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ANTIBACTERIAL AND PHYTOCHEMICAL SCREENING OF VARIOUS FRUITS EXTRACTS OF *ABELMOSCHUS MANIHOT* TRADITIONALLY USED FOR THE TREATMENT OF CHRONIC BRONCHITIS

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ABSTRACT

Abelmoschus manihot (*A. manihot*) is a flowering plant belong to the Malvaceae family that has been used traditionally in Oman to cure bronchitis, wound and toothache. Therefore, the goal of this present study is to prepare the various crude extracts from the fruits of the plant to identify the phytochemical constituents by using Gas Chromatography-Mass Spectrometry (GC-MS) and antibacterial activity by disc diffusion method. The powder of the fruits was extracted with methanol by using maceration method for 24 to 48 hours and then the extract was filtered by using Buchner apparatus. The methanol was removed under reduced pressure and the methanol crude residue was dissolved in a mixture of alcohol-water solution and successively fractionated with hexane, chloroform, ethyl acetate, and butanol to give the corresponding fractions. All solvents were removed from under reduced pressure and the crude various extracts was used to determined their antibacterial activity against *Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae), Proteus vulgaris (P. vulgaris), Streptococcus pneumoniae (S. pneumoniae), and Staphylococcus aureus (S. aureus)* using the disc diffusion method. In addition, the phytochemicals of each extract were identified by using GC-MS. The results showed that among the extracts hexane and water extracts had the highest antibacterial activity with the range of inhibition zone 0-10.6 mm against the applied bacterial strains. The primary phytochemicals found in the extracts were unsaturated fatty acids, terpenoids derivatives, normal and aromatic hydrocarbons, alkaloid, and flavonoids derivatives. In conclusion, the best antibacterial activity extract of the selected plant could be used for the development of antibacterial agents.

1.Introduction

Plants are undeniably important in our daily lives. Plants are used by many creatures to carry out their functions. Humans, as well as some animals, ingest plant components, which are then eaten by humans. Plants were also employed to construct homes and treat a variety of illnesses. Plants were once thought to be the primary and only therapy for a variety of ailments, long before synthetic drugs were developed. Originally, drugs were made from extracts of various plant parts such as seeds, leaves, fruits, and roots (Todarwal et al, 2011). In the past, all medical therapies involved

manipulating plants in a precise way, such as boiling, grinding, or drying a specific component of a medicinal plant to cure various illnesses. Medicinal plants have been used to prevent and treat ailments since ancient times because they are biological sources of various minerals and bioactive elements that are both useful and safe. As a result, traditional medicine is preferred by 80% of the world's population. Only in Kenya, nearly 70% of the population relies on traditional medicine and home remedies as a first line of treatment for their illnesses (Abdullahi, 2011). More than 50,000 plant species are used for medical purposes all around the world (Chen et al, 2016). *Abelmoschus manihot* (*A. manihot*) is one of the well-known medicinal plant, which have so many medicinal values. This plant can be used for many conditions as it contains many active ingredients that are useful as anti-inflammatory, antibiotic, antidiabetic, antioxidant and many other uses (Onakpa,2011 & Dwivedi,et al 2013).

A. manihot which is commonly known as okra, edible hibiscus or sunset muskmallow belongs to the Malvaceae (Wen, et al 2015, Dorr & Wiersema 2010) family and it is considered to be a flowering plant. In addition, it is considered herbaceous perennial plant. It has palmate leaves that are profoundly analyzed and dissected (Wen, et al 2015). It has also profound projections found deeply called lobes and they are five to nine in number. The leaves vary in their size at the base of the plant they are wide. Also, they have different shapes, pigmentation, and colors. The leaves are alternate in the direction, and they are one of the simplest types of leaves (Onakpa, 2013). The length of the petiole can be from 3 to 25 cm. The leaf blade of this plant is linear and contains deep lobed segments which can be 3 to 7 in number. The stem is branching, woody and erect. The root is 30 to 40 cm long and it is known to be shallow and adventitious. The flowers are bell shaped and they consist of overlapping five petals. The petals are yellow in color and the flower has red center. The fruit is ovoid capsule covered by hair and has a narrow tip (Onakpa, 2013, & Dwivedi, et al 2013).

