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Unraveling Phytochemical Diversity and Medicinal Potential of Sida rhombifolia Complex: A Chemotaxonomic Investigation from Northern India

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Abstract

The present study focuses on the collection and identification of Sida rhombifolia complex for its phytochemical diversity and chemotaxonomical investigations collected from two distinct regions in northern India, namely Uttar Pradesh (Saharanpur) and Uttrakhand (Dehradun). The objective of this research is to explore the medicinally potent phytochemicals inbuilt within this complex, using a chemotaxonomic approach. Ethyl acetate and methanolic extracts of leaf samples from each region were subjected to GC/MS analysis to distinguish the presence of ecologically significant metabolites. A comprehensive analysis exposed the identification of 96 distinct phytochemicals. This complex is characterized by Squalene, phytol, vitamine E, and methyl sterate. 4-Octadecenoic acid, methyl ester; 8,11,14-Docosatrienoic acid, methyl ester; 7-Hexadecenoic acid, methyl ester, fatty acids are new to S. rhombifolia complex. These newly identified chemical constituents can further be characterized by investigation through techniques such as NMR, QSAR, and in silico molecular docking to illuminate their potential medicinal properties. Subsequent exploration may facilitate the development of novel drug formulations for Ayurvedic medication. Furthermore, statistical analyses, including two-way random ANOVA, coupled with Partial Least Squares Discriminant Analysis (PLSDA) and Hierarchical Cluster Analysis (HCA), validate the substantial phytochemical diversity exhibited by S. rhombifolia var. scabrida compared to other profiles. This study emphasizes the unrevealed nature of S. rhombifolia var. scabrida, emphasizing the scarceness of finding elucidating its pharmacological properties. Additionally, comprehensive literature surveys, morphological identifications, and illustrative representations are provided to ensure the precision of morphological and chemotaxonomic categorization.

Key words S. rhombifolia Complex, Soxhlet, Phytochemicals, PLSDA, HCA, Random Forest, Venn diagram, SPSS, ANOVA

Highlights

1) Phytochemical diversity and chemotaxonomy of Sida rhombifolia complex.
2) Identified and characterized phytochemicals of Sida rhombifolia complex.
3) Emphasized the unrevealed nature of Sida rhombifolia var. scabrida.

1. Introduction

The genus Sida, includes approximately 247 species [1]. According to current estimate Sida comprises of 21 taxa with 17 species, 2 subspecies and 02 varieties [2] including 7 species from Uttar Pradesh [3]. Sida rhombifolia L. (Family Malvaceae) is a popular drug plant distributed throughout the tropical and sub-tropical regions of the world. The huge morphological variabilities have made this complex difficult to identify. About thirty binomials were proposed to this species by several taxonomists and often regarded this species as Sida rhombifolia complex [4]. Critical systematic studies based on the vegetative features from seedling stage to the adult plants and reproductive characters of these taxa resulted in the reduction of many species and the determination of three different plants as S. rhombifolia complex [5]. Within the Sida rhombifolia complex, S. rhombifolia ssp. retusa is the most studied subspecies, while S. rhombifolia var. scabrida is the least studied (Supplementary Table 1). Sida rhombifolia is considered as type species for genus Sida [6]. This ethnomedicinal plant is popularly called as 'Mahabala' arrowleaf Sida by natives and kurumthotti in Ayurvedic medicine. Traditionally, it was used in India in the form of extracts/powder/paste by tribal populations for treating common ailments like cough and cold, fever, stomach, kidney and liver disorders and inflammations [7].

