WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Isolation and Characterization of Pure Organic Compounds from Medicago Sativa (Alfalfa) Leaf

Nnawuihe, G.C. and Agoha, C.C.

Department of Chemistry/Biochemistry, Federal Polytechnic Nekede, Owerri, Imo State, Nigeria.
Email: Cnnawuihe@fpno.edu.ng

ABSTRACT

A good knowledge of the chemical composition of plant leads to a better understanding of its possible medicinal value. In this study, the isolation and characterization of pure organic compounds from Medicago sativa (Alfalfa) leaf was carried out. The matured fresh leaves of Medicago sativa were collected, washed, air-dried, ground to powder and extracted using methanol. The methanolic extract were subjected to Column chromatography and the eluates were isolated using thin layer chromatography, the pure isolate was further identified using GC-MS and FT-IR using the following solvents; ethanol, N-Hexane, Diethyl ether, Chloroform, Acetone, Ethyl acetate, Petroleum ether, Methanol, Dichloromethane and Isopropanol. The results obtained showed that Hexachloroethane has the highest percentage abundance in the organic component of the leaf as shown in the GC-MS result of the ten (10) different fractions of the pure isolate of Medicago sativa leaf. However, since the GC-MS revealed Hexacholoethane as the most prominent compound, it is a pure isolate of Medicago sativa leaf. However, the bioactive compounds present in the leaf has been found from literatures to be responsible for the following pharmacological properties; antibacterial, antifungi, antidiuretic, antimalarial, antiasthma, anticancer, cholinomimetic, antiarrhythmic, analgesic, antihperglycemic etc

Keywords: Bioactive compounds, isolate, pharmacological properties, characterization

INTRODUCTION

Within the field of Phytochemistry, the definition of natural products is usually restricted to organic compounds isolated from plant natural sources that are produced by the pathways of primary or secondary metabolism. Within the field of medicinal chemistry, the definition is often further restricted to secondary metabolites. Secondary metabolites are not essential for survival, but nevertheless provide plants that produce them a survival advantage (Jones and Kossel, 2018).

Plant chemistry is the basis of the therapeutic uses of herbs. A good knowledge of the chemical composition of plants leads to a better understanding of its possible medicinal value. Modern chemistry has described the role of primary plant metabolites in basic life functions such as cell division and growth, respiration, storage and reproduction (Bourgaud et al., 2019). They include the components of processes such as glycolysis, the Krebs or citric acid cycle, photosynthesis and associated pathways. Primary metabolites include small molecules such as sugars, amino acids, tricarboxylic acids, or Krebs cycle intermediates, proteins, nucleic acids and polysaccharides. Eventually, the primary metabolites are similar in all living cells (Bennets et al., 2019).

Secondary plant metabolites are numerous chemical compounds produced by the plant cell through metabolic pathways derived from the primary metabolic pathways. The concept of secondary metabolite was first defined by Albrecht Kossel, Nobel Prize winner for physiology or medicine in 1910 (Anbalahan, 2021).

Thirty years later, Czapek described them as end-products. According to him, these products are derived from nitrogen metabolism by what he called ‘secondary modifications’ such as deamination. In the middle of the twentieth century, advances of analytical techniques such as chromatography allowed the recovery of more and more of these molecules, and this was the basis for the establishment of the discipline of phytochemistry (Gomathi et al., 2017).

Secondary metabolites have shown to possess various biological effects, which provide the scientific base for the use of herbs in the traditional medicine in many ancient communities. They have been described as antibiotic, antifungal and antiviral and therefore are able to protect plants from pathogens. Besides, they constitute important UV absorbing compounds, thus preventing serious leaf damage from the light. It was noticed that some herbs as forage grasses such as Medicago sativa (alfalfa) can express estrogenic properties and interact with fertility of animals (Aganga and Tshwenyane, 2020).

Medicago sativa is used traditionally for the treatment of several ailments such as arthritis, kidney problems, fever, as diuretic, anti cancer, anti-rheumatic, diabetes, and in the treatment of boils. However, most of the compounds that are responsible for the pharmacological actions of this traditional remedy are not known. This has hindered the standardization and development of this herb and made its recognition, acceptance and utilization remain locally restricted. Therefore, it is necessary to isolate and characterize pure organic compounds from Medicago sativa leaves.

MATERIALS AND METHODS

Materials and Reagents

The materials used in this study were GC-MS machine model: GC system-7890B; MSD-5997A, Shimadzu FTIR–8400s Fourier transform infrared spectrophotometer, Japan, Chromatographic column etc. All reagents used were of Analytical grade (AG) so further purification were not required.

Sample Collection and Preservation

Leaves of Medicago sativa were harvested from the Akwete, Ezigaragu, Enyiogugu in Aboh Mbaise L.G.A, Imo State; on the 12th of June, 2023. The plant material was authenticated by Mr. Ibe Ndukwe of the Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria. The collected leaves of M. sativa were air-dried for thirty days and were ground into small particles using electric blender. The weight of the ground sample was 714 g.

Sample Extraction

Five hundred (500) grams of the ground M. sativa leaf was weighed and put into an amber coloured bottle. Four litres of methanol was poured into the sample and allowed to stay for forty eight hours. After which it was filtered using Whatman filter paper No.1 and other filtration apparatus. The methanol extract was subjected to distillation using rotary evaporator under a reduced temperature to get the crude sample. The Digital Heidolph Rotary Evaporator (4000 series) was set at 650C under reduced pressure. The sample flask of the rotary evaporator was filled to one-fourth of its capacity with the filtrate (methanol extract) and was fitted to the rotary machine. The flask was held at an angle and rotated rapidly in the heating bath which was maintained at a temperature of 65 0C. This action spreads the liquid in a film on the wall of the flask and provided a large surface for evaporation. Care was taken to ensure that bumping of the material did not occur by adjusting the knob provided, immediately the first sign of bumping was observed to let some air to enter the sample flask. The pressure of the rotary evaporator was maintained at 0.01 mmHg.

As the solvent vapour passed through the condenser, it condensed and was recovered in a second round bottomed flask; the solvent recovery flask. The two condensers in the thermoflask filled with ice block baths were also monitored to make sure that the extracted solvent did not suck into the pressure pump during the process. After recovering of the methanol in another container, the extract was allowed to stand so that the little methanol in the crude fraction could evaporate completely to get the actual weight of the sample. The extract collected weighed 50.37 g.

COLUMN CHROMATOGRAPHY

Partitioning of Methanol Extract between Chloroform and Water (1:1)

A separating funnel was washed with distilled water. It was then clamped to a retort stand with its tap closed. The methanol extract was put into a beaker and 200 ml of chloroform was measured into the beaker using measuring cylinder. The crude methanol extract was dissolved in the chloroform and gently poured inside the separating funnel. 200 ml of distilled water was also measured into the separating funnel. The separating funnel was corked and shaken for 30 minutes to mix the two liquids with the extract. Care was taken to release the tap at intervals to prevent build up of pressure in the separating funnel while shaking was going on. After which the separating funnel was clamped to a retort stand and allowed to stand. The substance in the separating funnel was observed to be dark green in colour. After 2 hours 35 minutes, the substance in the funnel was observed to be separated into two distinct layers, an upper aqueous layer and lower chloroform layer. Both chloroform and aqueous layer were collected in two separate beakers. The chloroform fraction was collected and reintroduced into the separating funnel and was washed with another 200 ml of distilled water, the separating funnel was allowed to equilibrate and the lower chloroform layer was also collected. The washing process was repeated severally until a clear aqueous layer was observed. The chloroform layer was collected and properly stored.

Column Packing

The column used was 280 mm in height and 35 mm in diameter. 4.2 g of the chloroform extract was weighed in a beaker. Thirty millilitre of chloroform was measured using the measuring cylinder and poured into the beaker. The mixture was stirred until a solution of the plant sample was obtained. Then, 40 g of silica gel was added to the above solution and mixed properly until slurry was obtained. The slurry was left to evaporate in the open laboratory. The dry slurry weighed 41.3 g.

The column previously washed, was rinsed with hexane. Cotton wool was used to plug the outlet of the column to about 3 cm, sand was also added. This acted as a supporting base so that the packing of silica gel did not wash out of the bottom of the column. The column of silica gel was deposited on the top of the supporting base in the form of slurry. The slurry was prepared in a separate container by adding the silica gel absorbent, a little at a time, with appropriate quantity of the solvent hexane. The slurry was swirled until it became homogenous and relatively free of entrapped air bubbles.

Having prepared the slurry, it was mixed and poured into the column. Care was taken while taping the column constantly and gently on the side, during the pouring operation, with a pencil fitted with rubber stopper. The tapping promoted even settling and mixing and gave evenly packed column free of air bubbles. This continued until all the materials had settled. Hexane was cycled through the column several times to ensure that settling was complete and that the column was firmly packed. Care was taken never to let the column run dry during packing. The already prepared dry slurry of the sample was poured into the packed column and then sealed with dry silica gel. Beakers were labeled 1-31 for collecting fractions eluted from different solvent mixtures. The column was clamped to a retort stand. Elution began with hexane (non polar solvent). The polarity of elution solvent mixture was increased

gradually by adding successively increasing quantities of moderately polar chloroform. After elution with hexane-chloroform, chloroform-methanol was used. Care was taken to run with a flow rate of 12-15 drops per minute. It is important to operate at this optimum flow rate because if the flow of solvent through the column is too rapid, the solutes will not have time to equilibrate with the adsorbent and pass down to the column (Donald et al., 1998). If the rate of flow is too slow or stop for a period, the solute band will diffuse in all directions. In either of these cases, separation will be poor (Donald et al., 1998). The process of column chromatography lasted for more than 72 hours during which various fractions were collected in labeled beakers for thin layer chromatography.

THIN LAYER CHROMATOGRAPHY (TLC)

Preparing Plate for Thin-layer Chromatography

Silica gel slurry was made by mixing and stirring one part of silica gel in three part of distilled water. The surface of the glass plate (5.5 cm x 16 cm) was wiped with clean cotton wool to clean and dry it. One surface of the plate was then coated with the slurry by carefully smearing the surface of the plate with the slurry gel to form a thin layer on the glass plate. The coated plates were left in the open laboratory to dry for three hours at room temperature after which they were activated in the oven at a temperature of 1100C. The plates were then ready to use.