A. manihot is considered to be native to southwestern Asia. It is cultivated as vegetables especially in tropical areas. The plant has also wide distribution in Eastern European countries and Asia like India, Indonesia and Southern China (Chen et al, 2016 & Onakpa 2013).

A. manihot is one of the most important medicinal plants as it contains many active ingredients that have valuable therapeutic benefits. Many studies revealed that the plant has been used in traditional medicine in the past and also in the modern medicine as it contains a lots of chemical constituents which made the plant so special. This plant is known to have anti-inflammatory (Huang et al 2008) antifungal (Grosvenor et al 1995), antibacterial (Zamrul et al, 2019) antidiabetic (Dubey& Mishra 2017, Chen,et al 2015, Alam et al 2019, Zhao et al 2020) antioxidant and other activities (Wang,et al 2020, Gul et al 2011, Tahseen et al 2010). For example, in traditional medicine this plant is still used for cuts specially the bark of the plant which is mixed with water to produce a paste and this paste that can be applied to wounds. The applied paste will keep the wound clean and accelerate wound healing process. Recently, the juice of flowers is used to treat chronic bronchitis in addition to its benefits in the treatment of toothache. Furthermore, the roots of *A. manihot* are used in Nipal for the treatment of sprains (Luan et al, 2020). The literature available nowadays reveals that there are many chemical components obtained from this plant, they are estimated by 128 including polysaccharides, steroids, amino acids, flavonoids, nucleosides, volatile oils and many additional chemicals. It is also proven that the plant contains alkaloids (Liu et al 2020). The *A. manihot* is known to have many active ingredients that can be involved in the production of many drugs like antibiotics, antiviral, antidiabetic, antioxidant, diuretics, anti-inflammatory and many other activities (Xue et al 2011). Also, many studies revealed that this plant can be used for wound healing process, as well as protection against osteoporosis. Another study has shown that the Okra has hepatoprotective properties (Wang et al 2020).

Antibacterial effects are the ability to kill or inhibit the growth of bacteria, and they can be detected by allowing bacteria (G+ and G-) to colonize and providing suitable incubation conditions for the bacteria to grow, and extracts of various polarities will be inserted into the plate, and then observations and calculations will be made according to the zones of inhibitions, which are the areas in which bacterial growth is inhibited (Luan et al 2020). There is no previous research on the antibacterial activity of the fruit of *A. manihot* in Oman but species from other countries have shown to have antibacterial activity. Therefore, the goal of this present study is to prepare the various crude extracts from the fruits of the plant and determine the phytochemicals and antibacterial activity by using GC-MS and disc diffusion method.

2. Materials and methods

2.1. Materials

All chemicals and solvents were of analytical quality and were utilized without additional purification. Chem Solute in Germany provided the ethanol absolute (99.5 percent). Methanol, hexane, chloroform, butanol, and DMSO were purchased from Fisher Scientific in the United Kingdom, while ethyl acetate was procured from Carbon Group in Ireland. The University of Nizwa's DARIS Center distilled the water. Scharlau, Chemie Company Whatman Grade 1 Qualitative filter papers, plastic petri plates, and nutritional agar

Three gram-negative bacterial strains such as *Escherichia coli (E. coli), Proteus vulgaris (P. vulgaris), and Klebsiella pneumoniae (K. pneumonia)* and two gram-positive bacterial strains *Staphylococcus aureus (S. aureus) and Streptococcus pneumoniae (S. pneumonia)* were obtained from Nizwa Hospital and those were cultured at the DARIS Center, University of Nizwa. The cultured bacterial strains were used for the determination of antibacterial activity.

2.1.1. Collection of the Samples

The fruits of the *A. manihot* shrub were gathered in the Dhofar mountains. It's an untamed variety. Dhofar is located in southern Oman and is ideal for medicinal plants. The plant was sent to the lab for further processing. Dr. Syed Abdullah Gilani, Department of Biological Sciences and Chemistry, College of Arts and Sciences, University of Nizwa, recognized the samples, and a voucher specimen was deposited in the university's herbarium. The fruits samples were first cleaned and dried on newspapers in the shade at room temperature. A ball mill was used to grind the dried materials into a coarse powder.

