (LCFAs) biodegradation intermediates of vegetable oil have been identified by GC-MS method [25]. The mechanism of degradation which have reported to possibly be initiated at the position of the double bonds would lead to different metabolites. Fig. 3 gives the GC-MS of the un-inoculated oil shows several absorption bands characteristics of vegetable oil [25]. Some of these absorption bands disappeared after inoculation and incubation with *Aspergillus niger* after four weeks (Fig. 4). Prominent peaks were noticed to be of fatty acids (Figs. 5 to 11) compounds which is hereby viewed to be due to the action of *Aspergillus niger* on the *Lagenaria siceraria* oil. The fragmentation of the triglycerides shows that the unsaturation is not on an even number of Carbon atom (Figs. 5 and 8) resulting in the propyl – like breakage with molecular weight of 42 (C_3H_7) whereas, Figs. 6, 7, 9, 10 and 11 show fragmentation on even carbon atom resulting in ethyl-like breakage with molecular weight of 28 (C_2H_2). Therefore, the present of double bonds in the acids could be said on the fifth carbon atom. Furthermore, the sizes of the absorption bands revealed the amount of the compounds corresponding to their functional groups produced by the biodegradation of the

Lagenaria siceraria oil. The GC-MS (Figs. 4 to 11) showed that acidic metabolites were produced more similar to the hydrolysis of vegetable oil by other agents [26-28] where sterol and fatty acids are produced usually in the ratio 1:3.

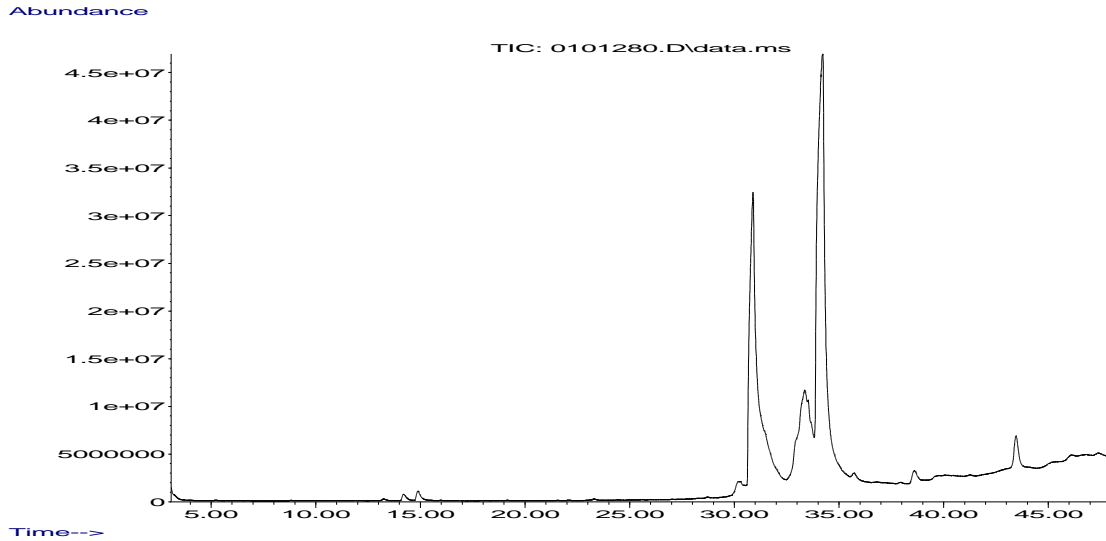


Fig. 4. GC absorption peaks of *Lagenaria siceraria* seed oil after four weeks of incubation with *Aspergillus niger*

