Chemical Profiling of *Lavatera cachemiriana*: An Important Ethno-medicinal Herb of Kashmir Himalayas

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ABSTRACT
Lavatera cachemiriana is an important medicinal herb being used in the Unani system of medicine, it possesses various ethno-medicinal properties. The current study was aimed to investigate phytochemical profiling of *L.cachemiriana* roots using classical and modern hyphenated techniques. The preliminary analysis has showed presence of varied classes of phytocompounds such as titerpenoids or sterols, cardiac glycosides, saponins, alkaloids, tannins or phenolics, flavonoids, resins, gums, mucilages, diterpenes, quinines and coumarins. There were 37 compounds identified during GC-MS analysis. The complex chemical profile offer a future opportunity to use *L.cachemiriana roots* as a source of lead bioactive molecules.

Keywords: Phytocompound, *Lavatera kashmiriana,* GC-MS analysis, *Lavatera cachemiriana*, Phytochemical analysis.

I. INTRODUCTION

Lavatera cachemiriana Cambess (*Malvaceae*) is an endemic and endangered plant of Kashmir Valley (Molur and Walker, 1998). It is a beautiful, semi-evergreen, perennial, tall mallow flowering herb of Kashmir which grows in humus rich soils in meadows, shrubberies and forest clearings (Ford, 1938; Sharma, 2003; Kaul, 1997; Vidyarthi, 2010). Traditionally used for various therapeutic purposes such as roots as laxative (Sharma, 2003), abdominal disorders and renal colic (Kaul, 2010), flowers for common cold and mumps and seeds as antiseptic etc. (Dar *et al*., 2002). *L. cachemiriana* C is an important ornamental and medicinal herb (Fig.1.2) endemic to Kashmir valleyoriginally (Molur and Walker, 1998; Kaul, 1977) and is currently found in western Himalya from Pakistan to Uttar Pradesh/Uttaranchal (Sharma, 2003; Kaul, 1977). It has a wiry stem which grows up to 2m tall with 3-5 lobed velvety textured heart shaped leaves, lower leaves with 5 round lobes and upper leaves with 3-5 lobes, central lobe being much longer than the side lobes. It has long blooming, funnel-shaped, silkytextured, clear, hermaphrodite, lilac-pink colored flowers which appears from July till September, the mode of propagation is by small, blackish, kidney shaped seeds (Molur and Walker, 1998; Sharma, 2003; Kaul, 1977). It is known as sazakul in Kashmiri language or reshkhatmi in persian (Vidyarthi, 2010), while as different names are being used locally for various parts of this plant species such as sazposh for flowers, sazamool for the roots (Kaul, 1997; Vidyarthi, 2010) and wan sotsal for leaves (Dar *et al*, 2002).

Lavatera cachemeriana Cambess or *Lavatera Kashmiriana* (Malvaceae) is an important ornamental and medicinal herb of Kashmir Himalaya, India (Molurand Walker, 1998); though being endemic to Kashmir valley originally (Molur and Walker, 1998;Kaul,1977); however, it is currently found in western Himalaya from Pakistan to Uttar Pradesh and Uttaranchal (Sharma, 2003;Kaul,1977). This is an attractive, semi-evergreen, perennial, tall mallow flowering herb (Fig.1) that which grows in humus rich soils, in meadows, shrubberies and forest clearings (Ford, 1938; Sharma, 2003; Kaul, 1997; Vidyarthi, 2010). The wiry stem beautifies it further which grows up to 2m tall with 3-5 lobed velvety textured heart shaped leaves, the lower leaves have 5 round lobes and upper leaves have 3-5 lobes, central lobe being much longer than the side lobes. It has long blooming, funnel-shaped, silky textured, clear, hermaphrodite, lilac-pink colored flowers which appear from July till September, the mode of propagation is by small, blackish, kidney shaped seeds (Molur and Walker, 1998; Sharma, 2003; Kaul, 1977). Locally it is known by the name of sazakul in Kashmiri language or reshkhatmi in persian (Vidyarthi, 2010) , while as different names are being used locally for various parts of this plant species such as sazposh for flowers, sazamool for Roots (Kaul, 1997; Vidyarthi, 2010) and wan-sotsal for leaves (Dar *et al*, 2002).

