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ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS, ANTI-MICROBIAL ACTIVITY AND MOLECULAR CHARACTERIZATION OF MEDICINAL WEED *SONCHUS OLERACEUS* L. USING RAPD MARKER

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Keywords:

RAPD, Antimicrobial, Bioactive compounds, Cluster analysis and *S. oleraceus*

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ABSTRACT: In present study, Himalayan weed *Sonchus oleraceus* extracts are screened for the occurrence of bioactive compounds, anti-microbial activity, and its molecular characterization using RAPD. The agar well diffusion method was used to detect anti-microbial activity of aqueous, methanol, chloroform and ethyl acetate extracts of *S. oleraceus*. The anti-microbial activity of different extract was checked at different concentrations i.e. at 150mg/ml, 200mg/ml, 250mg/ml and 300mg/ml against five bacteria namely *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi*. The anti-microbial activity of the different extracts of *S. oleraceus* was associated with the concentration. In phytochemical screening alkaloids, flavonoids, protein, carbohydrates, tannins, phenol, terpenoids and saponins were confirm in different extracts. The primers OPA-1, OPA-9, OPA-16, TC-1 and TC-3 were used in molecular characterization using RAPD against 14 samples collected from five districts namely Kangra, Una, Bilaspur, Hamirpur and Solan of Himachal Pradesh. Cluster analysis revealed that isolates H1 and H3, H2 and S2, U1 and U2 were showing maximum homology. In comparison to all the isolates, isolate K1 from the Kangra district showed least homology to all the other isolates from total covered five districts of Himachal Pradesh.

INTRODUCTION: The name *Sonchus oleraceus* was given in 1753 by Carolus Linnaeus. Sonchus is the Greek name for sow thistle, meaning a hollow stem, and epithet oleraceus mean kitchen vegetable. It is considered as one of the medicinal weeds in more than 55 countries [1].

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Commonly, *Sonchus oleraceus* is known as Dodak in Hindi, Sow thistle in English, Naayi Hakkarike in Kannada, Pathari in Marathi, Ratrinta in Telugu, Osithagarai in Tamil and Titaliya in Bihar [2].

S. oleraceus is native to Europe, North Africa and West Asia and then spread to North and South America, India, China, Southern Australia [3]. There are a significant number of invasive species in diverse environments in various districts of Himachal Pradesh, such as wastelands, protected areas, farmland, reserve forests, river banks, *etc*. [4-8, 10]. *S. oleraceus* is one of the medicinal invasive alien plants having the ability to establish, invade

and out-compete native species [11, 12]. Primarily, *S. oleraceus* recognized as a weed but later it was discovered to have great medicinal purposes. This weed mainly found in cultivated crops or around roadsides, and it play the important role in pharmaceuticals and animal nutrition [13]. The genus *Sonchus*belongs to tribe (Lactuceae), sub-tribe (Crepidinea) and family (Asteraceae) and includes more than 50 species. It is an annual, erect, leafy and glabrous herb with white milky juice, taproot is up to 1 m high [14]. For instance, traditionally, it is used asfolklore medicine for the treatment of gastrointestinal tract [15], its juices prevent from hemorrhage during childbirth [16], decoction with kopa, clover, and salt is administered to expel placenta worms [17, 18], It is also used as sedative, vermifuge and in the treatment of the liver disorder, healing wounds and ulcers [19], treats phthisis [20], treats gastric spasm, hepatitis, infections, inflammation, headaches, general pain and rheumatism [21, 22], help in the treatment of vitiligo [23].

It is also used as a cathartic, cancer treatment and used as a vermicide [24]. This plant contains a number of pharmacologically active compounds which are useful in the formation of herbal products including complex mixtures of leaves, stems, flowers, roots and seeds [25, 26]. The main components of this plant are terpenes, steroids, flavones, and coumarins [27]. Flavonoids are the secondary metabolites in plants which protects the plant from UV radiation and fungal infections [25, 26, 27, 28]. Flavonoids (luteolin, apigenin, kaempferol, and quercetin) and their glucoside derivatives were identified from whole plant extracts. Composition of carotenoids [29] and characterization of coumarin[30] from *S. oleraceus* was isolated and studied. Recently, caftaric acid is identified from the leaf extracts [31]. This plant is known for its high content of antioxidants and antioxidant activity [32-36]. The different extracts of *Sonchus* species indicate good anti-microbial activity. Methanolic extract of whole plant of *S. oleraceus* showed the antibacterial activity against six bacterial strains *i.e. Clavibacter michiganensis, Escherichia coli, Erwinia amylovora, Bacillus subtilis, Bacillus aquimaris* and *Pseudomonas syringae* by using minimal inhibitory concentration (MIC) methods [37]. The antibacterial property of aqueous extract of Chinese *S. olearceus* was also evaluated [38]. The antibacterial property from the roots of *S. oleraceus* was evaluated and ascribed to major compounds [39]. The different extracts of this plant has been confirmed against anti-plasmodial activity [37], larvicidal activity [40] and antifungal activity [41]. The genetic fingerprinting of 11 plant species including *S. oleraceus* of desert origin from various areas of Saudi Arabia were studied by using random amplified polymorphic DNA (RAPD) [42]. A very few molecular work has done by the researchers on this plant, so it is needed to explore this plant at molecular level. Due to some environmental conditions and habitat, some changes occurred in the plant at the morphological and genetic level. The genetic diversity of S. *oleraceus* in Himachal Pradesh has not been investigated so far. Therefore, the present study aimed to investigate the presence of bioactive compounds, anti-microbial potential, and genetic diversity of natural populations of *S. oleraceus* using RAPD marker.