Spotting, Developing and Visualizing

Solution of each fraction was made using appropriate solvents. A small capillary tube was used to collect some fractions from the beaker and spotted 1.5 cm above the base of the plate. The spotted plate was put in a Thin Layer Chromatography (TLC) tank, containing 100 ml of mixture of appropriate solvents for elution. The plate was placed in the development chamber (TLC tank) such that the spotted point did not dip inside the solvent. Care was taken to ensure that the spot was above the level of the solvent to prevent the spotted material from dissolving in the mixture of solvents rather than undergoing chromatography. The developing chamber was capped with care to avoid disturbing the movement of the solvent. The solvent advanced up the plate by capillary action. When the solvent had advanced to 70 – 75 % to the end of the coated surface, the plate was removed and position of the solvent front was marked. The developed plate was allowed to dry in the open laboratory, then treated with iodine (visualizing agent) in a covered tank and left for 30 minutes to allow iodine vapour to saturate the tank. When the spot became visible, the plate was removed from the iodine tank and the spot outlined. The retention factors (Rf) of the fractions from chromatography were determined.

R_f=(Distance moved by solute)/(Distance moved by solvent front)

GAS CHROMATOGRAPHY-MASS SPECTROSCOPY (GC-MS)

The GC/MS analysis was carried out in the laboratory of the department of Industrial Chemistry, School of Science and Technology, Covenant University, Otta, Ogun state, Nigeria using GC-MS machine model: GC system-7890B; MSD-5997A with the following solvents; ethanol, N-Hexane, Diethyl ether, Chloroform, Acetone, Ethyl acetate, Petroleum ether, Methanol, Dichloromethane and Isopropanol.

The extracts were injected into the column of the spectrometer at 250°C injector temperature. Temperature of the oven started at 70°C and held for 5 min. It was then raised at the rate of 10°C per min to 280°C without holding. Holding was allowed for 6 min at programmed rate of 5°C per min. Temperature of ion sources were maintained at 200°C. The injector temperature was set at 250°C and detector temperature was set at 250°C. The mass spectrum of compounds present in samples was obtained by electron ionization at 70eV and detector operates in scan mode 50 to 600 Da atomic units. The MS Table was generated through ACQ mode scan within 0.5 seconds of scan interval at the speed of 666 and fragments from 30 to 350 Da were maintained. Total running were 21 minutes.

FT-IR ANALYSIS

The sample was subjected to Fourier Transform Infrared Spectroscopic analysis using Shimadzu FTIR–8400s Fourier transform infrared spectrophotometer, Japan; at the Department of Industrial Chemistry, School of Science and Technology, Covenant University, Otta, Ogun State, Nigeria with the following solvents; ethanol, N-Hexane, Diethyl ether, Chloroform, Acetone, Ethyl acetate, Petroleum ether, Methanol, Dichloromethane and Isopropanol.

The samples were oven dried to get powders of the different solvent extracts used for FTIR analysis. The dried extracts powder (10 mg) was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample disc and analysis was carried out by scanning the samples through a wave number range of 400 to 4000 cm-1 with a resolution of 2cm-1. FTIR analyses were performed and the different peaks present and possible chemical interactions were examined.

RESULTS AND DISCUSSION

RESULTS

Table 1.1: GC-MS Result of Ethanol Fraction

S/N	Name	RT (min)	% Composition
1	Oxalyl chloride	2.22	1.01
2	Phosgene	2.42	0.43
3	Trichloromethane	2.67	0.56
4	Trichloromethane	3.10	4.65
5	Trichloronitromethane	3.36	1.13
6	Trichloronitromethane	3.43	4.58
7	Trichloroethylene	3.69	3.78
8	Ethane, hexachloro-	5.10	22.12
9	p-Benzoquinone, 2,3,5,6-tetrachloro-	14.18	0.78
10	p-Benzoquinone, 2,3,5,6-tetrachloro-	14.18	0.78
11	Benzene, hexachloro-	15.12	2.99
12	Benzene, hexachloro-	15.12	2.99
13	1,3-Cyclopentadiene, 1,2,3,4-tetrachloro-5-(dichloromethylene)-	15.70	1.65
14	Benzene, hexachloro-	16.01	3.71
15	Benzene, hexachloro-	16.72	1.30
16	3,3'-Dichloro-5,5'-difluorobiphenyl-4-ol	17.61	0.62
17	Phenol, 2,4-dibromo-6-nitro-	18.19	2.39
18	Benzene, pentachloro(trichloroethenyl)-	18.76	4.29
19	p-Terphenyl, 2,5-dichloro-	22.95	0.62
20	Naphthalene, octachloro-	25.49	1.47

Table 1.2: GC-MS Result of N-hexane Fraction

S/N	Name	RT (min)	% Composition
1	Oxalyl chloride	2.12	1.01
2	Phosgene	2.42	0.43
3	Trichloromethane	2.67	0.56
4	Trichloromethane	3.10	4.65
5	Trichloronitromethane	3.36	1.13
6	Trichloronitromethane	3.43	4.58
7	Trichloroethylene	3.69	3.78
8	Tetrachloroethylene	5.10	2.02
9	Tetrachloroethylene	5.34	1.03
10	Ethane, 1,1,2,2-tetrachloro-	6.31	0.53
11	Ethane, pentachloro-	7.26	2.75
12	Cyclotetrasiloxane, octamethyl-	7.33	0.78
13	Ethane, hexachloro-	8.86	21.08
14	1,3-Butadiene, 1,1,2,4,4-pentachloro-	9.37	1.14
15	1,3-Butadiene, 1,1,2,4,4-pentachloro-	9.83	2.74
16	1,3-Butadiene, 1,1,2,4,4-pentachloro-	9.90	0.74
17	1,3-Butadiene, 1,1,2,3,4,4-hexachloro-	10.63	8.26
18	Hexachlorocyclopentadiene	11.82	0.46
19	Benzene, pentachloro-	13.95	1.51

Table 1.3: GC-MS Result of Diethyl ether Fraction

S/N	Name	RT (min)	% Composition
1	Phosgene	2.13	0.88
2	Methylene chloride	2.42	0.70
3	Trichloromethane	3.14	3.07
4	Carbon Tetrachloride	3.40	0.95
5	Trichloronitromethane	3.46	4.75

6	Trichloroethylene	3.73	3.47
7	Tetrachloroethylene	5.10	9.67
8	Tetrachloroethylene	5.32	1.10
9	Ethane, 1,1,2,2-tetrachloro-	6.31	0.81
10	Ethane, pentachloro-	7.27	3.72
11	Cyclotetrasiloxane, octamethyl-	7.32	0.56
12	Ethane, hexachloro-	8.83	21.20
13	1,3-Butadiene, 1,1,2,4,4-pentachloro-	9.37	1.16
14	1,3-Butadiene, 1,1,2,4,4-pentachloro-	9.83	3.42
15	1,3-Butadiene, 1,1,2,4,4-pentachloro-	9.90	0.90
16	1,3-Butadiene, 1,1,2,3,4,4-hexachloro-	10.62	10.70
17	Hexachlorocyclopentadiene	11.81	0.65

Table 1.4: GC-MS Result of Chloroform Fraction

S/N	Name	RT (min)	% Composition
1	Ethane, pentachloro-	7.27	3.72
2	Cyclotetrasiloxane, octamethyl-	7.32	0.56
3	Ethane, hexachloro-	8.87	24.70
4	1,3-Butadiene, 1,1,2,4,4-pentachloro-	9.37	1.16
5	1,3-Butadiene, 1,1,2,4,4-pentachloro-	9.83	3.42
6	1,3-Butadiene, 1,1,2,4,4-pentachloro-	9.90	0.90
7	1,3-Butadiene, 1,1,2,3,4,4-hexachloro-	10.62	10.70
8	Hexachlorocyclopentadiene	11.81	0.65
9	Benzene, pentachloro-	13.95	1.86
10	p-Benzoquinone, 2,3,5,6-tetrachloro-	14.17	0.50
11	3,3'-Dichloro-5,5'-difluorobiphenyl-4-ol	17.09	0.45
12	3,3'-Dichloro-5,5'-difluorobiphenyl-4-ol	17.09	0.45
13	3,3'-Dichloro-5,5'-difluorobiphenyl-4-ol	17.61	0.74
14	3,3'-Dichloro-5,5'-difluorobiphenyl-4-ol	17.61	0.74
15	3,3'-Dichloro-5,5'-difluorobiphenyl-4-ol	17.92	0.44
16	Phenol, 2,4-dibromo-6-nitro-	18.18	2.82
17	Benzene, pentachloro(trichloroethenyl)-	18.732	4.02
18	2,6-Lutidine, 4-[[p-aminophenyl]thio]-3,5-dichloro-	22.94	0.72
19	Naphthalene, octachloro-	25.47	1.45

Table 1.5: GC-MS Result of Acetone Fraction

S/N	Name	RT (min)	% Composition
1	Phosgene	2.13	0.79
2	Methylene chloride	2.42	0.66
3	Trichloromethane	3.13	3.79
4	Carbon Tetrachloride	3.38	0.74
5	Trichloronitromethane	3.44	0.80
6	Trichloroethylene	3.71	3.56
7	Tetrachloroethylene	5.08	9.85
8	Tetrachloroethylene	5.31	1.08
9	Ethane, 1,1,2,2-tetrachloro-	6.30	0.90
10	Ethane, pentachloro-	7.27	3.69
11	Ethane, hexachloro-	8.81	23.35
12	1,3-Butadiene, 1,1,2,4,4-pentachloro-	9.37	1.06
13	1,3-Butadiene, 1,1,2,4,4-pentachloro-	9.83	3.44
14	1,3-Butadiene, 1,1,2,4,4-pentachloro-	9.90	0.87
15	1,3-Butadiene, 1,1,2,3,4,4-hexachloro-	10.62	11.15