2.1.2. Preparation of extracts

The coarse powder (130 gm) was placed into a beaker and extracted with methanol solvent (500 ml) for 48 hours to allow the extraction process to take place. The extract was then filtered by using a Buchner funnel, and the methanol solvent was removed under reduced pressure. The obtained semi-solid residue (8.2 gm; yield 8.2%) from the fruits coarse powder was suspended in an alcohol-water mixture (90:10) before fractionated. The solution was then placed into a separatory funnel. The relevant fractions, which comprise the remaining water fraction, were then obtained by adding hexane, chloroform, ethyl acetate, and butanol in order of increasing polarity. By evaporating all solvents with a rotary evaporator, hexane, chloroform, ethyl acetate, butanol, and water extracts were obtained. All of the extracts were subjected to antibacterial and phytochemical testing (Weli et al, 2020).

2.1.3. Antibacterial activity

The antibacterial activity of various plant fruits extracts of *A. manihot* was determined against a number of gram-positive and gram-negative bacterial strains. In this study, the disc diffusion method was employed to investigate activity. The stock solution of each extract was prepared by adding 10 mg of extract with 10 ml of DMSO solvent. From the stock solution of each extract, four different concentrations such as 1000, 500, 250 and 125 µg/ml were prepared using dilution method by adding DMSO. Petri dishes were made by adding agar to the dish, which formed some solid surface rich in nutrition for bacteria. A sterile cotton swab was then used to inoculate the germs onto the plates.

Discs (6 mm) were prepared from the filter paper and insert the discs in all prepared concentration of each extract. Then, extract-impregnated disc papers were carefully put onto the agar plate's surface. The paper discs were inserted in all prepared concentrations and kept for 30 minutes. Then, the disc was applied on the agar gel plate against the selected bacterial strains. As a positive and negative controls, the antibiotic levofloxacin and DMSO were used (Weli et al, 2018). All the plates were incubated inside the incubator for 24 hours at constant temperature 37 degrees Celsius. After 24 hours, all the plates were taken out and measured their inhibition by using the scale.

2.1.4. Phytochemicals of A. manihot

GC-MS analysis for phytochemicals was performed in Sultan Qaboos University, College of Agricultural and Marine Sciences, Central laboratories, on a Perkin Elmer Clarus, fitted with a SP-2560 Supelco capillary column (100 m × 0.250 mm i.d. × 0.2μm film thickness) coupled to Clarus 600C MS. As a carrier gas, helium (purity 99.9999%) was used at a constant flow of 1.0 ml/min. The temperatures for injection, transfer line and ion source were adjusted at 250, 240 and 240ºC, respectively. The ionizing energy was 70 eV. Electron multiplier (EM) voltage was obtained from autotune. All data were obtained by collecting the full-scan mass spectra within the scan range 35-500 amu. The sample (1 μl) was injected into the injector with a split ratio of 50:1. The oven temperature program was 60ºC (holds for 1 minutes) and accelerated at a rate of 8ºC/min until 280ºC hold for 25 minutes. The unknown compounds in the fruits crude extracts were identified by comparing the spectra obtained with mass spectrum libraries (NIST 2011 v.2.3 and Wiley, 9th edition) and further confirmed with C7-C30 saturated alkane's standard (cat. # 49451-U).

3.Results and discussion

As a sources of bioactive compounds, the medicinal plants are considered one of the main resources for the discovery of medicine either natural or synthetic drugs. In addition, medicinal plants play a significant role for the progress of human cultures all over the world. Due to the importance of medicinal plant the present study was undertaken to conduct one of the traditionally used medicinal plant *A. manihot* in Oman. Therefore, the selected plant was collected from the southern part of Oman. After necessary process the coarse powder samples was extracted with methanol by using a maceration method and afterwards fractionated by various polarities of solvents. The percentage of yield of each extract from the methanol extract was presented in Table 1.