MATERIALS AND METHODS:

Plant Collection and Identification: Twenty-five accessions of *S. oleraceus* whole plants were collected manually from different districts of Himachal Pradesh (H.P.) *viz*., Bilaspur, Hamirpur, Kangra, Solan, and Una. The botanical material was authenticated by Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni (Solan), H.P. in the month of January, 2016 (Dispatched No. 074/2916). The vegetative and reproductive parts of this plant was shown in **Fig. 1.**

Extract Preparation: Plant leaves were washed properly under running tap water, 70 percent ethanol, and cleaned to remove soil particles and moisture with the aid of tissue paper. The leaves were kept for some time for drying to remove the moisture content and then sterilized with 0.1% mercuric chloride for around 5 minutes. The rinsed leaves of this plant were dried in darkroom and crushed to get a fine powder. The plant extracts were prepared by using a cold percolation method. For the preparation of different extracts, 60 grams of fine powder from each plant was dissolved in 160 ml of respective absolute solvents (in the following order: aqueous, chloroform, methanol, and ethyl acetate) for three successive days at room temperature. The supernatant was filtered through Whatman filter paper No. 1, while the residues were used for subsequent extraction. The dissolved parts were filtered and stored in a glass bottle or in

petri plates. The filtrates were then evaporated under reduced pressure at 50 °C by using a rotary evaporator to yield the crude extract after the completion of third extraction. The yield was calculated using the equation 1:

Percentage Yield (%) = (Dry weight of extract ⁄ Dry weight of plant material) × 100

Phytochemical Analysis Qualitative: Preliminary phytochemical screening of *S. oleraceus* was performed as described previously [43].

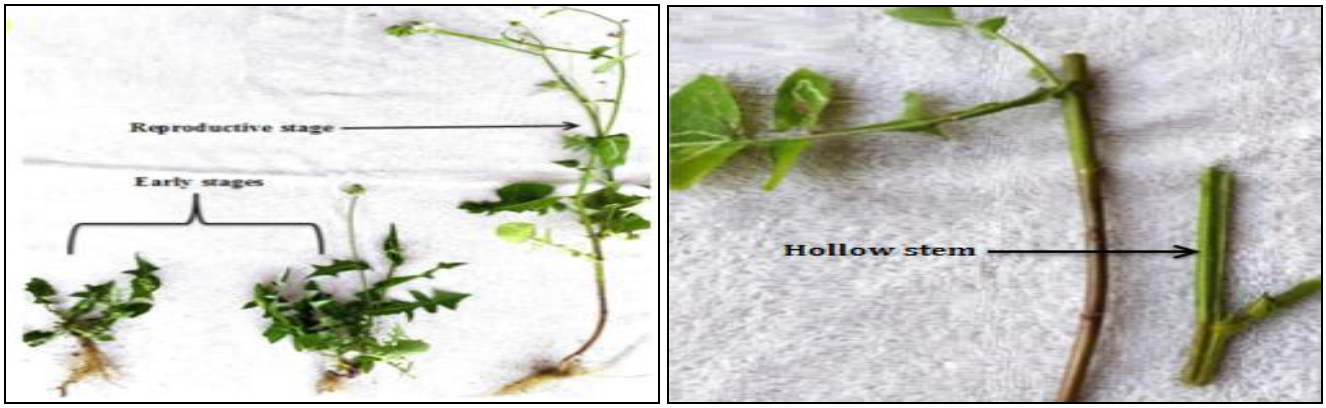

FIG. 1: THE ABOVE PICTURES ARE SHOWING (A) EARLY VEGETATIVE AND LATE REPRODUCTIVE STAGES (B) HOLLOW STEM OF *S. OLERACEUS* (SOW THISTLE)

Anti-microbial Assay: Five pathogenic bacterial strains, namely *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Salmonella typhi*, *Klebsiella pneumoniae* were collected from the microbiology lab of Shoolini University. The selected bacterial strains were carefully inoculated in all five test tubes containing nutrient broth and were incubated at 25-28°C under shaking conditions. The anti-microbial activity was performed with the help of the agar well diffusion method [44]. In brief, 50 µl of the bacterial inoculums was spread over plates containing nutrient agar and then 6 mm wells were created with the help of puncture on the plates. These were impregnated the 6mm discs of Whatman paper within the wells and check the anti-microbial activity by taking 50µl of each extracts in different concentration *i.e.* 150mg/ml, 200mg/ml, 250mg/ml and 300mg/ml against all pathogenic bacteria. Two controls were included in test *i.e.* the antibiotic ampicillin of 10µl at concentration of 100 mg/ml (positive control) and DMSO was taken as a negative control. The plates were kept in the incubator for 24-48 h at 37 °C. The obtained inhibition zones (mm) around the disc were measured.

Extraction of Genomic DNA: Total genomic DNA was extracted from the leaves of *S. oleraceus* by Cetyltrimethyl ammonium bromide method (CTAB) method as described previously [45].

