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Determination of Bioactive Compounds in *Agave amica* L. cv Arka Prajwal

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ABSTRACT: A study was carried out to identify bioactive compounds in tuberose and the compounds are dl-Glyceraldehyde dimer, Formic acid, 2-propenyl ester, 4H-Pyran-4-one,2,3-dihydro,3,5-dihydroxy-6-methyl, Dodecane, 5, 8-diethyl-, Sucrose, 3-Deoxy-d-mannoic lactone, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, 9,12-Octadecadienoic acid, ethyl ester, 9,12,15-Octadecadienoic acid (Z,Z,Z), Ethyl 9,12,15-octadecatrienoate, Hexanedioic acid, bis (2-ethylhexyl) ester. Among all the identified compounds Hexanedioic acid, bis (2-ethylhexyl) ester found to possess the highest retention time Ethyl 9,12,15-octadecatrienoate followed by 9,12,15-Octadecadienoic acid (Z,Z,Z), 9,12-Octadecadienoic acid, ethyl ester, Hexadecanoic acid, ethyl ester, n-Hexadecanoic acid, 3-Deoxy-d-mannoic lactone, sucrose, Dodecane, 4H-Pyran-4-one, 2,3-dihydro,3,5-dihydroxy-6-methyl, Formic acid, 2-propenyl ester, dl-Glyceraldehyde dimer. As a result of the intercropping, the effect of marigold in tuberose the compounds namely n-Hexadecanoic acid, 9,12,15-Octadecadienoic acid (Z,Z,Z), Hexanedioic acid, bis(2-ethyhexyl) esterbelonging to the palmitic acid group, carboxyl group, linoleic acid group were found with nematicidal property. The nematicidal property might be due to the influence of the marigold intercropped in between the sole crop tuberose. In future, the compounds possessing nematicidal property can be formulated and utilized as biocontrol against the infestation of the nematode prevailing in the roots of the commercially cultivated crop namely tuberose, Crossandra.

Keywords: GC-MS, *Polianthes tuberosa*, retention time, peak area, activity.

INTRODUCTION

Tuberose (*Agave amica* L.) belonging to the family Asparagaceae, a native of Mexico is perennial in nature with white flowers and are used for decoration in wedding and also used as loose flower with demand in the markets. It is one of the major growing tropical ornamental bulbous flowering plants mainly cultivated for its long lasting flower spikes. It is popularly known as Rajanigandha or Nishigandha and is a native of Mexico. The flowers are most attractive and elegant in its appearance with sweet fragrance. It has been popular for the aromatic oils extracted from the fragrant white flower. It widely used in the perfumery industry. Tuberose flower possess anti-microbial, nematicidal, pesticidal, mosquito larvicidal and anti-inflammatory properties. The plants with the aid of volatile organic compounds (VOCs) has consistent communication with the organisms present in the environment. The communication allows the plant-organism interaction and thus ending up in the tuning of the growth, development, defence, propagation and the life cycle which helps in the achievement of fitness to its maximum.

Volatiles, which are being produced from plants, belongs to the classes of chemicals namely terpenoids, benzenoids, phenylpropanoids and fatty acid-derivatives that includes volatiles of green leaves and

also possess nitriles, (ald)oximes, sulphides belonging to minor classes (Bouwmeester *et al.*, 2019). The scents from the floral parts helps in the high signal encoding specifically. The present study aimed at identification of the nature of the compounds present in the floral parts of tuberose responsible for their antioxidant activity. The GC-MS enables the bioactive compounds to separate and characterize easily.

The Gas Chromatography-Mass Spectrometry with Flame Ionization Detector and Thermal Desorber (GC-MS and GC-MS/TD) is widely used in the Identification and Quantification of root volatiles, plant metabolite, culture metabolite, plant volatiles, fatty acid profiling, soluble materials, profiling of essential oil, anti-microbial and anti-nematicidal VOCs, microbial VOCs (mVOCs), antimicrobial and anti-nematicidal meatbolites, VOC profile of fruits, vegetables, flowers, roots, leaves, metabolite profiling of pretreated Lignocellulosic biomass, identification of unknown VOC compounds namely solid, liquid and powder. The main aim of the study is the identification of the compounds present in tuberose. The major objective of the study was to elucidate the impact of intercropping with marigold in tuberose. The main aim was to provide an emerging perspective about the natural compounds and its effect on the nematode.

