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Effect of Physicochemical Properties of Soil on Secondary Metabolites of *Calotropis gigantea* (L) Collected from Shivalik hills of Himachal Pradesh, India

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ABSTRACT: Present investigation deals with to study the effects on physicochemical properties of soil on secondary metabolites of *Calotropis gigantea* (L) W. T. Aiton collected from five districts (Hamirpur, Una, Solan, Sirmour and Kangra) of Himachal Pradesh, India within the range of the Shivalik hills. The phytochemical analysis of latex extract of *C. gigantea* using GC-MS technique from all districts showed total 34 compounds out of which 21 were reported from Kangra and Sirmour district; 16 compounds were obtained from the Hamirpur and Solan districts whereas 15 were observed from Una districts. Results of physicochemical properties of soil showed non-significant variation in three parameters (particle size analysis, pH and organic carbon) and significant variations with four parameters (moisture content, available N, available K and CEC) from all districts. Physicochemical study concludes that soil of different districts of Himachal Pradesh has similar properties w.r.t. soil pH, organic carbon percentage and soil texture. Variation was observed only with moisture content, available K and cations exchange capacity, which concludes that these properties could be responsible for variation in the chemical profile of *C. gigantea* of five districts with other unknown ecological factors.

Keywords: *Calotropis gigantea*, Shivalik hills, secondary metabolites, phytochemical analysis, GC-MS, physicochemical analysis.

INTRODUCTION

The nature has blessed the humans with various medicinal plants which are the effective source for both traditional and modern medicines. These plants are used in different ways to treat various infectious diseases. *C. gigantea* (family Asclepiadaceae) is one of the medicinal plants distributed in the tropical and subtropical area of the world and throughout the India. The plant serves as pioneer in desert soil and shows xerophytic adaptation because of the presence of latex as well as extensively branched root system and thick leaves with waxy coverage. The plant flowers throughout the year, especially during the hot season. Flowers are primarily pollinated by bees, butterflies and wasps (Schmelzer and Gurib-Fakim, 2013).

The milky latex is the principle constituent present in *C. gigantea* leaf and stem which contains protease enzymes, calotropin DI and DII (Sen gupta *et al*., 1984), calotropain FI, calotropain FII (Abraham and Joshi, 1979), and uscharine, ascorbic acid, glutathione, calactin (Rastogi Ram, 2001), calotoxin, stigmasterol, caoutchouc and gigantin (Balakrishna *et al*., 1995). Additionally, taraxasterol, α-amyrin, ß-amyrin, ß-sitosterol and ψ- taraxasterol have also been isolated from the plant (Rastogi Ram and Mehrotra, 1991). Due to the presence of all these active constituents *C. gigantea* has been reported as a highly medicinal plant. Medicinally, plant has antifungal (Larhsini *et al*., 1997), analgesic (Mohsin *et al*., 1989), expectorant, anti-inflammatory (Kapur and Sarin, 1984) activities. Plant is also reported in the treatments of migraine, diarrhea and asthma (Sebastian and Bhandari, 1984).

Medicinal properties of plants are due to the presence of secondary metabolites like phenols, alkaloids, terpenoids, flavonoids, etc. Literature show that different conditions which affect the concentration of secondary metabolites are environmental conditions, geographic variations, genetic factors and evolution, age of the plant and also amount of plant material/space. Various works have been done by different researchers on medicinal plants to study the effect of geographic (altitudinal and latitudinal), climatic and genetic variations in the production of secondary metabolites (Zidorn, 2010; Lnping *et al.*, 2013; Aslam *et al.*, 2015).

Very little literature is available in which differences in secondary metabolite concentration are studied w.r.t. soil factors (soil texture, available NPK, soil moisture, etc.) as they are important to study the variation in production of secondary metabolites which indirectly affect the growth and properties of plants (Yang *et al*., 2018). Therefore, the aim of the present investigation was to study the effect of physicochemical properties of soil on the concentration of secondary metabolites of *C. gigantea.*

MATERIAL AND METHODS

The work was carried out in the laboratory of School of Biological and Environmental Sciences, Shoolini University, Solan.

A. Site selection

C. gigantea plants grow in different regions of lower Himalayan regions of Himachal Pradesh, India which comprises mostly Una, Sloan (Nalagarh), Sirmour (Nahan), Bilaspur, Hamirpur districts etc. The altitude of lower Shivalik hills vary from 350 meters to 1500 meters above the mean sea level whereas latitude extend from 29 °C-33 °C N to 74-80.5°C E. Therefore, three plants each of five districts (Una, Sirmour, Solan and Kangra) of Shivalik hills were selected for sample collection due to very less availability of the plant in the state. Identification of plants was done during flowering season (March-July). The collected, identified plants were used for herbarium preparation and herbarium sheets were deposited in the herbarium of Shoolni University, Solan with voucher numbers SUBMS/BOT-5225-A, 5225-B, 5225-C, 5225-D and 5225-E.

B. Phytochemical study

Sample collection. For phytochemicals study, latex of selected plants was collected using a syringe in a glass bottle containing known amount of methanol and stored at 4 °C in a refrigerator.

Extract preparation. Methanol extract of latex was prepared by taking 1ml of fresh latex in 1 ml of methanol (HPLC grade). The mixtures were placed in a shaker for overnight, filtered through the whatman's filter paper, stored at 4°C in airtight bottles (Khusro *et al*., 2014) and further used for GC-MS analysis.