S. rhombifolia ssp. retusa (L.) Bross., commonly known as 'Bala' holds a significant place in traditional medicinal practices owing to its diverse therapeutic properties (Supplementary Table 1). The plant has been used historically in many cultures as a treatment for a variety of illnesses, including cancer, skin maladies, inflammatory and respiratory disorders, as well as antibacterial and antioxidant-related health problems [8]. Its oil is utilized to nourish the nerves, brain, and spinal cord. Additionally, it is utilized as a rejuvenator to treat illnesses by encouraging tissue healing and reducing stiffness, discomfort, and inflammation [9]. The crude root extract of this species has a sedative effect and significantly increases mice's ability to sleep on pentobarbitone [10]. A thorough literature survey revealed that a very few work [11] has been done on S. rhombifolia var. scabrida. Accurate identification and differentiation among these taxa are vital due to their traditional use in herbal remedies and potential therapeutic applications. Two major problems with S. rhomifolia complex are the correct identification of different taxa and druggable phytochemicals from the complex. By combining macroscopic observations and chemical profiling, we aimed to provide a robust method for the accurate

identification of taxa within the S. rhombifolia complex (Supplementary Table 2 & 4). The current investigation provides ample amount of information in support of correct identification of the taxa under this complex using combination of morphological and phytochemical variations.

2. Materials and Methods

2.1 Sample Collection, Identification, and Illustration

S. rhombifolia and S. rhombifolia ssp. retusa were collected from Saharanpur (UP), while S. rhombifolia var. scabrida collected from Dehradun (UK). These taxa were identified after intense morphological study using Euromex CMEX 5 DC 5000C morphology microscope (Fig. 1). Each plant specimen was meticulously identified by legal deeds [3, 12-14]. Voucher specimens have been submitted in herbarium and museum of Botany Department, Chaudhary Charan Singh University, Meerut for future reference.

2.2 Extraction, detection and quantification of phytochemicals

Leaves from each taxa were air-dried and powdered. Thereafter, Soxhlet extraction was done with methanol and ethyl acetate solvents to obtain crude extracts rich in bioactive compounds. Extracted crudes with their relative solvents are denoted as:- E. rhombifolia = Ethyl acetate extract of Sida rhombifolia, E. retusa = Ethyl acetate extract of Sida rhombifolia ssp. retusa, E. scabrida = Ethyl acetate extract of Sida rhombifolia var. scabrida, M. rhombifolia = Methanolic extract of Sida rhombifolia, M. retusa = Methanolic extract of Sida rhombifolia ssp. retusa, M. scabrida = Methanolic extract of Sida rhombifolia var. scabrida. The extracts were analyzed by GC-MS [15] to identify phytochemicals. A high-resolution fused silica capillary column (SH-Rxi-5Sil MS, 30m x 0.25mm x 0.25μm, 5% biphenyl/95% dimethyl polysiloxane) was used with helium carrier gas (1.0 mL/min). The oven temperature program started at 50°C and ramped to 300°C at 5°C/min. Samples (1 μL in methanol) were scanned at m/z 50-650. Library searches and retention time comparisons along with peak spectra identified individual compounds. Peak areas provided their relative abundance.