Traditionally *L. cachemiriana* C. (Kashmiri tall mallow) is used for various therapeutic purposes such as roots as laxative (Sharma, 2003), for abdominal disorders, renal colic (Kaul, 2010; Handa, 2006); flowers have been reported to be used for common cold, mumps and seeds as antiseptic etc. (Dar *et al*., 2002; Jeelani *et al*., 2013; Malik *et al*., 2011). The root decoction of *L. cachemeriana* is being used as anti-dandruff agent and is believed to enhance hair growth; when used on scalp. It is used in Unani medicinal preparations e.g. in throat problems, as a mild laxative and its roots are sold as a crude drug in Kashmiri market (Kaul, 1997; Vidyarthi, 2010). During older days, it was famous agent against mumps in children; recently it been indicated to possess anti-inflammatory and analgesic properties (Parveen, 2013). The leaves and flowers were used to cure skin irritation in pregnant ladies (Hassan, *et al*., 2013; Kuishu, 2007), urinary disorders (Ballabh , *et al*., 2008) and seeds as antiseptic (Dar *et al*., 2002; Malik *et al*., 2011). Furthermore, strong fibre is obtained from stems of *L.Cachemiriana*; that is used to make strings, bags, paper etc. (Pfaf, 2016). Recent research on this species has revealed its various biological activities including anti-lipoxygenase (Khattak *et al*., 2005), as a protease inhibitor and antibacterial agent against both gram positive and gram negative species (Rakashanda *et al*, 2013; Parveen, 2013) and also possesses anticancer properties (Dar *et al*, 2004). As per literature survery, Parveen, 2013 reported that it contains various compounds like Sesterpene called Lavaterone identified as 11-(4,8,10-trimethyl decalinyl)-13,17-dimethyl decan-19-one, Lavaterepene, Lavateral, Lavaterosterol, Lavateronic acid. Furthermore Dar *et al;* 2004 has reported isolation of two diterpene compounds {ent-pimmaran 8(14),15-diene-19-oic acid and ent-pimmarane 7(8),9(11),15-diene-19 oic acid} from *L.cachemeriana.* The phytochemical profiling of different species of *lavatera* like *L.trimestris* has lead identification of various lead compounds such as dodecanoic acid, tetradecanoic acid, n-hexadecanoic acid , cis-trans-p-coumaric acid, cis-/trans-p-coumaric acid methyl ester, caffeic acid methyl ester, ferulic, p-hydroxybenzoic, protocatechuic, gallic, vanillic, isovanillic, syringic, ellagic, chlorogenic acids, kaempferol, hiperoside, ,quercitrine, rutoside, luteoline 7-glucoside (Wozniaka *et al*., 2007). The presence of diverse phytocompounds within Malvaceae family promises different biological properties such as antibacterial, anti-inflammatory, anti-viral, hepatoprotective, ant-malarial, analgesic etc. (Franz and Chladek, 1973).

Fig. 1. *Lavatera cachemeriana* Cambess field grown plant.

Due to over exploitation of roots, restricted distribution & continuous decline, *L. Cachemiriana* has been declared as endangered by IUCN -International Union for Conservation of Nature (Molur and Walker, 1998; Parveen, 2013; IUCN, 1970 and 1980), hence strategic conservation program of this medicinal herb is needed and the primary goal of conservation is to ensure survival of population which could be achieved by adaptation towards environmental changes (Frankel *et al.*, 1995). For potential conservation management and sustainable utilization of this evocative medicinal herb; it is also important to highlight its chemical profiling, so that strong recommendations and deliberations will be provided. for proper conservation and its sustainable utilization. The presence of diverse phytocompounds within Malvaceae family promises different biological properties such as antibacterial,anti-inflammatory, anti-viral, hepatoprotective, anti-malarial, analgesic etc. (Franz and Chladek, 1973). To the best of our knowledge, there was very scanty information available in the literature regarding chemical profiling of *L. Cachemiriana*. Though the plant is an endangered one and people use it for various therapeutic purposes that pose danger to its existence. Therefore, the key objectives of this study were to evaluate chemical profiling of roots of the collected sample. To the best of our knowledge, despite *L. Cachemiriana* has endangered status with fabulous traditional uses and people use it for various therapeutic purposes that pose danger to its existence; however, there is very scanty information available in the literature about its in-depth scientific studies including chemical profiling based on modern hyphenated techniques like GC-MS analysis (Dar *et al*, 2004). Therefore, the key objectives of present study were to evaluate preliminary phytochemical analysis and volatile profile.

