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An*invitro* Antibacterial and Anticandidalactivity of a Phytomedicine*perillafrutescence* (L.): A Future Herb for the Development of New Antimicrobial Drug

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ABSTRACT
A herb of interior and remote villages ofUttarakhandPerillafrutesenceis scientifically investigated first time in view of its traditional uses and folklores in treating various diseases and microbial infections.

In this study the chemical composition of the Perillafrutesencewas analyzed and the antimicrobial activity of different solvents from different parts of the plant was determined.The antimicrobial activity was detected in flower and seedextracts. Chemical composition analysis of seeds and flowers with GC-MS indicated the presence of saturated and unsaturated fatty acids.The plant seeds extracts presents12.11area% of saturated fatty acids and 19.04area %of unsaturated fatty acids while the flower extracts contains17.89 area % saturated and 2.15 area% of unsaturated fatty acid.The antimicrobial activities were performed using the agar diffusion methods.The flower extracts was active against Staphylococcus aureus (ATCC-6538),E.coli(ATCC-8739) and Candida albicans(ATCC-10231) followed by seed extract in concentration of 100 µl while pure fatty acids Linoleic,Linolenic and oleic acids were active against all test organisms in a concentration of 10 µl.

The results indicated that Perillafrutesenceshowed antibacterial and anticandidal activities and may be a promising potential candidate for the development of new antimicrobial Phytomedicine.

Keywords:Atimicrobial;anticandidalactivityphytomedicine,Perillafrutesence,Fattyacids,GC-MS

INTRODUCTION

The use of herbal and other natural substances is a part of the fabric of traditional medicines in different parts of the world. Medicinal plants have been found as a good source of therapeutic and novel compounds. Targeted screening of a large diversity of medicinal plants is expected to yield novel biological activities including problematic group of multidrug resistant bacterial pathogens [1].

The consistently increasing incidences of infectious diseases are due to microbial resistance against presently available antibiotic drugs [2]. Global deaths are increases due to these multidrug resistant microbes due to increasing infections in population [3]. The increasing resistance rate and reoccurrences of infections have great impact on our society [4-6]. The mortality rate due to antimicrobial resistance may be exceeded 10 million by 2050 leading to a increasing mortality due to infectious diseases [7].

In developing countries plant derived drugs are important to fight against serious diseases. The percentage of world population stillrelies on traditional medicines is 62-80% for the treatment of common illness [8-9]. Medicinal plants would be the best source of a variety of drugs says WHO [10]. Medicinal plants have a different variety of phytochemical compounds such as alkaloid, flavonoids,tannins&polyphenolic compounds showing antimicrobial properties [11-12]. Various medicinal plants have been mentioned in a number of phytotherapy manuals for treating urinary tract infections, GIT disorders, respiratory diseases and skin

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infections.For the development of new drugs scientists are working and increasing their focus on natural products to search new bioactive compounds which leads to develop better drugs against microbial infections and drug resistant microbes[13-15]. From plants, new antimicrobial chemical entities can be obtained having more curative properties than commercially available antibiotics therefore, plants antimicrobial compounds have more clinical values than the commercially available against drug resistant microbes[16].

The antimicrobial activity are due to the presence of secondary metabolites in plants called phytochemicals or plants bioactive compounds [17-18].

Perillafrutescens(L.) is an important herb of interior and remote villages of hilly region of Uttarakhand belongs to family Lamiaceae generally called as Bhanjira where peoples are using seeds as a food that also used as folklore medicine and remedy for various ailments such as cough, allergy, depression, anxiety, tumor, intoxication, and some intestinal problems ,cancers , infectious diseases and cardiovascular ailments **.** In northern regions of India the stem part of the plant is used as pain reliever and anti-abortive agent, the leaves are said to helpful for relief in asthma, colds and flu's[19]. To expel intestinal worms and healing the cuts, wounds the leaf juice of this plant is used in Dekhatbhuli, Nepal [20].The paste of *Perillafrutescens*(L.) root in combination with goat urine is used as a poultice to relief from rheumatoid arthritis [21].Chinese has been used for centuries as a medicinal plant for asthma, influenza, cough, chronic bronchitis and vomiting.