MATERIALS AND METHODS

The field trial was conducted as an open field experiment at Botanical Garden, Field. No. 10 at Horticultural College and Research Institute, Coimbatore. The treatment combinations of tuberose intercropped with marigold of the study are 3 rows of tuberose as the sole crop with 1 marigold as intercrop (T_1), 6 rows of tuberose as the sole crop with 1 marigold as intercrop (T_2), 9 rows of tuberose as the sole crop with 1 marigold as intercrop (T_3), 9 rows of tuberose as the sole crop with 1 marigold as intercrop (T_4), 12 rows of tuberose as the sole crop with 1 marigold as intercrop (T_5), 15 rows of tuberose without intercropping (T_6).

Preparation of the extract. Freshly excised petals of the tuberose flowers were weighed and the disruption of tissue was done by grinding with the solvent ethanol in a pre-chilled pestle and mortar. The sample was kept in a water bath for 45 minutes. The sample extracts were then centrifuged at 10,000 rpm for 10 minutes. The supernatant obtained was stored at 4°C and used for further experiments (Maiti *et al.*, 2014).

GC-MS analysis. The instrument used for the analysis is Perkin Elmer Clarus SQ8C system and the GC-MS was equipped by means of Elite-I fused with capillary column made up of silica with DB-5 MS Capillary Standard Non Polar Column of dimension 30 Mts, thickness of 0.25 mm carrier gas which flows constantly at the rate of 1 ml/minute and the volume was 1µL.

For the detection of compounds through GC-MS, ionization system of electron with the energy ionizing at the rate of 70eV was taken. Each component and its relative percentage was calculated with the average peak areas to that of the total areas. Turbomass is being used for the handling of mass spectra and chromatograms.

Identification of compounds

The GC-MS interpreted on mass spectrum was done with the aid of National Institute Standard Technology (NIST) database, which has more than 62,000. The spectrum of both the known and unknown compounds compared with the components in the NIST library. The detection of the compounds, its name, molecular weight and the structure was ascertained with the help of the test materials. The identification of compounds were done by comparing the spectrum of known and unknown compounds in the NIST library and available.

Heat map clustering analysis and interpretation. The heat map clustering is the pictorial representation of hierarchical clusters in data matrices. The visualization of the data matrix represented with the aid a rectangular grid that corresponds to the rows and columns present and the furnished the cells through means of different colours. The hierarchical clustering is an algorithm that possess objects similar in nature and grouped as clusters. The endpoint is a set of clusters in which each cluster is distinct from the objects within the clusters. There is an existence of similarity within the clusters in a broader way.

The dendrograms along the sides represents the independent clustering of variables and the rows. The data values for each row and the column is shown in the heat map as they have the same range of values and are standardized. It may indicate any kind of pattern and provides the association between the rows and columns. On the other hand, the modification of clustering may be done in order to generate different patterns. The rectangular area of same colour is the main pattern of look of the dendrogram. The heat map generated through R software belongs to the type row dendrograms and the properties are furnished below in Table 1 and 2; Fig. 1 and 2.

Row Dendrograms: As a result of clustering, the row dendrograms exhibit similarity between the row distance and the nodes.