II. RESEARCH METHODOLOGY

2.1. Plant material: Collection, authentication and processing

L. cachemeriana Cambess was collected from Gulmargh region of Kashmir, India (10,020 feet above sea level) during the month of June 2012 (Fig.1). The sample was authenticated at Centre for Biodiversity and Taxonomy, University of Kashmir herbarium (KASH) and voucher specimen was deposited under voucher number KASH-1726. The root portion was separated, shade dried, grind into fine powder using electric blender and then passed via mesh sieve. The root powder was designated as LCR (*L. cachemeriana* root) and kept in the light protected bottle at 5^0C till further analysis.

2.2. Solvents, chemicals and instruments

The solvents were of analytical grade (E. Merck Ltd., Mumbai, India) and chemicals were procured from Sigma Aldrich Co, St Louis, USA. GC-MS analysis was done using JEOL GC-Mate II mass spectrometer (USA).

2.3. Phytochemical extraction and analysis of extracts

2.3.1 Phytochemical extraction

The successive mode of phytochemical extraction of *L. cachemeriana* Cambess roots (40 grams) was done using five different solvents based on their polarity index i.e. starting from less polar towards more polar (Petroleum ether-PE, chloroform-CH, ethanol-EtOH, methanol-MeOH and aqueous-AQ). For PE, CH, EtOH and MeOH extractions, Soxhlets apparatus was (Tiwari *et al.*, 2011; Mir, *et al.*, 2011) while as for aqueous extraction; maceration was undertaken (Evans, 1996). The extracts were filtered using Whatman No. 1 filter paper and dried at 40^0C using rotary evaporator. The weight of each dried residue was recorded and percentage of yield of each extract was calculated. Also, extracts were stored in the labelled sterile brown colour screw capped bottles at 5^0C for subsequent use.

2.3.2. Preliminary Phytochemical Analysis

Preliminary phytochemical analyses was performed for detection of different phytocompounds in all the extracts (Titerpenoids or sterols, Cardiac glycosides, anthraquinone gylcosides, Saponins, Alkaloids, Tannins or Phenolics, Flavonoids, Resins or gums or mucilages, Diterpenes, Anthocynins, Quinones and Coumarins) using standard methods available in previous literature (Tiwari *et al.*, 2011; Mir, *et al.*, 201; Harborne, 1998; Kokate, 2010; Canell, 1998; Soni and Sosa, 2013); all these tests were carried out thrice (n=3).

2.3.3. Identification of phytocompounds using GC-MS analysis

The GC-MS analysis of *L.cachemiriana* petroleum ether root extract was performed (Anastasaki *et al.*, 2009) using Thermo Scientific TSQ 8000 mass spectrometer with capillary column (HP-5MS , column diameter 0.25mm, thickness 0.2mm and length 30m), mass detector (HP 5973) was scanned within mass range of 50–1000 m/z. The Ionization potential was 70eV with ionization source temperature at 200^0C.Also, carrier gas used was helium at 1ml/min with initial column temperature as 60^oC for 3 minutes, then raised to 180^oC at rate of 3°C /min temperature and finally raised at the rate of 8 °C/min till 250^oC and it was maintained at this temperature for 10 minutes. 1µl of each extract was injected manually using splitless mode and run time was 40 minutes. The unknown compounds were tentatively identified by gas chromatography based mass spectrometry (GC-MS) using NIST library and supportive literature.

2.4. Statistical analysis

All the measurements were done in triplicates and results are expressed as mean ± SD. P values <0.05 were considered statistically significant and $P<0.01$ considered as very significant.

III. RESULTS AND DISCUSSION

3.1. Crude extract yield and phytochemical analysis

In the current study, the shade dried root powder of *L.cachemiriana* (Fig.2) was successively used for the phytochemical extraction using hot extraction in Soxhlet apparatus and cold aqueous extraction; final yield from each solvent was expressed as % w/w (% Final weight/initial weight). The percentage of yield obtained from each extract (Table-1). The highest yield was found from petroleum ether extract i.e. 1.50 (% w/w), followed by chloroform as 1.25 % w/w, the other extracts showed lower but varying degrees of yield and this difference in the yield could be attributed due to the variation in the polarities of compounds extracted in each solvent (Tiwari *et al.*, 2011).