Table 1. % of yields of each extract from the methanol extract of *A. Manihot*

Name of extract	Amount(g)	%Yield
Hexane	10.70±0.15	8.20±0.11
Chloroform	5.63±0.22	4.33±0.72
Ethyl acetate	2.08±0.39	1.60±0.19
Butanol	3.90±0.09	3.00±0.10
Water	7.33±0.13	5.64±0.26

3.1. Antibacterial activity

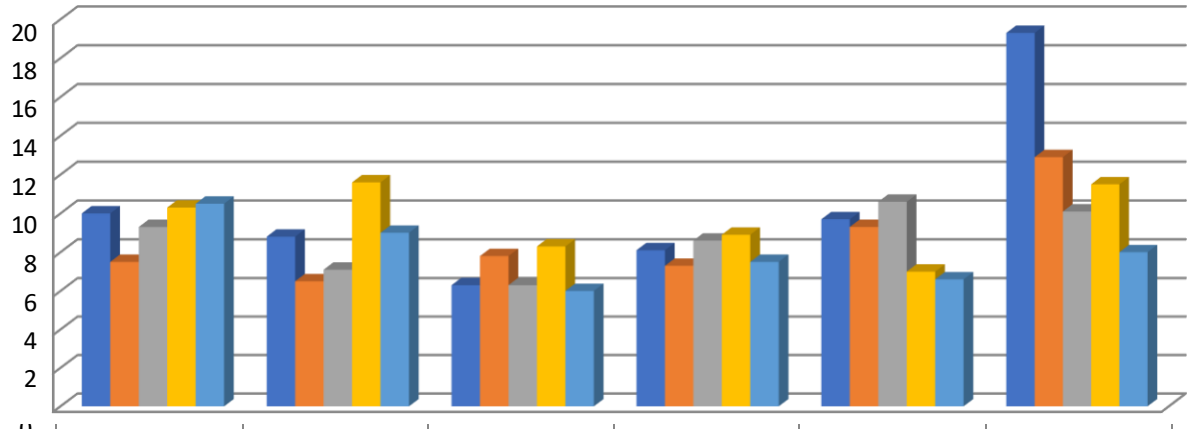
The antibacterial activity was evaluated by disc diffusion method describe by several authors (Weli et al 2018). All the various polarities fruits extracts of *A. manihot* at different concentrations were evaluated against the two-Gram positive bacterial strains *S. aureus* and *S. pneumonia* and three-Gram negative bacterial strains *E. coli, P. vulgaris,* and *K. pneumonia.* The results of antibacterial activity of the prepared various fruits extracts at different concentration was presented in Table 2. Based on the experimental results showed that in general all the extracts from the fruits of *A. manihot* gave the significant zones of inhibition

against the employed bacterial strains. The highest activity was observed in hexane extract against all tested bacteria with the range of 0-15 mm except *S. aureus* it gave moderate activity about 6.5 mm at the highest concentration of 1000µg/ml. The chloroform residue was most active against *K. pneumonia* with the zone of inhibition 12 mm at the highest concentration 1000µg/ml. The ethyl acetate residue showed comparatively best activity with the zone of inhibition 8.5 mm against *S. aureus* at the concentration 1000 µg/ml and weaker activity against other employed bacteria at all prepared concentrations. However, the butanol extract gave good inhibition against all applied Gram (– and +) bacteria strains with the range of 0-9 mm. In addition, the water extract residue also showed very good activity against Gram-negative tested bacteria strains and moderate to weak activity against Gram-positive tested bacteria strains Table 2.