GC-MS analysis. GC-MS analysis of latex methanol extract was done by following standard method (Kumar and Kalavathy, 2013). The latex extract was evaporated to dryness under nitrogen atmosphere using turbo evaporator. GC-MS analysis was performed on an Agilent gas chromatograph model 6890 N coupled to an Agilent 5973 N mass selective detector. Analytes were separated on an HP-5MS capillary column (30 m X 0.25 mm X 1.0 µl) by applying the following temperature program: 40°C for 5 minutes, 40–70°C at 2°C /minutes, 70°C for 2 minutes, 70-120°C at 3°C /minutes, 120-150°C at 5°C /minutes, 150-220°C at 10°C /minutes and then 220°C for 2 minutes. Transfer line temperature was 280°C. Mass detector conditions were: EI mode at 70 eV; source temperature: 230°C; scanning rate 2.88 scan second^{-1}; mass scanning range: m/z 29-540. Carrier gas was helium at 1.0 ml minute^{-1}. The tentative identification of volatile components was achieved by comparing the mass spectra with the data system library (NIST 98) and other published spectra 20, supported by retention index data, which were compared with available literature retention indices18. All compounds were quantified as 3-octanol equivalents.

B. Physicochemical analysis of rhizosphere

Sample collection. For physicochemical study of rhizosphere, soil samples were collected from each sampling sites of rhizosphere from three sampling depths 0-15 cm, 15-30 cm and 30-45cm known as topsoil (TS), subsoil (SS), sub-sub soil (SSS), respectively (Ololade *et al*., 2010). The samples were collected inside labeled polyethylene bags.

Physicochemical analysis. For physicochemical analysis, collected soil samples were dried, ground with mortar and pestle and sieved through 2 mm mesh before analysis. Soil at different depth of selected sites was analyzed for moisture content (Chauhan *et al*., 2011), particle size composition (Bouyoucos, 1961), organic carbon (Walkley and Black, 1934), available nitrogen (Chapman and Pratt, 1961), available potassium (Jackson and Villiers, 1967) and soil cations exchange capacity (Thomas, 1982). Soil pH of freshly collected samples was measured in soil water suspension (1:5) (Anderson and Ingram, 1993).

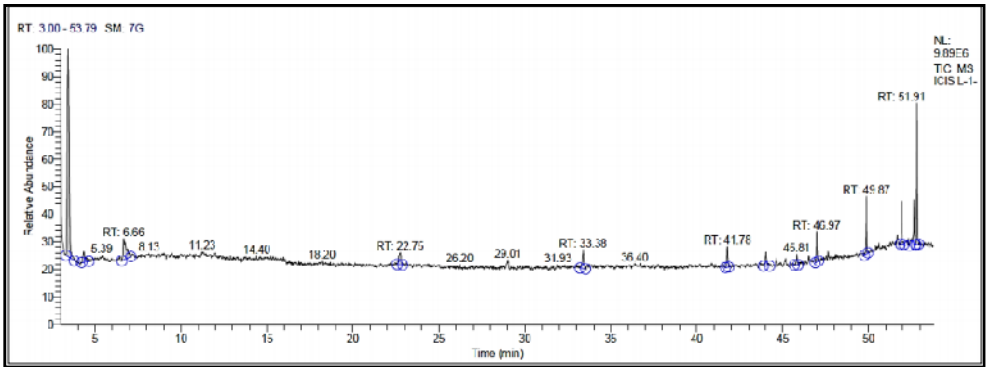
C. Statistical analysis

Statistical analysis of the soil data was done using PRISM software by calculating mean and standard deviation whereas Bonferroni multiple comparison test was used for the comparison of soil data between different districts in different soil depth.

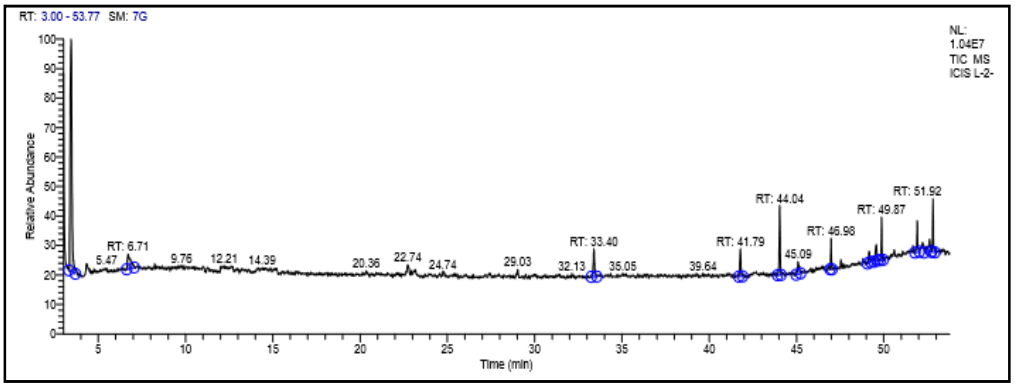
RESULTS

A. Phytochemical study

For phytochemical analysis, GC-MS technique was used to study the total compounds present in latex methanol extract of *C. gigantea* collected from five districts of Himachal Pradesh. Identification of compounds in this study was based on the peak area of the compound (which represents the percentage of that compound) (Fig. 1A-E), their molecular formulas and molecular weight. The major compounds observed in latex extract are shown in table 1.



A



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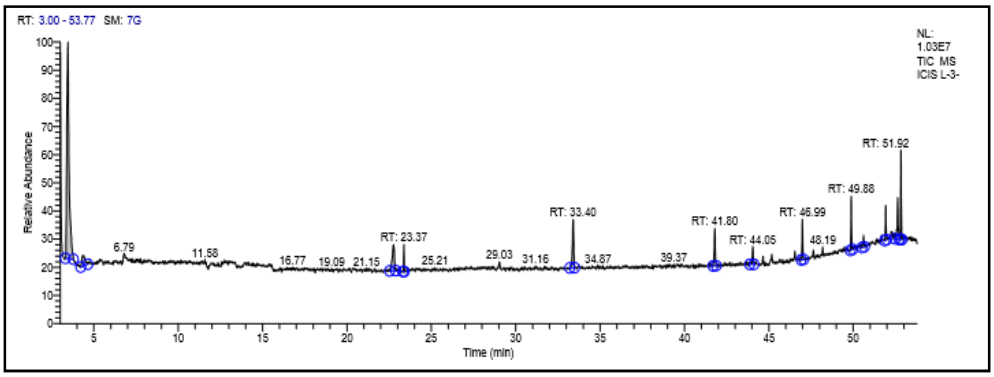