Random Amplified Polymorphic DNA (RAPD) Analysis: RAPD analysis was performed as described by Williams *et al.,* with some modifications in the protocol [46]. The genetic variation among was evaluated by using random decamer primers, including OPA-1(5'-CAGGCCCTTC-3'), OPA-3 (5'-AGTCAGCCAC-3'), OPA-9 (5'-GGG TAACGCC-3'), OPA-13 (5'-CAGCACCCAC-3'), OPA-14(5'-TCTGTGCTGG-3'), OPA-16(5'-AGC CAGCCAA-3'), TC-1(5'-GGAGTACTGG-3'), TC-2 (5'GGTCTAGAGG-3'), TC-3 (5'-GAGTCTCA GG-3'), and TC-4(5'-GGACTGCAGA-3'). Polymerase chain reaction (PCR) was carried out in final volume (25 µL) of reaction mixture consisted of 1 µL (~100 ng) of genomic DNA, 2 µL (10 pM) of RAPD primer, 2.5 µL of 2 mM dNTPs, and 0.3 µL (5 U µLG1) of Taq DNA polymerase (Bangalore Genei Pvt. Ltd.) with 10X PCR buffer (containing 15 mM $MgCl_2$). The PCR mixture was subjected to initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 36°C for 1 min and elongation at 72°C for 1 min with a final extension step at 72°C for 10 min. The final PCR products were analyzed by performing electrophoresis using 1.5% agarose gel. The samples were loaded into wells along with a DNA molecular marker of 1kb plus to know the molecular size of the DNA sample.

Data Analysis: The RAPD fragments obtained upon agarose gel electrophoresis were scored for presence (1) and absence (0) of amplification bands for each sample. The data of similarity coefficients were generated and used to construct the dendrogram indicating the genetic relatedness or differentiation among the different isolates of *S. oleraceus*. Data was compiled as a binary 0-1 matrix, '1' represents the presence of a band and '0' represents the absence of a band in the specified size range. All high and low-intensity bands were considered in statistical analysis. Dendrogram was produced from the distance matrix by UPGMA (Unweighted Pair-Grouped Method Arithmetic) method, constructed using online D-UPGMA (DendroUPGMA: A dendrogram construction utility) program, version-2015.

RESULTS: In the present research work entitled "Analysis of phytochemical constituents, anti-microbial activity and molecular characterization of *S. oleraceus* L. using RAPD marker'' was carried out during the year 2016. The study was aimed to check the anti-microbial components, presence of bioactive compounds, and genetic variation of different isolates of plant's samples of *S. oleraceus* collected from different regions of Himachal Pradesh, namely Solan, Una, Bilaspur, Kangra, and Hamirpur **Table 1**. The collected leaves *S. oleraceus* were of different sizes (Small and Large) and colour (Light green and Dark green). This plant was authenticated by Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni (Solan), H.P. in the month of January 2016.

TABLE 1: PLACES OF COLLECTION OF DIFFERENT ACCESSIONS OF *SONCHUS OLERACEUS* L.

S. no.	Accessions and district	Place of collection	Plant Description			Locality Geographical Coordinates		Elevation in Meters
			Used Part	Colour of Leaves	Size of Plant	Latitute (°N)	Longitude (°E)	
1	B1 (Bilaspur)	Chandpur, Himachal Pradesh, India	Leaves	Dark Green	Small	31° 21' 50" N	76° 44' 57" E	533
2	B2 (Bilaspur)	Kandrore, Himachal Pradesh, India	Leaves	Dark Green	Small	31° 23' 32" N	76° 45' 42" E	586
3	B3 (Bilaspur)	Ghumarwin, Himachal Pradesh, India	Leaves	Dark Green	Small	31° 29' 10" N	76° 40' 07" E	740
4	B4 (Bilaspur)	Naina Devi, Himachal Pradesh, India	Leaves	Light Green	Small	31° 17' 37" N	76° 30' 57" E	480
5	B5 (Bilaspur)	Kuthera, Himachal Pradesh, India	Leaves	Light Green	Large	31° 29' 56" N	76° 43' 13" E	751
6	H1 (Hamirpur)	Galore, Himachal Pradesh, India	Leaves	Light Green	Large	31° 40' 57" N	76° 30' 31" E	828
7	H2 (Hamirpur)	Nadaun, Himachal Pradesh, India	Leaves	Dark Green	Large	31° 46' 13" N	76° 18' 37" E	449
8	H3 (Hamirpur)	Bangana, Himachal Pradesh, India	Leaves	Light Green	Small	31° 36' 56" N	76° 17' 18" E	726
9	H4 (Hamirpur)	Shahtalai, Himachal Pradesh, India	Leaves	Light Green	Small	31° 27' 12" N	76° 31' 25" E	600
10	H5 (Hamirpur)	Hamirpur, Himachal Pradesh, India	Leaves	Light Green	Large	31° 41' 18" N	76° 33' 29" E	838
11	K1 (Kangra)	Baijnath, Himachal Pradesh, India	Leaves	Light Green	Small	23° 02' 54" N	76° 37' 14" E	907
12	K2 (Kangra)	Dharamshala, Himachal Pradesh, India	Leaves	Dark Green	Large	32° 09' 40" N	76° 13' 08" E	636
13	K3 (Kangra)	Nurpur, Himachal Pradesh, India	Leaves	Light Green	Small	32° 17' 05" N	75° 52' 28" E	467
14	K4 (Kangra)	Jawalamukhi, Himachal Pradesh, India	Leaves	Light Green	Small	31° 52' 08" N	76° 18' 08" E	490
15	K5 (Kangra)	Jawali, Himachal Pradesh, India	Leaves	Light Green	Small	32° 08' 12" N	76° 00' 44" E	435
16	S1(Solan)	Nauni, Himachal Pradesh, India	Leaves	Light Green	Small	30° 51' 46" N	73° 10' 32" E	1267
17	S2 (Solan)	Baddi, Himachal Pradesh, India	Leaves	Dark Green	Large	30° 58' 46" N	76° 41' 19" E	413
18	S3 (Solan)	Sultanpur, Himachal Pradesh, India	Leaves	Light Green	Small	30° 51' 58" N	77° 05' 38" E	1401

