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PHYTOCHEMICAL COMPOSITION OF *SIDA RHOMBIFOLIA* SSP. *RETUSA* (L.) *BROSS*. : A COMPREHENSIVE GC/MS ANALYSIS

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Abstract

Sida rhombifolia ssp. *retusa*, commonly known as "bala," is a traditional medicinal plant recognized for its diverse therapeutic properties. This paper explores the geographical variations in the phytochemical composition of Sida rhombifolia ssp. retusa across five distinct locations—Kurukshetra, Panipat, Meerut, Haridwar and Saharanpur of India. The study employs Gas Chromatography-Mass Spectrometry (GC-MS) to analyze of leaf samples, aiming to elucidate variations in phytochemical constituents and their potential impact on the plant's medicinal efficacy. Our findings reveal significant diversity in the secondary metabolite profiles, influenced by environmental factors of different regions. The analysis revealed a total of 76 Phytochemicals with substantial differences in the abundance and types of bioactive compounds, such as alkaloids, terpenoids, halogenated hydrocarbons tocopherols, sterols, fatty acids, alkanes, and hydrocarbons across these regions including 28 in Kurukshetra, 8 in Panipat, 5 in Saharanpur, 4 each in Meerut and Haridwar both. Statistical analyses, including Principal Component Analysis (PCA), highlight clustering based on geographical origin. Besides, environmental parameters offer preliminary insights, suggesting a connection between compound abundance and specific regional conditions. These variations have direct implications for the medicinal properties of *Sida rhombifolia* ssp. *retusa*, indicating the need for region-specific considerations in traditional medicine.

Keywords: *Sida rhombifolia* ssp. *retusa*, Geographical Variation, GC-MS Analysis, Phytochemical Composition, Medicinal Plants, Therapeutic Efficacy.

INTRODUCTION

Sida rhombifolia ssp. *retusa* (L.) Bross., commonly known as "bala," holds a significant place in traditional medicinal practices owing to its diverse therapeutic properties. Widely distributed across various geographical locations, this plant has been utilized for centuries to address an array of ailments [1], [2], [3]. However, the phytochemical composition of

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Sida rhombifolia ssp*. retusa* is known to exhibit considerable variability, influenced by factors such as climate, soil conditions, and geographical location. Understanding these variations is crucial for unlocking the full therapeutic potential of this medicinal plant.

The genus *Sida*, to which *Sida rhombifolia* ssp. *retusa* belongs, include 12 species in India [69] known for their pharmacological importance [1], [2], [3], [4], [5], [6]. *Sida rhombifolia* ssp*. retusa*, in particular, has been traditionally employed in different cultures as a remedy for conditions ranging from respiratory disorders and inflammatory ailments to skin diseases, cancer, antibacterial, antioxidant related health issues [14], [36], [24]. Its oil is used to nourish brain spinal cord, and nerves. It also used as a rejuvenator in case of ailments, by promoting tissue recovery and relieve inflammation, pain and stiffness [1]. Due to which, it is used in treatment of gout, osteoarthritis, paralysis, poliomyelitis, and sciatica [1]. Crude root extract of this plant had a sedative effect and also shows significant potentiation of pentobarbitone sleeping time in mice [2]. Several therapeutic uses like hepatoprotective activity [4]; Hypoglycemic and Hypolipidemic effect [3]; Cytotoxic, antibacterial, antitubercular and antimycotic [5]; and antioxidant [8], [6], [7] potentials has been decoded. This suggests that *Sida rhombifolia* ssp. *retusa* is a potent medicinal weed and can be used to treat animal ailments.

The plant is recognized for its rich reservoir of bioactive compounds including fatty acids, terpenoids, tocopherols, hydrocarbons and other secondary metabolites, which contribute to its medicinal efficacy [10], [12], [16], [17], [37].

Geographical factors have long been acknowledged as influential determinants of plant secondary metabolite production. Variations in temperature, humidity, altitude, and soil composition can lead to distinct adaptations in plant species, ultimately affecting the synthesis of bioactive compounds. Therefore, a comprehensive analysis of the phytochemical composition of *Sida rhombifolia* ssp*. retusa* across different geographical locations becomes imperative for a holistic understanding of its medicinal potential.

Gas Chromatography-Mass Spectrometry (GC-MS) emerges as an invaluable analytical tool for probing the intricate chemical composition of plant extracts. This technique facilitates the identification and quantification of a wide spectrum of compounds present in the samples, providing detailed insights into the plant's chemical profile. By applying GC-MS to leaf samples of *Sida rhombifolia* ssp. *retusa* collected from diverse locations, this study aims to elucidate the variations in the plant's phytochemical constituents and explore the potential impact of these variations on its therapeutic properties.

In this context, our research focuses on five distinct geographical locations—Kurukshetra, Panipat, Meerut, Haridwar, and Saharanpur. Each of these regions presents unique environmental conditions that may contribute to the divergence in the phytochemical profiles of *Sida rhombifolia* ssp. *retusa*. By systematically investigating these variations, we seek to pave the way for a nuanced understanding of the medicinal potential of *Sida rhombifolia* ssp*. retusa* and, by extension, contribute to the broader field of plant-based medicine and natural product research.