Table 2. Antibacterial activity of different polarities extracts of *A. manihot* against *S. pneumonia, S. aureus, P. vulgaris, K. pneumonia and E. coli*

Extracts	**Concentration µg\ml**	***S. pneumonia***	***S. aureus***	***P. Vulgaris***	***K. pneumonia***	***E. Coli***
Hexane	1000	10	6	15	13.5	9.5
	500	9	7	7.5	9	13
	250	11	6.5	9	9	10
	125	10	10.5	6	10	9.5
Levofloxacin	300	14	12	9.5	10	8
Chloroform	1000	7.5	7	6	12	9
	500	7	6	6	12	9
	250	11	7	10.5	10.5	6
	125	10	6	6	12	12
Levofloxacin	300	18.5	6.5	6	10	6
Ethyl acetate	1000	6	8.5	6	7	6
	500	6.5	8	6	9	6
	250	6.5	8	6	10	6
	125	6.5	7	7	7	6
Levofloxacin	300	15	10	8	10	6
Butanol	1000	8.5	8	8.5	8	9
	500	7.5	8	9	8.5	7.5
	250	8.5	7	7.5	9	7.5
	125	8	6	9.5	10	6
Levofloxacin	300	12.5	10	7	9	6
Water	1000	9.5	10	8.5	7	6
	500	9.5	9	16	7	6
	250	8.5	8.5	6	7	6.5
	125	10	8.25	12	7	8
Levofloxacin	300	17	13	10	7	6

From the results the order of inhibition zone against *S. pneumonia* hexane>water>chloroform>butanol>ethyl acetate, Zone of inhibition against *S. aureus*: water >ethyl acetate>hexane>butanol>chloroform, Zone of inhibition against *P. vulgaris*: water>hexane>butanol>chloroform>ethyl acetate, Zone of inhibition against *K. pneumonia*: chloroform>hexane>butanol>ethyl acetate>water, and inhibition zone against *E. coli*: hexane >chloroform>butanol >water>ethyl acetate Figure 1.



	Hexane	Chloroform	Ethyl acetate	Butanol	water	Levofloxacin
S. pneumonia	10	8.8	6.3	8.1	9.7	19.3
S.aureus	7.5	6.5	7.8	7.3	9.3	12.9
P. Vulgaris	9.3	7.1	6.3	8.6	10.6	10.1
K. pneumonia	10.3	11.6	8.3	8.9	7	11.5
E.Coli	10.5	9	6	7.5	6.6	8

Figure 1. Data analysis represented by average inhibition zones by mm of antibacterial potentials of different polarities extracts of *Abelmoschus manihot*

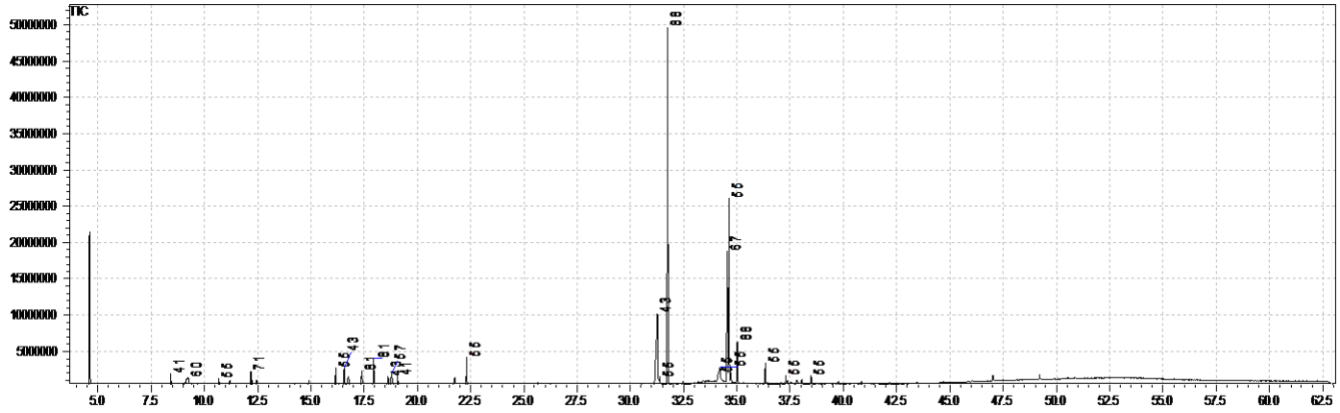
3.2. Phytochemicals

3.2.1. Hexane extract

The highest percentage phytochemicals in the hexane extract are octadecanoic acid, methyl ester, erucic acid, 9-octadecadienoic acid ethylester, n-hexadecanoic acid, 9,12-octadecadienoic acid and lower percent are 2-heptenal, hexanoic acid, 3-octen-2-one, 2,3'-bifuran, octahydro-, cyclopropane, octyl-, 2-decenal, 2,4-decadienal, 2,4-decadienal, dec-3-en-2-one, octane, 2,4,6- trimethyl-, 2-undecenal, nonanoic acid, 9-oxo-, ethyl ester, ethyl 9-hexadecenoate, ethyl ester, erucic acid, cis-10 nonadecenoic acid, Z-8-methyl-9-tetradecenoic acid. The percentage of each phytochemical are presented in Table 3 and Figure 2.