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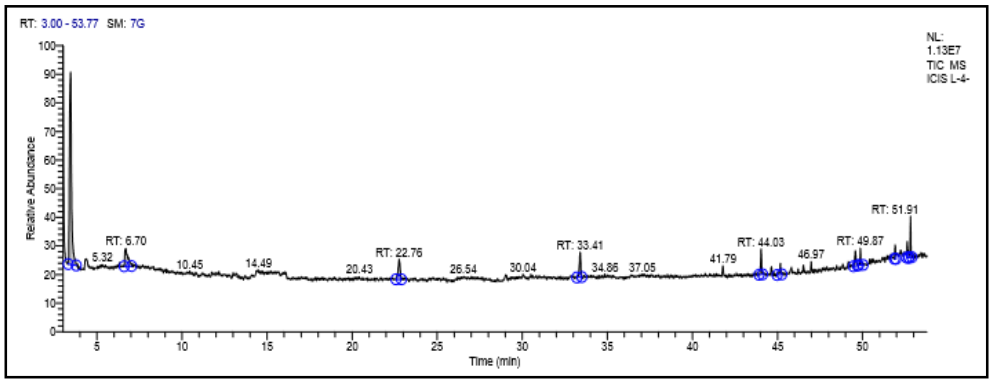
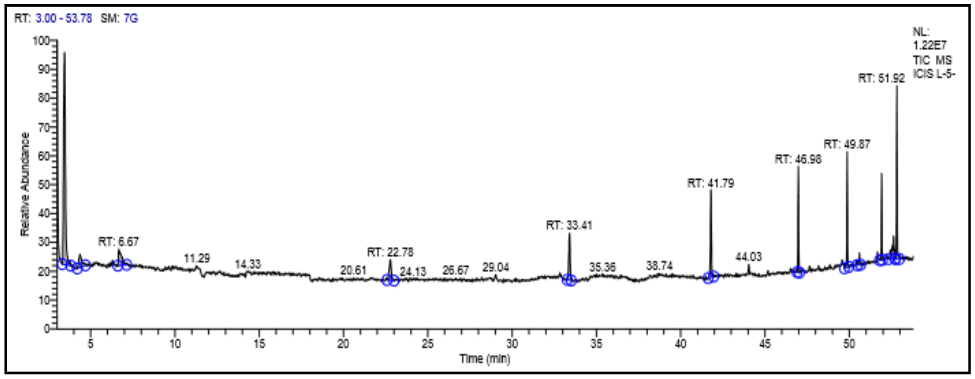


D

Fig. 1. **(A-D).** GC-MS chromatogram of methanolic extract of *Calotropis gigantea* latex extract of districts Sirmour (A), Kangra (B), Hamirpur (C), Una (D).



E

Fig. 1. **(E).** GC-MS chromatogram of methanolic extract of *Calotropis gigantea* latex extract of district Solan (E).

Study showed the compound Cyclohexane (C_6H_{12}) at retention time 3.45 with peak area 51.52 % as the major compound present in latex of *C. gigantea*. Additionally, Butane-2, 2-dimethyl (C_6H_{14}) and D-Mannose-1-phosphate sodium salt ($C_6H_{13}O_9P$) showed their presence at Rt- 6.71 and 46.98 with peak area 5.86 % and 2.74 %, respectively. Three other compounds were observed at RT- 52.82 which were 1-[(T-butyl) dimethyl silyl thin] butane ($C_7H_{15}F_3O_3SSi$), 9, 12, 15-Octadecatrienoic acid ($C_{19}H_{32}O_2$), methyl ester and Hexadecane ($C_{16}H_{34}$) with peak area 4.84 %. Similarly, three compounds [D-mannose ($C_6H_{12}O_2$), L-Glutamic acid ($C_5H_9NO_4$) and Phenol-2,5-bis (1-1-dimethyl-ethyl) ($C_{14}H_{22}O$)] were observed at 49-16 RT and three compounds [Oxadiazon ($C_{15}H_{18}$), Decane ($C_{10}H_{22}$) and Cholest-5-en-3-ol-24-propylidene (3.beta.) ($C_{29}H_{48}O_2$)] were at RT-41.79. Additionally, latex was also found to be rich in Phenol-2,5-bis(1,1-dimethylethyl) ($C_{14}H_{22}O$), Z-1,6-Tridecadiene ($C_{13}H_{24}$), at retention time 49.16 and 49.57.

Table 1: GC-MS analysis of methanolic extracts of *Calotropis gigantea* latex

Sr. No	Compound Name	Retention Time	Peak Area %	Molecular Formula	Molecular Weight
1	D-Mannose-1-phosphate sodium salt	46.98	2.74	$C_6H_{13}O_9PNa$	282.0
2	1-[(T-butyl)dimethyl silylthin butane	52.82	4.84	$C_7H_{15}F_3O_3SSi$	205.0
3	1-Hexadecyne	51.92	3.32	$C_{16}H_{30}$	221.0
4	2-Methoxy 4-vinyl phenol ethanone	6.71	5.86	$C_9H_{10}O_2$	149.0
5	5- Nonadecen-1-ol	33.40	4.64	$C_{19}H_{38}$	266.9
6	9,12,15- Octadecatricenoic acid, methyl ester	52.82	4.84	$C_{19}H_{32}O_2$	293.2
7	Butane-2,2-dimethyl	6.71	5.86	C_6H_{14}	86.24
8	Campesterol	49.87	3.50	$C_{28}H_{48}O$	401.0
9	Cholest-5-en-3-ol-24- Propylidene (3.beta.)	41.79	3.69	$C_{29}H_{48}O_2$	429.0
10	Cyclohexane	3.45	51.52	C_6H_{12}	84.0
11	Decane	41.79	3.69	$C_{10}H_{22}$	147.0
12	D-mannose	49.16	1.87	$C_6H_{12}O_6$	180.1
13	Eicosane	51.92	3.32	$C_{20}H_{42}$	281.0
14	Guanidine nitrate	45.09	2.49	$CH_6N_4O_3$	121.0
15	Hexadecane	52.82	4.84	$C_{16}H_{34}$	127.2
16	L- Glutamic acid	49.16	3.50	$C_5H_9NO_4$	147.0
17	Oxadiazon	41.79	3.69	$C_{15}H_{18}$	341.0
18	Pentacosane	33.40	4.64	$C_{25}H_{52}$	355.1
19	Phenol-2,5-bis(1-1-dimethyl-ethyl)	49.16	1.87	$C_{14}H_{22}O$	204
20	Phenol-3-isopropoxy-5-methyl	4.35	3.17	$C_{10}H_{14}O_2$	166.9
21	Tricosane	46.98	2.74	$C_{23}H_{48}$	326.9
22	Z-1,6-Tridecadiene	49.57	3.19	$C_{13}H_{24}$	130.3