Table 1.6: GC-MS Result of Ethyl acetate Fraction

S/N	Name	RT (min)	% Composition
1	Phosgene	2.13	0.79
2	Methylene chloride	2.42	0.66
3	Trichloromethane	3.13	3.79
4	Carbon Tetrachloride	3.38	0.74
5	Ethane, 1,1,2,2-tetrachloro-	6.30	0.90
6	Ethane, 1,1,2,2-tetrachloro-	6.30	0.90
7	Ethane, pentachloro-	7.27	3.69
8	Ethane, hexachloro-	8.94	25.14
9	Benzene, hexachloro-	15.72	1.99
10	Benzene, hexachloro-	16.04	3.73
11	Alpha,2,3,4,5,6-hexachlorotoluene	16.45	0.51
12	Benzene, hexachloro-	16.71	1.57
13	3-Chlorophenol, pentafluoropropionate	17.61	0.75
14	3,3'-Dichloro-5,5'-difluorobiphenyl-4-ol	17.92	0.49
15	Phenol, 2,4-dibromo-6-nitro-	18.19	3.17
16	Benzene, pentachloro(trichloroethenyl)-	18.74	4.00
17	2,6-Lutidine, 4-[[p-aminophenyl]thio]-3,5-dichloro-	22.94	0.74
18	Benzo[b]1,4-dioxane, 5,6,7-tribromo-8-methoxy-	25.47	1.67

Table 1.7: GC-MS Result of Petroleum ether Fraction

S/N	Name	RT (min)	% Composition
1	Methylene chloride	2.42	0.66
2	Trichloromethane	3.13	3.79
3	α-D-xylofuranoside, methyl 3-omethyl	3.38	0.74
4	Pentadecanoic acid, 1,14-methyl methyl ester	6.30	0.90
5	Hexadecanoic acid 14-methyl, methyl ester	6.30	0.90
6	7-Octadecenoic acid, methyl ester	7.27	3.69
7	Benzene, hexachloro-	15.72	1.99
8	Methyl Stearate	16.04	3.73
9	9-Octadecenoic acid methyl ester	16.45	0.51
10	Octadecanoic acid	16.71	1.57
11	Methyl Stearate	17.61	0.75
12	9-Octadecenoic acid methyl ester	17.92	0.49
13	Ethane, hexachloro-	18.19	23.17
14	Benzene, pentachloro(trichloroethenyl)-	18.74	4.00

Table 1.8: GC-MS Result of Methanol Fraction

S/N	Name	RT (min)	% Composition
1	Trichloromethane	3.13	3.79
2	Carbon Tetrachloride	3.38	0.74
3	Trichloronitromethane	3.44	0.80
4	Trichloroethylene	3.71	3.56
5	Tetrachloroethylene	5.08	10.85
6	Tetrachloroethylene	5.31	1.08
7	Ethane, 1,1,2,2-tetrachloro-	6.30	0.90
8	Ethane, pentachloro-	7.27	3.69
9	Ethane, hexachloro-	8.34	23.55
10	Diethyl-4,4-methylenedialllophanate	9.37	1.06
11	Phenylephrine	9.83	3.44
12	Pentadecanoicacid 14-methyl, methyl ester	9.90	0.87
13	2-formyl-9-[β-cl-ribofuranosyl]hypoxantine	10.62	11.15
14	9-Octadecenoic acid methyl ester	12.44	0.45
15	Phytol	12.67	0.33
16	Methyl Stearate	13.22	0.67

17	Hexadecanoic acid 14-methyl, methyl ester	15.18	1.09

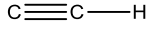
Table 1.9: GC-MS Result of Dichloromethane Fraction

S/N	Name	RT (min)	% Composition
1	Oxalyl chloride	2.22	1.01
2	Phosgene	2.42	0.43
3	Trichloronitromethane	3.43	4.58
4	Trichloroethylene	3.69	3.78
5	Tetrachloroethylene	5.10	11.12
6	p-Benzoquinone, 2,3,5,6-tetrachloro-	14.18	0.78
7	p-Benzoquinone, 2,3,5,6-tetrachloro-	14.18	0.78
8	Ethane, hexachloro-	15.12	22.99
9	1-propanol	15.18	2.99
10	Erythritol	15.70	1.65
11	2-dimethyl (prop-2-enyl)silyloxypropane	16.01	3.71
12	Threitol	16.72	1.30
13	2-o-methyl-D-mann opyranose	17.61	0.62
14	2-pentadecanoic-6,10,14-trimethyl	18.19	2.39
15	Pentadecanoic acid	18.76	4.29
16	n-hexadecanoic acid	22.95	0.62
17	9-octadecenoic acid	25.49	1.47
18	Phytol	25.88	0.34

Table 1.10: GC-MS Result of Isopropanol Fraction

S/N	Name	RT (min)	% Composition
1	Phosgene	2.13	0.79
2	Methylene chloride	2.42	0.66
3	Trichloromethane	3.13	3.79
4	Carbon Tetrachloride	3.38	0.74
5	Ethane, 1,1,2,2-tetrachloro-	6.30	0.90
6	Ethane, 1,1,2,2-tetrachloro-	6.30	0.90
7	Ethane, pentachloro-	7.27	3.69
8	Ethane, hexachloro-	7.59	25.44
9	Benzene, hexachloro-	15.72	1.99
10	1,3-Butadiene, 1,1,2,4,4-pentachloro-	16.04	3.73
11	1,3-Butadiene, 1,1,2,3,4,4-hexachloro-	16.45	0.51
12	Benzene, hexachloro-	16.71	1.57
13	3-Chlorophenol, pentafluoropropionate	17.61	0.75
14	7-Octadecenoic acid, methyl ester	17.92	0.49
15	Benzene, hexachloro-	18.19	3.17
16	Methyl Stearate	18.74	4.00
17	9-Octadecenoic acid methyl ester	22.94	0.74

Table 1.11: FT-IR Results of Ethanol fraction

Wavenumber (cm^{-1})	Functional groups
3749.7018	OH, Alcohol
2228.9480	 , Alkyene
1945.6703	C-H, Alkane
1994.1257	C-H, Alkane
2091.0365	C=C=C, Acetylene
2385.4962	C=N, Amine
2646.4098	C-H, Alkane
2728.4113	C-H, Alkane
3071.3263	C-H, Alkane
3127.2364	OH, Alcohol

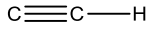
3749.7018	OH, Alcohol
2228.9480	 , Alkyene
1945.6703	C-H, Alkane
1994.1257	C-H, Alkane
2091.0365	C=C=C, Acetylene
2385.4962	C=N, Amine
2646.4098	C-H, Alkane
2728.4113	C-H, Alkane
3071.3263	C-H, Alkane
3127.2364	OH, Alcohol

Table 1.12: FT-IR Results of N-Hexane fraction

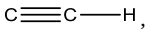
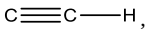
Wavenumber (cm^{-1})	**Functional groups**
3652.7910	OH, Alcohol
3395.6047	 , Alkyene
3168.2371	=C-H, Alkene
3116.0544	=C-H, Alkene
2855.1407	H-C-H, Alkane
2925.9602	H-C-H, Alkane
1546.8452	N-H, Amine
1285.9315	C-O, Ether
972.8351	C=C, Alkene
1360.4783	C-O, Ether
3652.7910	OH, Alcohol
3395.6047	 , Alkyene
3168.2371	=C-H, Alkene
3116.0544	=C-H, Alkene
2855.1407	H-C-H, Alkane
2925.9602	H-C-H, Alkane
1546.8452	N-H, Amine
1285.9315	C-O, Ether
972.8351	C=C, Alkene
1360.4783	C-O, Ether

Table 1.13: FT-IR Results of Diethyl ether fraction

Wavenumber (cm^{-1})	**Functional groups**
2094.7638	C=C=C, Acetylene
1543.1178	N-H, Amine
2661.3192	C-H, Alkane
3078.7810	C-H, Alkane
3339.6947	C-H, Alkane
693.2848	C-Cl, Chloride group
1282.2042	C-O, Ether
2855.1407	H-C-H, Alkane
1654.9380	C=O, Ketone
1356.7509	C-O, Ether
2094.7638	C=C=C, Acetylene
1543.1178	N-H, Amine
2661.3192	C-H, Alkane

3078.7810	C-H, Alkane
2855.1407	H-C-H, Alkane
2925.9602	H-C-H, Alkane
1546.8452	N-H, Amine
1285.9315	C-O, Ether
972.8351	C=C, Alkene
1360.4783	C-O, Ether

Table 1.14: FT-IR Results of Chloroform fraction

Wavenumber (cm^{-1})	Functional groups
3552	O-H stretching vibration (free)
2945.30	CH_3 asymmetry stretching
2887.44	C-H stretching vibration
2360.87	NH component
2054	$-NH^{3+}$ stretching vibration; broad
1654.92	C=C stretching vibration
1411.89	C-H deformation vibration
1163.08	CH_3 rocking vibration
927.76	C-C skeletal vibration
858.32	C-C skeletal vibration

Table 1.15: FT-IR Results of Acetone fraction

Wavenumber (cm^{-1})	Functional groups
3612.67	O-H stretching vibration (free)
2931.80	CH_2 asymmetry stretching
2858.51	C-H stretching vibration
2401.38	N-D stretching vibration
1743.65	C=O of esters
1544.98	N-H deformation vibrations
1463.97	C-H deformation vibration
1219.01	C-C skeletal vibration
771.53	C-H out-of-plane bending vibration for substituted benzenes ring
677.01	C-H wagging vibration
422.41	Aromatic C-OH in-plane bending vibration

Table 1.16: FT-IR Results of Ethyl acetate fraction

Wavenumber (cm^{-1})	Functional groups
3408.66	O-H stretch, Hydroxy group, H-bonded
2950.22	C-H stretch, Aliphatic
2840.76	C-H stretch
2522.99	O-H stretch, Acidic group
2075.38	Multiple bonding
1643.53	C=O stretching vibration, Ketone group
1460.46	C=C-C, Aromatic ring stretch
1405.75	O-H bend, Alcoholic group
1108.62	C-O stretch, , Ether group
1019.80	Phosphate ion
663.47	C-Br stretch

Table 1.17: FT-IR Results of Petroleum ether fraction

Wavenumber (cm^{-1})	Functional groups
3391.27	O-H stretch, Polymeric OH
2924.14	Asymmetric stretching of –CH (CH_2) vibration,
2856.81	Symmetric stretching of –CH (CH_2) vibration,

1729.40	C=O stretch, Carbonyl group
1630.17	C=O stretching vibration, Ketone group
1507.20	C=C-C, Aromatic ring stretch
1460.14	C=C-C, Aromatic ring stretch
1375.65	O-H bend, Alcoholic group
1261.45	CN stretch
895.22	P-O-C stretch,
768.55	C-Cl stretch,
724.23	C-Cl stretch,
611.95	C-Br stretch