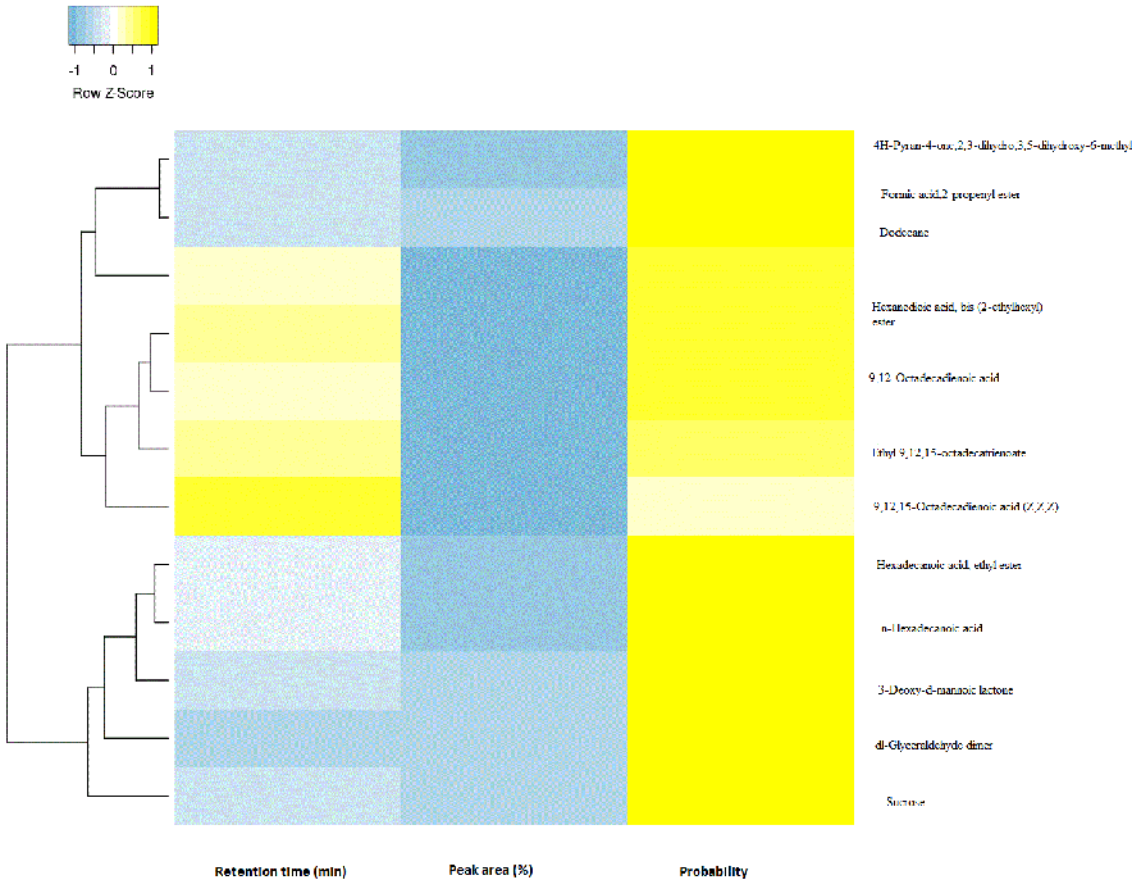


Fig. 1. Hierarchical clustering analysis of *P. tuberosa* L. cultivar Arka Prajwal.

RESULTS AND DISCUSSION

The prevailing compounds present in the extract of ethanol obtained from the floral parts of *Agave amica* L. was identified as the result of analysis through GC-MS. The retention time (RT), molecular formula, molecular weight (MW), structural representation, area (%), the probability of the occurrence of the specific compounds, the significance into the plants were found and discussed in detail. The best separation method and technique for identification were Gas Chromatography and Mass Spectrometry and also one of the vital quantification method for volatile compounds, semi-volatile compounds (Grover and Patni 2013).