Table 3. Compounds present in the hexane extract

#	Compound name	R.Time (min)	Area	Area %	Cal KI	NIST KI
1	2-Heptenal, (Z)-	8.421	3838514	1.01	963.1365	932
2	Hexanoic acid	9.246	4605197	1.22	993.7184	973
3	3-Octen-2-one	10.679	2526867	0.67	1045.451	1015
4	2,3'-Bifuran, octahydro-	12.186	5089419	1.34	1099.856	1079
5	Cyclopropane, octyl-	16.163	5617027	1.48	1249.533	1115
6	2-Decenal, (Z)-	16.579	5941056	1.57	1265.72	1227
7	2,4-Decadienal, (E,E)-	17.395	5588290	1.48	1297.471	1288
8	2,4-Decadienal	17.95	8848750	2.34	1320.082	1270
9	Dec-3-en-2-one	18.624	3143083	0.83	1347.705	1233
10	Octane, 2,4,6-trimethyl-	18.793	4685995	1.24	1354.631	1277
11	2-Undecenal	19.07	2987578	0.79	1365.984	1350
12	Nonanoic acid, 9-oxo-, ethyl ester	22.311	8893830	2.35	1505.068	1507
13	n-Hexadecanoic acid	31.265	51134796	13.51	1953.911	1942
14	Ethyl 9-hexadecenoate	31.38	2398319	0.63	1960.335	1955
15	Octadecanoic acid, ethyl ester	31.754	121128966	31.99	1981.229	2181
16	Erucic acid	34.195	7019802	1.85	2124.085	2546
17	9,12-Octadecadienoic acid (Z,Z)-	34.543	43987128	11.62	2145.305	2095
18	(E)-9-Octadecenoic acid ethyl ester	34.632	60218239	15.9	2150.732	2174
19	Octadecanoic acid, ethyl ester	35.019	13250251	3.5	2174.329	2167
20	cis-10-Nonadecenoic acid	36.332	6649612	1.76	2256.815	2256
21	Z-8-Methyl-9-tetradecenoic acid	38.476	3712100	0.98	2397.086	2104



F**igure 2.** GC-MS chromatogram of Hexane fruit extract of *Abelmoschus manihot*

3.2.2. Chloroform extract

Nonanoic acid was the major constituent in the chloroform extract about (20.5%) followed by dimethyl sulfoxide, hexanoic acid, 8-nonynoic acid, hexadecanoic acid, ethyl ester and linoleic acid ester up to 3.63% and to lesser amounts from other components that are presented in Table 4 and Figure 3.