The preliminary phytochemical analysis of different root extracts of *L.cachemiriana* have revealed the presence of important phytoconstituents i.e. triterpenoids and sterols, cardiac glycosides, phenolics and tannins, saponins, fats and lipids, alkaloids , flavonoids, diterpenes, quinones , saponins, coumarins and resins (Table-2). The highest number of compounds is released in aqueous extract as 7, followed by 6, 5, 3 and 3 in each methanol, ethanol, chloroform and petroleum ether extracts respectively. The variation in the number compounds in different extracts depends upon the method of extraction, solvent polarity and polarity of the compounds in each sample extract (Tiwari *et al.*, 2011).The results have showed presence of diverse classes of compounds which have possesses possible potential biological activities including anti-microbial, anti-oxidant, anti-ulcer, anti-hyperlipedemic, anti-hyperglycemic, anti-cancer, anti-proliferative, anti-inflammatory etc. (Atanassova and Bagdassarian, 2009; Porwal *et al.*, 2012). Different ethno-medicinal properties of this medicinal herb against common cold (Rakashanda *et al.*, 2013), mumps (Jeelani *et al.*, 2013; Malik *et al.*, 2011), urinary disorders (Ballabh *et al.*, 2008), antiseptic (Dar *et al.*, 2002), anti-lipoxygenase (Khattak *et al.*, 2005), protease inhibitor, antibacterial agent (Rakashanda *et al.*, 2013) and anticancerous properties (Dar *et al.*, 2004) could be because of presence diverse class of compounds reported in this species. There are important phytocompounds found by earlier researchers in other species of *Malvaceae* such as mucilage polysaccharides, flavanoids; glycosides; fat coumarins; tannins, polyphenols, sterols, alkaloids, proanthocyanidins, viopudial, polysachharides, urosolic acid (Franz, 1966 and 1973; Kiessoun *et al.*, 2010).

Since the traditional medicine use is widespread across the world as an alternative system of medicine and this is an evidence that plants still play role as source of different novel active biological compounds with various pharmacological activities such as anti-bacterial, anti-inflammatory, cardio protection, anti-fungal, anti-viral, anti-bacterial, anti-cancer etc. (Yan, *et al.*, 2002) and plant based medicines offer cure without any dreaded side effects as compared to costlier synthetic drugs which have usually more toxic side effects; the public has also become more aware about safer and cost effective mode of traditional medicines as compared to costly synthetic medicines that causes more adverse reactions (Montoro *et al.*, 2012). Therefore, existence of diverse phytochemical constituents of *L.*cachemiriana root extracts offers us an opportunity to assess its important biological properties and also to validate its ethno-medicinal usage, this is because plant based secondary metabolites acts as promising source of preventive agents against different diseases (Razia *et al.*, 2013).

The volatile profile based on GC-MS analysis of petroleum ether root extract has revealed diverse number of compounds (Tabl-3). The compounds were tentatively identified based on comparison of their mass spectra with existing NIST library mass spectra as well as using supportive literature. The total ion chromatogram (TIC) obtained along with their respective retention time (RT) is reported under Fig.2. The peak area % of each compound was determined using TIC based ratio of peak area of each compound to the total area of all the peaks. We have identified total 37 compounds which are having significant differences in their peak area percentage (P<0.05) and are spread across different retention times I.e. 6.45-37.76. Among all these compounds, nine compounds have showed higher peak areas i.e. {3}Nonanoic acid, {12} n-Hexadecanoic, {16}Methyl 8,11,14-heptadecatrienoate {20} Menthol, 1'(butyn-3-one-1-yl),(1R,2S,5R), {37} Beta Sitosterol {14} Methyl 7,8octadecadienoate , {4} Nonynoic acid, {35} Benzoic acid, 3,5-dicyclohexyl-4-hydroxy, and {17}Octadecanoic acid with % peak areas as 12.29%, 11.07%, 9.94%, 7.62%, 6.18%, 5.95% , 4.93%, 4.76 % and 4.2% respectively. We have also observed five compounds which peak area between 2.10-2.44% and rest below <1.53%. As per authors knowledge, this would be the first ever study about GC-MS based compound identification in root extract of *L.cachemiriana*. Various lead phytocompounds have been isolated previously in other species of *Malvaceae* (Ramasubramaniaraja,2011; Razia *et al.*, 2013), the differences in the composition in each plant species could be attributed because of various phases of biosynthesis, accumulation, organ type, stages of development (Dulf *et al.*, 2013). The compounds identified by the current study such as Dodecanoic acid, Tetradecanoic acid and n-Hexadecanoic acid possesses various biological properties including antioxidant, antimicrobial, larvicidal etc. (Alam *et al.*, 2014). The haunt for essential medicinal plants has led to the overexploitation of many such plants, this demands immediate balance by cultivating more number of such plants and to design the research strategies for different scientific amelioration methodologies . A proper harnessing in cultivation and trade of medicinal plants would preserve the biodiversity along with economic upliftment of nations with diverse medicinal flora (Wiersum *et al.*, 2006). Therefore, the diverse volatile profile observed in *L.cachemiriana* petroleum ether extract indicates its potential to be a lead source of phytocompounds for aromatic, food and medicinal industries.