Keeping in view of the traditional uses and folklores in treating various microbial diseases a study is designed to explore the detailed scientific investigation of *Perillafrutescens*(L.).

Considering the vast potential of Indian medicinal plants as an anti-infective agent, we have selected the plant on the basis of their traditional uses, ethanopharmocological data and local availability.

In this study, we have investigated the *in vitro* antimicrobial activity in flowers and seeds of *Perillafrutescens*(L.) as development of resistance by microbes against the available drugs/ antibiotics has deepened the concerns for the identification of new and natural non-antibiotics/ natural antimicrobial compounds.

EXPERIMENTAL

COLLECTION OFMATERIAL

The plant of the *Perillafrutescens*(L.) was collected for this study from local farmers of village Sahiya near Chakarata,Dehradun,Uttarakhand,India. The Plant was authenticated by Dr.MayaramUniyal, (Ex Herbs Advisor, Government ofUttarakhand,India) and voucher specimen HDC-0520 was deposited to Pharmacogonosy department of The Himalaya Drug Company, Dehradun, Uttarakhand,India. The Flowers and seeds were dried in shade. After drying foreign matters and dust and dirt were removed. The seeds were crushed in mortar and pestle while flowers were converted into powder with help of a grinder.The method of Zurera [22] was used with little modifications.

SAMPLE PREPARATION

SAMPLE PREPARATION FOR GC-MS ANALYSIS

The powder of flowers and crushed seeds were extracted in methanol and filtered using whatmann filter paper no-1. The extract was evaporated to dryness and stored at 4°C for further analysis.

GC–MS analysis of extract was in methanol and injected in a Shimadzu GCMS-QP2010Ultra system coupled with mass selective detector with an ion source having temperature 230°C and interface temperature 270^{O}C. The operating conditions of the GC–MS set for the analysis were as follows: Oven temperature 140 °C for 5 min then 280 °C at 10 °C/ min and held for 30 min. The sample injection was 1.0µL and the carrier gas was helium with 41.6cm/seconds of linear velocity. Identification of compounds/ Components were identified on the basis of retention time (RT) for GC and interpretation of mass spectrum was done by comparing spectral fragmentation obtained, to the database provided by WILEY8LIB and National Institute Standard and Technology (NIST11LIB).

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ANTIMICROBIAL ACTIVITYDETERMINATION

CULTURE MEDIAAND INOCULUM PREPARATION

Nutrient broth/ Agar and Muller–Hinton broth/ agar (Hi-Media Pvt. Ltd., Mumbai, India) were used to grow the test bacteria at appropriate temperature 30-37 °C for 18hrs and then appropriately diluted in sterile 0.8% saline solution to obtain a cell suspension of 10^5 –10^6 CFU/ml and SCDA/SCD broth for *Candida albicans.*

ANTIMICROBIAL ASSAY

The agar well/disc diffusion method (Perez et al. 1990) as adopted earlier (Ahmad and Beg 2001) was used. 0.1 ml of diluted inoculum (10^5 CFU/ml) of test organisms was spread on Muller-Hinton agar plates. Wells of 8 mm diameter were punched into the agar medium and filled with 100µl of plant extract of 10mg/ml concentration and solvent blank (DMSO) separately. The plates were incubated at 37 °C, over night. The antibiotic (chloramphenicol) at 100µg/ml conc. was used in the test system as positive control. Zone of inhibition of bacterial growth around each well was measured in mm.Similarly sterilized filter paper disc were soaked in test sample and were placed on the inoculated media and incubated overnight at 37°C.

RESULTS & DISCUSSION

FATTY ACIDDETERMINATION BY GAS CHROMATOGRAPHY(GC-MS)

The values obtained by gas chromatography for the chemical composition of fatty acids in the crude extracts are presented in Table 1 and Figure 1&2. The Methanolic extracts of *P.frutesecnce*seedscontained both saturated and unsaturated fatty acids. The saturated fatty acidswere primarilyPalmiticacid,Stearic acid and its methyl esters while unsaturated fatty acids included Linoleic acids,Linolenic,alpha-Linolenic and its methyl esters. The methanolic extract of the flowers had a high content of saturated fatty acids (17.89%) and unsaturated fatty acids (1.98%). Saturated fatty acids in flowers included Palmitic acid andStearicacids, and unsaturated fatty acids including Linoleic and Linolenic acids.