Table 2: Comparative GC-MS analysis of methanolic extracts of *Calotropis gigantea* latex collected from five districts [Solan (S); Sirmour (Si); Una (U), Kangra (K), Hamirpur (H); Present (+); Absent (-)]

Sr. No.	Compound Name	Mol. Formula	S	Si	U	H	K
1.	Cyclohexane	C_6H_{12}	+	+	+	+	+
2.	2-Methoxy-4-vinyl phenol ethanone	$C_{15}H_{13}N$	+	+	-	+	+
3.	Butane-2,2-dimethyl	C_6H_{14}	+	+	+	-	+
4.	Pentacosane	$C_{25}H_{52}$	-	-	+	-	+
5.	5-Nonadecen-1-ol	$C_{19}H_{38}$	+	+	+	+	+
6.	Cholest-5-en-3-ol,24,Propylidene(3.beta.)	$C_{29}H_{48}O_2$	+	+	-	+	+
7.	Oxadiazon	$C_{15}H_{18}$	-	-	-	-	+
8.	Decane (alkanehydrocarbon)	$C_{10}H_{22}$	+	+	+	+	+
9.	Guanidine; nitric acid	$CH_6N_4O_3$	+	-	-	-	+
10.	Tricosane (paraffin hydrocarbon)	$C_{23}H_{48}$	-	+	-	+	+
11.	D-Mannose-1-phosphate sodium salt	$C_6H_{13}O_9PNa$	-	-	-	-	+
12.	D-Mannose	$C_6H_{12}O_6$	-	-	-	-	+
13.	Campesterol	$C_{28}H_{48}O$	+	+	+	+	+
14.	Phenol-1,2,5-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	-	-	-	-	+
15.	Z-1,6-tridecadiene	$C_{13}H_{24}$	-	-	+	-	+
16.	L-Glutamic acid	$C_5H_9NO_4$	+	+	+	+	+
17.	1-Hexadecyne	$C_{16}H_{30}$	+	+	+	+	+
18.	Eicosane	$C_{20}H_{42}$	-	+	+	+	+
19.	9,12,15-Octadecatricenoic acid, methyl ester	$C_{19}H_{32}O_2$	-	-	-	+	+
20.	Hexadecane	$C_{16}H_{34}$	+	+	-	+	+
21.	1-[(T-butyl)dimethyl silylthin]butane	$C_7H_{15}F_3O_3SSi$	-	-	-	-	+
22.	4-Methyl-2 phenylindole	$C_{15}H_{13}N$	+	-	+	+	-
23.	β- Tocopherol	$C_{25}H_{48}O_2$	+	-	-	-	-
24.	Phenol-3-isopropoxy-methyl	$C_{10}H_{14}O_2$	-	+	+	-	-
25.	3-Ethyl-1-tetradecene	$C_{16}H_{32}$	-	-	-	-	-
26.	9,19-Cyclolanost-24-en-3-ol(3-beta)	$C_{30}H_{50}O$	-	+	-	-	-
27.	8-Ethyl-7,8,9,10-tetrahydro-1,6,8,11-tetrahydroxy	$C_{20}H_{18}O_6$	+	+	+	+	-
28.	(E,E)-4,8,12-Trimethyl-3,7,11-tridecatriene-1-ol	$C_{16}H_{26}$	-	+	-	-	-
29.	1-Octanol,3,7-dimethyl	$C_{10}H_{22}O$	-	+	-	-	-
30.	2-Hydroxyhexadecyl butanoate	$C_6H_{12}O_3$	-	+	-	-	-
31.	Levomenol	$C_{15}H_{26}O$	+	+	+	+	-
32.	Vitamin E	$C_{29}H_{50}O_2$	-	-	+	-	-
33.	Pentadecanoic acid 14-methyl-,methyl ester	$C_{17}H_{34}O_2$	-	+	-	-	-
34.	β- Sitosterol	$C_{29}H_{50}O$	-	-	-	+	-