Table 1.18: FT-IR Results of Methanol fraction

Wavenumber (cm^{-1})	**Functional groups**
3396.30	O-H stretch,
2946.99	C-H stretch, Aliphatic
2836.87	C-H stretch, Ether group
2524.35	O-H stretch, Carboxylic group
2042.41	Multiple bonding
1648.88	C=O stretching vibration, Ketone group
1452.51	C=C-C, Aromatic ring stretch
1409.17	O-H bend, Alcoholic group
1023.87	Phosphate ion
636.29	C-Br stretch, Aliphatic

Table 1.19: FT-IR Results of Dichloromethane fraction

Wavenumber (cm^{-1})	**Functional groups**
3874.86	Non bonded, O-H stretch
3762.92	Non bonded, O-H stretch
3440.82	O-H stretch, H bonded
2922.12	Asymmetric stretching of –CH (CH_2) vibration
2855.09	Symmetric stretching of –CH (CH_2) vibration,
2640.90	S-H stretch
2218.60	Carbon-Carbon triple bond
2118.61	Carbon-Carbon triple bond
2027.59	Carbonyl compound frequency
1931.27	Carbonyl compound frequency
1722.95	C=O stretch
1637.29	C=O stretching
1512.64	C=C-C, Aromatic ring
1462.82	C=C-C, Aromatic ring stretch
1265.99	CN stretch
1100.76	PO_3 stretch
676.68	C-Br stretch
445.35	S-S stretch

Table 1.20: FT-IR Results of Isopropanol fraction

Wave number (cm^{-1})	**Functional groups**
1243	S=O, Sulfon/Chloride
1420	OH, Alcohol
1630	C=C, Alkene
1631	C=C, Alkene
1880	C-H, Aromatic compounds

2059	N=C=S, Isothiocyanate
2493	S-H, Thiol
2766	C-H, Alkane

DISCUSSION

Table 1.1 shows the GC-MS Result of the Ethanol fraction of the pure isolate of M. sativa leaf. The result showed that Hexachloroethane has the highest percentage composition of 22.12% with a retention time of 5.10 minutes. The result also revealed that a total of 20 compounds were identified from the pure isolates which indicate that the other 19 compounds were trace compounds from the isolates. However, the highest abundance of Hexachloroethane in the ethanol fraction showed that it is a pure isolate.

Table 1.2 shows the GC-MS Result of the N-Hexane fraction of the pure isolate of M. sativa leaf. The result showed that Hexachloroethane has the highest percentage composition of 21.08% with a retention time of 8.86 minutes. The result also revealed that a total of 19 compounds were identified from the pure isolates which indicate that the other 18 compounds were also trace compounds from the isolates.

Table 1.3 shows the GC-MS Result of the Diethyl ether fraction of the pure isolate of M. sativa leaf. The result showed that Hexachloroethane has the highest percentage composition of 21.20% with a retention time of 8.83 minutes. The result also revealed that a total of 17 compounds were identified from the pure isolates which indicate that the other 16 compounds were also trace compounds from the isolates.

Table 1.4 shows the GC-MS Result of the Chloroform fraction of the pure isolate of M. sativa leaf. The result showed that Hexachloroethane has the highest percentage composition of 24.70% with a retention time of 8.87 minutes. The result also revealed that a total of 19 compounds were identified from the pure isolates which indicate that the other 18 compounds were also trace compounds from the isolates.

Table 1.5 shows the GC-MS Result of the Acetone fraction of the pure isolate of M. sativa leaf. The result showed that Hexachloroethane has the highest percentage composition of 23.35% with a retention time of 8.81 minutes. The result also revealed that a total of 15 compounds were identified from the pure isolates which indicate that the other 14 compounds were also trace compounds from the isolates.

Table 1.6 shows the GC-MS Result of the Ethyl acetate fraction of the pure isolate of M. sativa leaf. The result showed that Hexachloroethane has the highest percentage composition of 25.14% with a retention time of 8.94 minutes. The result also revealed that a total of 18 compounds were identified from the pure isolates which indicate that the other 17 compounds were also trace compounds from the isolates.

Table 1.7 shows the GC-MS Result of the Petroleum ether fraction of the pure isolate of M. sativa leaf. The result showed that Hexachloroethane has the highest percentage composition of 23.17% with a retention time of 18.19 minutes. The result also revealed that a total of 14 compounds were identified from the pure isolates which indicate that the other 13 compounds were also trace compounds from the isolates.

Table 1.8 shows the GC-MS Result of the Methanol fraction of the pure isolate of M. sativa leaf. The result showed that Hexachloroethane has the highest percentage composition of 23.55% with a retention time of 8.34 minutes. The result also revealed that a total of 17 compounds were identified from the pure isolates which indicate that the other 16 compounds were also trace compounds from the isolates.

Table 1.9 shows the GC-MS Result of the Dichloromethane fraction of the pure isolate of M. sativa leaf. The result showed that Hexachloroethane has the highest percentage composition of 22.99% with a retention time of 15.12 minutes. The result also revealed that a total of 18 compounds were identified from the pure isolates which indicate that the other 17 compounds were also trace compounds from the isolates.

Table 1.10 shows the GC-MS Result of the Isopropanol fraction of the pure isolate of M. sativa leaf. The result showed that Hexachloroethane has the highest percentage composition of 25.44% with a retention time of 7.59 minutes. The result also revealed that a total of 17 compounds were identified from the pure isolates which indicate that the other 16 compounds were also trace compounds from the isolates while the other function groups indicates the trace compounds in the isolate.

Table 1.11 shows the FT-IR Result of the Ethanol fraction of the pure isolate of M. sativa leaf. The result revealed the presence of OH group from an Alcohol which correspond to the absorption frequency of 3749.7018 cm-1, the correspond to the Alkyene group, the C-H correspond to the Alkane group, the C=C=C correspond to the Acetylene group while the C=N correspond to the Amine group. The presence of the alkane group in the ethanol fraction agrees with the GC-MS result of which hexachloroethane had the highest percentage abundant as the pure isolate.

Table 1.12 shows the FT-IR Result of the N-Hexane fraction of the pure isolate of M. sativa leaf. The result revealed the presence of OH group from an Alcohol, the correspond to the Alkyene group, the =C-H and C=C correspond to the alkene group, the H-C-H correspond to the Alkane group, the N-H correspond to the Amine group while the C-O correspond to the Ether group. The presence of the alkane group in the N-Hexane fraction also agrees with the GC-MS result of which hexachloroethane had the highest percentage abundant as the pure isolate.

Table 1.13 shows the FT-IR Result of the Diethyl ether fraction of the pure isolate of M. sativa leaf. The result revealed the presence of the C=C=C correspond to the Acetylene group, the correspond to the Alkyene group, the C=O correspond to the Ketonegroup, the H-C-H correspond to the Alkane group, the N-H correspond to the Amine group, the C-O correspond to the Ether group while the C-Cl correspond to the chloride group. The presence of

the alkane and Chloride group in the diethyl ether fraction also agrees with the GC-MS result of which hexachloroethane had the highest percentage abundant as the pure isolate.

Table 1.14 shows the FT-IR Result of the Chloroform fraction of the pure isolate of M. sativa leaf. The result revealed the presence of the C=C=C correspond to the Acetylene group, the correspond to the Alkyene group, the C=O correspond to the Ketonegroup, the H-C-H correspond to the Alkane group, the N-H correspond to the Amine group, the C-O correspond to the Ether group while the C-Cl correspond to the chloride group. The presence of the alkane and Chloride group in the diethyl ether fraction also agrees with the GC-MS result of which hexachloroethane had the highest percentage abundant as the pure isolate.

Table 1.15 shows the FT-IR Result of the Acetone fraction of the pure isolate of M. sativa leaf. The result revealed the presence O-H which corresponds to an alcohol, CH3 C-C and C-H group which correspond to an alkane, N-H and -NH3+ of amine. However, the presence of the alkane group in the Chloroform fraction also agrees with the GC-MS result of which hexachloroethane had the highest percentage abundant as the pure isolate.

Table 4.16 shows the FT-IR Result of the Acetone fraction of the pure isolate of M. sativa leaf. The result revealed the presence O-H which corresponds to an alcohol, CH2 of an Alkene, C-C and C-H group which correspond to an alkane, C=O of esters and C-OH of aromatic vibration. However, the presence of the alkane group in the Acetone fraction also agrees with the GC-MS result of which hexachloroethane had the highest percentage abundant as the pure isolate.

Table 4.17 shows the FT-IR Result of the Ethyl acetate fraction of the pure isolate of M. sativa leaf. The result revealed the presence O-H which corresponds to an alcohol, C-C and C-H group which correspond to an alkane, C=O of ketone, C=C-C of aromatic ring, C-O of ether, Phosphate ion and C-Br of bromide group. However, the presence of the alkane and the bromide group in the Ethyl acetate fraction also agrees with the GC-MS result of which hexachloroethane had the highest percentage abundant as the pure isolate.

Table 4.18 shows the FT-IR Result of the Petroleum ether fraction of the pure isolate of M. sativa leaf. The result revealed the presence O-H which corresponds to an alcohol, C-C and C-H group which correspond to an alkane, C=O of ketone, C=C-C of aromatic ring, C-N group of Cyanide, P-O-C of phosphate group, C-O of ether, Phosphate ion, C-Br of bromide group and C-Cl group. However, the presence of the alkane, Chloride and the bromide group in the Petroleum ether fraction also agrees with the GC-MS result of which hexachloroethane had the highest percentage abundant as the pure isolate.

Table 1.18 shows the FT-IR Result of the Methanol fraction of the pure isolate of M. sativa leaf. The result revealed the presence O-H which corresponds to an alcohol, C-H group which correspond to an alkane, C=O of ketone, C=C-C of aromatic ring, Phosphate ion and C-Br of bromide from group. However, the presence of the alkane and the bromide group in the Methanol fraction also agrees with the GC-MS result of which hexachloroethane had the highest percentage abundant as the pure isolate.

Table 4.19 shows the FT-IR Result of the Dichloromethane fraction of the pure isolate of M. sativa leaf. The result revealed the presence O-H which corresponds to an alcohol, -CH2 and C-C group which correspond to an alkane, C=O of ketone, C-N of Cyanide, C=C-C of aromatic ring, S-S stretch and S-H group of thiols, Phosphate ion and C-Br of bromide group. However, the presence of the alkane and the bromide group in the Dichloromethane fraction also agrees with the GC-MS result of which hexachloroethane had the highest percentage abundant as the pure isolate.

