Therapeutic potential of aromatic phyto-constituents derived from *Anisochilus carnosus* (L.) Wall

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Abstract
Now a days plant derived constituents are emphasized for drug formation. The family Lamiaceae has been described to be rich in essential oils. The glandular trichomes distributed over the aerial and vegetative organs of plants belonging to Lamiaceae, are recognized as the site of essential oil biosynthesis. *Anisochilus carnosus* (L) Wall. Belonging to the family (Lamiaceae-Mint family) grows on rocks, an annual herb distributed in the various high altitude regions of Western Ghats, Tamil Nadu, Maharashtra and Rajasthan. It is locally known as Induparni in Sanskrit, Panjiri-ka patta in Hindi and Kapurli in Marathi and extensively used in folk medicine for the treatment of gastric ulcer and skin diseases. The plant study revealed the occurrence of Tetraethylenepentamine (1.81%), Methamphetamine (7.32%), α-Muurolene (1.09%), Methyl hexadecyl ether (25.73%), Benzenamine, 2-methoxy (14.37%), Warfarin (4.41%), Nonyne (20.22%), Oxymesterone (2.94%), Seneciphylline (20.22%), that have therapeutic potential, It also aunthenticates the claims made by traditional practitioners in curing various diseases.

Key words: Lamiaceae, *GC*-MS analysis, aromatic oil, *Anisochilus carnosus*

1. Introduction
Medicinal plants have bio-active compounds which are used for curing various human diseases. More than 7,500 species of medicinal plants grow in India Aromatic plants have a pleasant, characteristic fragrant smell. The fragrance of these plants is carried in the essential oil fraction obtained from different plant material like flowers, buds, seeds, leaves, twigs, bark, wood and roots [1] (Zamfirache, 2010). Aromatic phyto-constituents are natural products that have an ecological role in regulating the interactions between plants and their environment. A prominent group of natural products is the terpenes and their derivatives [2] (Gershenzon *et al.*, 1999).

Anisochilus carnosus belongs to the family Lamiaceae, which is otherwise known as thick-leaved lavender in English and Karpuravalli in Telugu, Marathi and Tamil [3] (Ignacimuthu *et al.*, 2006). It is an annual erect herb, stems quadrangular, sparsely pubescent, brownish from prolonged exposure to sun. Leaves fleshy, broadly ovate, deeply crenate, obtuse or acute, base rounded, verrucose above and pubescent beneath. The whole plant is used as diaphoretic, stimulant, expectorant in liver disorders, cough and cold [4] (Rabe *et al.*, 1997). Leaves are used for cough, dropsy, indigestion and sores in the leg fingers [5] (Ravikumar *et al*., 2008).

2. Material and methods
2.1 Plant collection
Anisochilus carnosus leaves were collected in the month of August-September 2014 from Naranala forest Maharastra India. The frequent visits were made to the wild habitat for collection of Fresh and healthy leaves. The plant was identified with the help of floras [6] (Dhore, 2002).

2.2 Preparation of acetone extract
Fresh and healthy leaves were used for extraction. All these leaves were thoroughly washed with tap water to remove the soil and dust particles and further shade-dried up to one week further plant leaves were grinded to fine powder. 5 g of the powder was extracted exhaustively with acetone by hot extraction process (Soxhlet extraction). Extract filtered with what men filter paper and concentrated up to 10 ml.

2.3 Preliminary phytochemical analysis
5 g of the powder was extracted exhaustively with acetone by hot extraction process (Soxhlet extraction). Extract concentrate up to 100 ml. Phytochemical test was performed using the standard methods [7] (Harborne, 1998)

2.4 Gas Chromatography-Mass Spectroscopy (GC-MS) analysis.
For GC-MS analysis acetone extract was used. The GC-MS analysis of leaves extract isolated from *Anisochilus carnosus* was carried out using gas chromatography – high resolution mass spectrophotometer. 2 µl of sample employed for GC-MS Analysis. Analysis was carried out using Alegent Hp 7880 with coloum 25m. Helium gas was used as carrier gas at constant flow rate and estimation of aromatic phyto-constituents done with the help of NIST online libray.