19	S4 (Solan)	Kumarhatti, Himachal Pradesh, India	Leaves	Light Green	Small	30° 53' 11" N	77° 03' 16" E	1712
20	S5 (Solan)	Nalagarh, Himachal Pradesh, India	Leaves	Dark Green	Small	30° 02' 14" N	76° 44' 22" E	498
21	U1 (Una)	Amb, Himachal Pradesh, India	Leaves	Dark Green	Large	31° 41' 03" N	76° 07' 26" E	484
22	U2 (Una)	Haroli, Himachal Pradesh, India	Leaves	Dark Green	Large	31° 24' 45" N	76° 13' 12" E	469
23	U3 (Una)	Gagret, Himachal Pradesh, India	Leaves	Dark Green	Large	31° 38' 08" N	76° 03' 11" E	513
24	U4 (Una)	Chintpurni, Himachal Pradesh, India	Leaves	Dark Green	Large	31° 48' 59" N	76° 03' 42" E	780
25	U5 (Una)	Una, Himachal Pradesh, India	Leaves	Dark Green	Large	31° 24' 53" N	76° 13' 08" E	512

Phytochemical Studies: For phytochemical studies, various extracts, *i.e.*, methanol extract, chloroform extract, ethyl acetate, and aqueous extract of this plant, were prepared by using a cold percolation method. The yield (g) of these extracts was measured in **Table 2**. After phytochemical analysis of all prepared extracts, it was found that alkaloids were confirmed in all the extracts. The carbohydrates were not present in chloroform extract, and flavonoids were present in all the extracts. Tannins were not present in the chloroform extract, but in chloroform extract, the phenols were confirmed. Terpenoids were present in all the extracts except methanol extract. Saponin was only present in the aqueous extract, and the amino acids were not present in the methanol extract **Table 3**. The qualitative phytochemical screening was done to detect the various bioactive compounds by different tests **Fig. 2.**

TABLE 2: YIELDS (IN G) OF DIFFERENT EXTRACTS OF *S. OLERACEUS*

S. no.	Extracts	Weight of extracts (in g)
1	Methanol	0.969g
2	Aqueous	0.654g
3	Chloroform	0.336g
4	Ethyl acetate	0.128g

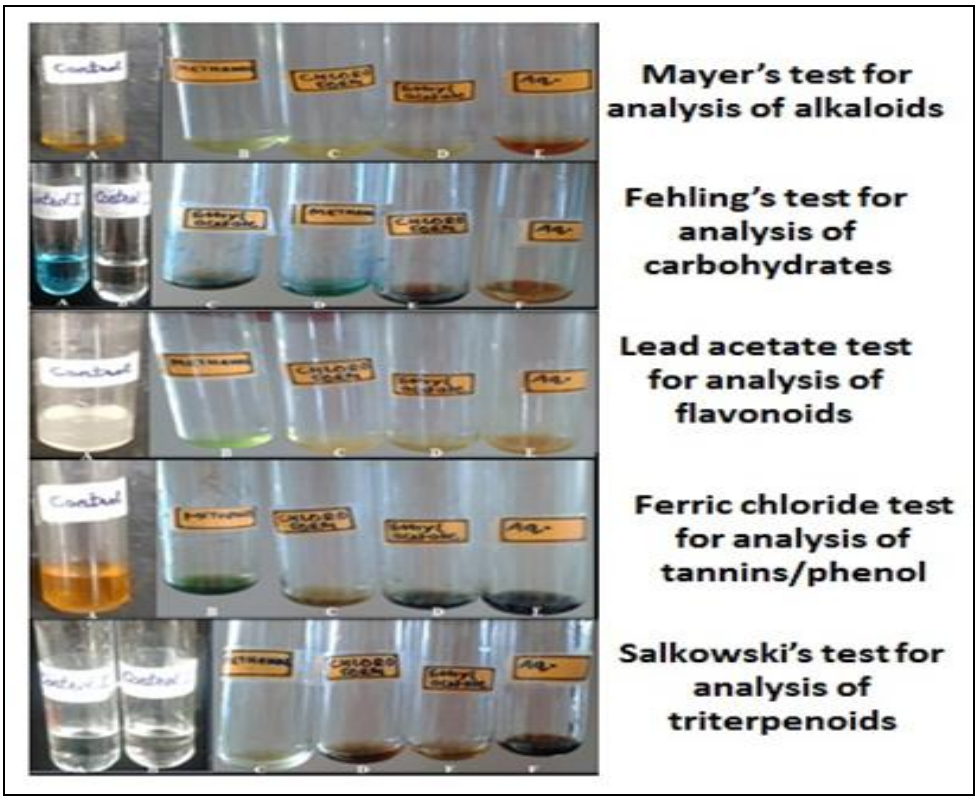

FIG. 2: DIFFERENT TEST FOR ANALYSIS OF ALKALOIDS, CARBOHYDRATES, FLAVONOIDS, TANNINS/PHENOLS AND TRITERPENOIDS IN ETHYL ACETATE, METHANOL, CHLOROFORM AND AQUEOUS EXTRACT; A-CONTROL 1 (CHLOROFORM), B-CONTROL 2 (CONC. H_2SO_4) C-METHANOL EXTRACT, D-CHLOROFORM EXTRACT, E-ETHYL ACETATE EXTRACT AND F-AQUEOUS EXTRACT