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METHODOLOGY

1. **Sample Collection and identification:** *Sida rhombifolia* ssp*. retusa* were collected from five different geographical locations viz. Kurukshetra: 29° 52’ to 30° 12’; Panipat: 29° 23’ 56.1408”N and 76° 58’ 37.4916”E; Meerut: 28° 57’ to 29° 02’N and 77° 40’ to 77° 45’E; Saharanpur: 22.9680° north, 77.5552° E; Haridwar: 29.58° N and 78.13° E. Each plant specimen was meticulously identified by legal deeds [69], [70]. The voucher specimens were submitted in herbarium and museum of botany department of Chaudhary Charan Singh University, Meerut for future reference.
2. **Preparation of Plant Extracts:** The collected plant material, comprising whole plant thoroughly cleaned, each plant part separated, air-dried, and only leaves were ground into a fine powder for further analysis. The powdered samples were subjected to extraction using methanol in soxhlet, to obtain crude extracts rich in bioactive compounds.
3. **Preliminary phytochemical screening:** We performed preliminary phytochemical test on methanolic extract of leaves. It show positive test for alkaloids, fats, carbohydrates, proteins, steroids and for terpenoids (table 1).
4. **Gas Chromatography-Mass Spectrometry (GC-MS) Analysis:** The extracted samples underwent Gas Chromatography-Mass Spectrometry (GC-MS) analysis to discern and quantify the presence of chemical constituents. Employing a high-resolution instrument featuring a fused silica capillary column, the GC-MS system utilized the SH-Rxi-5Sil MS column (composed of 5% biphenyl and 95% dimethyl polysiloxane) with dimensions of 30 meters length, 0.25 mm inner diameter, and a 0.25 µm film thickness. The temperature range for the analysis was set at 320-350 °C. The mobile phase, helium (He), was maintained at a flow rate of 1.0 ml/min. In the gas chromatography segment, the temperature program (oven temperature) initiated at 50°C and incrementally rose to 300°C at a rate of 5°C/min. The injection volume was 1 micro liter. Samples, dissolved in methanol, were comprehensively analyzed within a mass-to-charge ratio (m/z) range of 50-650. The obtained results were then compared using the integrated chemical library search program. The identities of individual compounds based on their retention times and mass spectra (table 2) was done. Peak integration and area normalization were performed to quantify the relative abundance (fig. 4) of each compound in the sample.

Table 1: Preliminary Phytochemical Screening

Phytochemical test				**Occurrence**
Alkaloid test	I)Dragandroff	II) Wanger	III) Mayer	Present
Steroids	I) Libermann- Buchard Test			Present
Fatty acid	I) Spot test	II) Sudan III		Present
Terpenoids	I) Salkowaski test			Present
Protein	I) Ninhydrin test	II) Biuret test		Present
Carbohydrates	I) Molish test	II) Iodine test		Present

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5. **Identification of Compounds:** Compounds were tentatively identified by comparing their mass spectra with existing databases such as NIST (National Institute of Standards and Technology) and Pubchem, supplemented by relevant literature (table 4).
6. **Statistical Analysis:** Principal Component Analysis (PCA) was conducted on 76 distinct chemicals extracted from GC/MS profiles of *Sida rhombifolia* ssp. *retusa* (table 2).

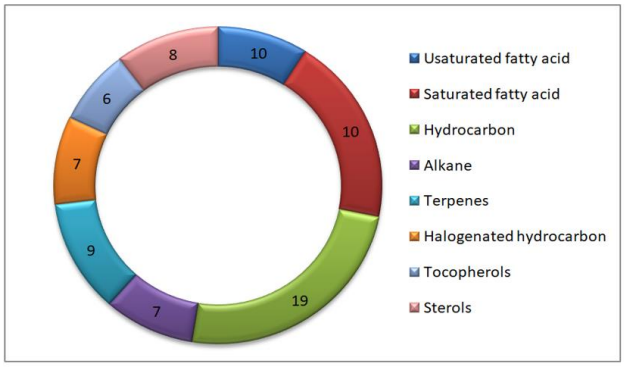

Figure 1: Relative abundance of different phytochemicals

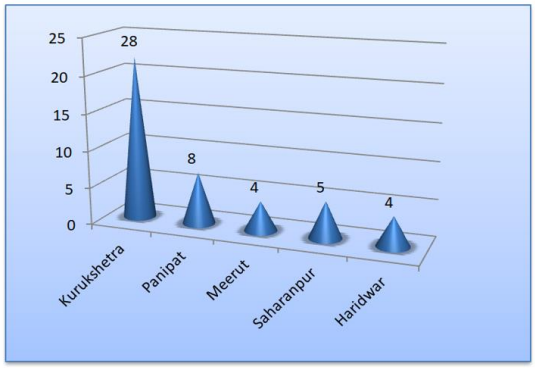

Figure 2: Chemical diversity among different localities

RESULTS & DISCUSSION

1. **Geographical Variation in Phytochemical Composition:** The GC-MS analysis revealed significant variations in the phytochemical composition of *Sida rhombifolia* ssp. *retusa* across the five geographical locations: Kurukshetra, Panipat, Meerut, Haridwar, and Saharanpur. The chromatograms (fig.5) displayed diverse peaks corresponding to different compounds, suggesting that environmental factors play a pivotal role in shaping the secondary metabolite profile of the plant.
2. **Halogenated hydrocarbons.** Distinctive phytochemicals unique to each locality are documented (table 3), with a total of 76 such compounds identified across all profiles. The relative abundance of these chemicals exhibits variability among different locations (fig.4). This suggests that specific environmental conditions may play a role in influencing the synthesis of particular bioactive molecules.