2.5 Evaluation of aromatic phyto-constituents

$$\text{Relative percentage of compound} = \frac{\text{area covered by single phytoconstituents}}{\text{total peak area of all phytoconstituents}} \times 100$$

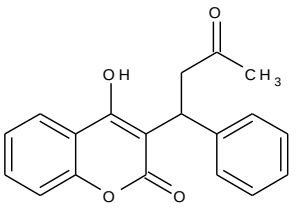
3. Observation table

Test	Solvent			
	Petrolium ether	Acetone	Benzene	Water
1) Alkaloids				
a)Mayer's reagent	++	++	+	++
b)Wagner's reagent	++	++	++	+++
2) flavonoids				
a) Alkaline reagent	-	+	-	++
b) Lead acetate	-	++	++	++
3) Tannins				
a) Iodine test	-	++	-	++
b) Ferric chloride	++	+	-	++
4) Essential- oil				
a) Sudan red-III	+++	+++	+++	+++
b) Tincture alkana test	+	++	-	+++
5) Phenol				
a) Gelatine	-	++	++	++
b) Nitric acid	+ +	-	-	+++
6) Terpenoids				
a) Salkowki's	-	++	++	++
7) Cardiac glycosides	-	+++	++	-
a) Keller Kelliani's	+	+	++	+
8) Quinones				
a) HCL test	++	+	+	++
9)Anthraquinones				
a)H2 SO4 test	++	++	-	-
10) coumarine				
a) Alcoholic NaOH test	++	++	-	++

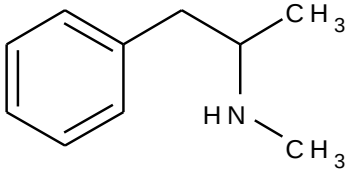
(+++ highly present, ++ moderately present, + presence, - absence of phytoconstituents.)

Preliminary phytochemical analysis of *Anisochilus carnosus* in acetone solvent.
Aromatic phytoconstituents in leaves in acetone extract.

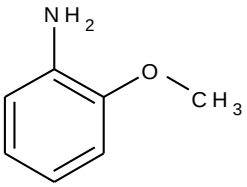
Sr. No.	R.T.	Compound	Peak Area	M.W.	M.F.
1.	10.45	Tetraethylenepentamine	1.81%	189.19	$C_8H_{23}N_5$
2.	12.99	Methamphetamine	7.32%	149.12	$C_{10}H_{15}N$
3.	13.66	α-Muurolene	1.09%	204.21	$C_{15}H_{24}$
4.	18.44	Methyl hexadecyl ether	25.73%	256.27	$C_{17}H_{36}O$
5.	21.35	Benzenamine, 2-methoxy.	14.37%	123.152	C_7H_9NO
6.	21.97	Warfarin	4.41%	308.33	$C_{19}H_{16}O_4$
7.	23.27	Nonyne	20.22%	124.14	C_9H_{16}
8.	25.80	Oxymesterone	2.94%	318.22	$C_{22}C_{28}0_6$
9.	31.70	Seneciphylline	20.22%	333.3789	$C_{18}H_{23}NO_5$