TABLE 3: RESULT OF PHYTOCHEMICAL CONSTITUENTS PRESENT IN DIFFERENT EXTRACTS I.E. METHANOL EXTRACT, ETHYL ACETATE EXTRACT, CHLOROFORM EXTRACT AND AQUEOUS EXTRACT OF *S. OLERACEUS*

Phytochemical Constituents	Aqueous extract	Methanol Extract	Chloroform extract	Ethyl acetate extract
Alkaloids	+	+	+	+
Carbohydrates	+	+	-	+
Flavonoids	+	+	+	+
Tannins	+	+	-	+
Terpenoids	+	-	+	+
Phenols	-	-	+	–
Saponin	+	-	-	-
Xanthoprotein	+	-	+	+

Note= Sign + (for presence) and Sign – (for absence)

Anti-microbial Potential: The anti-microbial activity of the aqueous, methanol, chloroform, and ethyl acetate extracts of *S. oleraceus* was checked against the bacterial strains of *K. pneumoniae, S. aureus, P. aeruginosa, E. coli, and S. typhii* at different concentrations (150mg/ml, 200mg/ml, 250mg/ml and 300mg/ml) **Fig. 4.** The inhibition zones (mm) of varying sizes against all bacteria's were obtained in **Table 4**. The graphical representation of anti-microbial activity of all extracts against all respective bacterias was shown in **Fig. 3.** The zone of inhibition of 20 mm was observed around the aqueous extract and 15 mm around the methanol extract. The chloroform and ethyl acetate extract showed no inhibition zone against the different extracts of *S. oleraceus* against *K. pneumoniae.* The aqueous extract indicated the maximum inhibition zone against *K. pneumoniae* at 300 mg/ml. The methanol extract showed the minimum inhibition zones than the aqueous extract. Ethyl acetate and the chloroform extract did not show any inhibition zone against *K. pneumoniae.* The ethyl acetate and chloroform extract did not show any inhibition zone at any concentration. Ethyl acetate extract indicated the maximum inhibition zones against *S. aureus* at 300 mg/ml. The aqueous extract indicated the maximum inhibition zones against *E. coli* at 300 mg/ml. The chloroform extract showed the minimum inhibition zones than the aqueous extract. The ethyl acetate extract showed very less inhibition zone at the concentration of 300 mg/ml that aqueous extract indicated the maximum inhibition zones against *S. typhi* at 300 mg/ml. The ethyl acetate extract and chloroform did not show any inhibition zone against *S. typhi* at different concentration. The aqueous extract indicated the maximum inhibition zone against *P. aeruginosa* at 300 mg/ml. The methanol extract showed the minimum Inhibition zones than the aqueous extract. The ethyl acetate showed very less inhibition zone of 18 mm against *P. aeruginosa*. By comparing the diameters of the inhibition zones, the various extracts of *S. oleraceus* showed less or same activity than standard antibiotic. *S. aureus* was the most susceptible bacteria amongst all bacterial strains. The anti-microbial activity of different extracts of *S. oleraceus* was associated with the concentration **Fig. 4.** With the higher concentration of extracts, the growth of bacteria was low.

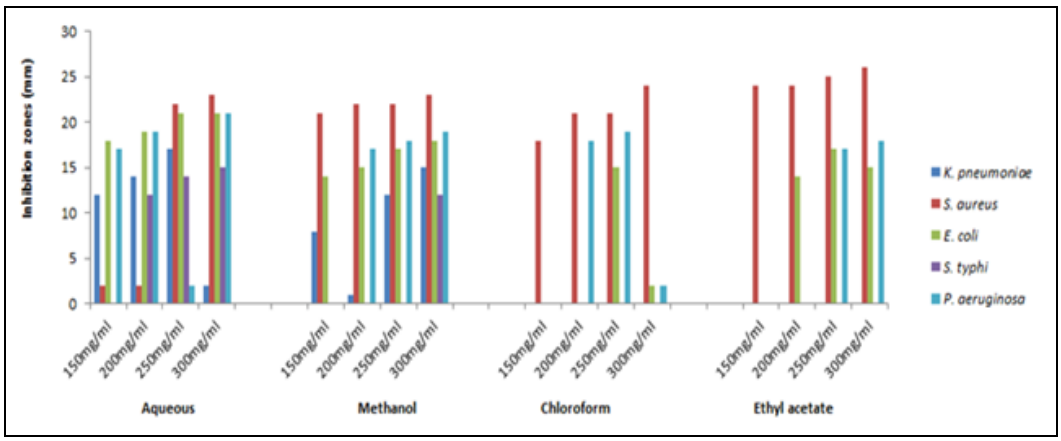

FIG. 3: THE GRAPHICAL REPRESENTATION OF ANTI-MICROBIAL POTENTIAL OF DIFFERENT EXTRACTS AT DIFFERENT CONCENTRATIONS

TABLE 4: INHIBITION ZONES OF DIFFERENT EXTRACTS *VIZ*. AQUEOUS, METHANOL, CHLOROFORM AND ETHYL ACETATE EXTRACT OF *S. OLERACEUS* AGAINST ALL BACTERIAS AT DIFFERENT CONCENTRATIONS