2.3 Identification of Compounds and Metabolomics Data Processing

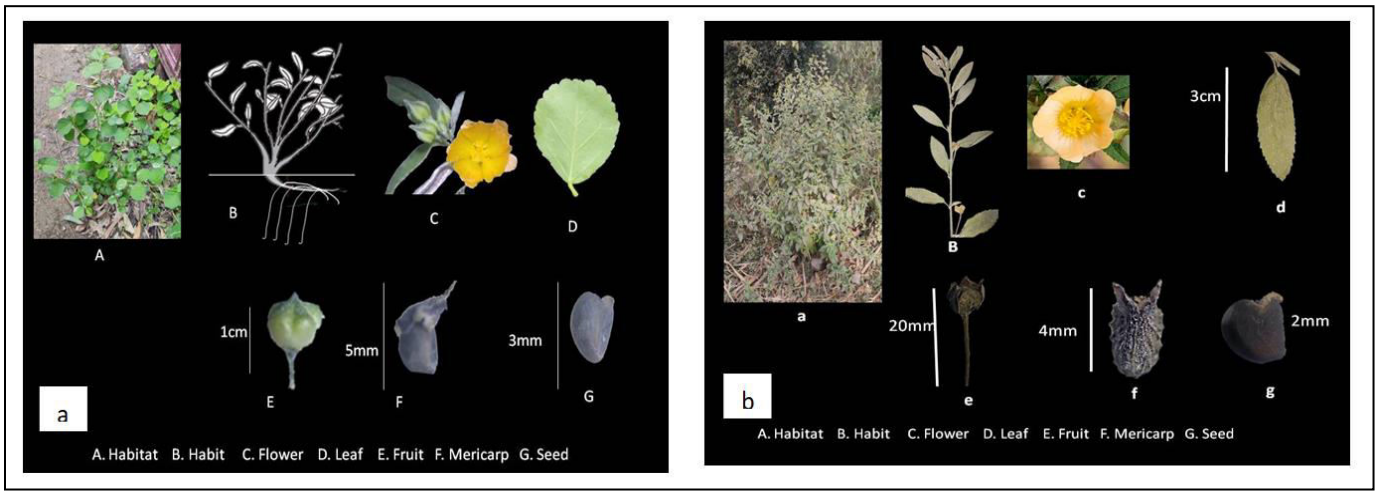
Compounds were tentatively identified by comparing their mass spectra with existing databases such as NIST (National Institute of Standards and Technology) (https://www.nist.gov/) and Pubchem [16] supplemented by relevant literature. Raw data (MS Excel) was converted to .csv file format and imported into MetaboAnalyst 6.0 for PLSDA, HCA, and Random Forest analyses [17] to characterize metabolites. After GC-MS analysis of the S. rhombifolia complex, data was normalized and analyzed using MetaboAnalyst 6.0 to identify principal components (PCs) through

PLSDA, HCA, and Random Forest. Furthermore, 2 way random ANOVA performed in MS excel, after data analysis through Kurtious distribution in spss software for studying phytochemical variation among different taxa.

3. Results and Discussions

3.1 Morphological studies

To minimize identification errors among closely related species, we employed a morphological analysis considering more than thirty contrasting characters (Supplementary Table 4). S. rhombifolia ssp. retusa is characterized by its prostrate habit and variable leaves with retuse and truncate apices. In contrast, S. rhombifolia and S. rhombifolia var. scabrida are erect, with differences primarily in peduncle articulation, mericarp number, and awn size. Specifically, S. rhombifolia has middle articulation, whereas S. rhombifolia var. scabrida has articulation at the lower base of the pedicle [18].



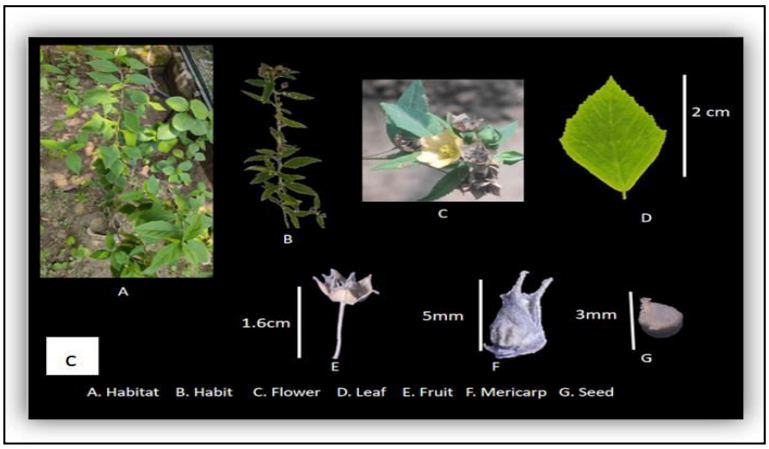


Fig.1 Illustrations of S. rhombifolia Complex whereas a) S. rhombifolia b) S. rhombifolia ssp. retusa c) S. rhombifolia var. scabrida