Table 1.20 shows the FT-IR Result of the Isopropanol fraction of the pure isolate of M. sativa leaf. The result revealed the presence O-H which corresponds to an alcohol, C=C group which correspond to an alkene, S=O of sulfon/Chloride, C-H of aromatic compound, N=C=S of Isothiocynanate, S-H group of thiols and C-H of alkane group. However, the presence of the alkane group in the Isopropanol fraction also agrees with the GC-MS result of which hexachloroethane had the highest percentage abundant as the pure isolate.

CONCLUSION

The results of this research work revealed that Medicago sativa has a lot of Phytochemicals which could be used as raw material for pharmaceutical industries. The findings therein showed that Hexachloroethane has the highest percentage abundance in the organic component of the leaf as shown in the GC-MS result of the ten (10) different fractions of the pure isolate of Medicago sativa. However, since the GC-MS revealed Hexacholoethane as the most prominent compound, it is a pure isolate of Medicago sativa leaf. Based on the findings therein, it is necessary that the following recommendations should be strictly adhered to.

Further researches should be carried out on the isolation and characterization of pure compounds from Medicago sativa using other solvents other than the ones used in this study.

More work should be done on the pharmacodynamics and pharmacokinetics of the pure compound isolated.

The synthesis of the isolated pure compound should also be encouraged.

Researches should be carried out on the industrial applications of the isolated pure compound.

ACKNOWLEDGEMENT

The authors are grateful to the Management of Tertiary Education Trust Fund (TETFUND) for supporting this research and sponsoring it 100% through Federal Polytechnic Nekede for 2023 (Merged) TETFUND Intervention in Research Project

REFERENCES

Adaay, M.H., Al-Dujaily, S.S. and Khazzal, F.K. (2022). Effect of aqueous extract of Medicago sativa and Salvia officinalis mixture on hormonal, ovarian and uterine parameters in mature female mice. J Mater Environ Sci, 4(4):424-433.

Aganga, A.A. and Tshwenyane, S.O. (2020). Lucerne, Lablab and Leucaena leucocephala forages: Production and utilization for livestock production, Pakistan Journal of Nutrution, 2:46-53.

Ahmad, N., Rahman, Z.U., Akhtar, N., Ali, S., Ahmad, M. and Ahmad, I. (2017). Ethanolic extract of Medicago sativa plants exhibits estrogen like activity, increases serum total proteins and decreases serum total cholesterol in immature female rats. Int J Agric Biol, 15:297-300

Aldo, T. and Luciano, P. (2017). Volatiles from Medicago sativa complex flowers. Phytochemistry, 45:1145-1148.

Al-Dosari, M.S. (2018). In vitro and in vivo antioxidant activity of alfalfa (Medicago sativa L) on carbon tetrachloride intoxicated rats, Am J Chin Med, 40(4):779-793.

Amann, R. and Peskar, B.A. (2018). Anti-inflammatory effects of aspirin and sodium salicylate, European Journal of Pharmacology, 447(1):1-9

Amberger, J.S. and Hamosh, A. (2017). Searching Online Mendelian Inheritance in Man (OMIM): a Knowledgebase of Human Genes and Genetic Phenotypes. Curr. Protoc. Bioinformatics, 58, 1–12.

Amraie, E., Farsani, M.K., Sadeghi, L., Khan, T.N., Babadi, V.Y. and Adavi, Z. (2019). The effects of aqueous extract of alfalfa on blood glucose and lipids in alloxan-induced diabetic rats. Interv Med Appl Sci ; 7(3):124-128.

Anbalahan, N. (2021). Pharmacological activity of mucilage isolated from medicinal plants, International Journal of Applied and Pure Science and Agriculture, 3(1):98-113.

AOAC (2006). Acyclic and incompletely cyclized triterpene alcohols in the seed oils of Medicago sativa, Lipids. 34(11):1151-1175

Asif, H.M., Akram, M., Saeed, T.M., Khan, I., Akhtar, N. and Rehman, R. (2021). Review paper carbohydrates. International Research Journal of Biochemistry and Bioinformatics, 1(1):001-005

Avato, P., Bucci, R., Tava, A., Vitali, C., Rosato, A., Bialy, Z. and Jurzysta, M. (2018). Antimicrobial activity of saponins from Medicago sp.: Structure-activity relationship. Phytother Res, 20(6):454-457.

Avorn, J., Monane, M., Gurwitz, J.H., Glynn, RJ, Choodnovskiy, I. and Lipsitz, L.A. (2021). Reduction of bacteriuria and pyuria after ingestion of Medicago sativa, Journal of the American Medical Association, 271(10):751-754

Babatunde, S., Fahy, E., Gupta, S., Sud, M., Byrnes, R.W. and Cotter, D. (2014). Bioinformatics and systems biology of the lipidome, Chemical Reviews, 111(10):6452-6490.

Bader, G.D. and Hogue, C.W.V. (2013). An automated method for finding molecular complexes in large protein interaction networks. BMC Bioinformatics, 4:2.

Barnig, C., Frossard, N. and Levy, B.D. (2018). Towards targeting resolution pathways of airway inflammation in asthma. Pharmacol. Ther. 186, 98–113.

Beauchemin, K.A. and Rode, L.M. (2018). Compressed baled alfalfa hay for primiparous and multiparous dairy cows. Are naringenin and quercetin useful chemicals in pest-management strategies, Journal of Pest Science, 87(1):173-180

Bennets, H.W., Underwood, E.J. and Shier, F.L. (2019). A specific breeding problem of sheep in subterranean clover pastures in Western Australia, Australian Veterinary Journal, 22(1):2-12

Benowitz, N.L. (2021). Pharmacology of nicotine: Addiction, smoking-induced disease, and therapeutics, Annual Review of Pharmacology and Toxicology, 49:57-71.

Bora, K.S. and Sharma, A. (2018). Evaluation of anxiolytic effect of Medicago sativa in mice, Pharm Biol, 50(7):878-882.

Bora, K.S. and Sharma, A. (2019). Evaluation of antioxidant and cerebroprotective effect of Medicago sativa Linn against ischemia and reperfusion insult. Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine,45-87.

Bourgaud, F., Gravot, A., Milesi, S. and Gontier, E. (2019). Production of plant secondary metabolites: A historical perspective, Plant Science, 161:839-851

Bruno, F., Spaziano, G., Liparulo, A., Roviezzo, F., Nabavi, S. M. and Sureda, A. (2018). Recent advances in the search for novel 5-lipoxygenase inhibitors for the treatment of asthma. Eur. J. Med. Chem.153, 65–72.

Bui, T.T., Piao, C.H., Song, C.H., Lee, C.H., Shin, H.S. and Chai, O.H. (2017). Baicalein, wogonin, and Scutellaria baicalensis ethanol extract alleviate ovalbumin-induced allergic airway inflammation and mast cell-mediated anaphylactic shock by regulation of Th1/Th2 imbalance and histamine release. Anat. Cell Biol. 50,124–134.

Burley, S.K., Berman, H.M., Kleywegt, G.J., Markley, J.L., Nakamura, H. and Velankar, S. (2017). Protein Data Bank (PDB): the Single Global Macromolecular Structure Archive. Methods Mol. Biol. 1607, 627–641.

Catarino, M.D., Silva, A.M.S., Cruz, M.T. and Cardoso, S.M. (2022). Antioxidant and anti-inflammatory activities of Medicago sativa, Food & Function, 8(9):3355-3365.

Caunii, A., Pribac, G., Grozea, I. and Gaitin, D. (2020). Design of optimal solvent for extraction of bioactive ingredients from six varieties of Medicago sativa, Chemistry Central Journal, 6:123.

Chao, J., Dai, Y., Verpoorte, R., Lam, W., Cheng, Y.C. and Pao, L.H. (2017). Major achievements of evidence-based traditional Chinese medicine in treating major diseases. Biochem. Pharmacol. 139, 94–104.

Chavan, S.S., Jadhav, R.S., Khemnar, K.S. and Tambe, V.B. (2017). Evaluation of antibacterial activity and phytochemical screening of Medicago sativa leaves. Int J Curr Res Acad Rev, 3(5):308-313.

Chegini, H., Oshaghi, M., Boshagh, M.A., Foroutan, P. and Jahangiri, A.H. (2020). Antibacterial effect of Medicago sativa extract on the common bacteria in sinusitis infection. Int J Bio Med Public Health, 1(1):1-5.

Chen, X., Zhou, H., Liu, Y.B., Wang, J.F., Li, H. and Ung, C.Y. (2016). Database of traditional Chinese medicine and its application to studies of mechanism and to prescription validation. Br. J. Pharmacol. 149, 1092–1103.

Choi, K.C., Hwang, J.M., Bang, S.J., Kim, B.T., Kim, D.H., Chae, M., Lee, S.A., Choi, G.J., Kim, D.H. and Lee, J.C. (2019). Chloroform extract of alfalfa (Medicago sativa) inhibits lipopolysaccharide-induced inflammation by down-regulating ERK/NF-κB signaling and cytokine production, J Med Food, 16(5):410-420.

Contero, F., Abdo, S., Vinueza, D., Moreno, J., Tuquinga, M. and Paca, N. (2021). Estrogenic activity of ethanolic extracts from leaves of Ilex guayusa loes and Medicago sativa in Rattus norvegicus, Pharmacology Online, 2:95-99.

Daina, A., Michielin, O. and Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep. 7:42717

Dixit, V.P. and Jain, P. (2018). Hypolipidemic effects of Medicago sativa seed extracts (50%) EtOH) in rabbits under experimental conditions. Ancient Science of Life, 10(1):52-55.

Doss, A., Parivuguna.V., Vijayasanthi, M. and Surendran, S. (2020). Antibacterial evaluation and phytochemical analysis of Medicago sativa L. against some microbial pathogens, Indian Journal of Science and Technology, 4(5):550-552.

Eric, Y. (2016). Botanical medicines for the urinary tract, World Journal of Urology, 20(5):285-293

Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M. and Garapati, P. (2018). The Reactome Pathway Knowledgebase. Nucleic Acids Res. 46, 649–655.

Fang, S., Dong, L., Liu, L., Guo, J., Zhao, L. and Zhang, J. (2020). HERB: A high through put experiment and reference-guided database of traditional Chinese medicine. Nucleic Acids Res. 49, 1197–1206.

Flood-Page, P., Menzies-Gow, A., Phipps, S., Ying, S., Wangoo, A. and Ludwig, M. S. (2013). Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. J. Clin. Invest. 112, 1029–1036.