Inhibition zones of *S. oleraceus* against *K. pneumoniae*				
Plant extracts	**150mg/ml**	**200mg/ml**	**250mg/ml**	**300mg/ml**
Aqueous	12	14	17	20
Methanol	8	10	12	15
Chloroform	–	–	–	–
Ethyl acetate	–	–	–	–
Inhibition zones of *S. oleraceus* against *S. aureus*				
Plant extracts	**150mg/ml**	**200mg/ml**	**250mg/ml**	**300mg/ml**
Aqueous	20	20	22	23
Methanol	21	22	22	23
Chloroform	18	21	21	24
Ethyl acetate	24	24	25	26
Inhibition zones of *S. oleraceus* against *E. coli*				
Plant extracts	**150mg/ml**	**200mg/ml**	**250mg/ml**	**300mg/ml**
Aqueous	18	19	21	21
Methanol	14	15	17	18
Chloroform	–	–	15	20
Ethyl acetate	–	14	17	15
Inhibition zones of *S. oleraceus* against *S. typhi*				
Plant extracts	**150mg/ml**	**200mg/ml**	**250mg/ml**	**300mg/ml**
Aqueous	–	12	14	15
Methanol	–	–	–	12
Chloroform	–	–	–	–
Ethyl acetate	–	–	–	–
Inhibition zones of *S. oleraceus* against *P. aeruginosa*				
Plant extracts	**150mg/ml**	**200mg/ml**	**250mg/ml**	**300mg/ml**
Aqueous	17	19	20	21
Methanol	–	17	18	19
Chloroform	–	18	19	20
Ethyl acetate	–	–	17	18

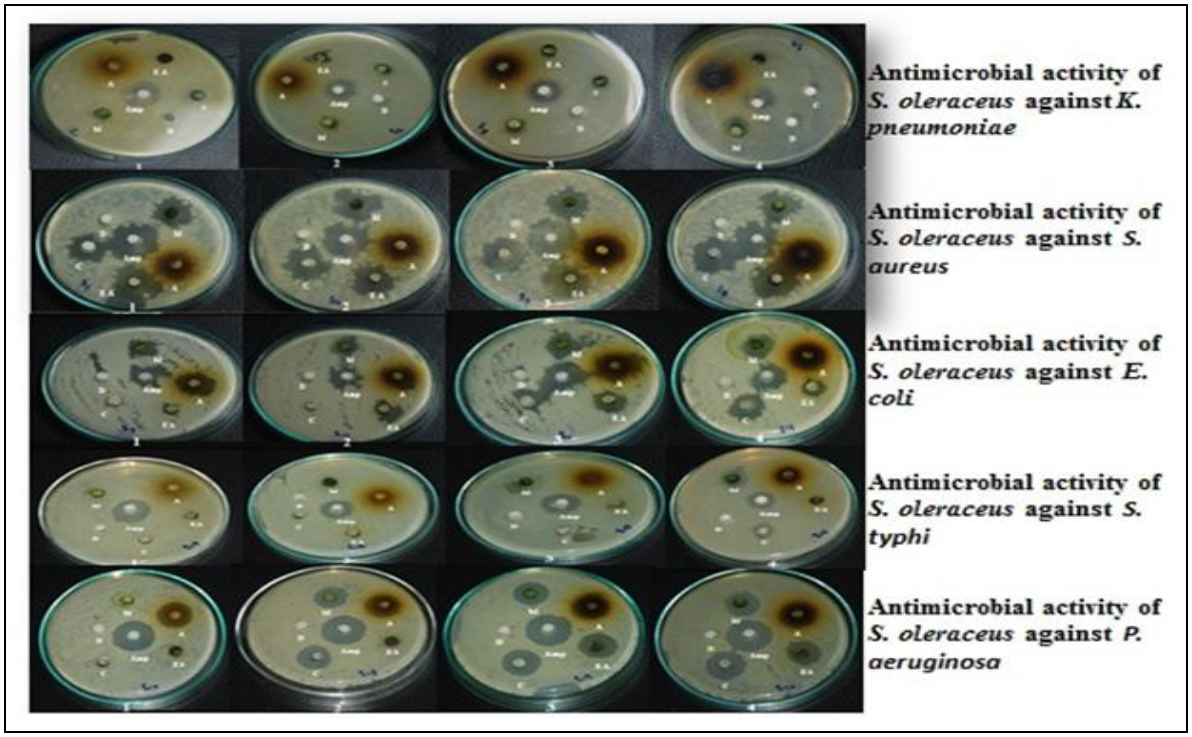

FIG. 4: ANTI-MICROBIAL ACTIVITY OF *S. OLERACEUS* IN DIFFERENT EXTRACTS I.E., EA-ETHYL ACETATE, A-AQUEOUS, C-CHLOROFORM, D-DMSO, M-METHANOL AND AMP-AMPICILLIN AT VARYING CONCENTRATIONS viz., (1) 150mg/ml CONC. (2) 200mg/ml CONC. (3) 250mg/ml CONC. (4) 300mg/ml CONC. AGAINST ALL BACTERIAS