Warfarine



Methamphetamine



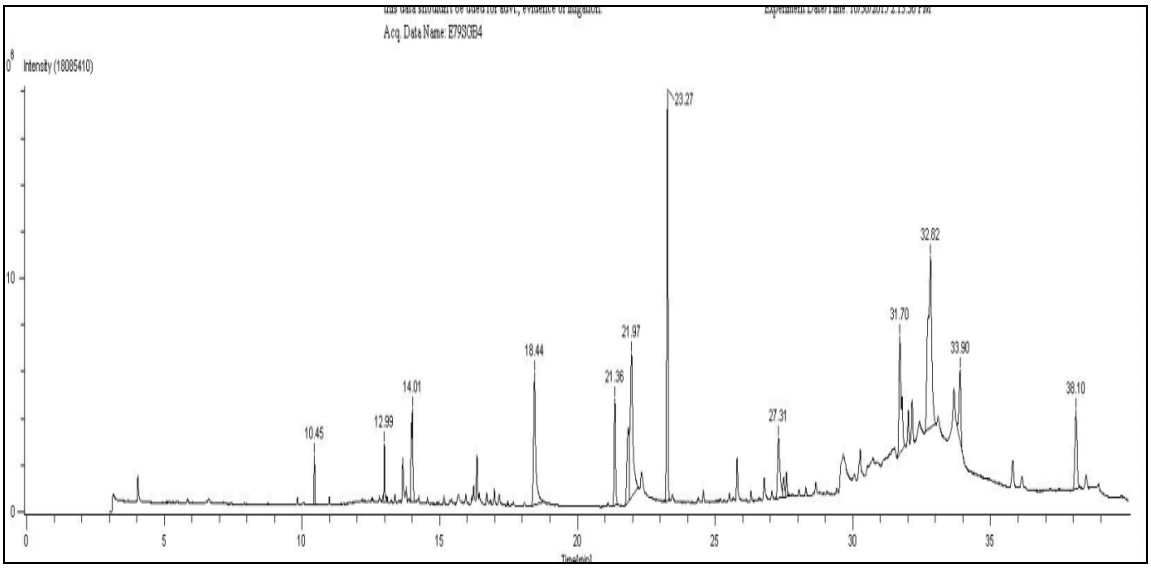
2-Methyl benzednamine

$CH_3\text{-}(CH_2)_{15}\text{-}O\text{-}CH_3$

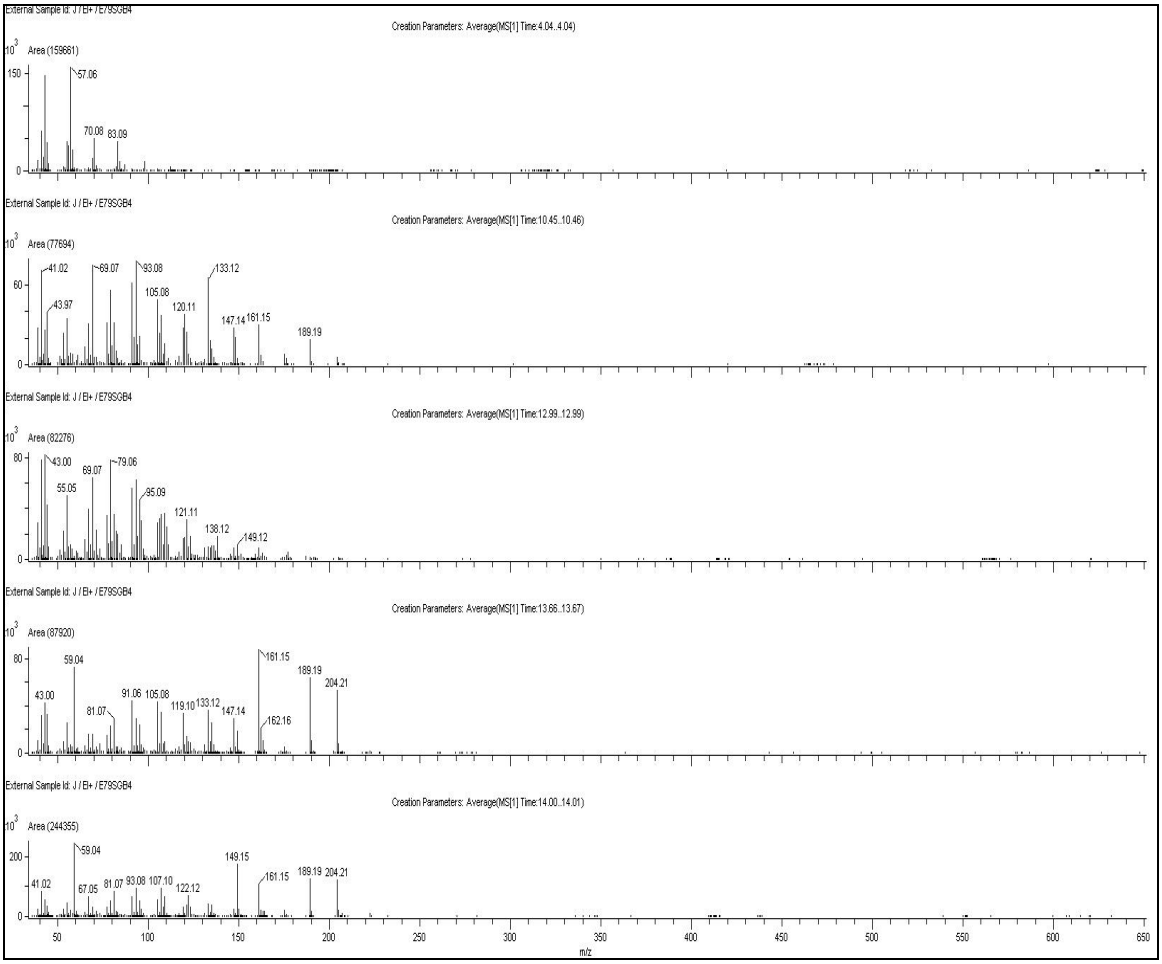