Multiple drug resistance in pathogenic microbes has emerged as important problem in many countries of the world. There are now increasing case reports documenting the development of clinical resistance to newer and broad spectrum antibacterial drugs like Fluroquinolone (Norfloxacin, Ciprofloxacin, Oflaxacin etc.) in many pathogenic bacteria. In the present study, test organisms*Staph. aureus*,*E. coli*, and *Candida albicans* were used.

In the present study, Seeds and flowers of *P.frutescence* were selected on the basis of their traditional uses in treatment of different diseases in India and in the world. Only alcoholic extracts of plant material have been used as the alcohol was found suitable solvent for the extraction of antimicrobially active constituents from plants [23, 24].

The antimicrobial activity test was performed with the different solvent extractsof*P.frutesence*as described in (Table-2) in which methanolic extract of flowers showed strong inhibition(16mm diameter of zone of inhibition) against the *Staph aureus* followed by ethanol extract.While Ethanol extract of flower is more effective showed a zone of inhibition of 18mm against *Candida albicans.* The Hexane extract of flowers is second more active extractagainst*Staph.aureus* and *Candida albicans*.In seeds hexane extract is the more active extract with a zone of inhibition of 15 mm against *Staph.aureus* and 14mm against *Candida albicans*.Regarding the antimicrobial activity of pure omega fatty acids Linolenic acid was found more active pure fatty acid against *Staph aureus* and *Candida albicans*as in(Table-3.) followed by Linoleic and Oleic acids as shown in plate 1-5.

Our findings are correlated with reports of earlier workers [25-28]similarilyanticandidal activity of this plant demonstrated that the plants could exhibit varying level of activity (Table-2). Highest activity in terms of diameter of zone of inhibition was recorded in methanolicextractfollowed by ethanolicextract.Chloroform extract of seedshave not shownany antibacterial/anticandidal activity.

GC-MS analysis of active methanolicplant extracts was made for the presence of major Fatty acids as depicted in Table-1. The differences in these fatty acids might be responsible for varied activity. Thus our antimicrobial results also justify the traditional uses of these plants in differentailments and localized skin infections caused by *Staph.aureus*, *E.coli*, and *Candida albicans*.

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CONCLUSION

*P.frutesence*seedsand flowers present great medicinal importance in the deep and remote areas where this plant is used to cure various diseases and microbial infections. The obtained methanolic extracts from the flowers and seeds and pure omega fatty acids showed antibacterial/anticandidal activity against the human pathogens studied. The Gas Chromatographic analysis (GC-MS) identified the fatty acids: lauric, myristic, palmitic, stearic, oleic and linoleic and linolenic acids. Therefore, this study concludes that *P.frutesence* present potential pharmaceutical and technological applications due to the presence of bioactive compounds with antibacterial /anticandidalactivity and has brought forward new information on the development ofpotential new antimicrobial phytomedicinefrom this plant of Uttarakhand India.

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Table-1:**Fatty acid composition (%) of the methanol extracts of seeds& flower from *P.frutesence***

Sr.No.	Name of fatty acid	synonym	Area % (Methanolic extract of Seeds)	Area % (Methanolic extract of Flower)	Type of fatty acids
1.0	9,12-OCTADECADIENOIC ACID (Z,Z)-, METHYL ESTER	Linoleic acid, methyl ester	2.16	0.31	Unsaturated fatty acid
2.0	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Linolenic acid; α-Linolenicacid;methyl ester, (Z,Z,Z)	5.61	0.78	Unsaturated fatty acid
3.0	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Linolenic acid; α-Linolenic acid;	11.16	--	unsaturated fatty acid
4.0	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	Linolenic acid; α-Linolenicacid;ethyl ester, (Z,Z,Z)-	--	0.89	unsaturated fatty acid
5.0	n-Hexadecanoic acid	Palmitic acid	9.57	13.16	Saturated fatty acids
6.0	Octadecanoic acid	Stearic acid	0.63	4.53	Saturated fatty acids
7.0	OCTADECANOIC ACID, METHYL ESTER	Stearic acid METHYL ESTER	0.49	--	Saturated fatty acid
8.0	Hexadecanoic acid, methyl ester	Palmitic acid, trimethylsilyl ester	1.42	0.2	Saturated long-chain fatty acid
9.0	1,2-BENZENEDICARBOXYLIC ACID, BIS(2-METHYLP	Phthalic acid, diisobutyl ester	9.57	24.41	Aromatic carboxylic acid
10.0	9-OCTADECENAMIDE	Oleic acid amide	0.11	0.17	Unsaturated fatty acid