3.2 Comparative Phytochemical Profiling of S. rhombifolia complex

Phytochemical analyses include GC/MS of 6 profiles of three taxa (Fig.2). Each peak in the chromatogram allow for visual identification of phytochemicals based on their retention times. Each peak represents a specific compound, with its relative abundance (Supplementary Table 2). The profiles exhibit variability and highlight potential differences in phytochemical composition. Interestingly, squalene, phytol, vitamine E, and methyl sterate are consistently present across all profiles. GC/MS analysis of extracts identified 96 distinct metabolites. Among these, 72 exhibited specificity for this complex, while the remaining 24 showed similarity among taxa (Supplementary Table 2). These compounds have various biological activities like antimicrobial, antidibetic, antifungal etc. (Supplementary Table 1). Analysis of identified metabolites revealed the presence of various categories, including fatty acids, tocopherols, terpenoids, alkanes, alkenes, sterols, delta lactones, and hydrocarbons. Metabolites with their respective peak% are given in Fig. 3. S. rhombifolia and S. rhombifolia var. scabrida have 25 distinct phytochemicals while, S. rhombifolia ssp. retusa have 22 different biological compounds. Interestingly, S. rhombifolia ssp. retusa from Saharanpur exhibited the lowest number of identified metabolites (Supplementary Table 3).