The comparative GC-MS analysis showed total 34 compounds from the methanol extract of latex (Table 2). Out of 34 compounds, 21 were reported from *C. gigantea* latex collected from Kangra and Sirmour districts; 16 compounds were observed from Hamirpur and Solan districts whereas 15 were observed from Una district. On the other hand, 14 compounds i.e., 4-Methyl-2 phenylindole ($C_{15}H_{13}N$), β-Tocopherol ($C_{25}H_{48}O_2$), Phenol-3-isopropoxy-methyl ($C_{10}H_{14}O_2$); 3-Ethyl-1-tetradecene ($C_{16}H_{32}$), 9,19-Cyclolanost-24-en-3-ol(3-beta) ($C_{30}H_{50}O$), 8-Ethyl-7,8,9,10 tetrahydro-1,6,8,11-tetrahydroxy ($C_{20}H_{18}O_6$), (E,E)-4,8-12-Trimethyl-3,7,11-tridecatriene-1-ol-octanol, 3,7-dimethyl ($C_{16}H_{26}$), 2-Hydroxyhexadecylbutanoate ($C_6H_{12}O_3$), Levomenol ($C_{15}H_{26}O$), Vitamin E ($C_{29}H_{50}O_2$), Pentadecanoic acid 14-methyl-,methyl ester ($C_{17}H_{34}O_2$), β- Sitosterol ($C_{29}H_{50}O$) were totally absent in latex of Kangra district plants whereas in other districts these were found in very least quantity. Seven compounds [Cyclohexane (C_6H_{12}), 5-Nonadecen-1-ol ($C_{19}H_{38}$), Decane (alkanehydrocarbon) ($C_{10}H_{22}$), Campesterol ($C_{28}H_{48}O$), L-Glutamic acid ($C_5H_9NO_4$), 1-Hexadecyne ($C_{16}H_{30}$), Eicosane ($C_{20}H_{42}$)] were observed from plants collected from all the districts including Kangra district. Additionally, four compounds Oxadiazon ($C_{15}H_{18}$), D-Mannose-1-phosphate sodium salt ($C_6H_{13}O_9PNa$) and 1-[T-butyl) dimethylsilylthin] butane ($C_7H_{15}F_3O_3SSi$) were only observed from Kangra district plants.

Like variations observed in plants of Kangra districts, such types of variations were also observed from plant of other districts. For example, 2-Methoxy-4-vinylphenolethaone ($C_{15}H_{13}N$) and Cholest-5-en-ol-24-pyropylidene (3beta) ($C_{29}H_{48}O_2$) were reported from all districts plants except Una district. Similarly, Butane-2,2-dimethyl (C_6H_{14}) was found in all district plant except Hamirpur. Vitamin-E ($C_{22}H_{50}O_2$) and β-sitosterol ($C_{29}H_{50}O$) were observed only in plants of Una district and absent in other district plants. Five unique compounds like 3-Ethyl-1-tetradecane (C_6H_{22}), 9,19-Cyclolanost-24-en-3-ol(3beta) ($C_{30}H_{50}O$), (E,E)-4,8,12-Trimethyl 3,7,11-tridecatriene-1-ol ($C_{16}H_{26}$), 1-Octanol,3,7-dimethyl ($C_{10}H_{22}O$), 2-Hydroxyhexadecyl butanoate ($C_6H_{12}O_3$) and Pentadecanoic acid-14-methyl-,methyl ester ($C_{17}H_{34}O_2$) were only observed only from plants of Sirmour district. The presence and absence of other compounds are shown in Table 2.

B. Physicochemical analysis of rhizosphere

Particle size analysis/ Soil texture. Particles size analysis of the rhizosphere of *C. gigantea* from all the districts is presented in Table 3. Results showed similar soil texture of all the layers of rhizosphere of all the districts. Out of five districts four (Hamirpur, Sirmour, Solan and Una) had Sandy loam soil, whereas Kangra district soil was observed as Silt loam. Only the percentage of sand, silt and clay vary between soils of different districts.

Table 3: Soil particle size analysis from five districts (Hamirpur, Sirmour, Solan, Una and Kangra) of lower Himalaya at various soil depths (0-15 cm; 15-30 cm and 30-45 cm).

Horizons (cm)	Sand (%)	Silt (%)	Clay (%)	Soil texture
Hamirpur				
0-15	66.6±0.32	17.3±0.23	16.2±0.05	**Sandy loam**
15-30	65.4±0.2	17.1±0.1	17.6±0.05	
30-45	64.8±1.0	17.5±0.06	19.8±1.0	
Sirmour				
0-15	66.0±0.3	16.6±0.46	17.4±0.11	**Sandy loam**
15-30	67.4±1.3	17.1±0.41	17.7±2.00	
30-45	64.7±1.67	17.5±0.57	17.9±1.50	
Solan				
0-15	56.1±0.55	28.5±0.2	17.8±0.7	**Sandy loam**
15-30	55.8±0.34	28.3±0.17	17.5±0.51	
30-45	54.63±0.75	27.63±0.75	17.06±0.05	
Una				
0-15	65.3±0.43	16.8±0.55	17.9±0.30	**Sandy loam**
15-30	65.2±0.2	17.6±0.90	17.2±0.80	
30-45	65.6±0.2	16.8±0.90	17.6±0.83	
Kangra				
0-15	34.7±0.23	56.3±0.28	8.9±0.05	**Silt loam**
15-30	38.3±0.75	54.13±0.60	7.5±0.25	
30-45	39.06±0.11	53.43±0.06	7.6±0.17	

(Mean ± SD)

Moisture content. The results of moisture content of the rhizosphere of *C. gigantea* from all the selected districts are presented in Fig. 2A. In different sites and soil depths, moisture content varied from 0.94% to 16.7%. Hamirpur district was observed with highest moisture content at all three depths (TS-11.8%; SS-14.5%; SSS-16.7%), whereas lowest moisture content (TS-0.94%; SS-1.04%; SSS-1.04%) was observed from Una district. The moisture content increased from top soil profile (0-15 cm) to sub-soil profile (30-45 cm).

Statistical analysis of different soil depths and districts showed both significant and non-significant variations. Bonferroni multiple comparison tests revealed that the interaction between different soil depths were not same. Hamirpur vs Sirmour, Sirmour vs Solan, Solan vs Una, Solan vs Kangra and Una vs Kangra showed non-significant variation in all depths, except the lower soil profile of Hamirpur vs Sirmour and top soil profile of Solan vs Una. On the other hand, Hamirpur vs Solan, Hamirpur vs Una, Hamirpur vs Kangra, Sirmour vs Una, Sirmour vs Kangra and Solan vs Una showed significant variations. Most significant variation was observed in Hamirpur vs Una and Hamirpur vs Kangra districts.