Molecular Characterization: There were a collection of 25 samples of *S. oleraceus* from five districts of Himachal Pradesh namely Una, Kangra, Hamirpur, Bilaspur and Solan **Table 1**. A panel of 14 different villages of all districts was used, namely (U1, U2, U3) from Una, (H1, H2, H3) from Hamirpur, (B1, B2, B3) from Bilaspur, (K1, K2, K3) from Kangra, S1, S2 from Solan of Himachal Pradesh for molecular characterization **Table 5**. The genomic DNAs and RAPD banding patterns of a panel of 14 regions of each district for molecular characterization were isolated and amplified with the random polymorphic five RAPD primers **Fig. 5.** The obtained data of the number and size of amplified fragments with RAPD primers were evaluated after PCR amplification. A total of 48, 72, 57, 54 and 23 bands were scored by using specific primers OPA-1, OPA-9, OPA-16, TC-1 and TC-3 respectively **Table 6.** The number and size of the amplified products resulted from random primers were varied between 23-72 and 200-3000 base pairs, respectively**.** Clustering based on RAPD fingerprints data revealed formation of a single group which constituted nine independent branches on the similarity scale value of 0.25 (~71% similarity level) **Fig. 6**. Cluster analysis revealed that isolates Galore (H1) and Bangana (H3), Nadaun (H2) and Baddi (S2), Amb (U1) and Haroli (U2) were showing maximum homology. However, at similarity scale value of 0.2 (~57% similarity level) few of the isolates got divided into two independent groups, which were distantly related to each other. The first group comprises of isolates Chandpur (B1), Ghumarwin (B3), Galore (H1), and Bangana (H3), and the second group comprises of isolates Nurpur (K3), Kandrore (B2), Nadaun (H2), Baddi (S2), Amb (U1) and Haroli (U2). The isolate Baiznath (K1) from Kangra district was found to be distantly related to the other two samples from the same district, viz., Dharamshala (K2) and Nurpur (K3). While, the isolate Nauni (S1) from Solan district was also found to be distantly related to the other Baddi (S2) isolate from the same district.

Similarly, the isolate Amb (U1) from the Una district was found to be distantly related to the other Haroli (U2), and Gagret (U3) isolates from the same district. However, the isolates Chandpur (B1) and Ghumarwin (B3) from the Bilaspur district were found to be closely related to each other, but were both distantly related to the isolate Kandrore (B2) from the same district. In comparison to all the isolates, isolate Baijnath (K1) from the Kangra district showed the least homology to all the other isolates from the total covered five districts of Himachal Pradesh.

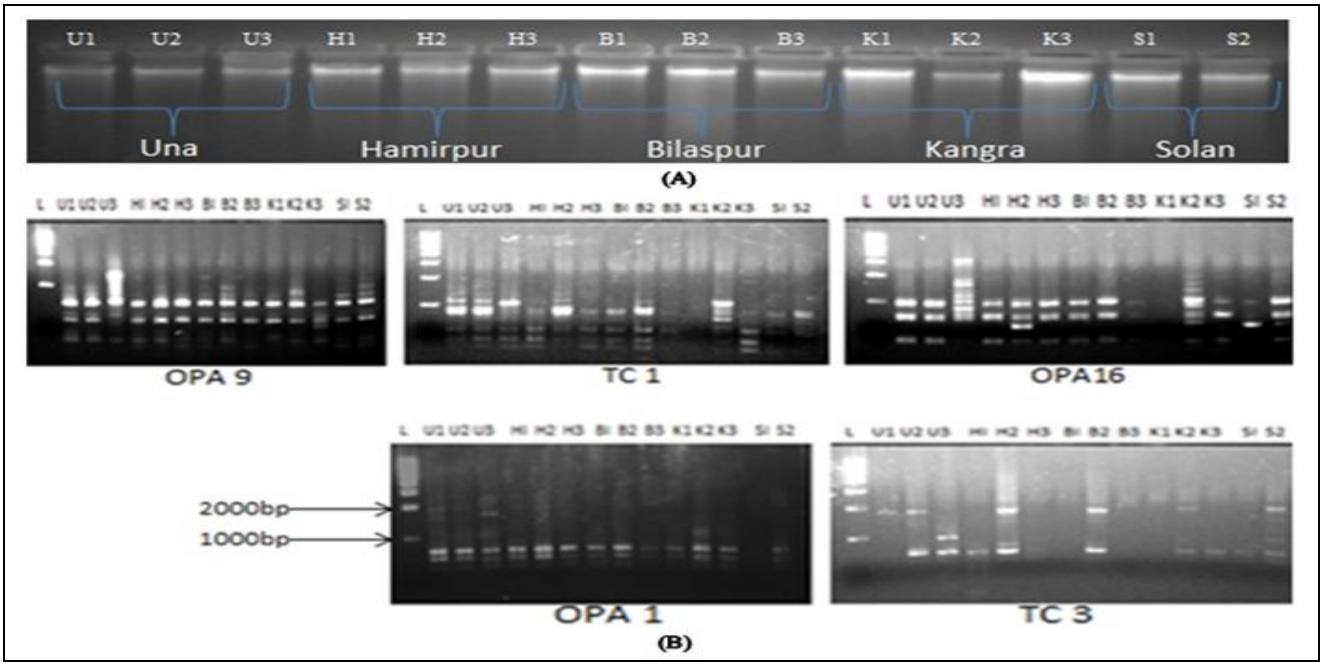

FIG. 5: (A) GENOMIC DNAS ISOLATION FROM A PANEL OF 14 SAMPLES OF *S. OLERACEUS* NAMELY: (U1, U2, U3) FROM Una, (H1, H2, H3) FROM HAMIRPUR, (B1, B2, B3) FROM BILASPUR, (K1, K2, K3) - FROM KANGRA AND (S1, S2) FROM SOLAN RESPECTIVELY (B) RAPD BANDING PATTERNS OF EACH REGION OF A PANEL WITH RAPD PRIMERS AFTER PCR AMPLIFICATION, WHEREAS L STANDS LADDER (1kb)