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Table 2: List of 76 different bioactive components decoded through GC/MS

			Localities with retention time				
Name	**MF**	**MW**	**A**	**B**	**C**	**D**	**E**
Undecane	C11H24	156.31	7.952	0	0	0	0
Tetradecanoic acid	C14H28O2	228.37	16.732	0	16.72	0	16.78
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2	C11H16O3	196.243	17.031	0	17.02	0	0
(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-en-1-yl)cyclohex-2-enone	C13H18O3	222.28	17.169	17.31	0	17.32	0
Neophytadiene	C20H38	278.516	17.442	0	17.42	0	0
2-Pentadecanone, 6,10,14-trimethyl-	C18H36O	268.478	17.517	17.57	0	17.57	17.52
Acetic acid, 10,11-dihydroxy-3,7,11-trimethyl-dodeca-2,6-dienyl ester	C17H30O4	298.4	17.789	17.91	0	0	0
3-Methyl-2-(3,7,11-trimethyldodecyl) furan	C10H14O	318.4	17.889	0	0	0	0
Hexadecanoic acid, methyl ester	C17H34O2	270.451	18.353	18.39	18.73	18.383	18.35
n-Hexadecanoic acid	C16H32O2	256.424	18.778	18.8	18.75	18.815	18.76
9,12-Octadecadienoic acid (Z,Z)-, methyl este	C19H34O2	294.47	19.972	0	19.94	0	0
8,11,14-Docosatrienoic acid, methyl ester	C23H40O2	348.6	20.03	0	20	0	0
Phytol	C20H40O	296.53	20.139	0	20.11	20.19	20.13
Methyl stearate	C19H38O2	298.5	20.265	20.27	20.23	20.258	20.24
(9E,11E)-Octadecadienoic acid	C18H32O2	280.45	20.399	20.09	0	19.976	0
9-Octadecenoic acid, 1,2,3-propanetriyl ester,	C57H104O	885.432	20.451	0	0	0	0
Octadecanoic acid	C18H36O2	284.48	20.645	0	0	0	0
Methyl 18-methylnonadecanoate	C21H42O2	326.56	22.03	0	0	0	0
Cyclooctane, tetradecyl-	C22H44	308.585	22.195	0	0	0	0
Tetratetracontane	C44H90	619.185	23.417	0	0	0	0
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ester	C19H38O4	330.503	23.605	0	0	0	0
Docosanoic acid, methyl ester	C23H46O2	354.61	23.657	0	0	0	0
Dotriacontane	C32H66	450.87	24.19	0	0	0	0
Hexatriacontane	C36H74	506.97	24.936	0	0	0	0
Octacosanoic acid, methyl ester	C29H58O2	438.77	25.163	0	0	0	0
Squalene	C30H50	410.718	25.764	25.77	25.73	25.767	25.74

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.delta.-Tocopherol	C27H46O2	402.65	26.729	0	0	0	0
.beta.-Tocopherol	C28H48O2	416.68	27.317	0	0	0	0
.gamma.-Tocopherol	C28H48O2	416.68	27.429	0	0	0	0
Tetrapentacontane	C54H110	759.451	27.659	0	0	0	0
Vitamin E	C29H50O2	430.706	27.93	27.98	27.91	27.96	27.93
Cholesterol	C27H46O	386.65	27.986	0	0	0	0
Campesterol	C28H48O	400.69	28.719	0	0	0	0
Stigmasterol	C29H48O	412.69	28.906	0	28.89	28.93	28.89
.gamma.-Sitosterol	C29H50O	414.707	29.391	0	29.37	29.42	29.38
.beta.-Amyrone	C30H48O	426.73	29.537	29.55	0	29.52	0
.beta.-Amyrin	C30H50O	426.73	29.804	0	0	0	0
4,22-Stigmastadiene-3-one	C29H46O	410.67	29.984	0	0	0	30
9,19-Cyclolanostan-3-ol, 24-methylene-, (3.be	C31H52O	440.744	30.42	0	0	0	0
.gamma.-Sitostenone	C29H48O	412.691	30.578	0	0	0	0
Pregn-4-ene-3,20-dione, 17-(acetyloxy)-16-me	C24H32O4	372.498	30.766	0	0	0	0
Olean-12-en-28-oic acid, 3-hydroxy-, methyl e	C33H52O4	468.71	31.773	0	0	0	0
Phytyl linoleate	C23H42O2	279.4	33.589	0	0	0	0
Phytyl tetradecanoate	C34H66O2	506.887	33.862	0	0	0	0
Phytyl stearate	C38H74O2	563	37.552	0	0	0	0
Phenol, 3,5-bis(1,1-dimethylethyl)-	C14H22O	206.33	7.952	13.95	0	0	0
Loliolide	C11H16O3	196.25	0	17.17	0	17.18	17.08
1-Methyl-1-n-octyloxy-1-silacyclobutane	C13H28OSi	536	0	17.37	0	0	0
7-Tetradecen-1-ol, (E)-, TMS derivative	C17H36OSi	284.6	0	17.49	0	0	0
2-Pentadecanone, 6,10,14-trimethyl-	C18H36O	268.478	0	17.57	0	17.57	17.52
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,	C17H24O3	276.37	0	18.27	0	18.28	18.23
6-Octadecenoic acid, methyl ester, (Z)-	C19H36O2	296.5	0	20.09	0	20.03	20.01
Sulfurous acid, pentadecyl pentyl ester	C20H42O3S	292.5	0	20.19	0	0	0
Methyl stearate	C19H38O2	298.5	0	20.27	0	0	20.24
6-Bromohexanoic acid, 5-ethyl-3-octyl ester	C16H31BrO2	223.11	0	22.03	0	0	0
3-methyl-5-(2,6-dimethylheptyl)-1,5-Pent-2-enol	C15H26O2	238.366	0	22.34	0	22.35	0
Bis(2-ethylhexyl) phthalate	C6H4(CO2C8H17)2	391	0	23.8	0	0	0
alpha.-Tocospiro B	C29H50O4	462.705	0	26.01	25.96	26.01	0
1,2-Dichlorododecane	C10H20Cl2	239.22	0	29.47	0	0	0