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Table: 2-Mean diameter of growth inhibition in (mm) of Test pathogens in Antimicrobial susceptibility tests using the solvent extracts (concentration: 100 microlitre) from *P.frutesence*

S.No.	*Perilla frutescence*	**Diameter of the inhibition halo (mm)**		
		TEST ORGANISMS		
		Staph.aureus	*E.coli*	*Candida albicans*
	Flowers			
1.0.	Methanol extract	16	12	14
2.0.	Acetone extract	13	12	13
3.0.	Hexane extract	14	11	16
4.0.	Chloroform extract	13	12	15
5.0.	Ethanol extract	15	14	18
	Seeds			
1.0.	Methanol extract	10	11	13
2.0.	Acetone extract	10	10	11
3.0.	Hexane extract	15	12	14
4.0.	Chloroform extract	ND	ND	ND
5.0.	Ethanol extract	10	10	12

Table:3Antimicrobial activity of pure omega Fatty acids against test pathogens

S.No.	**Omega Fatty acids**	**Diameter of the inhibition halo (mm)**		
		TEST ORGANISMS		
		Staph.aureus	*E.coli*	*Candida albicans*
1.0.	Linoleic acid	12	11	12
2.0.	Linolenic acid	16	14	14
3.0.	Oleic acid	11	10	12

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Plates: Antibacterial activity of pure fatty acids against *Staph.aureus* and *E.coli*