TABLE 5: A PANEL OF DIFFERENT REGIONS OF EACH DISTRICT *VIZ*. UNA, HAMIRPR, BILASPUR, KANGRA WERE USED FOR MOLECULAR CHARACTERIZATION

S. no.	A Panel of different regions of each district in molecular characterization				
	Una	Hamirpur	Bilaspur	Kangra	Solan (S)
1	Amb (U1)	Galore (H1)	Chandpur (B1)	Baijnath (K1)	Nauni (S1)
2	Haroli (U2)	Nadaun (H2)	Kandrore (B2)	Dharamshala (K2)	Baddi (S2)
3	Gagret (U3)	Bangana (H3)	Ghumarwin (B3)	Nurpur (K3)	-

TABLE 6: PRIMER NAME, NUMBER AND SIZE OF FRAGMENTS AMPLIFIED BY RAPD PRIMERS

S. no.	Primers	Sequence 5'-3'	Amplified Fragments	Maximum Band Size (bp)	Minimum Band Size (bp)
1	OPA-1	(5'-CAGGCCCTTC-3')	48	1800	600
2	OPA-9	(5'-GGGTAACGCC-3')	72	1300	300
3	OPA-16	(5'-AGCCAGCCAA-3')	57	3000	400
4	TC-1	(5'-GGAGTACTGG-3')	54	1800	250
5	TC-3	(5'-GAGTCTCAGG-3')	23	1900	200

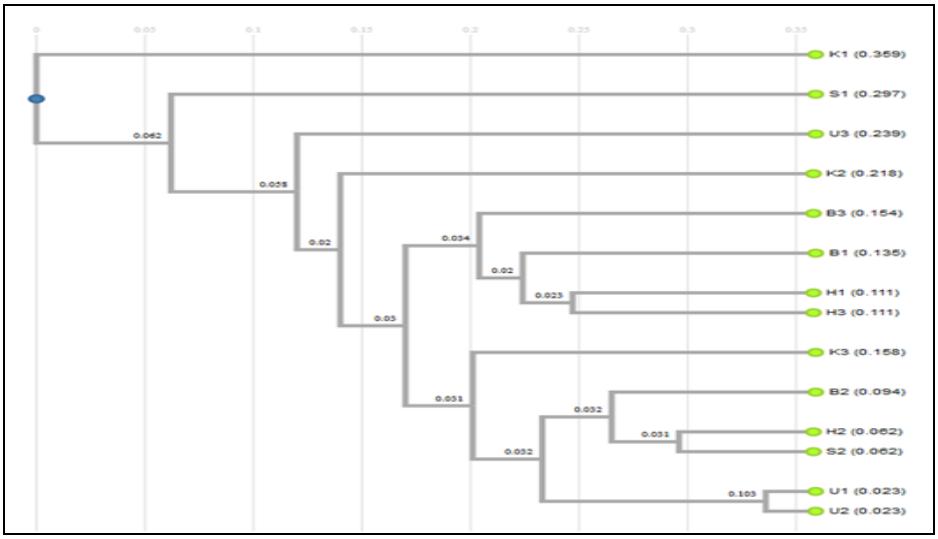

FIG. 6: DENDROGRAM OF *S. OLERACEUS* SPECIES DERIVED FROM RAPD FINGERPRINTS GENERATED BY USING FIVE DIFFERENT PRIMERS PRODUCED FROM THE DISTANCE MATRIX BY UPGMA (UNWEIGHTED PAIR-GROUPED METHOD ALIGNMENT) BY ARITHMETIC AVERAGE, CONTAINED IN THE SOFTWARE PACKAGE NTsys 2.2 VERSION

CONCLUSION: In the present project entitled "Analysis of anti-microbial potential and molecular characterization of *S. oleraceus* from different districts of Himachal Pradesh was carried out during the year, 2016. The present study revealed that ethyl acetate extract showed the maximum anti-microbial activity against *S. aureus.* This plant contains various bioactive compounds (flavonoids, saponins, tannins, phenols, and alkaloids) and contributes to various pharmaceutical responses. Molecular characterization using RAPD revealed the genetic variance between the fourteen samples selected from the total of twenty-five samples from five districts of HP. RAPD was done with the help of different primers and clustering based on RAPD fingerprints data revealed formation of a single group which constituted nine independent branches on the similarity scale value of 0.25 (~71% similarity level). Cluster analysis revealed that isolates H1 and H3, H2 and S2, U1 and U2 were showing maximum homology. However, at a similarity scale value of 0.2 (~57% similarity level) few of the isolates got divided into two independent groups, which were distantly related to each other. RAPD markers exhibit reasonable speed, cost, and efficiency compared with other methods, and RAPD can be done in a moderate laboratory. Therefore, despite its reproducibility problem, it will probably be important until better techniques are developed in terms of cost, time, and labour. The study on this plant can be useful in germplasm characterization and animal plant-microbe inter-actions. This work can be useful in the future to know the anti-microbial potential and explore phytochemical constituents quantitatively and their role against various pathogen.

The various phytochemicals present in stems and flowers can also be checked against the protection from many diseases. Hence, further study is needed to explore the benefits and the ability of *S. oleraceus* extracts as one of the therapeutic raw material in food, nutraceutical and pharmaceutical industries in future

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