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24-Noroleana-3,12-diene	C29H46	394.676	0	29.82	29.76	29.79	29.87
Tris(2,4-di-tert-butylphenyl) phosphate	C42H63O4P	662.92	0	31.55	0	0	0
N-Methoxy-N-methylacetamide	C4H9NO2	103.12	0	0	2.598	0	0
Glutarimide, N-(3-pentyl)-	C10H17NO	183.248	0	0	21.18	0	0
Cycloheptanone, 3-butyl-	C10H18O	154.25	0	0	22.19	0	0
(.+/-.)-.alpha.-Tocopherol acetate	C31H52O3	472.74	0	0	28.17	0	0
Silane, methyldiethoxymethoxy-	C11H26O3Si	118.25	0	0	0	4.054	0
7-Hexadecenoic acid, methyl ester, (Z)-	C17H32O2	268.435	0	0	0	20.09	0
Tridecane, 1,13-dibromo-	C13H26Br2	342.15	0	0	0	22.03	0
Hexadecyl isopropyl ether	C19H40O	340.6	0	0	0	23.71	0
(R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12	C28H48O2	416.68	0	0	0	27.47	0
Boscartol F	C10H16O	not found	0	0	0	0	17.32
2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl	C13H18O3	222.28	0	0	0	0	17.21
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296.53	0	0	17.86	17.47	17.44
9,12-Octadecadienoic acid, methyl ester	C19H34O2	294.47	0	0	19.94	0	19.95
9(11)-Dehydroergosteryl benzoate	C35H46O2	394.63	0	0	0	0	26.93
Stigmast-4-en-3-one	C29H48O	412.69	0	0	0	0	28.89

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(Abbreviation: Mf= Molecular formula; MW= Molecular weight; A=Kurukshetra; B=Panipat; C=Meerut; D=Saharanpur; E=Haridwar.)

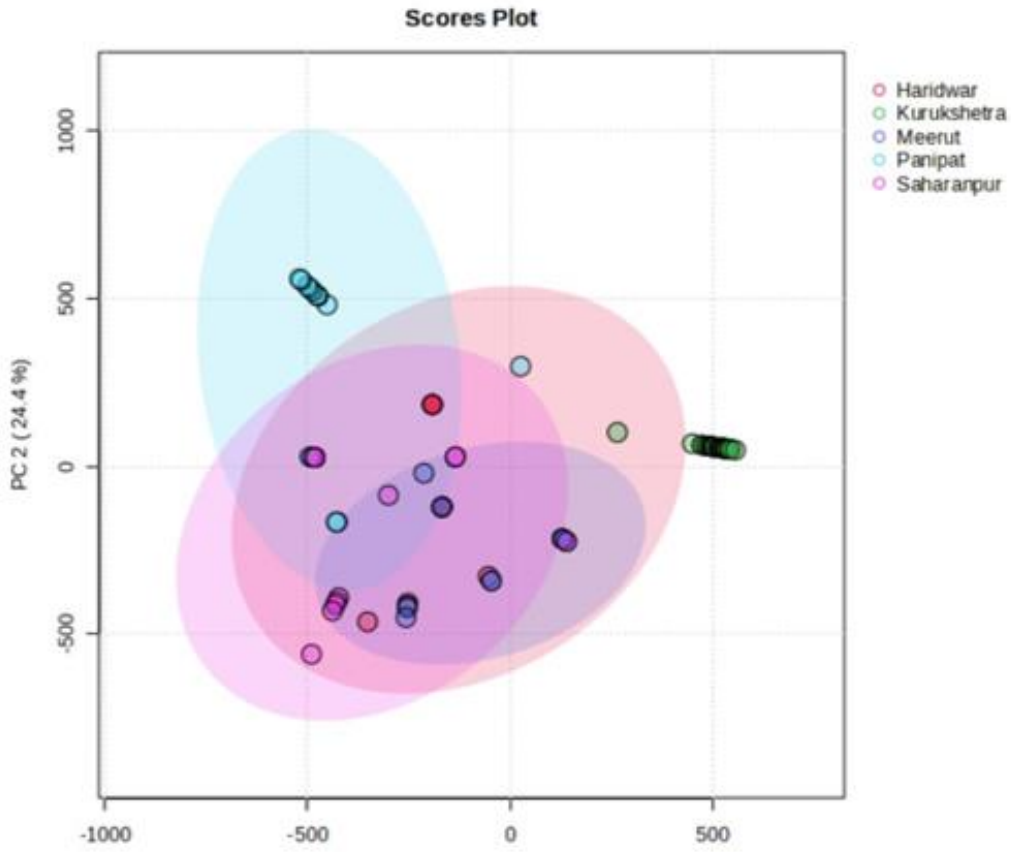

Figure 3: Principal components analysis

3. **Bioactive compound Profiling:** A total of 150 chemicals were identified across all five profiles, revealing 76 distinct chemicals upon comparing GC/MS profiles from different localities. Chromatograms (fig.5) and relative retention times (table 2) for these chemicals are provided. Notably, Squalene, Hexadecanoic acid, and n-Hexadecanoic acid methyl ester are consistently present in all profiles. A cluster bar graph (fig.4) has been constructed for the 76 chemicals, representing their relative concentrations based on height percentages. n-Hexadecanoic acid exhibits the highest concentration (fig.4), followed by Squalene, Vitamin E, 6-Octadecanoic acid, Phytol, Methyl sterate, 9,11 Octadecanoic acid, γ-Sitosteron, β-amyrone, Stigmasterol, Cholesterol, and Stigamstadiene-3-one, which are present in elevated concentrations among all phytochemicals. Across all profiles, the *Sida rhombifolia* ssp. *retusa* locality in Kurukshetra demonstrates greater chemical diversity. A cone graph (fig.2) visually represents this diversity, indicating that Kurukshetra contains 28 different chemicals, Panipat has 8, Saharanpur has 5, and both Haridwar and Meerut each have 4 distinct chemicals. Additionally, a doughnut-shaped graph

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(fig.1) has been prepared, illustrating common phytochemical classes among the profiles.