Soil pH. The results of soil pH analysis of the rhizosphere of *C. gigantea* from all the districts showed that the maximum soil pH from all the three soil depth (TS- 8.37; SS-8.55; SSS-8.69) was observed from the Sirmour district whereas minimum (TS-7.7; SS-7.73; SSS-7.77) from Kangra district.

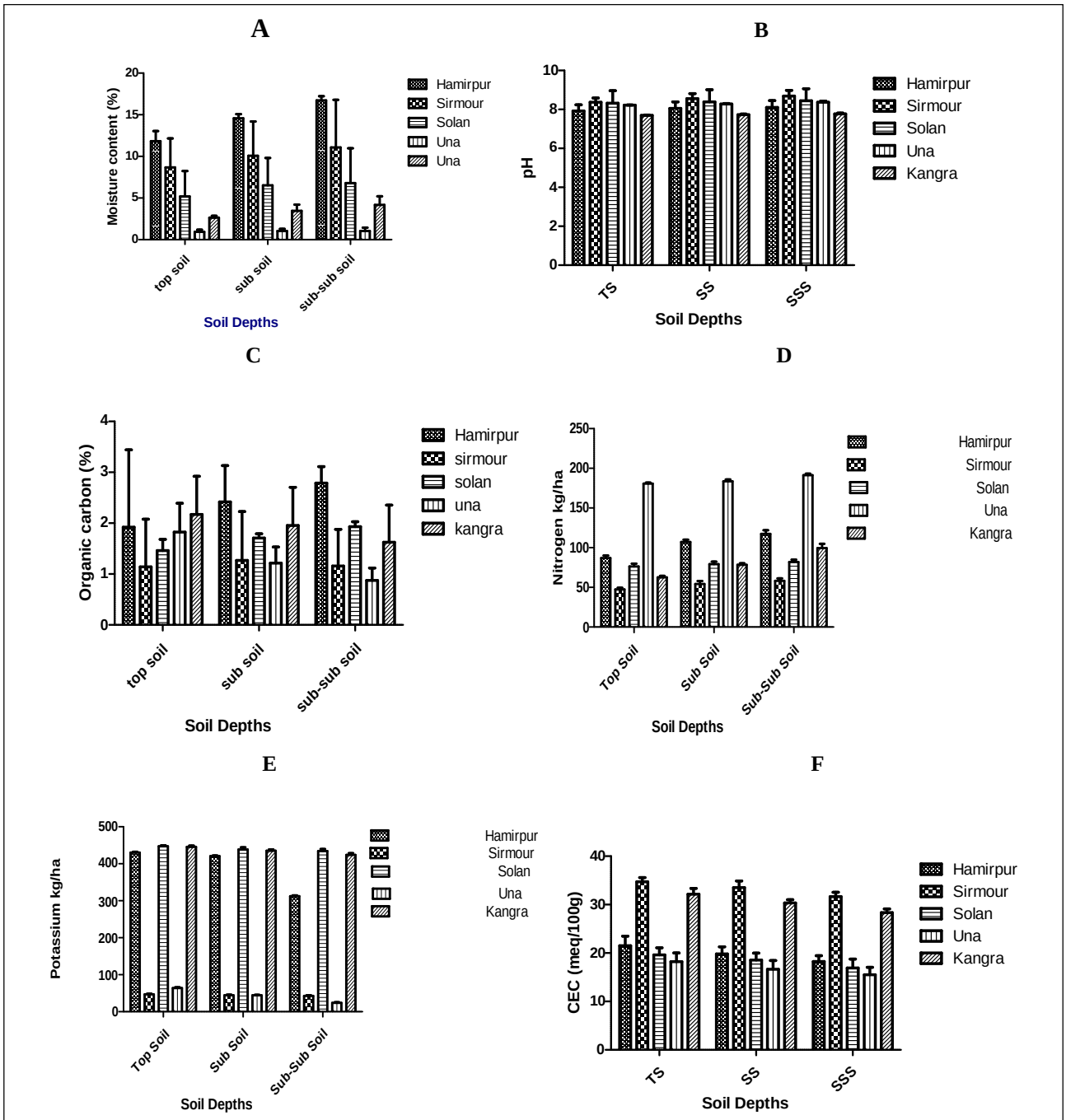


Fig. 2. 'A' Soil moisture content (%); 'B' Soil pH; 'C' Soil available nitrogen (kg/ha); 'D' Soil available potassium (kg/ha); 'E' Soil organic carbon (%); 'F' Soil cations exchange capacity (meq/100g) in five districts of lower Himalaya from various soil depths (0-15 cm; 15-30 cm and 30-45 cm); values were analyzed by two- way ANOVA followed by Bonferroni's multiple comparison test.

It was also found to increase from top soil profile (0-15 cm) to sub-soil profile (30-45 cm) (Fig. 2B) Statistical analysis (Bonferroni's multiple comparison tests) showed non-significant variation between different soil depths in all districts. Only Sirmour vs Kangra showed lest significant variation in sub soil and sub-sub soil layer.

Organic carbon. Organic carbon of the rhizosphere of *C. gigantea* from all the districts is presented in Fig. 2C. All five districts and three soil depths showed variation of organic carbon between 2.78% to 0.87%. Maximum organic carbon from all the three soil depth (TS-1.92%; SS-2.41%; SSS-2.78%) was observed from the Hamirpur district whereas minimum (TS-1.82%; SS-1.21%; SSS-0.87%) was observed from Una district. It was found to increased from top soil profile (0-15 cm) to sub-soil profile (30-45 cm). Statistical analysis from different soil depths and sites showed non-significant variation. Bonferroni's multiple comparison tests revealed that the interaction between different soil depths were statistically non-significant for most of the districts. Only Hamirpur vs Sirmour and Hamirpur vs Una showed significant variation in sub-sub soil.