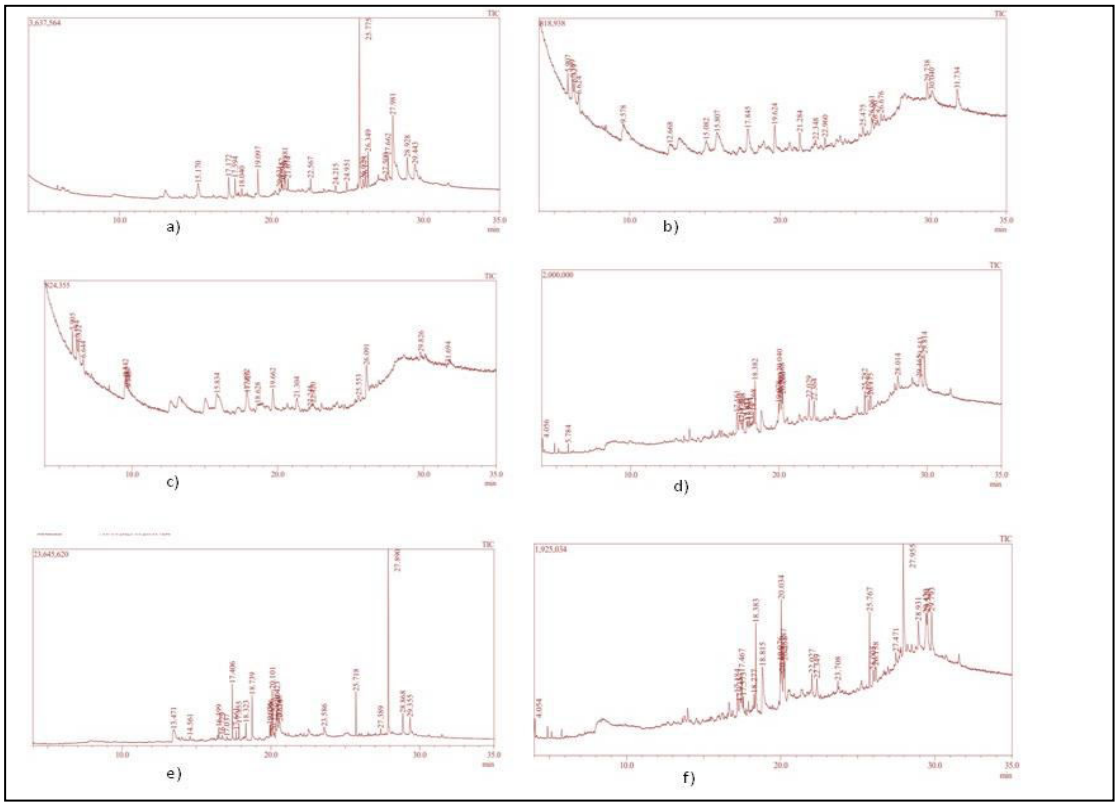


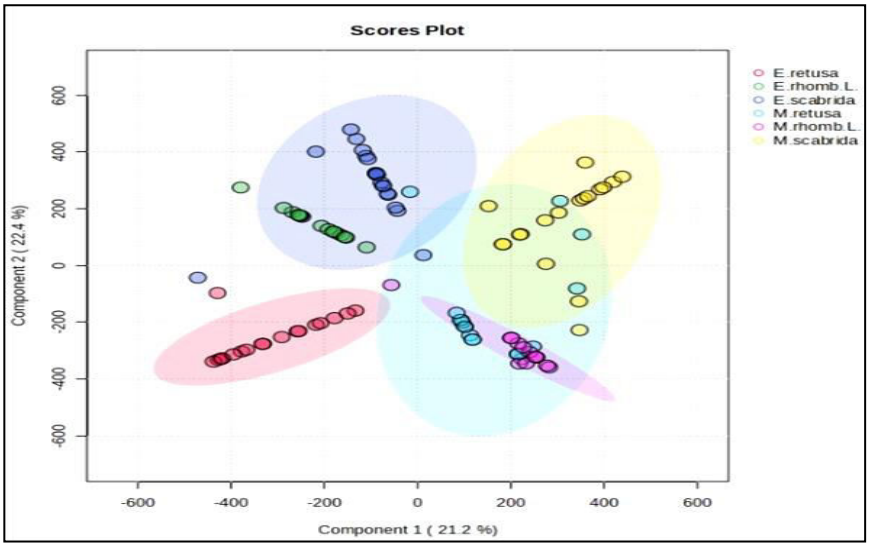
Fig. 2 Chromatograms of S. rhombifolia complex: Ethyl acetate extracts are; a) S. rhombifolia b) S. rhombifolia ssp. retusa c) S. rhombifolia var. scabrida; Methanolic

extracts are: d) S. rhombifolia e) S. rhombifolia ssp. retusa f) S. rhombifolia var. scabrida.

4. **Statistical Analyses**: Statistical analysis includes two-way random ANOVA, coupled with Partial Least Squares Discriminant Analysis (PLSDA) and Hierarchical Cluster Analysis (HCA), validate the substantial phytochemical diversity exhibited by S. rhombifolia var. scabrida compared to other profile.

4.1 The partial least square-discrimation analysis (PLS-DA)

The results of PLS-DA showed a substantial amount of variance in PCA1 (21.2%) and PCA2 (22.4%) accounting for 43.6% of the variation in the whole. The PLS-DA model was well-validated using a permutation test with $p < .001$ after 1000 permutations (Fig. 4) [19]. The presence of phytochemicals with their corresponding solvents (methanol and ethyl-acetate extracts of S. rhombifolia complex leaves) leads to the clustering of the samples. Their phytochemicals exhibit correlation in five distinct groupings. Phytochemicals recovered from the methanolic extract of the S. rhombifolia ssp. retusa shows substantial overlap with all PlSDA plots, while, S. rhombifolia var. scabrida exhibit highest variance among the studied groups.



E. retusa = Ethyl acetate extract of *Sida rhombifolia* ssp. *retusa*
E. Scabrida= Ethyl acetate extract of *Sida rhombifolia* ssp. *rhombifolia* var. *scabrida*
E. rhombifolia = Ethyl acetate extract of *Sida rhombifolia* L.
M. retusa = Methanolic extract of *Sida rhombifolia* ssp. *retusa*
M. Scabrida= Methanolic extract of *Sida rhombifolia* ssp. *rhombifolia* var. *scabrida*
M. rhombifolia = Methanolic extract of *Sida rhombifolia* L.