4. **Identification of specific Compounds:** Numerous compounds were identified through GC-MS analysis, including Fatty acids, Tocopherols, Terpenoids, Alkanes, Sterols, Hydrocarbons and halogenated hydrocarbons. Kurukshetra contains 28 different chemicals, Panipat has 8, Saharanpur has 5, and both Haridwar and Meerut each have 4 distinct chemicals. This analysis reveals that *Sida rhombifolia* ssp. *retusa* collected from Kurukshetra region shows high Bioactive potential among all different locations (table 3).

7. **Statistical Analysis:** Principal Component Analysis (PCA) revealed clustering of samples based on their geographical origin, supporting the hypothesis that the phytochemical composition of *Sida rhombifolia* ssp. *retusa* is influenced by the geographical location. The initial two PCA components, denoted as PCA1 and PCA2, exhibit significant variance compared to the remaining components. These first two principal components collectively account for 81.7% of the total variation, with PC1 contributing 57.3% and PC2 contributing 24.4%. The phytochemical principal components for five different localities of the same species are observed to form four distinct clusters. Notably, the Kurukshetra and Panipat accessions display considerable variation relative to each other when compared to the other three accessions. Among the latter—Panipat, Haridwar, Meerut, and Saharanpur—some similarities are evident, as indicated by the overlapping covariance plots (fig.3).

Table 3: Specific Phytochmeicals among five localities

Sr. no.	Phytochemicals	Locality name	Bioactive component classes
1	Undecane	Kurukshetra	Alkane
2	3-Methyl-2-(3,7,11-trimethyldodecyl) furan	Kurukshetra	Halogenated hydrocarbon
3	9-Octadecenoic acid, 1,2,3-propanetriyl ester,	Kurukshetra	Unsaturated fatty acid
4	Methyl 18-methylnonadecanoate	Kurukshetra	Saturated fatty acid
5	Octadecenoic acid	Kurukshetra	Saturated fatty acid
7	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)	Kurukshetra	Saturated fatty acid
8	Octacosanoic acid, methyl ester	Kurukshetra	Unsaturated fatty acid
9	Cyclooctane, tetradecyl-	Kurukshetra	Alkane
10	Tetratetracontane	Kurukshetra	Alkane
11	Docosanoic acid, methyl ester	Kurukshetra	Saturated fatty acid
12	Dotriacontane	Kurukshetra	Alkane
13	Hexatriacontane	Kurukshetra	Alkane
14	.delta.-Tocopherol	Kurukshetra	Tocopherol
15	.beta.-Tocopherol	Kurukshetra	Tocopherol
16	gamma.-Tocopherol	Kurukshetra	Tocopherol
17	Cholesterol	Kurukshetra	Sterol
18	Campesterol	Kurukshetra	Sterol
19	.beta.-Amyrin	Kurukshetra	Terpenoid
20	.gamma.-Sitostenone	Kurukshetra	Hydrocarbon

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21	9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta)	Kurukshetra	Hydrocarbon
22	Pregn-4-ene-3, 20-dione, 17-(acetyloxy)-16-methyl-, (16.alpha.)-	Kurukshetra	Sterol
23	Phytyl tetradecanoate	Kurukshetra	Saturated fatty acid
24	Olean-12-en-28-oic acid, 3-hydroxy-, methyl ester	Kurukshetra	Terpenoid
25	Phytyl linoleate	Kurukshetra	Unsaturated fatty acid
26	Phytyl tetradecanoate	Kurukshetra	Saturated fatty acid
27	Phytyl stearate	Kurukshetra	Hydrocarbon
28	Tetrapentacontane	Kurukshetra	Alkane
29	Phenol, 3,5-bis(1,1-dimethylethyl)-	Panipat	Hydrocarbon
30	1-Methyl-1-n-octyloxy-1-silacyclobutane	Panipat	Hydrocarbon
31	7-Tetradecen-1-ol, (E)-, TMS derivative	Panipat	Hydrocarbon
32	Sulfurous acid, pentadecyl pentyl ester	Panipat	Halogenated hydrocarbon
33	6-Bromohexanoic acid, 5-ethyl-3-octyl ester	Panipat	Halogenated hydrocarbon
34	1,2-Dichlorododecane	Panipat	Halogenated hydrocarbon
35	Tris(2,4-di-tert-butylphenyl) phosphate	Panipat	Halogenated hydrocarbon
36	Bis(2-ethylhexyl) phthalate	Panipat	Hydrocarbon
37	N-Methoxy-N-methylacetamide	Meerut	Hydrocarbon
38	Glutarimide, N-(3-pentyl)-	Meerut	Hydrocarbon
39	Cycloheptanone, 3-butyl-	Meerut	Hydrocarbon
40	(.+/-.)-.alpha.-Tocopherol acetate	Meerut	Tocopherol
41	Silane, methyldiethoxymethoxy-	Saharanpur	Halogenated hydrocarbon
42	7-Hexadecenoic acid, methyl ester, (Z)-	Saharanpur	Unsaturated fatty acid
43	Tridecane, 1,13-dibromo-	Saharanpur	Halogenated hydrocarbon
44	Hexadecyl isopropyl ether	Saharanpur	Hydrocarbon
45	(R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12	Saharanpur	Hydrocarbon
46	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl	Haridwar	Hydrocarbon
47	Boscartol F	Haridwar	Terpenoid
48	9(11)-Dehydroergosteryl benzoate	Haridwar	Sterol
49	Stigmast-4-en-3-one	Haridwar	Sterol

5. Implications for Medicinal Properties

The observed variations in the phytochemical composition of *Sida rhombifolia* ssp. *retusa* have direct implications for its medicinal properties. Different compounds have distinct pharmacological activities, and the geographical variation in their abundance may contribute to variations in the therapeutic efficacy of the plant in different regions (table 4).