IV. CONCLUSION

The current study was attempted to study phytochemical profile of *L. cachemeriana* roots. Conclusively, roots of this species appears to be important repository of potential phytocompounds, these compounds have potential to act as bioactive small molecules and tackle different therapeutically challenging diseases such as those related to oxidative stress or chemoprevention including cancer. The proper scientific exploitation of any species is important, owing to the very fact that the knowledge of medicinal and aromatic plant based bioactive compounds is essential to define the standardized herbal extracts and more number of economically important plants needs to be explored phytochemically as this will help to avoid the chances of any health problem because of unstandardized crude plant based extracts and it will pave a way for preservation of biodiversity at large. Therefore, it is recommended that different populations of this species could be regenerated in huge numbers using macro- and micro-propagation technique and awareness among general public about need for preservation and sustainable utilization of biodiversity that will result in everlasting benefits for the human kind.

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CONFLICT OF INTEREST

All the authors confirm that there is no conflict of interest.

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Fig.2: Roots of *Lavatera cachemiriana*.

Table 1: Percentage yield extract values (% w/w) of *Lavatera cachemiriana.*

Solvents	Yield (%W/W)
Petroleum ether	1.50
Chloroform	1.25
Methanol	0.713
Ethanol	0.6
Water	0.57

Table 2: Preliminary phytochemical analysis of root extracts of *Lavatera cachemiriana.*

S.No	Phytoconstituents	Leaf extracts				
		PE	CH	ME	EH	AQ
1	*Titerpenoids/Sterols*	+	+	-	-	-
2	*Cardiac glycosides*	-	-	-	+	+
3	*Anthraquinone gylcosides*	-	-	-	-	-
4	*Saponins*	-	-	-	-	+
5	*Alkaloids*	+	+	+	-	+
6	*Tannins/Phenolics*	-	-	+	+	-
7	*Flavonoids*	-	-	+	+	+
8	*Resins/gums/mucilages*	-	-	-	-	+
9	*Diterpenes*	-	-	+	+	-
10	*Anthocynins*	-	-	-	-	-
11	*Quinones*	+	-	-	+	-
12	*Coumarins*	+	-	+	+	+

[Note: (+) Positive; (-) Negative;PE - Petroleum ether, CH Chloroform, ME - Methanol, EH Ethanol, AQ Aqueous (n=3)]

Table 3: GC-MS based volatile profile of *L.cachemiriana* petroleum ether extract.

S.No.	RT	Compound Name	Molecular formula	Peak Area%
1	6.45	1-Nonyne	C9H16	2.26
2	9.13	2-Dodecanone	C12H24O	0.32
3	10.7	Nonanoic acid	C9H18O2	12.29
4	11	Nonynoic acid	C9H14O2	4.93
5	12.97	Isoshyobunone	C15H24O	0.23
6	14.43	Ethyl hydrogen suberate	C10H18O4	2.1
7	16.33	Pentadecanoic acid	C15H30O2	1.04
8	16.59	10Heneicosene (c,t)	C21H42	0.58
10	17.19	tertHexadecanethiol	C16H34S	0.35
11	17.96	Hexadecanoic acid, methyl ester	C17H34O2	0.83
12	18.45	n-Hexadecanoic acid	C16H32O2	11.07
13	18.91	Isopropyl palmitate	C19H38O2	0.39
14	19.54	Methyl 7,8octadecadienoate	C19H34O2	5.95
15	19.85	2Octylcyclopropene1heptanol	C18H34O	1.52
16	20.16	Methyl 8,11,14heptadecatrienoate	C18H30O2	9.94
17	20.31	Octadecanoic acid	C18H36O2	4.2
18	20.47	9Octadecenal,(Z)	C18H34O	2.13
19	20.97	E,E,Z1,3,12Nonadecatriene5,14diol	C19H34O2	0.86
20	21.43	Menthol, 1'(butyn3one1yl) ,(1R,2S,5R)	C14H22O2	7.62
21	21.77	Spiro[4.5]decan7one, 1,8dimethyl8,9epoxy4isopropyl	C15H24O2	0.95
22	21.99	Ethyl isoallocholate	C26H44O5	1.02
23	22.19	1Decanol,2octyl	C18H38O	2.38
24	22.79	Dasycarpidan1methanol, acetate (ester)	C20H26N2O2	1.3
25	23	Eicosane	C20H42	0.68
26	23.77	Tetratriacontane	C34H70	0.92
27	24.54	Tetracosane	C24H50	0.83
28	24.95	Stigmast-4-en-3-one	C29H48O	2.44
29	25.4	Octacosane	C28H58	1.28
30	26.42	Hexatriacontane	C36H74	0.97
31	27.62	Hexatriacontane	C36H74	0.57
32	29.07	Tetratetracontane	C44H90	0.31
33	29.19	Cholesta4,6dien3ol, (3á)	C27H44O	0.44
34	29.9	17(1,5Dimethylhexyl)10,13dimethyl2,3,4,7,8,9, 10,11,12,13,14,15,16,17tetradecahydro1Hcyclopenta[a]phenanthren3ol	C27H46O	0.61
35	31.89	Benzoic acid, 3,5dicyclohexyl4hydroxy, methylester	C20H28O3	4.76
36	32.62	Stigmasterol	C29H48O	0.48
37	34.08	beta Sitosterol	C29H50O	6.18