Methylhexadecyl ether

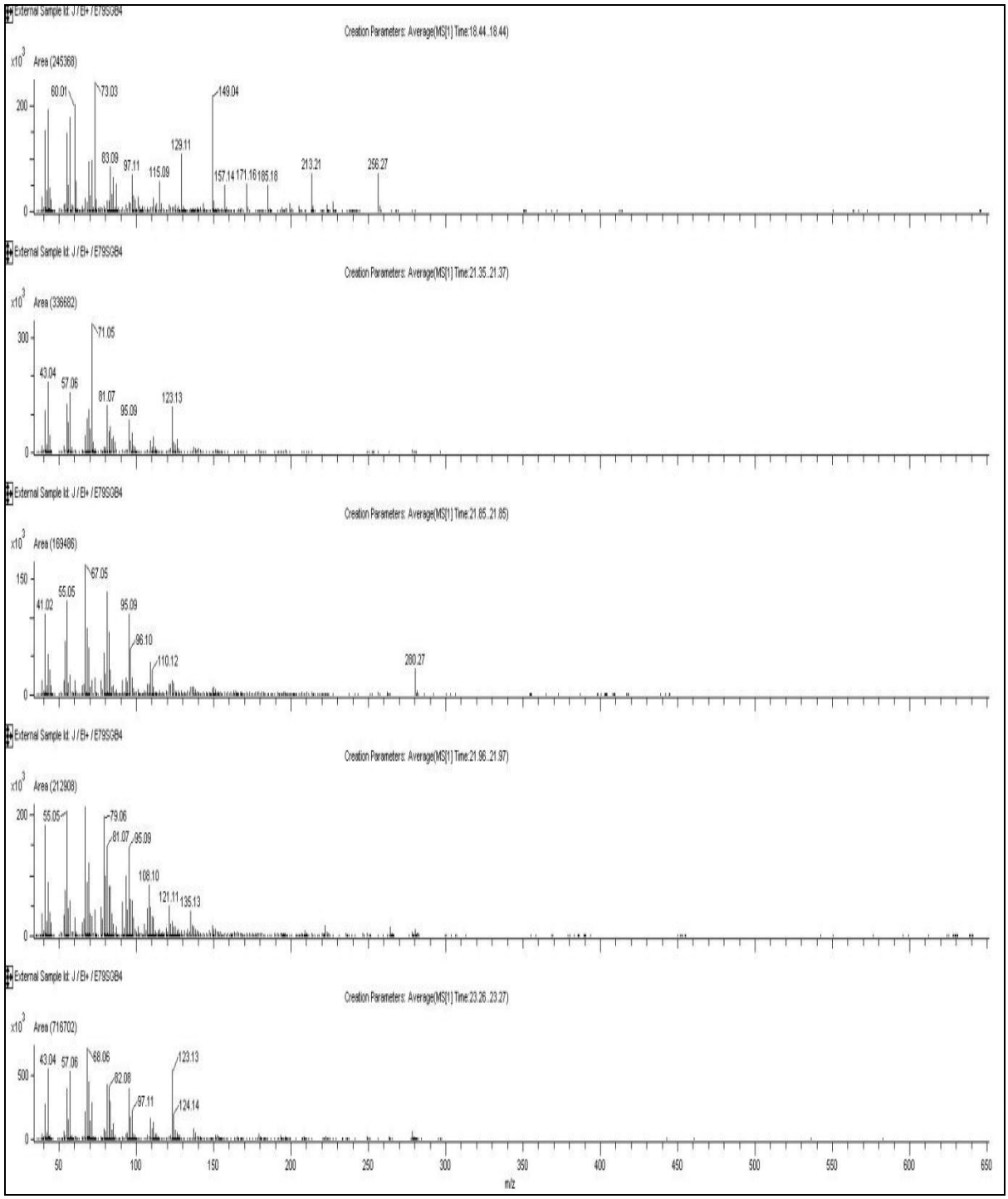
$H_2N\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_2\text{-}NH\text{-}NH_2$