6. Future Directions

This study opens avenues for further research to explore the pharmacological relevance of the identified compounds. In-depth investigations into the bioactivity of specific compounds and their potential synergistic effects will enhance our understanding of the medicinal potential of *Sida rhombifolia* ssp. *retusa* across diverse geographical locations.

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CONCLUSION

The comprehensive Gas Chromatography-Mass Spectrometry (GC-MS) analysis of leaf samples from *Sida rhombifolia* ssp. *retusa* collected across diverse geographical locations—Kurukshetra, Panipat, Meerut, Haridwar, and Saharanpur—has provided valuable insights into the phytochemical diversity of this medicinal plant. The study aimed to understand the impact of geographical factors on the synthesis of bioactive compounds and, consequently, on the potential medicinal properties of *Sida rhombifolia* ssp. *retusa*.

The results of the GC-MS analysis revealed pronounced variations in the phytochemical composition of *Sida rhombifolia* ssp*. retusa* among the studied locations Kurukshetra, Panipat, Meerut, Haridwar, and Saharanpur. Fatty acids, hydrocarbons and terpenoids, exhibited diverse profiles among other secondary metabolites (fig.4). This variation underscores the dynamic interplay between environmental factors and the plant's biochemical pathways, influencing the synthesis of bioactive compounds.

Principal Component Analysis (PCA) provided a visual representation of the clustering patterns of samples based on their geographical origin. Results of clustering states that species collected from Panipat and Kurukshetra display considerable variation relative to each other when compared to the other three accessions. Thus these accessions could be a distinct chemotype. Among the Panipat, Haridwar, Meerut, and Saharanpur—some similarities are evident, as indicated by the overlapping covariance plots (fig.3). The clustering observed in these analyses supports the hypothesis that geographical factors significantly contribute to the diversification of the phytochemical profile of *Sida rhombifolia* ssp*. retusa*.

The implications of these findings extend to the medicinal properties of *Sida rhombifolia* ssp. *retusa*. The observed variations in the phytochemical composition across different locations indicate potential differences in the therapeutic efficacy of the plant. Certain compounds, known for their pharmacological activities, may be more abundant in specific regions, influencing the plant's effectiveness in addressing particular health concerns.

In conclusion, this research contributes significantly to the understanding of the geographical variation in the phytochemical composition of *Sida rhombifolia* ssp. *retusa*. The findings underscore the importance of considering the environmental context in studies related to medicinal plants and natural products. Future research should examine deeper into the pharmacological implications of the identified compounds, exploring their synergistic effects and potential applications in medicine. The knowledge generated by this study lays the foundation for sustainable harvesting practices and targeted cultivation strategies, ultimately enhancing the utilization of *Sida rhombifolia* ssp. *retusa* in traditional and modern healthcare systems.

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Table 4: This table shows therapeutic uses of all bioactive components decoded thorough GC/MS profile

Sr. no.	Name of Compound	Therapeutic Uses	References
1	Undecane	Anti-inflammtory & Anti-allergic	[9]
2	Tetradecanoic Acid	Larvicidal & Repellant activity	[10]
3	Neophytadiene	Neuropharmacological effects	[11]
4	Phytol	Antimicrobial, Anti-cancer activities	[12]
5	Methyl stearate	Nematicidal Effect	[13]
6	Tetratetracontane	Anti-cancer & Anti-microbial;	[14], [36]
7	Dotriacontane	Antifungal	[15]
8	Squalene	Human Cancer Prevention; industry as an anti-wrinkle agent	[16]; [66]
9	delta-Tocopherol	Anti-oxidant	[17]
10	beta-Tocopherol	Anti-oxidant	[17]
11	gamma-Tocopherol	Anti-oxidant; anti-inflammatory	[17], [67]
12	Vitamin E	Anti-oxidants	[18]
13	Obtusifoliol	apoptotic effects on breast cancer	[19]
14	gamma-Sitosterol	Anti-diabetic; anti-inflammatory	[20], [24]
15	beta-amyrone	Antibacterial Activity, anti-inflammatory activity	[21]
16	beta-Amyrin	Anti-inflammatory; combat acute pancreatitis	[22], [66]
17	gamma-sitostenone	Antidiabetic (Inhibits alpha-glucosidase)	[23]
18	(9E,11E)-octadecadienoic acid	endogenous antiinflammatory factor,	[25]
19	Boscorto F	cytotoxicity, which may be the basis of its liver protection	[67]
20	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2	Attenuate inflammation induced by LPS.	[27]
21	(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-en-1-yl)cyclohex-2-enone	Nematocidal activity	[28]
22	9-Octadecenoic acid, 1,2,3-propanetriyl ester,	Anti-spasmodic and immune modulators	[65]
23	Tetratetracontane	Antioxidant and cytoprotective activities	[30]
24	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)	Hemolytic, pesticide, flavour, antioxidant	[31]
25	Docosanoic acid, methyl ester	production of biodiesel	[32]
26	Hexatriacontane	Road surfacing, paraffin wax and in candles	[56]
27	Octacosanoic acid, methyl ester	anti-inflammatory activity	[33]
28	Tetrapentacontane	fragrant molecules found in juvenile agarwood	[36]
29	Cholesterol	integrity, permeability, and fluidity of plasma membranes	[37]