The ethanol extract of *Agave amica* L. with the aid of GC-MS exhibited twelve similar compounds in both the best treatments and control. The compounds identified are dl-Glyceraldehyde dimer, Formic acid,2-propenyl ester, 4H-Pyran-4-one,2,3-dihydro,3,5-dihydroxy-6-methyl, Dodecane, 5, 8-diethyl-, Sucrose, 3-Deoxy-d-mannoic lactone, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, 9,12-Octadecadienoic acid, ethyl ester, 9,12,15-Octadecadienoic acid (Z,Z,Z), Ethyl 9,12,15-octadecatrienoate, Hexanedioic acid and bis (2-ethylhexyl) ester. Among all the identified compounds Hexanedioic acid, bis (2-ethylhexyl) ester found to possess the highest retention time (RT in minutes) as 29.77, followed by Ethyl 9,12,15-octadecatrienoate (26.33), 9,12,15-Octadecadienoic acid (Z,Z,Z) (25.70), 9,12-Octadecadienoic acid, ethyl ester (25.55), Hexadecanoic acid, ethyl ester (23.121), n-Hexadecanoic acid (22.24) while the lowest retention time was found in Formic acid, 2-propenyl ester (4.68) followed by dl-Glyceraldehyde dimer (3.40) respectively. The compounds identified has highest percentage namely 5-Hydroxymethylfurfuryl (19.82%), hexadecanoicacid, ethyl ester (Palmitic acid ester) with 6.23%, 2-Hydroxy-gamma-butyrolactone (12.16%) exhibited a wide range of potential bioactivities. The above phytochemicals are responsible antioxidant and antimicrobial activities which can be applied in pharmacological actions (Tapiero *et al.* (2002).

The compounds 9,12-Octadecadienoic acid, methyl ester are grouped under unsaturated fatty acids plays a vital role with growth of the cell, lowering the level of blood cholesterol and lubrication of the skin and improving its quality (Igwe and Okwa 2013; Okwa and Morah 2006).

The characterization and evaluation of bioactive compounds was formulated by Edy *et al.* (2017) in which the compounds namely with great abundance was registered by three compounds namely Neophytadine, followed by 9,12,15-octadecatrineoic acid-methyl ester and hexadecanoic acid exhibited the maximum retention time of 43.88%, 13.45% and 13.24% respectively.

The maximum scavenging capacity was reported in *Ficus septica*, *Cordiline* sp., *Celotia argantea*, *Melostoma polyanthum* (Mu'nisa *et al.*, 2018). Bindu

and Udayan (2018) registered that there was prevailing compounds namely hexadecanoic acid, 9,12-Octadecanoic acid and the report was considered as the first report on the bioactive compounds identification extracted from the tubers of *Polianthes tuberosa* by means of methanol. The above study is in accordance with the present study.

The identification of six compounds possessing insectifuge property *viz.*, hexadecanoic acid methyl ester, 9,12,15-octadecatrienoic acid (Z,Z,Z), 2,4-Di tert-butylphenol, tetradecanoic acid, squalene, octadecanoic acid was registered by the authors Karthikeyan *et al.* (2016); Gorane *et al.* (2018); Mahalakshmi and Thangapandian in 2019.

The compounds identified *viz.*, dodecanoic acid, octadecanoic acid, methyl ester, 9,12-octadecanoic acid (Z,Z) and 2,4-Di-tert-butylphenol were found with antiviral property against human viral diseases was reported by Karthikeyan *et al.* (2016); Leila *et al.*, (2020) and Ramya *et al.*, (2020). The three compounds 9,12-octadecanoic acid, octadecanoic acid and squalene were reported in cotton in which anti-viral property was used against leaf curl (Abdullah *et al*., 2019).

The wild genus of *Carica papaya* possessed the following compoundsnamely 9,12, octadecanoic acid (Z,Z), octadecanoic acid, dodecanoic acid and squalene which has the activity against the attack of plant viral diseases.

Vijaykumar *et al.* (2021) reported that the GC-MS report revealed hexadecanoic acid, octadecanedioic acid were found in successful grafts of papaya thus helped in the plant promotion activity and was in accordance with the report given by Jishma *et al.* (2017).