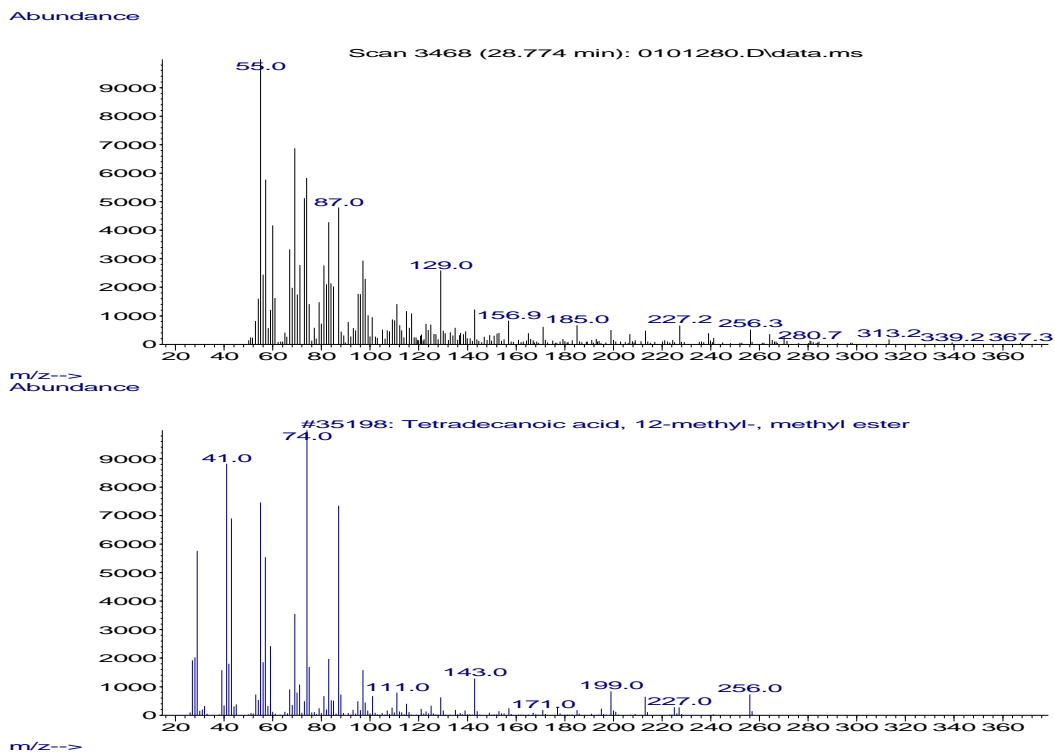


Fig. 5. MS of tetradecanoic acid and 12-methyl-methyl ester *Lagenaria siceraria* seed oil after four weeks of incubation with *Aspergillus niger*