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30	Campesterol	Reflect changes in body cholesterol homeostasis with atherogenic potential.	[38]
31	Stigmasterol	antiosteoarthritic, antihypercholestrolemic, cytotoxicity, antitumor,	[39]
32	4,22-Stigmastadiene-3-one	potential biomarker in soy bean	[40]
33	Loliolide	Antiapoptosis and Antiscratching	[41]
34	1-Methyl-1-n-octyloxy-1-silacyclobutane	Yellow Mosaic Virus Resistance	[42]
35	Acetic acid, 10,11-dihydroxy-3,7,11-trimethyl-dodeca-2,6-dienyl ester	insecticidal and antimicrobial properties	[44]
36	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,	skin diseases, gonorrhea, migraine, intestinal parasites, and warts	[45]
37	6-Octadecenoic acid, methyl ester, (Z)-	Antioxidant, Antimicrobial	[46]
38	alpha.-Tocospiro B	Anti-Inflammatory, Anti-Diabetic, and Cytotoxic Activities	[47]
39	Bis(2-ethylhexyl) phthalate	plasticizer used in medical products made with polyvinyl chloride	[48]
40	Tris(2,4-di-tert-butylphenyl) phosphate	anti-oxidant	[68]
41	N-Methoxy-N-methylacetamide	biological material or organic compound for life science related research	[32]
42	(.+/-.)-.alpha.-Tocopherol acetate	Protective effect for UVB irradiation	[50]
43	7-Hexadecenoic acid, methyl ester	antimicrobial	[51]
44	(R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12	Highly Potent, Orally Effective, and Metabolically Stable 5-LOX Inhibitor that Limits Inflammation	[52]
45	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Fragrance material	[53]
46	9,12-Octadecadienoic acid, methyl ester	Anti-inflammatory, Nematicide, Insectifuge,	[54]
47	Stigmast-4-en-3-one	Hypoglycaemic effect	[55]
48	Octadecanoic acid	Neuroprotective effect	[64]s
49	3-Methyl-2-(3,7,11-trimethyldodecyl) furan	Keratinocyte growth promoter	[56]
50	n-Hexadecanoic acid & Hexadecanoic acid methyl ester	Antiviral	[57]
51	8,11,14-Docosatrienoic acid, methyl ester	No previous record found	
52	Phytyl linoleate	used in the biosynthesis of prostaglandins and cell membranes	[56]
53	Pregn-4-ene-3, 20-dione, 17-(acetyloxy)-16-methyl-, (16.alpha.)-	Progestational activity, potent antiinflamatory	[59]
54	Phytyl stearate	Wax preprations	[60]
55	Phenol, 3,5-bis(1,1-dimethylethyl)-	anticancerous	[61]

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56	6-Bromohexanoic acid, 5-ethyl-3-octyl ester	New chemical compound	
57	Methyl 18-methylnonadecanoate	New chemical compound	
58	Cyclooctane, tetradecyl-	New chemical compound	
59	2-Pentadecanone, 6,10,14-trimethyl-	Antibacterial, anti-nociceptive	[62], [43]
60	9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta)	No previous record found	
61	Phytyl tetradecanoate	No previous record found	
62	Sulfurous acid, pentadecyl pentyl ester	No previous record found	
63	Olean-12-en-28-oic acid, 3-hydroxy-, methyl ester	No previous record found	
64	3-methyl-5-(2,6-dimethylheptyl)-1,5-Pent-2-enol	No previous record found	
65	1,2-Dichlorododecane	No previous record found	
66	24-Noroleana-3,12-diene	Pyrolytic product	[63]
67	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	No previous record found	
68	7-Tetradecen-1-ol, (E)-, TMS derivative	No previous record found	
69	Cycloheptanone, 3-butyl-	No previous record found	
70	Silane, methyldiethoxymethoxy-	No previous record found	
71	Tridecane, 1,13-dibromo-	No previous record found	
72	Hexadecyl isopropyl ether	No previous record found	
73	Glutarimide, N-(3-pentyl)-	No previous record found	
74	9(11)-Dehydroergosteryl benzoate	No previous record found	

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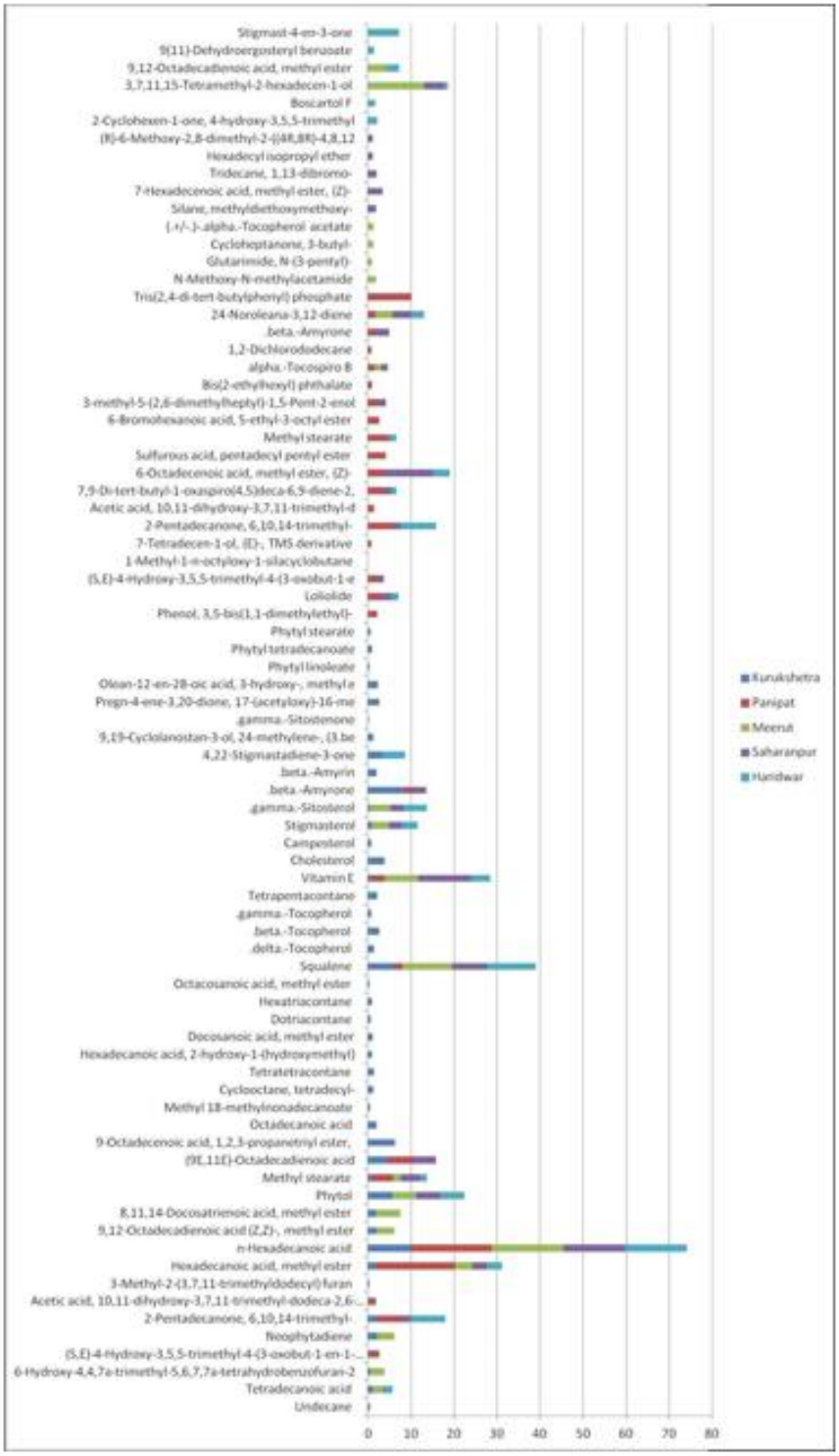