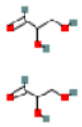
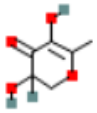
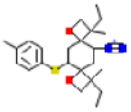
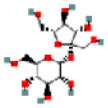
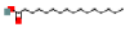
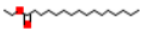
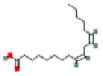
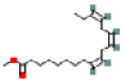
In accordance to the significance of the above compounds in relevance to that of the plants in terms of the nematicidal, pesticidal, 5-alpha reductase inhibitory, anti-microbial activities. The other properties of these compounds include mosquito larvicide, anti-inflammatory, haemolytic activity, anticancer, hepatoprotective, Anti-arthritic, anti-asthama, diuretic, anti-arthritic, anti-asthama, diuretic, antihistaminic, antieczemic, insectifuge, antiproliferative. The retention of emitted VOC has proven to be suitable for the identification of a wide range of floral released compounds. The rate of major volatile emission was found to be high during night time**.** Isoprenoid and phenylpropanoid pathways consists of major volatile (9 pm and 3 am). Mono terpenoids and benzonoidsis predominant for floral development. Methyl esters is found in abundance in the late stages of floral maturation.

The compounds, peak area, probability, their significance, molecular weight, structure present in the extract is furnished below the Tables 1 and 2.

Table 1: The activity of the compounds identified from the floral parts of *Polianthes tuberosa* L.

Sr. No.	Retention time (min)	Name of the compound	Peak area (%)	Probability	Activity	Reference
1.	3.409	dl-Glyceraldehyde dimer	7.152	75.7	Sugar moiety and Preservative	(Saravanan*et al.*, 2014).
2.	4.684	Formic acid,2-propenyl ester	1.050	32.1	Helps in rapid conversion of formic acid to COZ, then followed by the fixation of CO_2.	(www.webbook.nist.gov).
3.	7.050	4H-Pyran-4-one,2,3-dihydro,3,5-dihydroxy-6-methyl	1.050	32.1	Scavenge free radicals, acts as a strong antioxidant in glucose	Chen *et al.* (2021)
4.	7.880	Dodecane	0.668	12.3	Used as a solvent, distillation chaser and scintillator component.	https://pdbj.org; https://www.chempoint.com; https://www.spectrumchemical.com
5.	11.437	Sucrose	7.227	54.0	Aids in major transport form photoassimilated carbon.	https://www.arquivo.ufv.br
6.	14.578	3-Deoxy-d-mannoic lactone	4.077	73.5	Antimicrobial activity.	Ghosh *et al.* (2015); ncbi.nlm.nih.gov
7.	22.241	n-Hexadecanoic acid	0.858	70.9	Anti-microbial, Hypochlolesterolemicnematicide, pesticide, 5-alpha reductase inhibitor, mosquito larvicide, anti-inflammatory, antioxidant, haemolytic properties.	Khadim *et al.* (2017)
8.	23.121	Hexadecanoic acid, ethyl ester	1.512	74.4	Anti-microbial activity.	Kim *et al.* (2020)
9.	25.552	9,12-Octadecadienoic acid	1.940	36.5	Antimicrobial, Anticancer, Hepatoprotective, Anti-arthritic, anti-asthama, diuretic	Kim *et al.* (2020)
10.	25.702	9,12,15-Octadecadienoic acid (Z,Z,Z)	3.959	16.8	Antioxidant, antimicrobial activity, anti-inflammatory, nematicide, antihistaminic, antieczemic, insectifuge.	Khadim *et al.* (2017)
11.	26.338	Ethyl 9,12,15-octadecatrienoate	2.425	59.4	Antimicrobial activity, antioxidant, antiproliferative.	Kim *et al.* (2020)
12.	29.779	Hexanedioic acid, bis (2-ethylhexyl) ester	0.421	28.8	Antioxidant, Hemolytic, Hypocholesterolemic, Flavor, Nematicide, Anti-androgenic,Antimicrobial activity, Antiproliferative.	Phuong *et al.* (2018)

Table 2: The structural arrangements in *Agave amica* L. floral parts using Gas Chromatography-Mass Spectrometry.