García-Menaya, J. M., Cordobés-Durán, C., García-Martín, E., and Agúndez, J. A. G. (2019). Pharmacogenetic Factors Affecting Asthma Treatment Response. Potential Implications for Drug Therapy. Front. Pharmacol. 10:520.

Gatouillat, G., Magid, A.A., Bertin, E., Okiemy-Akeli, M.G., Morjani, H., Lavaud, C. and Madoulet, C. (2020). Cytotoxicity and apoptosis induced by alfalfa (Medicago sativa) leaf extracts in sensitive and multidrug-resistant tumor cells, Nutr Cancer, 66(3):483-491.

Gaweł, E. (2019). Chemical compositions of lucerne leaf extract (EFL) and its applications as a phytobiotic in human nutrition, Acta Sci Pol Technol Aliment, 11(3):303-309.

Gehm, B.D., McAndrews, J.M., Chien, P. and Jameson, J.L. (2020). Resveratrol, a polyphenolic compound found in Medicago sativa, is an agonist for the estrogen receptor, Proceedings of the National Academy of Sciences of the United States of America, 94(25):14138-14143

Golawska, S., Lukasik, I., Kapausta, I. and Janda, B. (2017). Analysis of flavonoids content of Alfalfa, Echological Chemistry and Engineering, 17:2-3.

Gomathi, R., Vijipriya, M. and Usha, K. (2017). Cardioprotective effect of ethanolic extract of Medicago sativa stem on isoproterenol induced myocardial infarction in Wistar Albino rats, International Journal of Pharmacy and Pharmaceutical Sciences, 6:839-842.

Gray, A.M. and Flatt, P.R. (1997). The traditional plant treatment, Medicago sativa, exhibits insulin-like and insulin-releasing actions in vitro. J., Nutri., 78:325-334

Hagerman, A.E. and Butler, L.G. (2019). The specificity of proanthocyanidin-protein interactions, The Journal of Biological Chemistry, 10(256):4494-4497

Harbone, A. (1973). Interaction of alfalfa saponins with components of the erythrocyte membrane in hemolysis, Biochimica et BiophysicaActa, 307(1):83-91

Hong, Y.H., Chao, W.W., Chen, M.L. and Lin, B.F. (2021). Ethyl acetate extracts of alfalfa (Medicago sativa L) sprouts inhibit lipopolysaccharide-induced inflammation in vitro and in vivo, J Biomed Sci,16:64.

Hong, Y.H., Huang, C.J., Wang, S.C. and Lin. B.F. (2019). The ethyl acetate extract of alfalfa sprout ameliorates disease severity of autoimmune-prone MRL-lpr/lpr mice. Lupus, 18(3): 206-215.

Hong, Y.H., Wang, S.C., Hsu, C. and Lin, B.F. (2018). Phytoestrogenic compounds in alfalfa sprout (Medicago sativa) beyond coumestrol. J Agric Food Chem, 59(1):131-137.

Iwualewa, E.O. Adewumi, C.O. Omisore, N.O. Adebanji, O.A and Azike, C.K. (2015). Proantioxidant effects and crytoprotective potentials.

John, O. (2014). lupane triterpene from frankincense Medicago sativa, Phytochemistry, 62:537-541.

Jones, M.E. and Kossel, A. (2018). A biographical sketch, Yale Journal of Biology and Medicine, 26(1):80-97

Joy, G.S. and George, P. (2018). Phytochemical analysis of alfalfa (Medicago sativa) seed extract by soxhlet extraction using different solvents, American Journal of Advanced Drug Delivery, 2(2):145-152.

Joy, G.S. and George, P. (2019). Antimicrobial screening of alfalfa (Medicago sativa) in various bacterial strains. IJPDA, 2(1):65-69.

Karimi, E., Oskoueian, E., Oskoueian, A., Omidvar, V., Hendra, R. and Nazeran, H. (2019). Insight into the functional and medicinal properties of Medicago sativa (Alfalfa)leaves extract, Journal of Medicinal Plants Research, 7(7):290-297.

Khayatzadeh, J., Farhoodi, M. and Rafiei, H. (2020). The wound healing effect of Medicago sativa extract on pinna rabbit cartilage. Arak Medical University Journal (AMUJ), 12(3):29-38.

Krakowska, A. Rafińska, K. Walczak, J. Kowalkowski, T. and Buszewski, B. (2020). Comparison of various extraction techniques of Medicago sativa : Yield, antioxidant activity, and content of phytochemical constituents. J. AOAC Int, 100(6):1681-1693.

Levy, L.F. and Fox, F.W. (2019). Antiscorbutic value of Lucerne, South Biochem, J., 29(4):884–888.

Little, E.L, Woodburg, R.O. and Wadsworth, F.H (2020). Trees of Puerto Rico and the virgin islands

Liu, X.G., Lv, M.C., Huang, M.Y., Sun, Y.Q., Gao, P.Y. and Li, D.Q. (2017). A network pharmacology study on the triterpene saponins from Medicago sativa L for the treatment of neurodegenerative diseases, J Food Biochem, 43(8): 12955.

Madambu, R. Grubben, G.J.H, Asante, I.K. and Akromah, R. (2016). Plant Resources of tropica Africa.

Martínez, R., Kapravelou, G., Porres, J.M., Melesio, A.M., Heras L, Cantarero, S., Gribble, F.M., Parker, H., Aranda, P. and López-Jurado, M. (2022). Medicago sativa L, a functional food to relieve hypertension and metabolic disorders in a spontaneously hypertensive rat model, Journal of Functional Foods, 26:470-484.

Masotti, V., Juteau, F., Bessiere, J.M. and Viano, J. (2019). Seasonal and phenological variations of the essential oil from the narrow endemic species Medicago sativa and its biological activities, Journal of Agricultural and Food Chemistry, 51:7115-7121

Massiot, G., Lavaud, C. and Guillaume, D. (2019). Reinvestigation of the sapogenins and prosapogenins from alfalfa (Medicago sativa), J Agric Food Chem, 36:902-909.

Mbaze, L.M., Lado, J.A., Wansi, J.D., Shiao, T.C., Chiozem, D.D. and Mesaik, M.A. (2018). Oxidative burst inhibitory and cytotoxic amides and lignans from the stem bark of Medicago sativa, Phytochemistry, 70(11-12):1442-1447.

Mehranjani, M., Shariatzadeh, M., Desfulian, A., Noori, M., Abnosi, M. and Moghadam, Z. (2017). Effects of Medicago sativa on nephropathy in diabetic rats. Indian Journal of Pharmaceutical Sciences, 69(6):768.

Mölgaard, J.V., Schenck, H. and Olsson, A.G. (2017). Alfalfa seeds lower low density lipoprotein cholesterol and apolipoprotein B concentrations in patients with type II hyperlipoproteinemia, Atherosclerosis, 65(1-2):173-179.

Montanaro, A. and Bardana, E.J. (2018). Dietary amino acid-induced systemic lupus erythematosus, Rheum Dis Clin North Am, 17(2):323–332.

Montanher, A.B., Zucolotto, S.M., Schenkel, E.P. and Fröde, T.S. (2018). Evidence of anti-inflammatory effects of Medicago sativa in an inflammation model, Journal of Ethnopharmacology, 109(2):281-288

Moudi, M., Go, R., Yong, S.C. and Nazre, M. (2021). Vinca alkaloids, International Journal of Preventive Medicine, 4(11): 1231-1235

Nowacki, E., Jurzysta, M. and Dietrych-Szstak, D. (2018). Biosynthesis of medicagenic acid in germinating alfalfa, Biochem Physiol Pflanzen, 156:183-186.

Okwu, H. and Ndu, T. (2006). Composition of the essential oil of Medicago sativa from Turkey, Journal of Medicinal Plants Research, 4(23):2587-2589

Oleszek, W.A. (2018). Composition and quantitation of saponins in alfalfa (Medicago sativa) seedlings, J Agric Food Chem, 46(3):960-962.

Oskoueian, E., Abdullah, N., Ahmad, S., Saad, W.Z., Omar, A.R. and Ho, Y.W. (2020). Bioactive compounds and biological activities of Medicago sativa L kernel meal extract, Int J MolSci, 12:5955-5970.

Oskoueian, E., Abdullah, N., Hendra, R. and Karimi, E. (2019). Bioactive compounds, antioxidant, xanthine oxidase inhibitory, tyrosinase inhibitory and anti-inflammatory activities of selected agro-industrial by-products, Int J Mol Sci, 12: 8610-8625.

Pazyar, N., Yaghoobi, R., Ghassemi, M.R., Kazerouni, A., Rafeie, E. and Jamshydian, N. (2018). Medicago sativa in dermatology: A succinct review. GiornaleItaliano di Dermatologia e Venereologia, 148(6):687-691

Pelczar, M.J., Chan, E.C.S. and Krieg, N.R. (2018). Control of microorganisms, the control of microorganisms by physical agents. In: Microbiology. New York, McGraw-Hill International, 469-509

Pichersky, E., Noel, J.P. and Dudareva, N. (2019). Biosynthesis of plant volatiles: Nature's diversity and ingenuity, Science, 311(5762):808-811

Puneet, G., Aditi, S., Vinayak, K., Anant, G.N., Samir, D. and Nikhil, M. (2018). Medicago sativa: Implications and role in preventing dental decay, Pharmacognosy Reviews, 7(14):152-156.

Rana, M.G., Katbamna, R.V., Padhya, A.A., Dudhrejiya, A.D., Jivani, N.P. and Sheth, N.R. (2017). In vitro antioxidant and free radical scavenging studies of alcoholic extract of Medicago sativa L. Rom J Biol (Plant Biology), 55(1):15-22.

Rezaei, M., Azadpour, M. and Romiani, R. (2019). Evaluation of antibacterial effects of aqueous alfalfa (Medicago sativa L) extract in comparison with gentamicin, ciprofloxacin and penicillin. Medicinal plants, 15(2):61-65.

Richard, D., Kefi, K., Barbe, U., Bausero, P. and Visioli, F. (2021). Polyunsaturated fatty acids as antioxidants, Pharmacological Research, 57(6):451-455.

Sadowska, B., Budzynska, A., Wieckowska-Szakiel, M., Paszkiewicz, M., Stochmal, A., Moniuszko-Szajwaj, B., Kowalczyk, M. and Rozalska, B. (2019). New pharmacological properties of Medicago sativa and Saponaria officinalis saponin-rich fractions addressed to Candida albicans. Journal of Medical Microbiology, 63:1076-1086.