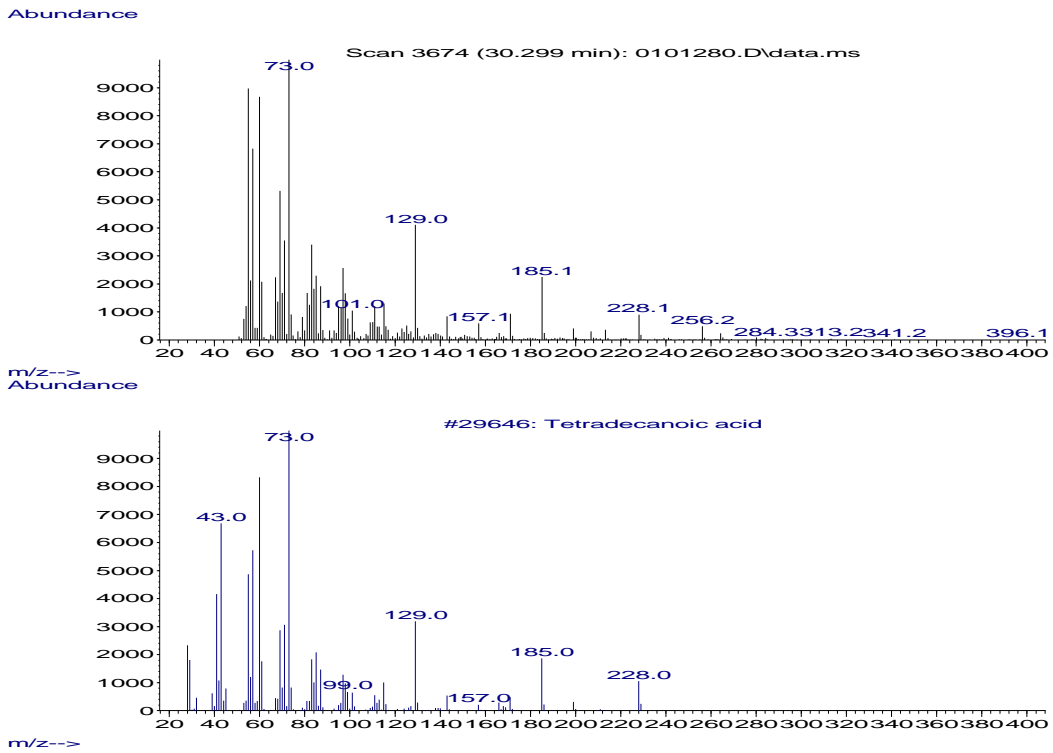


Fig. 6. MS of tetradecanoic in *Lageneria siceraria* seed oil identified after four weeks of incubation with *Aspergillus niger*

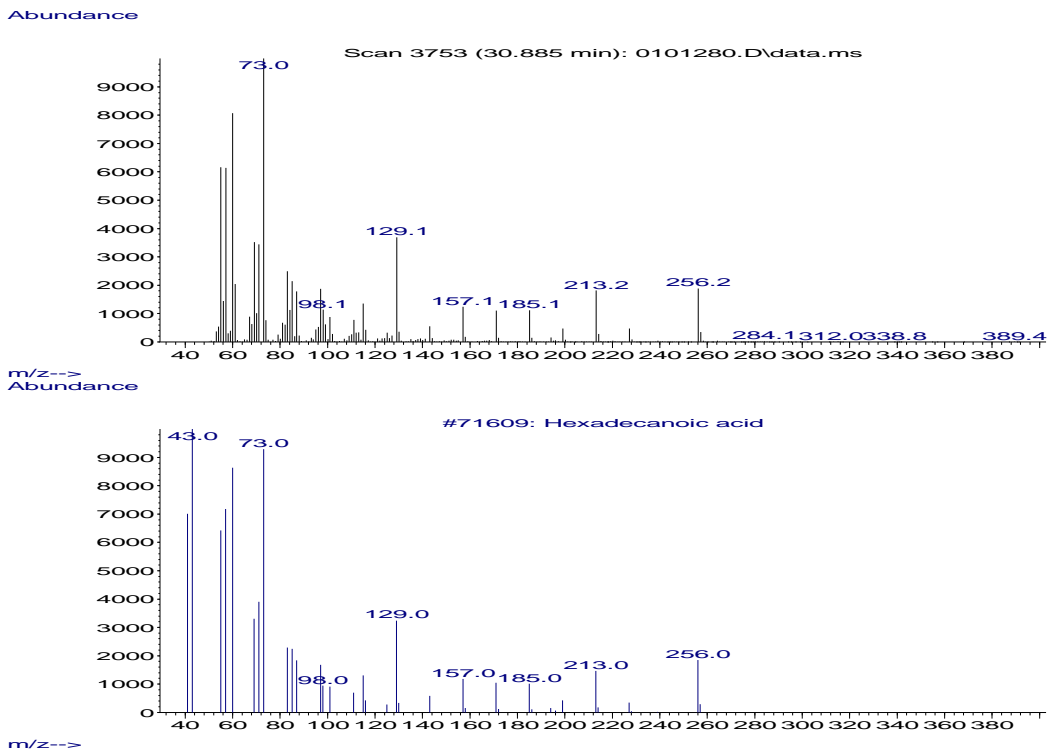


Fig. 7. MS of hexadecanoic acid in *Lageneria siceraria* seed oil identified after four of incubation with *Aspergillus niger*