Table 4. Compounds present in the chloroform extract

#	Compound name	R. Time (min)	Area	Area%	Cal KI	NIST KI
1	Hexanal	4.623	1877661	3.96	808.2288	806
2	Dimethyl Sulfoxide	5.265	8670103	18.29	831.9188	820
3	Dimethyl sulfone	7.434	2649863	5.59	926.7159	914
4	2-Heptenal, (Z)-	8.426	1243096	2.62	963.321	932
5	Hexanoic acid	9.113	3883513	8.19	988.6716	973
6	Heptanoic acid	11.603	390294	0.82	1078.809	1073
7	Nonanal	12.459	460385	0.97	1109.963	1081
8	Octanoic acid	14.208	762521	1.61	1174.741	1173
9	Pantolactone	14.876	313822	0.66	1199.481	1148
10	2-Decenal, (Z)-	16.578	1124355	2.37	1265.681	1229
11	Nonanoic acid	16.8	9708212	20.5	1274.319	1268
12	8-Nonynoic acid	17.361	2542464	5.36	1296.148	1270
13	2,4-Decadienal, (E,E)-	17.954	370391	0.78	1320.246	1288
14	1-Undecene, 8-methyl-	18.627	895560	1.89	1347.828	1124
15	gamma.-Nonalactone	19.105	1230906	2.6	1367.418	1325
16	Undec-10-ynoic acid	19.658	763741	1.61	1390.082	1469
17	9-Oxononanoic acid	21.714	1832321	3.87	1478.87	1483
18	Nonanoic acid, 9-oxo-, ethyl ester	22.312	291758	0.62	1505.114	1507
19	Ethyl hydrogen suberate	23.391	910306	1.92	1554.384	1553
20	Z-10-Tetradecen-1-ol acetate	28.084	330310	0.7	1782.437	1787
21	Hexadecanoic acid, ethyl ester	31.725	2303600	4.86	1979.609	1985
22	9,12-Octadecadienoic acid (Z,Z)-	34.532	705093	1.49	2144.634	2095
23	(E)-9-Octadecenoic acid ethyl ester	34.615	537516	1.13	2149.695	2185
24	Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Z,Z,Z)-	42.068	1720786	3.63	2652.09	2721
25	U.I	42.748	1875650	3.96	2702.923	

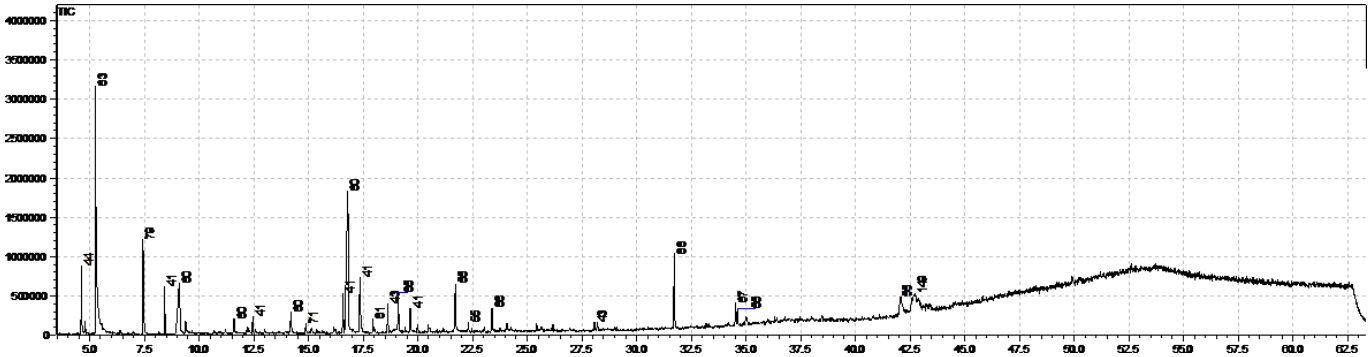


Figure 3. GC-MS chromatogram of Chloroform fruit extract of *Abelmoschus manihot*

3.2.3. Ethyl acetate extract

In the chromatogram **Figure 4**, it is clearly showed that no peaks appeared in the ethylaceate extract. It could be due to the compounds are not sensitive or not separated in the SP-2560 Supelco capillary column. In addition, inside the ethyl acetate extract might be all compounds are high molecular weight.

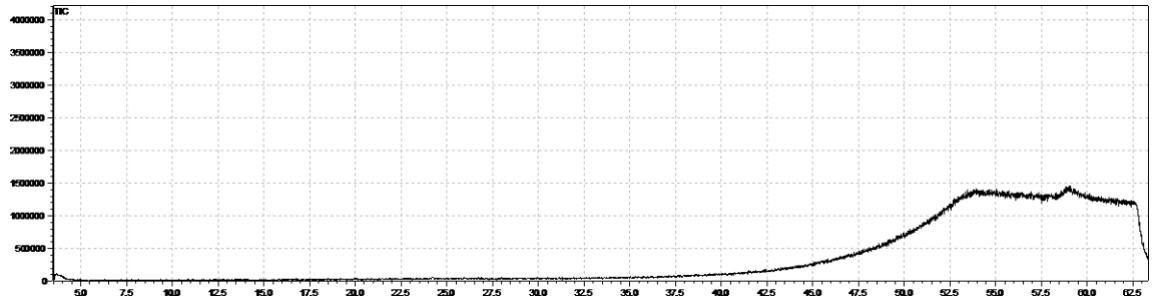


Figure 4. GC-MS chromatogram of ethyl acetate fruit extract of *Abelmoschus manihot*