Saeidnia, S., Gohari, A.R., Mokhber-Dezfuli, N. and Kiuchi, F. (2020). A review on phytochemistry and medicinal properties of the Medicago sativa, Daru, 19(3):173-186

Sargazi, A., Sargazi, A., Nadakkavukaran, P.K., Srgaz, A., Aali, N., Sargolzaeiaval, F. and Sepehri, Z. (2017). Effect of alfalfa (Medicago sativa L) extract on undesired hair growth in human: A safe remedy for cosmetic procedures. Der Pharmacia Lettre, 8 (19):351-356.

Seida, A., El-Hefnawy, H., Abou-Hussein, D., Mokhtar, F.A. and Abdel-Naim, A. (2019). Evaluation of Medicago sativa L sprouts as antihyperlipidemic and antihyperglycemic agent. Pak J Pharm Sci, 28(6):2061-2074.

Selvan, A.T., Prithvi, S., Suthakaran, R., Ravali, R., Kumar, G.M. and Ravali, R. (2019). Anxiolytic effect of Medicago sativa seeds extract on rodents. Indo American Journal of Pharmaceutical Research, 4(1):406-415.

Serafini, M., Peluso, I. and Raguzzini, A. (2018). Flavonoids as anti-inflammatory agents. The Pro-ceedings of the Nutrition Society, 69(3):273-278.

Servatyari, K., Ahmadi, A., Kashefi, H., Menbari, M.N., Rostami, A. and Moloudi, M.R. (2017). The effect of hydroalcoholic extract of Medicago sativa on liver function tests, blood biochemical factors and coagulation system in male rats, Scientific Journal of Kurdistan University of Medical Sciences, 86:16-26.

Sharma, P.R., Shanmugavel, M., Saxena, A.K. and Qazi, G.N. (2018). Induction of apoptosis by a synergistic lignan composition from Cedrusdeodara in human cancer cells, Phytotherapy Research, 22(12):1587-1594.

Sofowora, K. (1984). Saponins: Properties, applications and processing, Critical Reviews in Food Science and Nutrition, 47:231-258.

Spiller, F., Alves, M.K., Vieira, S.M., Carvalho, T.A. and Leite, C.E. (2021). Anti-inflammatory effects of Medicago sativa on carrageenan- and antigen-induced inflammation, The Journal of Pharmacy and Pharmacology, 60(4):473-478.

Stochmal, A. Simonet, A.M., Macias, F.A. and Oleszek, W. (2019). Alfalfa (Medicago sativa L.) flavonoids. 2. Tricin and chrysoeriol glycosides from aerial parts.J Agric Food Chem ; 49(11):5310-5314.

Stochmal, A., Piacente, S., Pizza, C., De-Riccardis, F., Leitz, R. and Oleszek, W. (2018). Alfalfa (Medicago sativa L.) flavonoids. 1. Apigenin and luteolin glycosides from aerial parts. J Agric Food Chem, 49(2):753-758.

Story, J.A., LePage, S.L., Petro, M.S., West, L.G. and Cassidy, M.M. (2020). Lightfoot FG and Vahouny GV. Interactions of alfalfa plant and sprout saponins with cholesterol in vitro and in cholesterol-fed rats. Am J Clin Nutr, 39(6):917-929.

Susana, J., Beatriz, G.M., Fabiana, C.M., Maria, A.D. and Moacir, G.P. (2019). Antifungal activity of five species of Polygala, Brazilian Journal of Microbiology, 42(3):1065-1075.

Trease, H. and Evans, U. (1989). Recent research on oriental medicinal plants. ln: Wanger H, Hiniko H, Farnsworth NR, editors. Economic and Medicinal Plant Research, 53-85

Vyas, S., Collin, S.M., Bertin, E., Davys, G.J. and Mathur, B. (2017). Leaf concentrate as an alternative to iron and folic acid supplements for anaemic adolescent girls: A randomized controlled trial in India. Public Health Nutrition, 13(3):418-423.

Wang, S., Dong, X. and Tong, J. (2018). Optimization of enzyme-assisted extraction of polysaccharides from alfalfa and its antioxidant activity. Int J BiolMacromol, 62:387-396.

Winiarska, H., Dworacka, M., Borowska, M., Bobkiewicz-Kozłowska, T., Górecki, P., Mścisz, P. and Mrozikiewicz, P.M. (2018). The effects of plant extracts of Medicago sativa and Trigonella foenum-graecumon postprandial glucose levels in type 2 diabetic rats. Herba Polonica, 53(4):35-44.

Xie, Y., Wang, L., Sun, H., Wang, Y., Yang, Z., Zhang, G. and Yang, W. (2019). Immuno-modulatory, antioxidant and intestinal morphology-regulating activities of alfalfa polysaccharides in mice, Int J Biol Macromol, 133:1107-1114.

Xu, L., Wu, Y., Zhao, X. and Zhang, W. (2022). The study on biological and pharmacological activity of coumarins. Asia-Pacific Energy Equipment Engineering Research Conference; (AP3ER 2015). 135-138

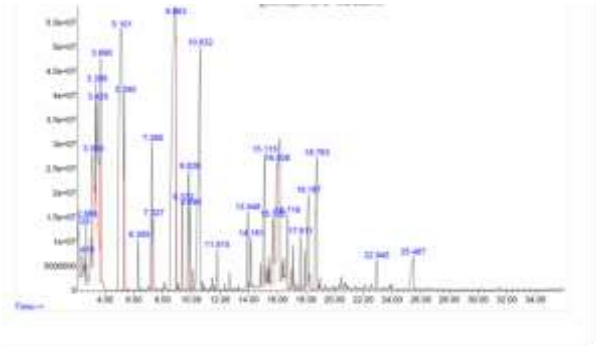
Yi, Z., Wang, Z., Li, H. and Liu, M. (2021). Inhibitory effect of Medicago sativa on chemically induced differentiation of human leukemia K562 cells, Toxicology Letters, 147(2):109-119

Zadeh, M.Z., Mirzaei, A., and Fazilati, M. (2019). Effects of hydro-alcoholic extract of Medicago sativa on the chemical burn injuries in rats. World Journal of Pharmacy and Pharmaceutical Sciences, 3(2):910-918.

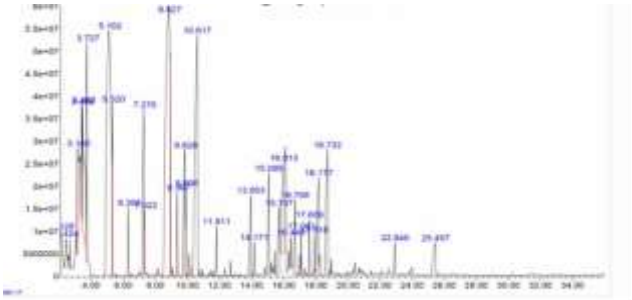
Zbigniew, S., Beata, Ż., Kamil, J., Roman, F., Barbara, K. and Andrzej, D. (2022). Antimicrobial and antiradical activity of extracts obtained from leaves of three species of the Medicago sativa, Microbial Drug Resistance, 20(4):337-343.

APPENDIX

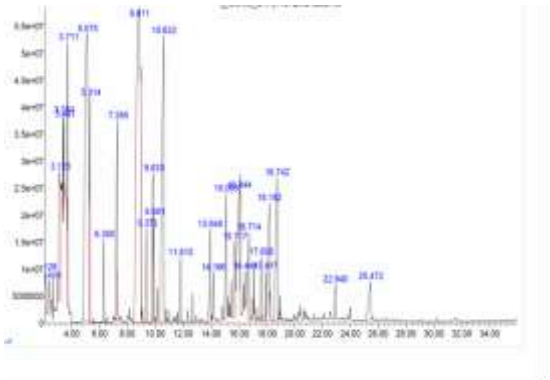
Appendix I: GC-MS Spectrum of Ethanol Fraction



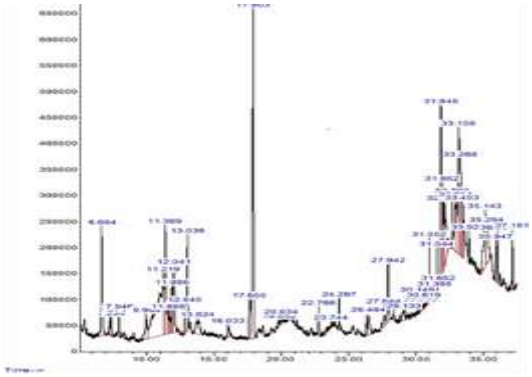
Appendix II: GC-MS N-hexane Fraction



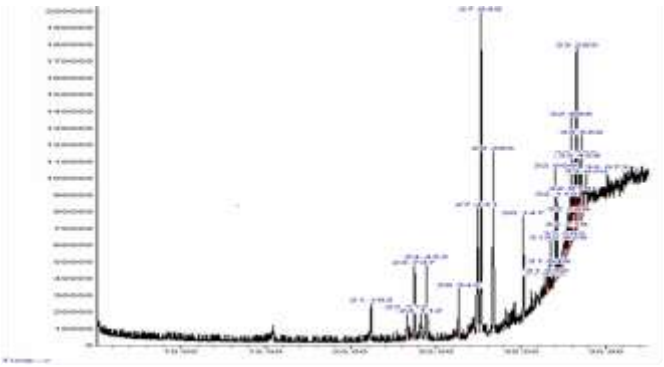
Appendix III: GC-MS Spectrum of Diethyl ether Fraction



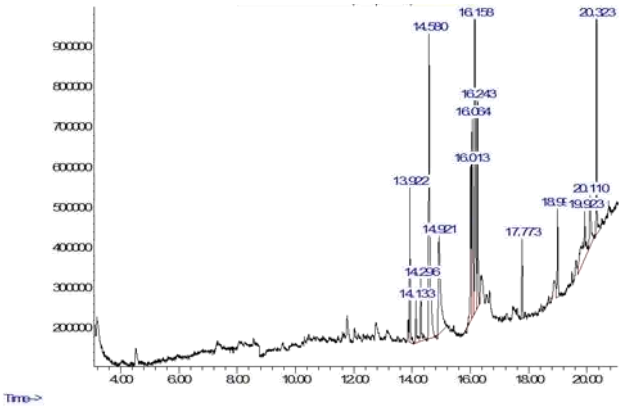
Appendix IV: GC-MS Spectrum of Chloroform Fraction