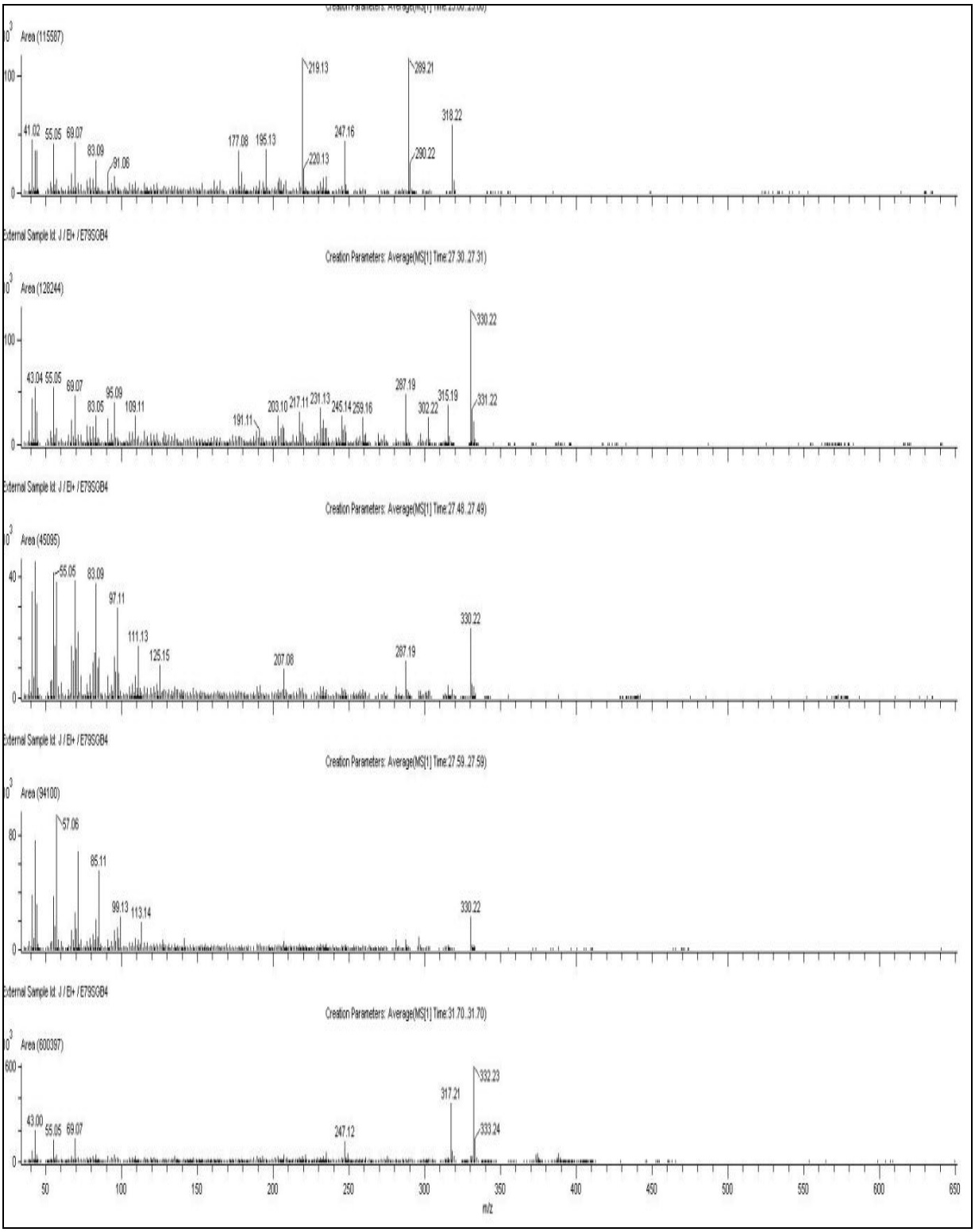
Tetraethylenepentamine

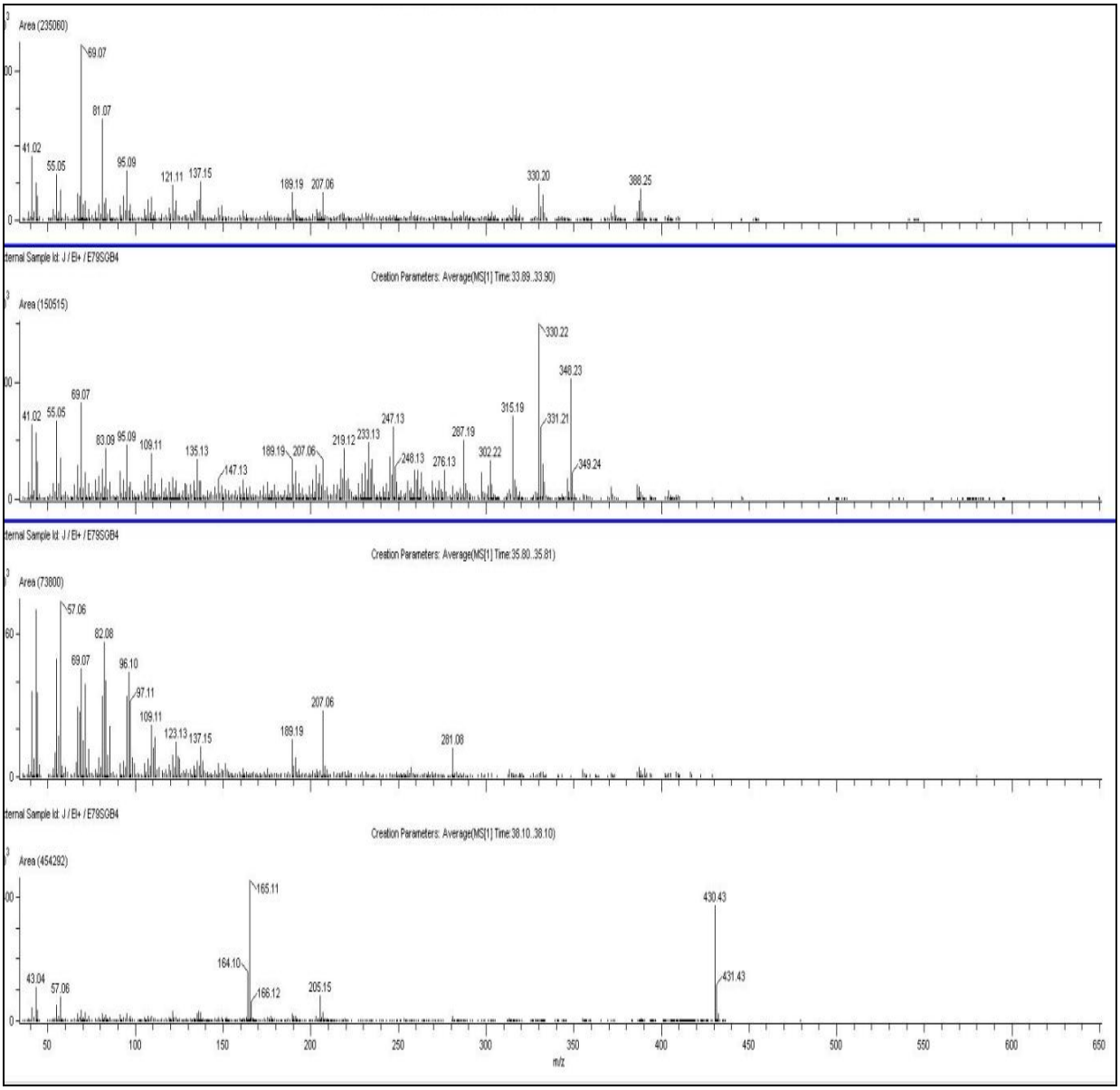


Chromatogram of *Anisochilus carnosus* in acetone solvent.