Fig.4 PLS-DA score plot showing sample clustering in S. rhombifolia complex

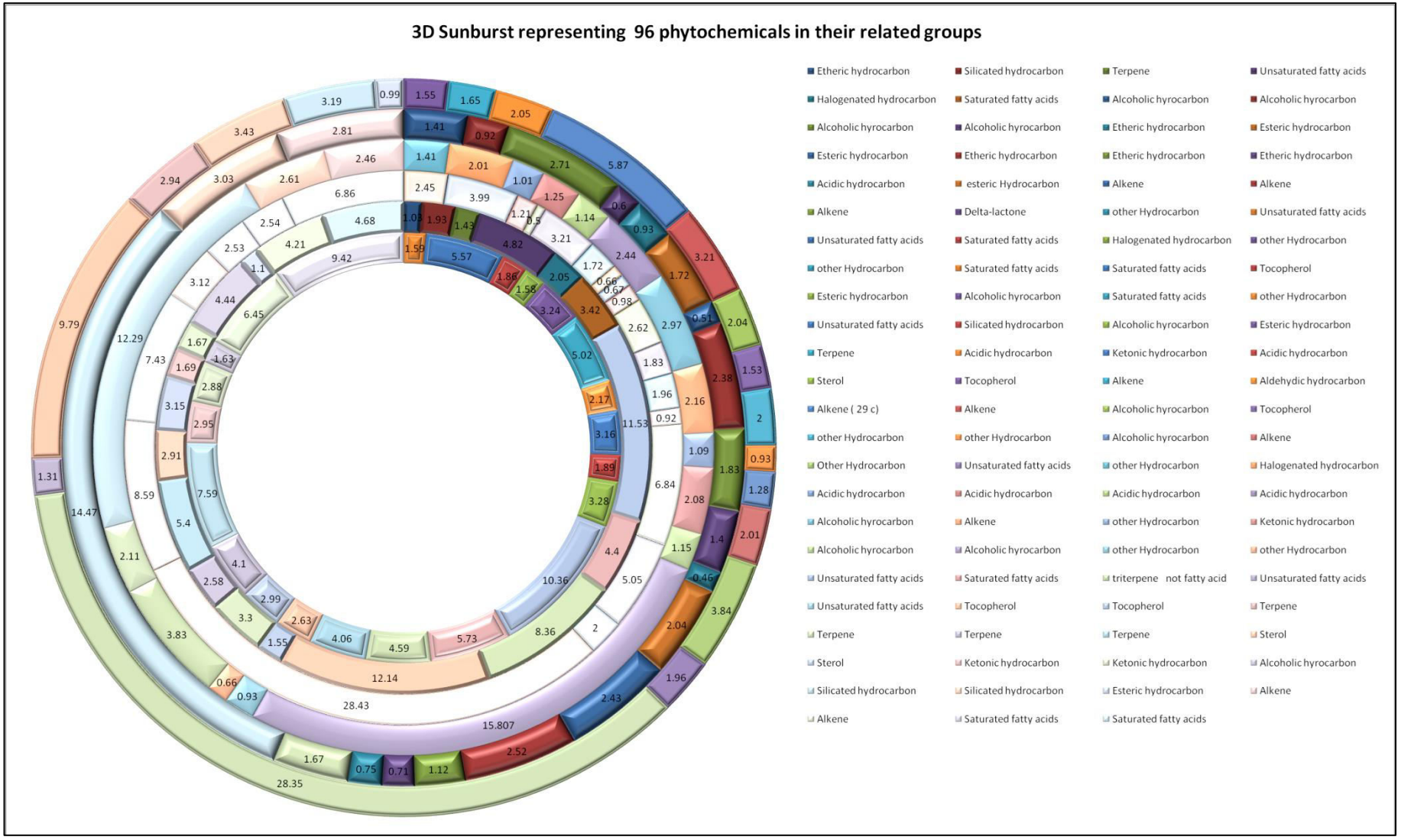


Fig.3 3D Sunburst (Wong et al., 2020) depicting percentage of the 96 putatively identified metabolites from each of the metabolite groups. 1st, 2nd, and 3rd layers representing methanol extract of S. rhombifolia, S. rhombifolia ssp. retusa & S. rhombifolia var. scabrida respectively. 4th, 5th, 6th layers representing ethyl acetate extract of S. rhombifolia, S. rhombifolia ssp. retusa & S. rhombifolia var. scabrida respectively.