Sr. No	Name of the compound	IUPAC name	Structure	Chemical formula	Molecular weight (g/mol)
1.	dl-Glyceraldehyde dimer	2,3-dihydroxypropanal		$C_6H_{12}O_6$	180.16
2.	Formic acid,2-propenyl ester	prop-2-enyl formate		$C_4H_6O_2$	86.09
3.	4H-Pyran-4-one,2,3-dihydro,3,5-dihydroxy-6-methyl	prop-2-enyl formate		$C_6H_8O_4$	144.12
4.	Dodecane	1,8-diethyl-1,8-dimethyl-6-(4-methylphenyl)sulfanyl-3,10-dioxadispiro[3.2.37.24]dodecane-12-diazonium		$C_{23}H_{33}N_2O_2S^+$	401.6
5.	Sucrose	(2R,3R,4S,5S,6R)-2-[(2S,3S,4S,5R)-3,4-dihydroxy-2,5-bis(hydroxymethyl)oxolan-2-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol		$C_{12}H_{22}O_{11}$	342.3
6.	3-Deoxy-d-mannoic lactone	3,5-dihydroxy-6-(hydroxymethyl)oxan-2-one		$C_6H_{10}O_5$	162.14
7.	n-Hexadecanoic acid			$C_{16}H_{32}O_2$	256.42
8.	Hexadecanoic acid, ethyl ester	ethyl hexadecanoate		$C_{18}H_{36}O_2$	284.5
9.	9,12-Octadecadienoic acid	(9Z,12Z)-octadeca-9,12-dienoic acid		$C_{18}H_{32}O_2$	280.4
10.	9,12,15-Octadecadienoic acid (Z,Z,Z)	Octadeca-9,12,15-trienoic acid		$C_{19}H_{32}O_2$	292.5
11.	Ethyl 9,12,15-octadecatrienoate	Adipic acid, bis(2-ethylhexyl) ester, Adipol 2EH; Bis(2-ethylhexyl) adipate; Bisoflex DOA; Di(2-ethylhexyl) adipate		$C_{22}H_{42}O_4$	370.6
12.	Hexanedioic acid, bis (2-ethylhexyl) ester	Adipic acid		$C_6H_{10}O_4$	146.14

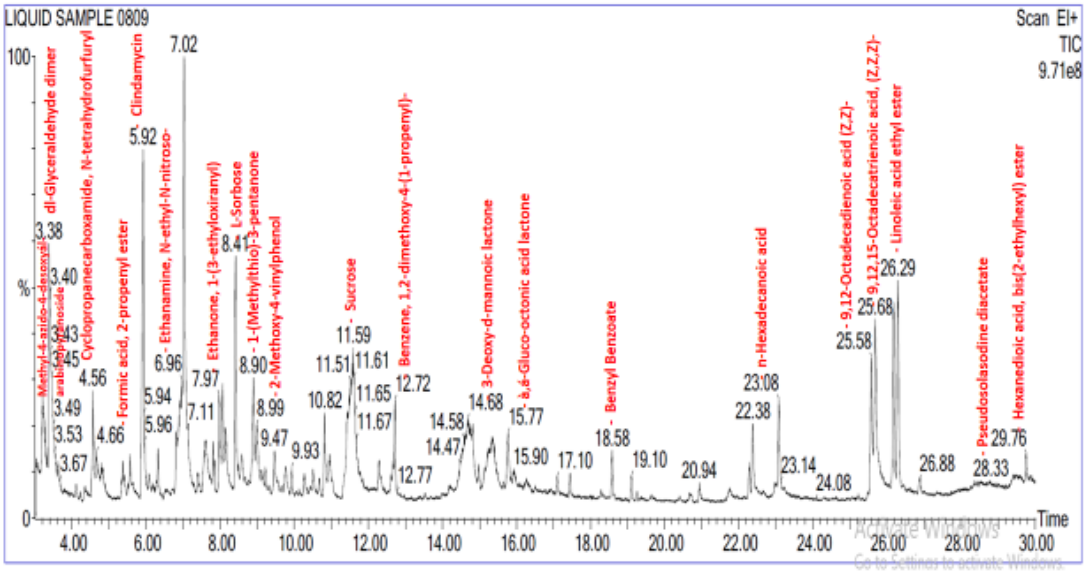


Fig. 2. Gas Chromatography Mass Spectrometry (GC-MS) profiling in Arka Prajwal of different treatments with marigold as an intercrop in tuberose.