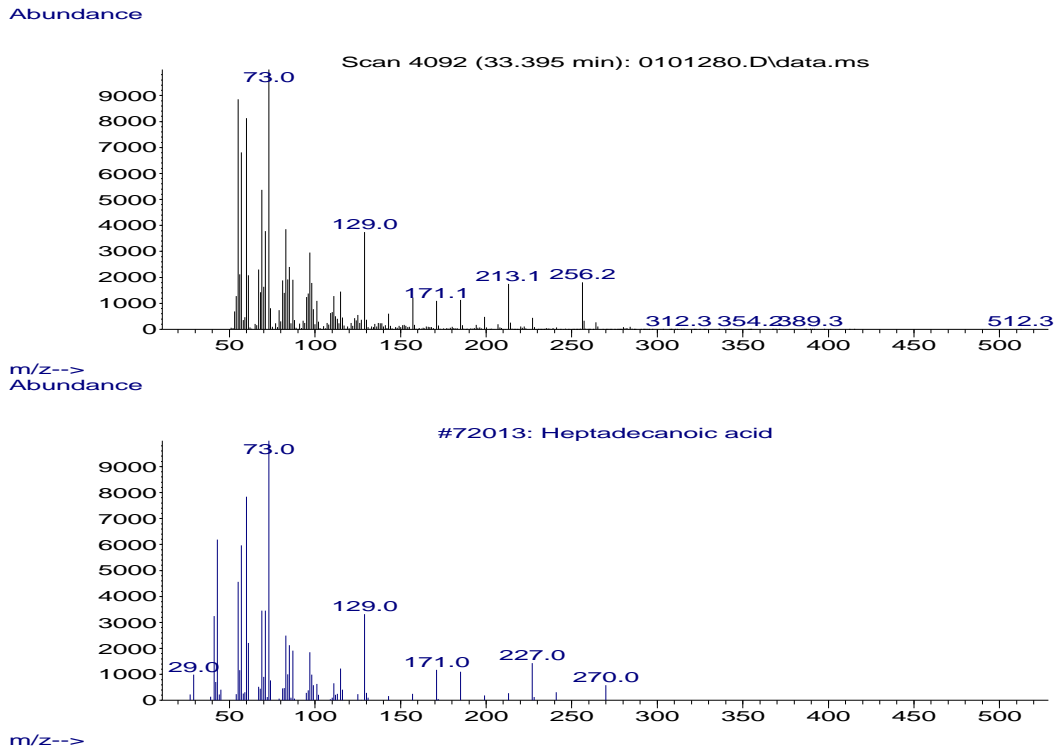


Fig. 8. MS of heptadecanoic acid in *Lageneria siceraria* seed oil identified after four weeks of incubation with *Aspergillus niger*