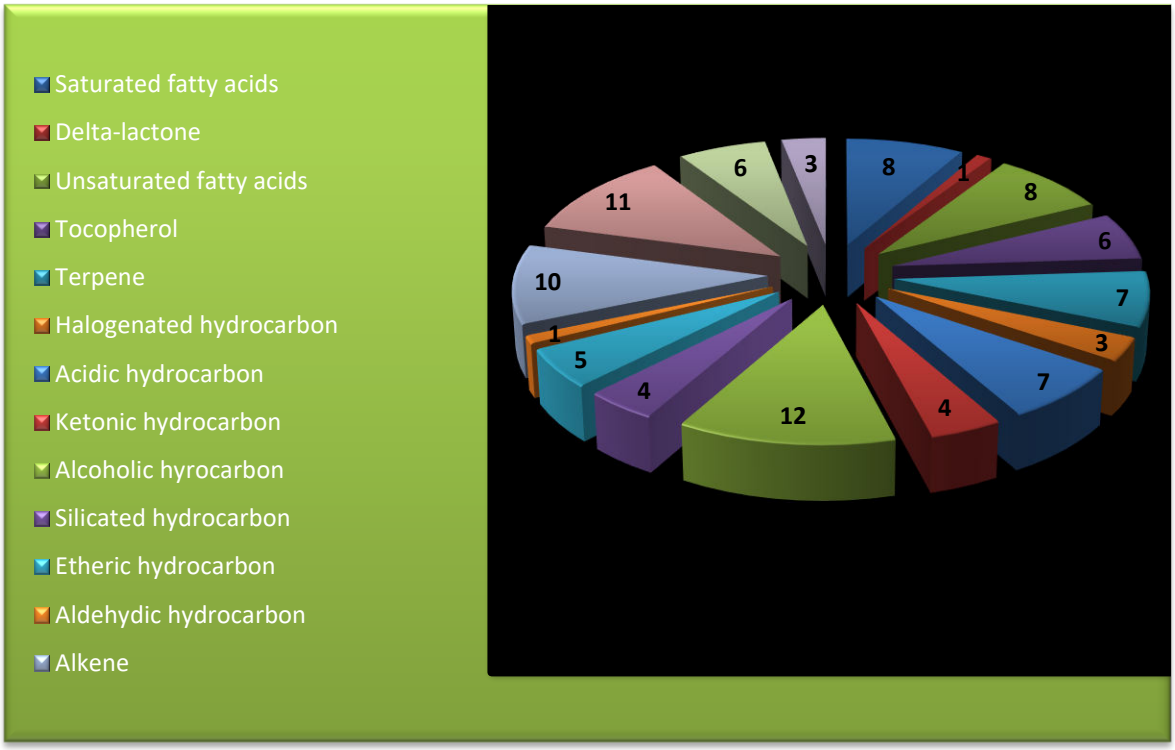


Fig.5 3D-pie graph illustrating relative composition of each secondary metabolite in S. rhombifolia complex.

4.2 Relative composition Phytometabolites in S. rhombifolia complex

The relative abundance of each type of metabolites found in the GC/MS profile of S. rhombifolia complex revealed the greatest contributions from alcoholic hydrocarbons, followed by halogenated hydrocarbon, acidic hydrocarbon, alkenes, unsaturated fatty acids, saturated fatty acids and others (Fig. 5). S. rhombifolia var. scabrida having highest diversity of alcoholic hydrocarbons than S. rhombifolia & S. rhombifolia ssp. retusa.

4.3 2D cluster bar graph is prepared through a statistical calculator by datatab (https://datatab.net/statistics-calculator/cluster/hierarchical-cluster-analysis-calculator). Mean of all phytochemicals according to their particular taxa and the solvents that go along with them is represented by this cluster bar chart. The methanolic extract S. rhombifolia var. scabrida had the highest concentration of tocopherols while, it is absent in ethanolic extract of S. rhombifolia ssp. retusa and S. rhombifolia. Besides, these unsaturated fatty acid shows highest concentration in E. retusa (Fig. 6). Relative abundance of each secondary

metabolites are jointly presents more in both extracts of Sida rhombifolia var. scabrida than Sida rhombifolia and Sida rhombifolia ssp. retusa.