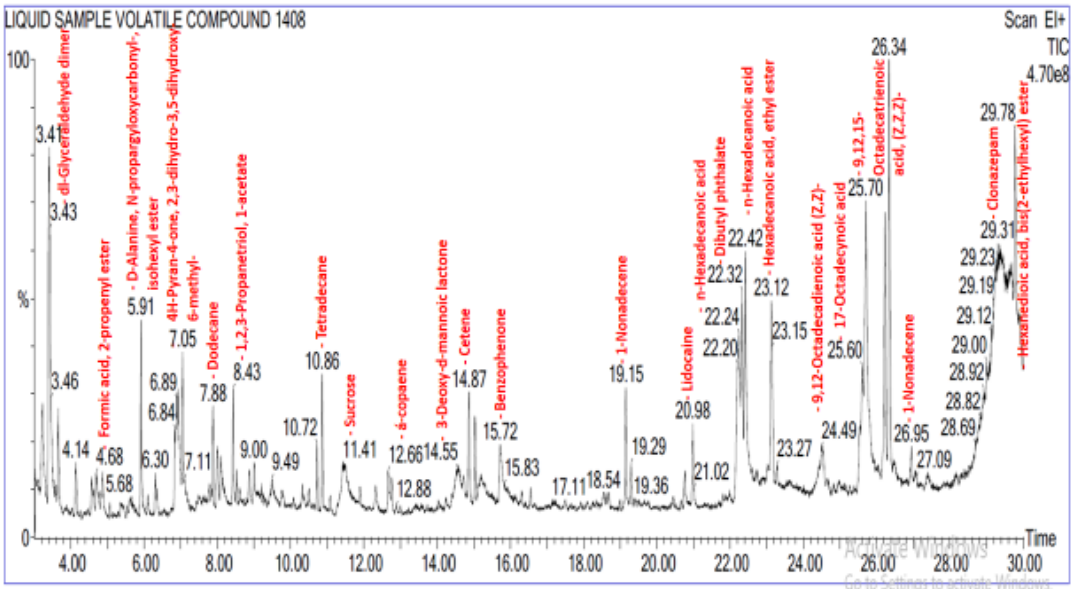


Fig. 3. Gas Chromatography Mass Spectrometry (GC-MS) profiling in Arka Prajwal without marigold as intercrop in between the sole crop.

CONCLUSION

In the undergone study, the compounds present in the tuberose has been taken to known the effect of the intercropping of marigold in tuberose and the impact on the nematode infestation. The treatments in which marigold the roots of marigold which has been imposed as the intercrop in the five treatments, whereas the intercropping with marigold was imposed treatment combinations possess nematicidal properties that plays a vital role in natural effect on the root knot nematode infestation. The GC-MS analysis is the basis for the understanding of the active principles present and the nature of the compounds can be detected. The identified compounds from *Agave amica* L. obtained from the ethanolic extract from the floral parts possess medicinal properties namely antioxidant, anticancer, antimicrobial activities. The GC-MS analysis revealed that presence of secondary metabolites especially the compounds namely n-Hexadecanoic acid, 9,12,15-Octadecadienoic acid (Z,Z,Z) with nematicidal activity which can be utilized in the industrial application. By the isolation of phytochemical constituents individually may help in fruitful results and can be used for the preparation of drugs in pharmacological research. In the future,

individual isolation of phytochemical constituent and its biological activity can be subjected for research in forthcoming research projects.

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Conflict of Interest. None.

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