Available nitrogen. The results of available nitrogen (N) analysis of the rhizosphere of *C. gigantea* from all the districts are presented in Fig. 2D. In different districts, the soil nitrogen percentage at different soil depths, varied from 47.44 kg/ha to 191.3 kg/ha. Maximum N from all the three depth (TS- 180.6 kg/ha; SS-183.6 kg/ha; SSS-191.3 kg/ha) was observed from the Una district and minimum (TS-47.44 kg/ha; SS-54.17 kg/ha; SSS-58.16 kg/ha) from Sirmour district. It was observed to increase from top soil profile (0-15 cm) to sub-soil profile (30-45 cm). Statistical analysis at different soil depths and sites showed significant variations. Non-significant variation was observed only between Solan and Kangra districts.

Available potassium. Available potassium (K) of three layers of rhizosphere of *C. gigantea* from all the districts are presented in Fig.2E. In different districts and soil depths the value of K in soil varied from 47.44 kg/ha to 191.3 kg/ha. Maximum K from all the three depth (TS- 445.8 kg/ha; SS-435.0 kg/ha; SSS-423.7 kg/ha) was observed from the Kangra district whereas minimum (TS-64.14 kg/ha; SS-44.24 kg/ha; SSS-24.11 kg/ha) was observed from Una district sub-sub soil and all layers of Sirmour district (TS-46.79kg/ha; SS-44.20kg/ha; SSS-46.79 kg/ha). It was observed to be decreased from top soil profile (0-15 cm) to sub-soil profile (30-45 cm). Statistical analysis at different soil depths and sites showed significant variations, non-significant variation was observed only in Solan vs Kangra districts.

Cations exchange capacity. Fig. 2F showing the results of cations exchange capacity (CEC) of the rhizosphere of *C. gigantea* from all the selected districts. In different districts and soil depths the soil CEC varied from 15.53 meq/100g to 34.73 meq/100g. Maximum CEC from all the three depth (TS-34.73 meq/100g; SS-33.53 meq/100g; SSS-31.66 meq/100g) was observed from the Sirmour district whereas minimum (TS-18.23 meq/100g; SS-16.66 meq/100g; SSS-15.53 meq/100g) was observed from Una district. It was found to decreased from top soil profile (0-15 cm) to sub-soil profile (30-45 cm). Statistical analysis at different soil depths and sites showed both significant and non-significant variations. Significant variation was observed in Hamirpur vs Sirmour, Hamirpur vs Kangra, Sirmour vs Solan, Sirmour vs Una, Solan vs Kangra and Una vs Kangra districts.

DISCUSSION

The variation in secondary metabolite constituents depends on the genotype of plant, ecological factors, environmental conditions, stress conditions (Nuwamanya *et al*., 2014) and interaction of plants with microorganisms etc. Research work have been done by different scientists to observe the effect of ecological and environmental conditions on secondary metabolites particularly alkaloids and phenolic compounds (Arena *et al*., 2017; Yang *et al*., 2018). Very little literature is found in which the role of soil's physicochemical properties has been studied in relation to secondary metabolites. Therefore, present investigation has tried to find out the effect of physicochemical properties in secondary metabolites of *C. gigantea*.

Soil is a natural medium on which agricultural, medicinal, timber and other plants grow (Shah *et al*., 2011). Its fertility depends on concentration of soil nutrients, organic and inorganic materials and water. The soil physicochemical properties which influence the soil fertility are being classified as physical, chemical and biological properties (Ramaru *et al*., 2000). Different parameters of soil fertility studied during present work were particle size analysis, moisture content, pH of the soil, organic carbon, available nitrogen (N), available potassium (K) and cations exchange capacity (CEC), etc.

The results of particle size analysis showed similar nature of soil of five districts of the Shivalik hills. Soil of four districts (Hamirpur, Una, Solan and Sirmour) was observed with sandy loam in texture, whereas the Kangra district had silt loamy soil in texture (Table 3). According to Suhakar Rao *et al*. (1991) the soil of foothills and valley areas up to an elevation of approximately 800 meters above sea level (Shivalik hills) has sandy loam in texture with scattered loamy patches.

They also stated that such type of soil has very low water retention capacity due to the higher percentage of sand. Moisture content is the amount of water associated with a given volume or mass of soil. It is a highly variable property and the results of moisture content showed that maximum moisture in soil was observed from the district Hamirpur (TS- 11.8%; SS- 14.5 %; SSS- 16.7%) whereas, minimum (TS- 0.94%; SS- 1.04%; S.S.S- 1.04%) was observed from district Una (Fig. 2). In all districts, moisture was observed to increase from top soil profile (0-15 cm) to sub-soil profile (30-45 cm). Significant variation was observed between Hamirpur vs Solan, Hamirpur vs. Kangra and Hamirpur vs. Una, Sirmour vs. Kangra and Sormour vs. Una districts. Phytochemical study showed 21 compounds from Kangra and Sirmour, 16 from Hamirpur and Solan and 15 form only Una (Table 2). Therefore, study showed the effect of moisture content on the amount of chemical constituents in plants. As in Una and Hamirpur, very less moisture content was found which create plants under water stress. According to Jaffer *et al*. (2012) water stress affect the production of secondary metabolites (phenols, flavonoids and anthocyanin) in plants. Various studies have reported the increase in the amount of secondary metabolites in a variety of medicinal plants, e.g., artemisinin in *Artemisia annua* (Charles *et al*., 1993), ajmalicine in *Catharanthus roseus* (Jaleel *et al*., 2008) and hyperforin in *Hypericum perforatum* (Zobayed *et al*., 2005) under water stress. According to Herms and Mattson under high water stress, there is a limit on the translocation of carbon to its sink, with the remaining carbon accumulates as carbohydrates, which leads to an increase in the carbon pool that would be allocated for secondary metabolism, with little or no competition with growth and development (Herms and Mattson, 1995).