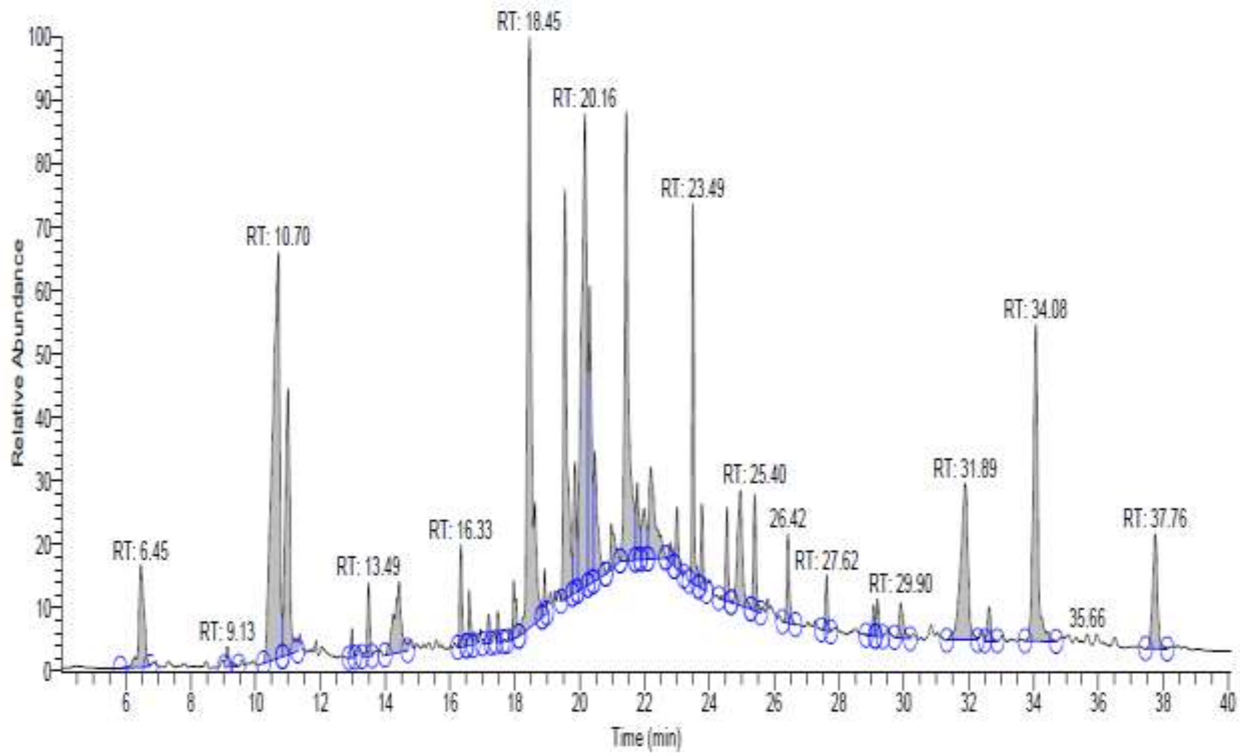

Fig 3: Total ion chromatograms of *Lavatera cachemiriana* petroleum ether extract.