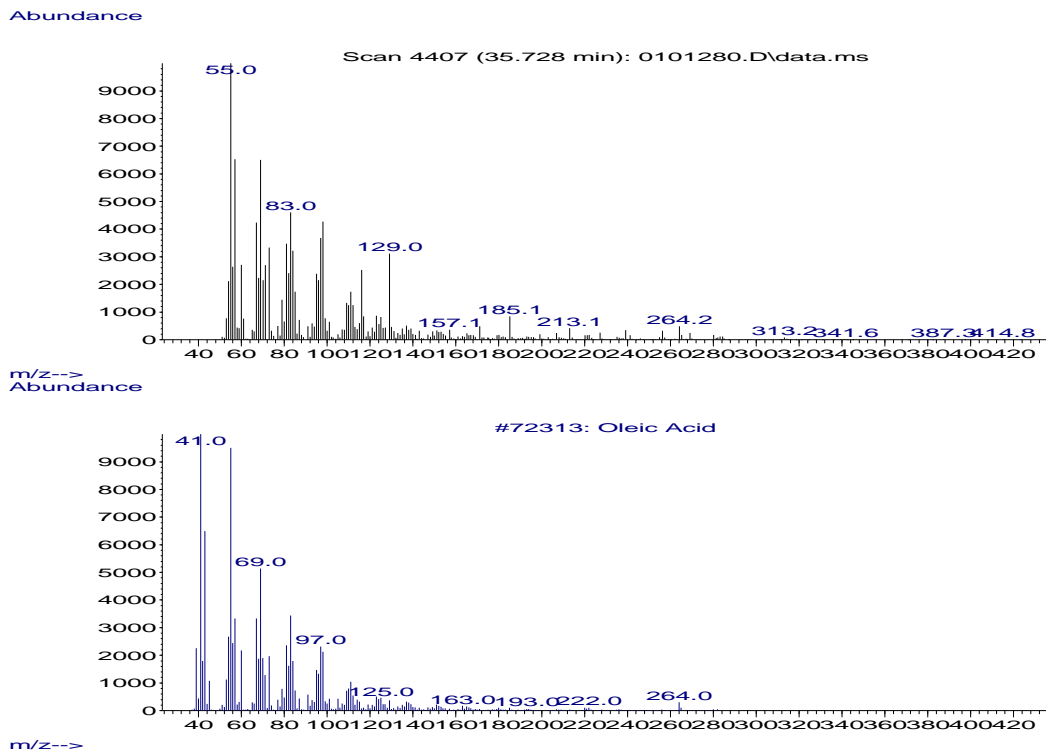


Fig. 9. MS of oleic acid in *Lageneria siceraria* seed oil identified after four weeks of incubation with *Aspergillus niger*

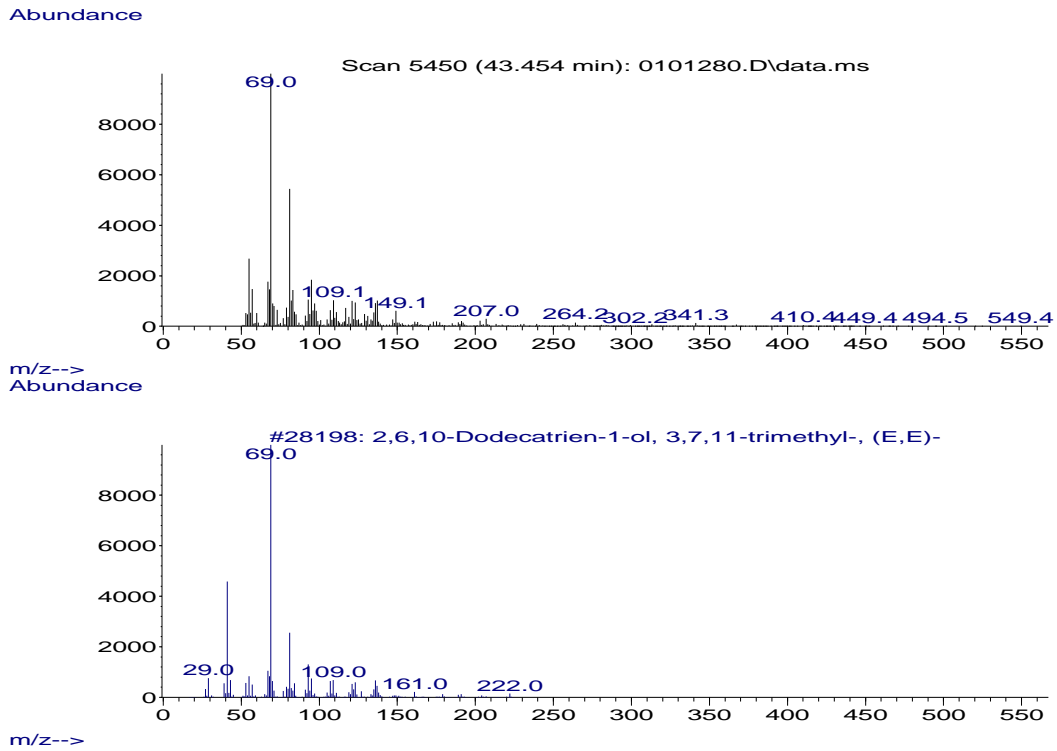


Fig. 10. MS of 2,6,10-dodecatrien-1-ol and 3,7, 11-trimethyl-(E,E) *Lageneria siceraria* seed oil after four weeks of incubation with *Aspergillus niger*