Figure 4: Stacked Bar Graph Shows Relative Presence of Chemical Compound

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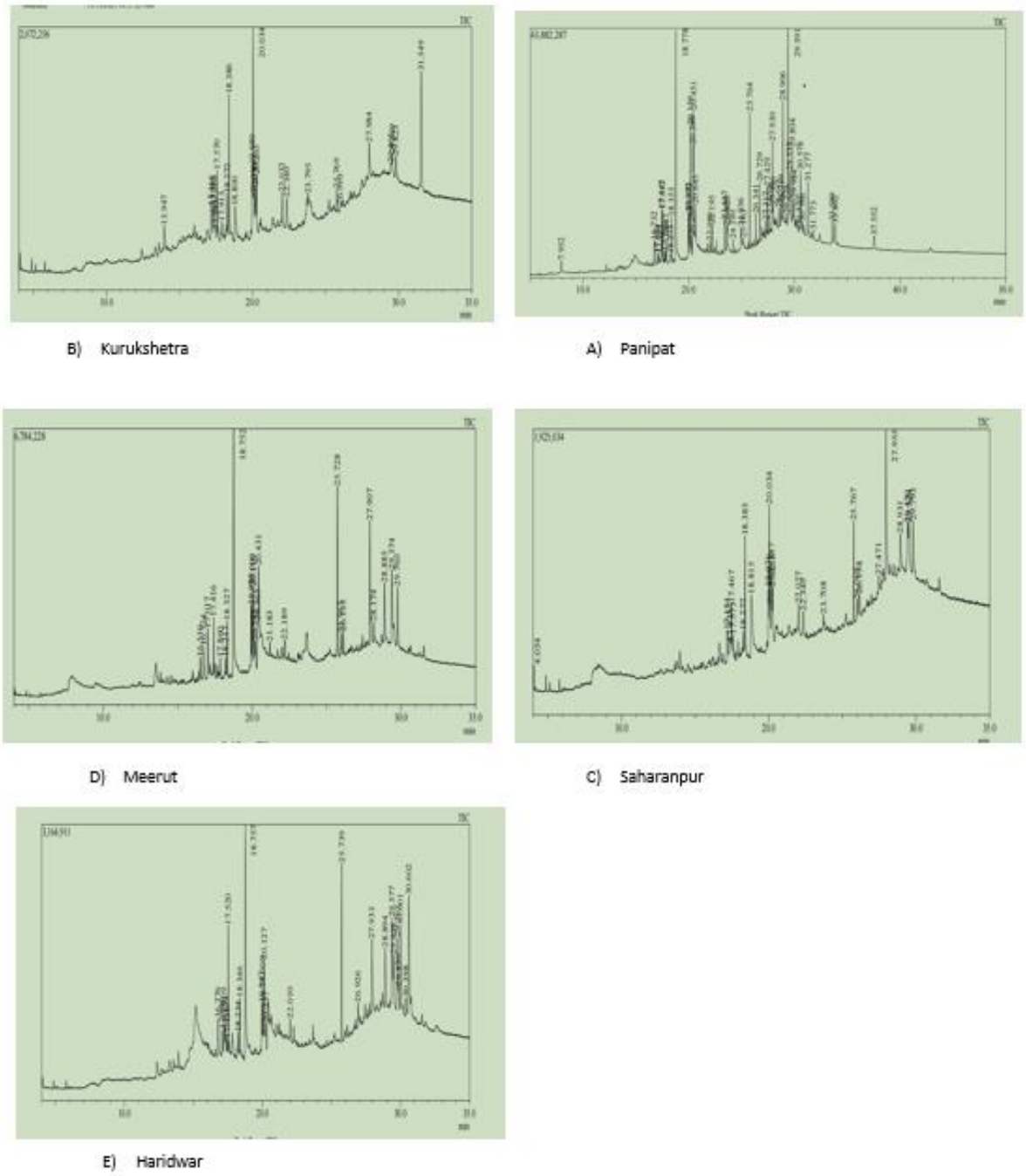

Figure 5: Chromatograms of all Five Localities

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