The pH is one of the most important factors in soil quality management. It indicates the acidity, neutrality and alkalinity of soil. Soil pH of all the districts was observed within the range of 7 to 9 where is was maximum in Sirmour district (8.69) and minimum in Kangra district (7.7) (Fig. 2B). Result showed that it increased as depth of soil increased. Similar results obtained by Reddy and McLatchey, (1991) where he has also observed that soil pH increases with the increase in depth.

The organic soil matter includes all the dead plant materials and live or dead animals which are more in the top layer of the soil compared to the sub-soil and sub-sub soil. Similar to results of pH of the soil, organic carbons from five districts showed variation from 0.87 % to 2.78 %. It was again maximum in Hamirpur district, whereas, minimum in Una district (Fig. 2C). Organic carbon also found to be decreased from top to bottomin soil layers of all districts. Sheik *et al*. (2002) also observed decrease pattern of organic carbon with the increasing depth of soil. The variation in secondary metabolites constituents between Hamirpur district and Una district is insignificant which concludes that organic carbon has little and no effect on the production of secondary metabolites or either it is the cumulative effect of genotype of the plant and edaphic factors.

N and K are essential plant nutrients. They are key components in plant proteins and chlorophyll. They are available in soil either natural or man-made sources and helpful in better plant growth. The available N content showed significant variation in all the district. Maximum N content (TS- 180.6 kg/ha; SS-183.6 kg/ha; SSS-191.3 kg/ha) was observed from Una district in all the soil depth and minimum (TS-47.44 kg/ha; SS-54.17 kg/ha; SSS-58.16 kg/ha) was observed from Sirmour district (Fig. 2D). It was found to increase from top soil profile to sub-sub soil profile. On the other hand, results of phytochemical analysis showed total 15 compounds from Una district and 21 compounds from Sirmour district, which concluded that higher N content in soil decreased the concentration of secondary metabolites. According to Zhong and Wang (1998) and Hahn *et al*. (2003) nitrogen level in soil affects the alkaloid content of the plant. Whereas Zamani *et al*. (2014) confirmed that with an increase in nitrogen in soil, secondary metabolites particularly alkaloids and flavonoids increased. The present plant was found to be rich in fatty acids, phenolic compounds, hydrocarbons, sterols, sesquiterpene and alcohol whereas alkaloids were observed in very less amount in *C. gigantea* through GC-MS plants which suggests that nitrogen content have little role in secondary metabolite production in *C. gigantea* collected from five districts of Himachal Pradesh. Wen-Hua *et al*. (2009) concluded that the effects of nitrogen availability on the accumulation of secondary metabolites in the plant are affected by genetic and environmental variation.

The results of available potassium showed a variation from 47.44 kg/ha to 191.3 kg/ha. The present study showed the decreased pattern of K from top soil profile (0-15 cm) to sub-soil profile (30-45 cm). Similarly, Zeng (1999) also observed the decreased pattern of K along with the depth of soil. The maximum K during study was observed from the Kangra district (TS- 445.8 kg/ha; SS- 435.0 kg/ha; SSS- 423.7 kg/ha) and minimum (TS- 64.14 kg/ha; SS- 44.24 kg/ha; SSS- 24.11 kg/ha) from the Una district (Fig. 2E). Secondary metabolites identified from the Una district were 15 and from the Kangra district were 21 which showed that soil available potassium content has a significant role in the production of secondary metabolites.

Mudau *et al*. (2007) demonstrated that N.P.K nutrition, increases total polyphenols in bush tea leaves. Ibrahim *et al*. (2012) also confirmed that high potassium fertilizer enhanced the carbohydrate content that simultaneously increased the production of secondary metabolites in *Labisia punila*.

CEC is the ability of the soil to hold or store cations. Negatively charged soil particles repel anions (negatively charged ions) and attract and hold cations (positively charged ions) therefore stopping them from being leached down the soil profile. CEC of the rhizosphere of *C. gigantea* from all the districts was observed to be varied from 15.53 meq/100g to 34.73 meq/100g (Fig. 2F). Statistical analysis at different soil depths and sites showed both significant and non-significant variations. Significant variation was observed in districts Hamirpur vs Sirmour, Hamirpur vs Kangra, Sirmour vs Solan, Sirmour vs Una, Solan vs Kangra and Una vs Kangra. According to Brady and Weil (1999) CEC is used as a measure of soil nutrient retention capacity. Plants obtained many of their nutrients from the soil by an electrochemical process called cations exchange which is a key method to understand the soil fertility. Higher the CEC of a soil, the more nutrients it is likely to hold and the higher will be the fertility level (Fullen and Catt, 2004). In the present study, maximum CEC (TS- 34.73 meq/100g; SS- 33.53 meq/100g; SSS-31.66 meq/100g) was observed from the Sirmour district, whereas minimum (TS-18.23 meq/100g; SS-16.66 meq/100g; SSS-15.53 meq/100g) was observed from Una district which could be the reason the variation in the secondary metabolite constituents observed from Sirmour and Una district (Table 2).

Additionally, soil with high CEC has high ability to hold water (e.g. clay soils) while soils with low CEC have low ability to hold water (e.g. sandy soils) (Efretuei *et al*., 2016). Present study observed maximum CEC from Sirmour district with higher moisture content, i.e. 8-11% and lower CEC was observed from Una district with less moisture content (1%) which concludes that Sirmour district soil is more fertile than Una district. Literature also shows that soils in the low CEC group typically have values <10 meq/100g while soils in the high CEC group have values greater than 10 meq/100g. Therefore, overall study revealed that soil of Shivalik hills has high fertility value because of greater value of CEC than 10 meq/100g.

CONCLUSION

GC-MS analysis shows the existence of various unique compounds with variable chemical structures in latex of *C. gigantea* collected from different districts. Physicochemical study shows that soil of different region or districts of Himachal Pradesh has similar properties w.r.t. pH and percentage of organic carbon available in soil and soil texture. Variation was observed only with moisture content, available K and cations exchange capacity, which concludes that these properties could be responsible for variation in the chemical profile of *C. gigantea* of five districts with other unknown ecological factors.

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