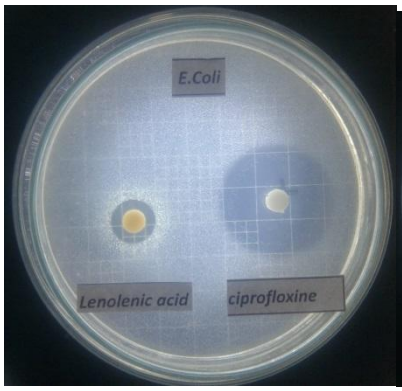

Plate-1

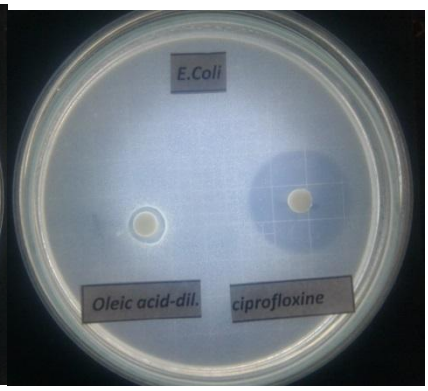

Plate-2

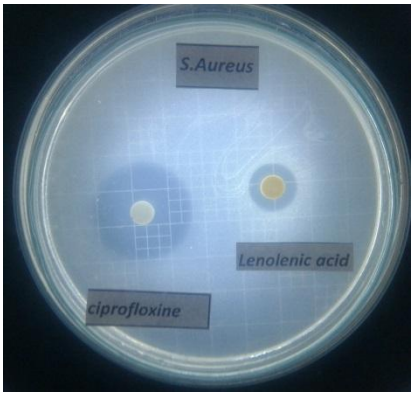

Plate-3

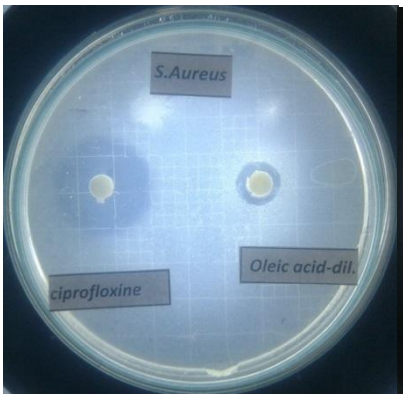

Plate-4

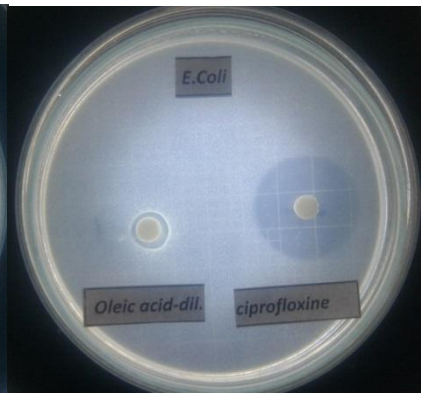

Plate-5

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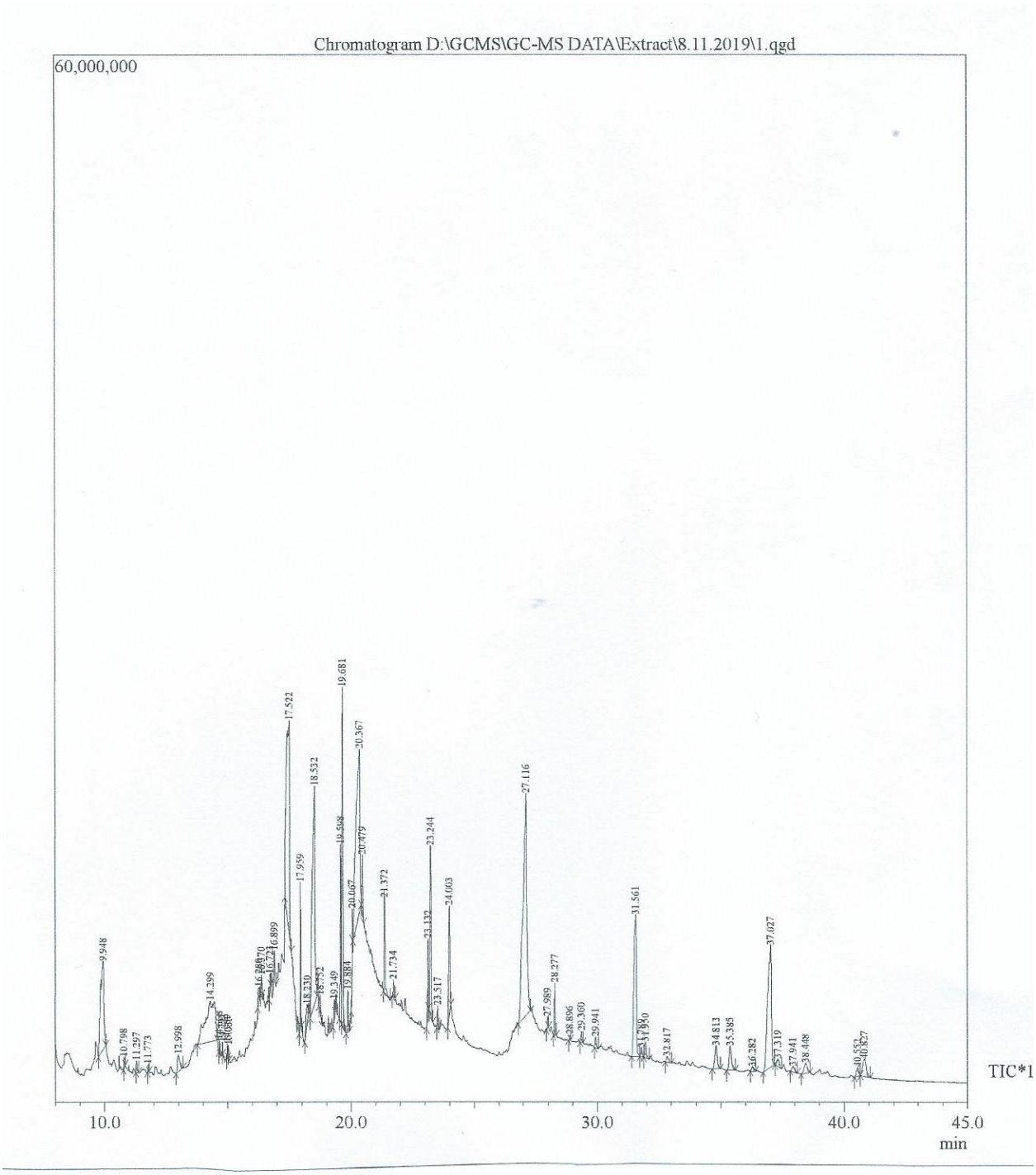