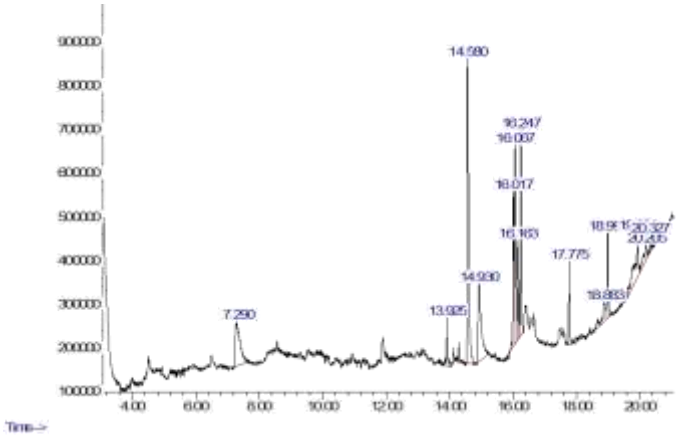
Appendix V: GC-MS Spectrum of Acetone Fraction



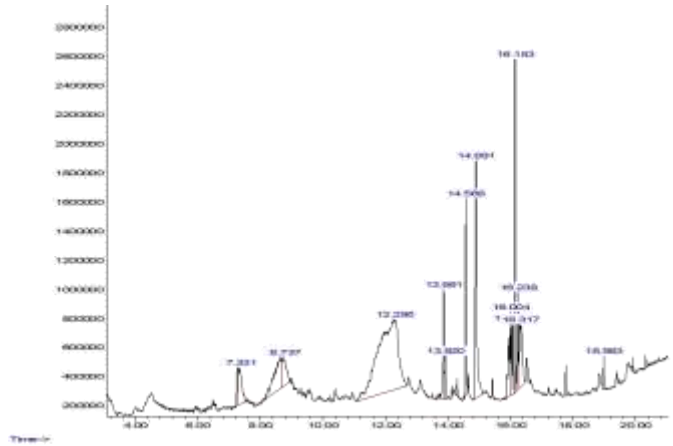
Appendix VI: GC-MS Spectrum of Ethyl acetate Fraction



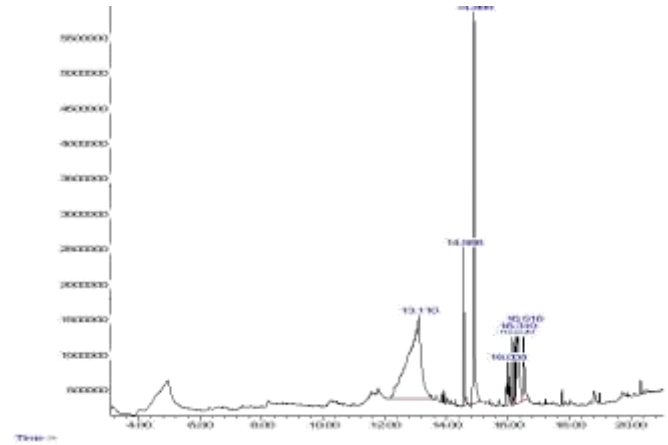
Appendix VII: GC-MS Spectrum of Petroleum ether Fraction



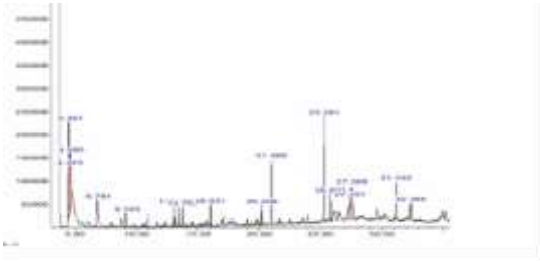
Appendix VIII: GC-MS Spectrum of Methanol Fraction



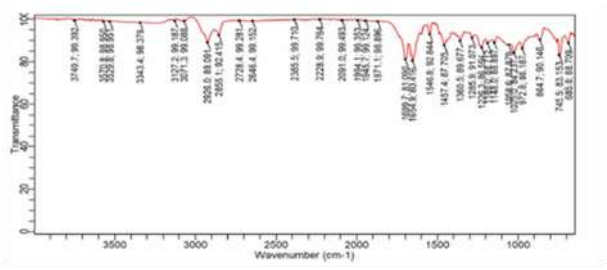
Appendix IX: GC-MS Spectrum of Dichloromethane Fraction



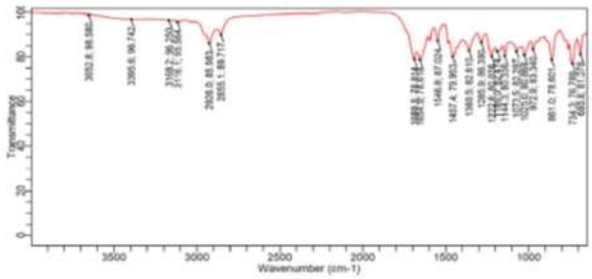
Appendix X: GC-MS Spectrum of Isopropanol Fraction



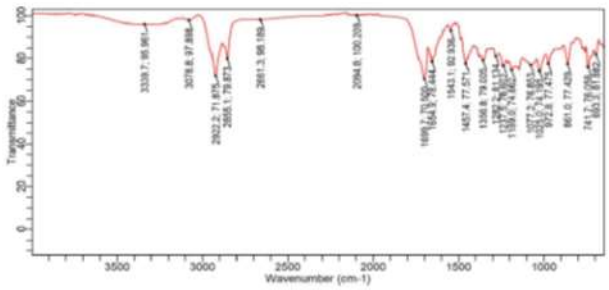
Appendix XI: FT-IR Spectrum of Ethanol fraction



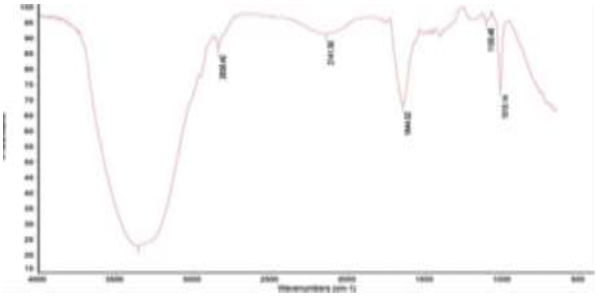
Appendix XII: FT-IR Spectrum of N-Hexane fraction



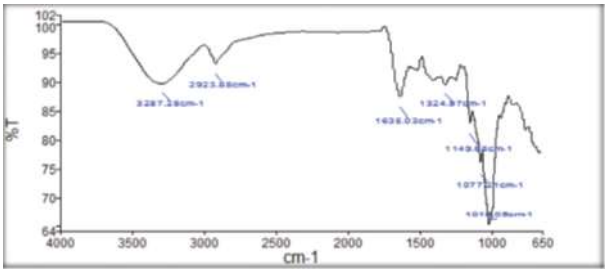
Appendix XIII: FT-IR Spectrum of Diethyl ether fraction



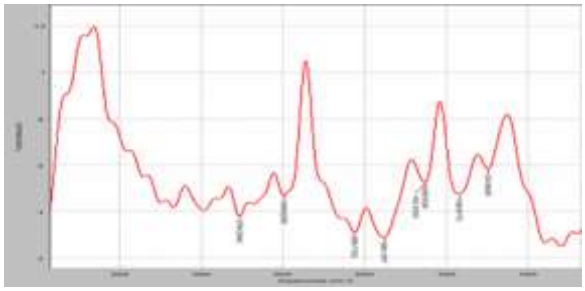
Appendix XIV: FT-IR Spectrum of Chloroform fraction



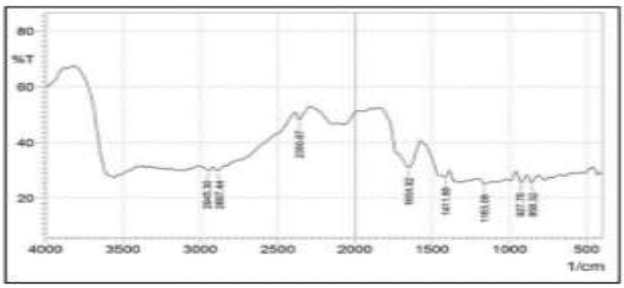
Appendix XV: FT-IR Spectrum of Acetone fraction



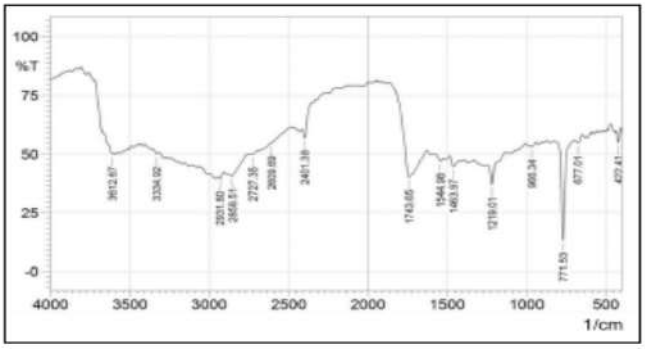
Appendix XVI: FT-IR Spectrum of Ethyl acetate fraction



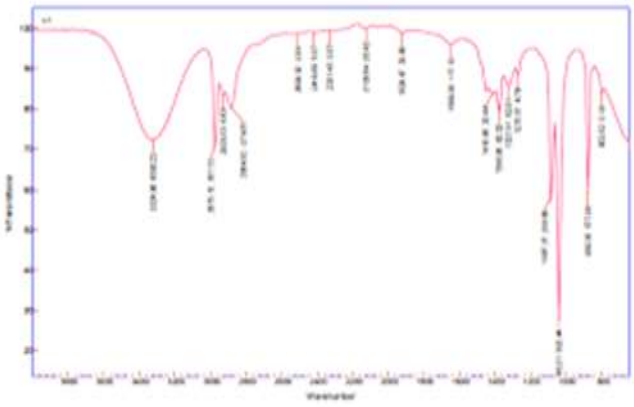
Appendix XVII: FT-IR Spectrum of Petroleum ether fraction



Appendix XVIII: FT-IR Spectrum of Methanol fraction



Appendix XIX: FT-IR Spectrum of Dichloromethane fraction



Appendix XX: FT-IR Spectrum of Isopropanol fraction