External Sample Id: J / Et+ / E79SGB4
Creation Parameters: Average(MS[1] Time:18.44..18.44)
x10^3 Area (245368)
60.01
73.03
149.04
83.09
97.11
129.11
115.09
157.14
171.16
185.18
213.21
256.27
External Sample Id: J / Et+ / E79SGB4
Creation Parameters: Average(MS[1] Time:21.35..21.37)
x10^3 Area (336682)
71.05
43.04
57.06
81.07
95.09
123.13
External Sample Id: J / Et+ / E79SGB4
Creation Parameters: Average(MS[1] Time:21.85..21.85)
x10^3 Area (169486)
67.05
55.05
41.02
95.09
96.10
110.12
280.27
External Sample Id: J / Et+ / E79SGB4
Creation Parameters: Average(MS[1] Time:21.96..21.97)
x10^3 Area (212908)
55.05
79.06
81.07
95.09
108.10
121.11
135.13
External Sample Id: J / Et+ / E79SGB4
Creation Parameters: Average(MS[1] Time:23.26..23.27)
x10^3 Area (716702)
43.04
57.06
68.06
123.13
82.08
97.11
124.14
m/z


Area (115587)
219.13
289.21
318.22
41.02
55.05
69.07
83.09
91.06
177.08
195.13
220.13
247.16
290.22
External Sample Id: J / EI+ / E79SGB4
Creation Parameters: Average(MS[1] Time:27.30..27.31)
Area (128244)
330.22
43.04
55.05
69.07
83.05
95.09
109.11
191.11
203.10
217.11
231.13
245.14
259.16
287.19
302.22
315.19
331.22
External Sample Id: J / EI+ / E79SGB4
Creation Parameters: Average(MS[1] Time:27.46..27.49)
Area (45095)
55.05
83.09
97.11
111.13
125.15
207.08
287.19
330.22
External Sample Id: J / EI+ / E79SGB4
Creation Parameters: Average(MS[1] Time:27.59..27.59)
Area (94100)
57.06
85.11
99.13
113.14
330.22
External Sample Id: J / EI+ / E79SGB4
Creation Parameters: Average(MS[1] Time:31.70..31.70)
Area (600397)
332.23
317.21
43.00
55.05
69.07
247.12
333.24
m/z



Mass –spectra *of Anisochilus carnosus* in acetone solvent.

4. Results and discussion

Preliminary chemical examination of *Anisochilus carnosus* done with four solvents petroleum ether, acetone, benzene and water according to increasing polarity revealed the presence of essential oil in leaves. It was found that water and acetone is best solvent for extraction, it showed presence of essential oil and Alkaloids. Sudand Red III test confirmed the presence of essential oil in leaves.

The solvent extract through acetone that was used for GC-MS analysis shows the presence of important aromatic phyto-constituents in leaves of *Anisochilus carnosus.* The percentage of phyto-constituents was calculated by the use of gas chromatogram, retention time, base-peak and mass peak.

Leaves contain Tetraethylenepentamine (1.81%), Methamphetamine (7.32%), α-Muurolene (1.09%), Methyl hexadecyl ether (25.73%), Benzenamine, 2-methoxy (14.37%), Warfarin (4.41%), Nonyne (20.22%), Oxymesterone (2.94%), Seneciphylline (20.22%).

Warfarin is an anticoagulant normally used in the prevention of thrombosis. It is the most widely prescribed oral anticoagulant drug in North America [8] (Schep *et al.*, 2010). Benzenamine has a fruity odor, similar to pineapple. It occurs naturally in many fruits, commonly used in flavors and fragrances. Methamphetamine is a strong central nervous system (CNS) stimulant that is mainly used as a recreational drug. Methyl hexadecyl ether find its use in paints and cleaners. Seneciphylline is hepatic drug due to traditional medicinal practitioner used this plant against gastric ulcer.

5. Acknowledgement

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6. References

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