Figure-1: GC-MS Spectra of methanol extract of seeds

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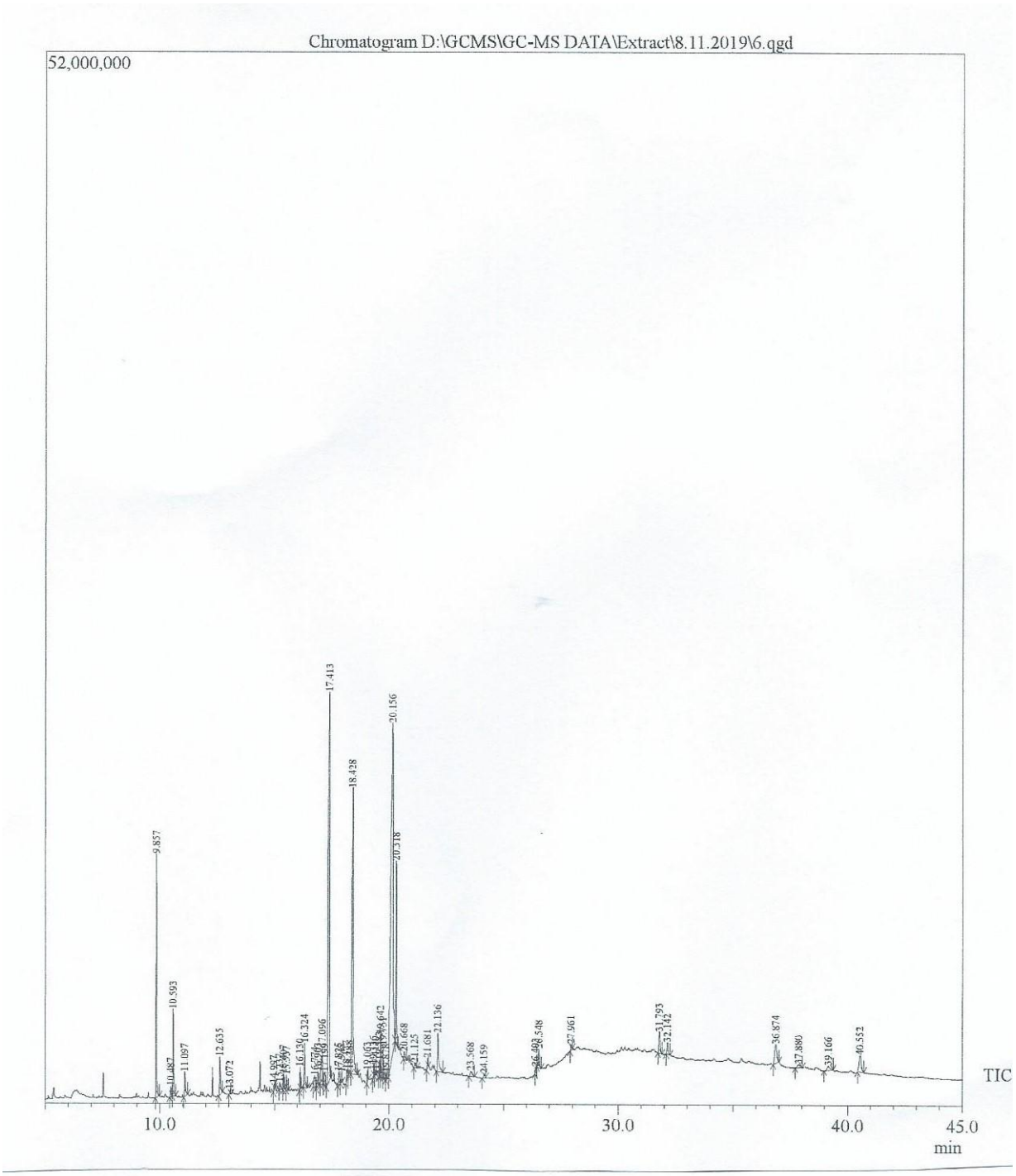

Figure-2: GC-MS Spectra of methanol extract of flowers