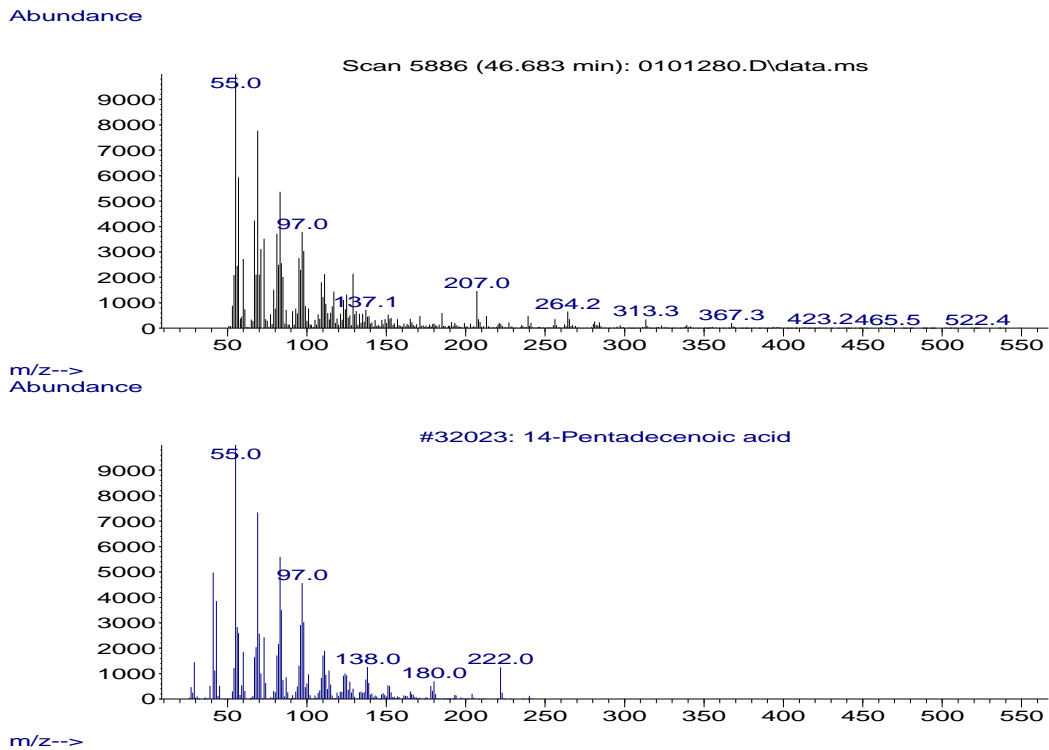


Fig. 11. MS of 14- pentadecenoic acid in *Lageneria siceraria* seed oil after four weeks of incubation with *Aspergillus niger*

4. CONCLUSION

Vegetable oil was extracted from the matured fruit seeds of *Lagenaria siceraria* with 53% oil yield/100 g. Although, the acid value, 2.52 mg KOH/g obtained was low suggesting that the oil will be stable against oxidation, degradation of the oil by *Aspergillus niger* after four weeks of incubation was attained. Biodegradation by products were identified by GC-MS spectroscopic measurement. These correspond to aliphatic saturated hydrocarbons, esters, organic acids and carbonyls. Although, the low acid value suggested stability of the oil to oxidation but its high iodine value indicated a rather high level of unsaturation i.e. presence of double bonds. This was therefore considered as possible sites of attack by the microorganisms as they break the oil in order to consume it. The extent of biodegradation could be easily be ascertained if the degraded oil is subjected to physicochemical analysis and results compared to that of the un-degraded oil. However, GC-MS method of the *Lagenaria siceraria* seeds oil has shown that it is a good method of elucidation of biodegradation and information of the composition of both the virgin and degraded oil was obtained. Furthermore, it is hereby recommended that as a result of the quality and quantity of the oil from *Lagenaria siceraria* seeds, some possible applications are in food, as lubricants and paints production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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