3.2.4. Butanol extract

Butanoic acid, butyl ester, 2-heptanol, 2-methyl and docosanoic acid, docosyl ester were present in high percent in the butanol extract 28.5, 28.2 and 16.64 percent respectively. In addition to palmitic acid, ethyl ester about 8.86% that are presented in Table 5, Figure 5.

Table 5. Compounds present in the butanol extract

#	Compound name	R.Time (min)	Area	Area %	Cal KI	NIST KI
1	Propanoic acid, 2-methyl-, butyl ester	8.345	692258	3.84	960.3321	952
2	Propanoic acid, 2 methyl-, 2-methylpropyl ester	8.405	1383514	7.67	962.5461	925
3	Butanoic acid, butyl ester	9.494	5139690	28.5	1002.671	969
4	2-Heptanol, 2-methyl-	12.975	5086154	28.2	1129.074	919

5	Butane, 1,1-dibutoxy-	16.409	753347	4.18	1259.105	1229
6	Palmitic acid, methyl ester	30.543	381329	2.11	1913.575	1908
7	Palmitic acid, ethyl ester	31.723	1598305	8.86	1979.497	1968
8	Docosanoic acid, docosyl ester	49.955	3000779	16.64	3304.206	4547

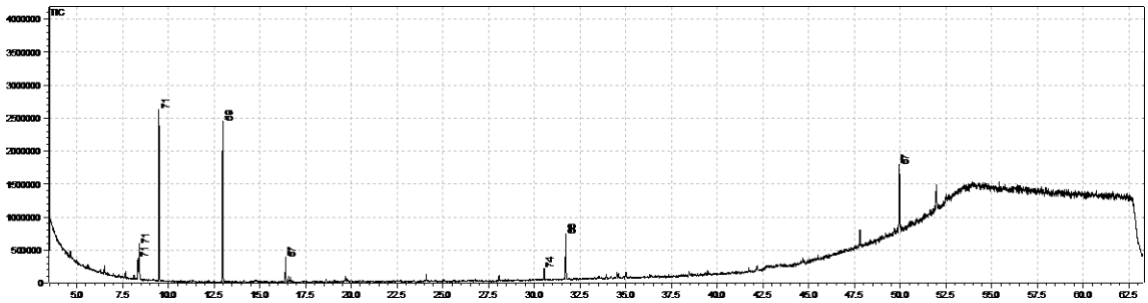


Figure 5. GC-MS chromatogram of Butanol fruit extract of *Abelmoschus manihot*

4. Conclusions

The antimicrobial activity and phytochemicals of each fruits extract were determined by disc diffusion method and GC-MS. In this study, the highest antibacterial activity was observed in hexane and water extracts with the range of inhibition zone 0-10.6 mm against the applied bacterial strains. Unsaturated fatty acids, terpenoids derivatives, normal and aromatic hydrocarbons, alkaloid, and flavonoids derivatives were the main phytochemicals detected in the different polarity extracts. Finally, the selected plant's extract with the highest antibacterial activity might be employed to develop antibacterial agents.

Major compounds found in butanol extract are butanoic acid, butyl ester, 2-heptanol, 2-methyl. In the chloroform extract nonanoic acid was the major constituent (20.5%). The hexane extract showed the presences of octadecanoic acid, methyl ester (31.99%), erucic acid, E- 9-octadecadienoic acid ethyl ester (15.9%), n-hexadecanoic acid (13.51%), and 9,12-Octadecadienoic acid (Z,Z) (11.62%). However, the ethyl acetate extract did not show the presences of any compounds.

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