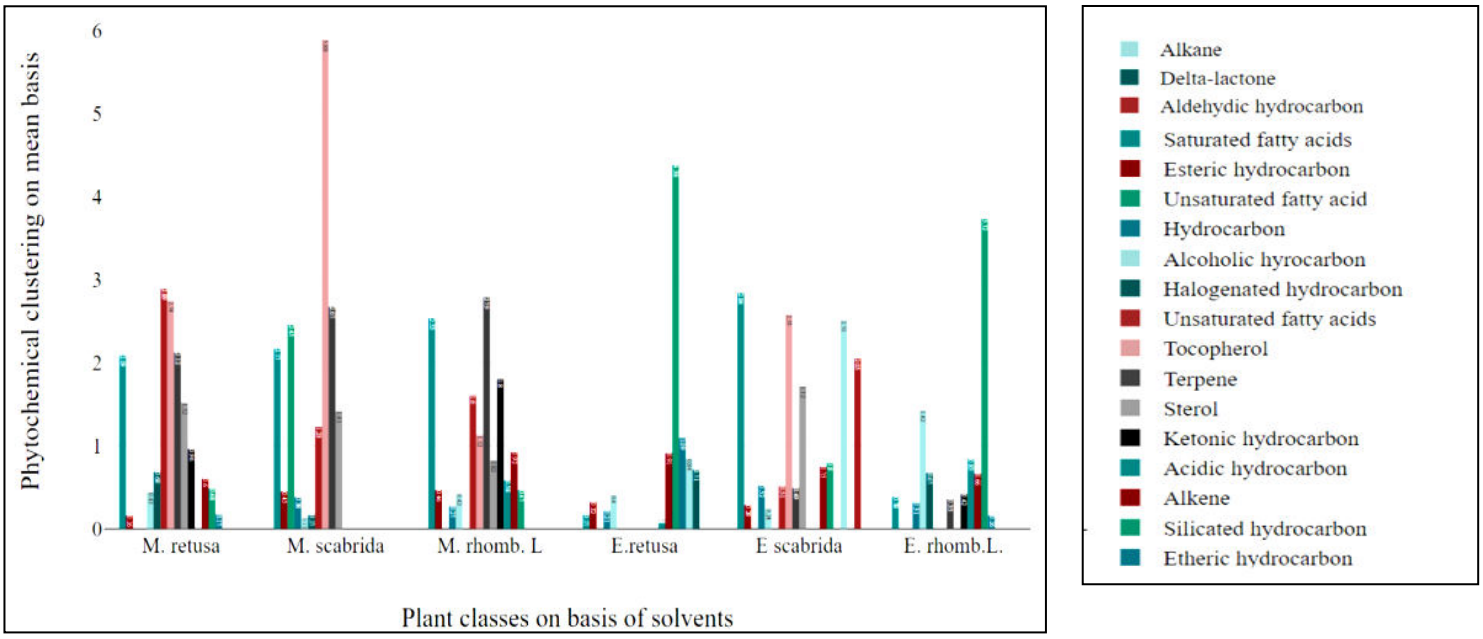


Fig.6 2 D bar graph representing phytochemical clustering of plant classes.

4.4 Hierarchial cluster analysis (HCA): Presentation of PLS-DA has been depicted in the form of dendrogram in HCA. A dendrogram based on Euclidean distances is prepared and average clustering by ward algorithm showed very distinct grouping of E. rhombifolia biological compounds, while biological compound in M. scabrida and M. retusa (Fig. 4) are little bit more mixed (Fig. 7). This mixing is due to presence of outliners. Top 5 outliners graph is prepared through random forest method (Fig.8). This HCA clustering clearly illustrates that Sida rhombifolia var. scabrida shows more variability as compare to Sida rhombifolia and Sida rhombifolia ssp. retusa.

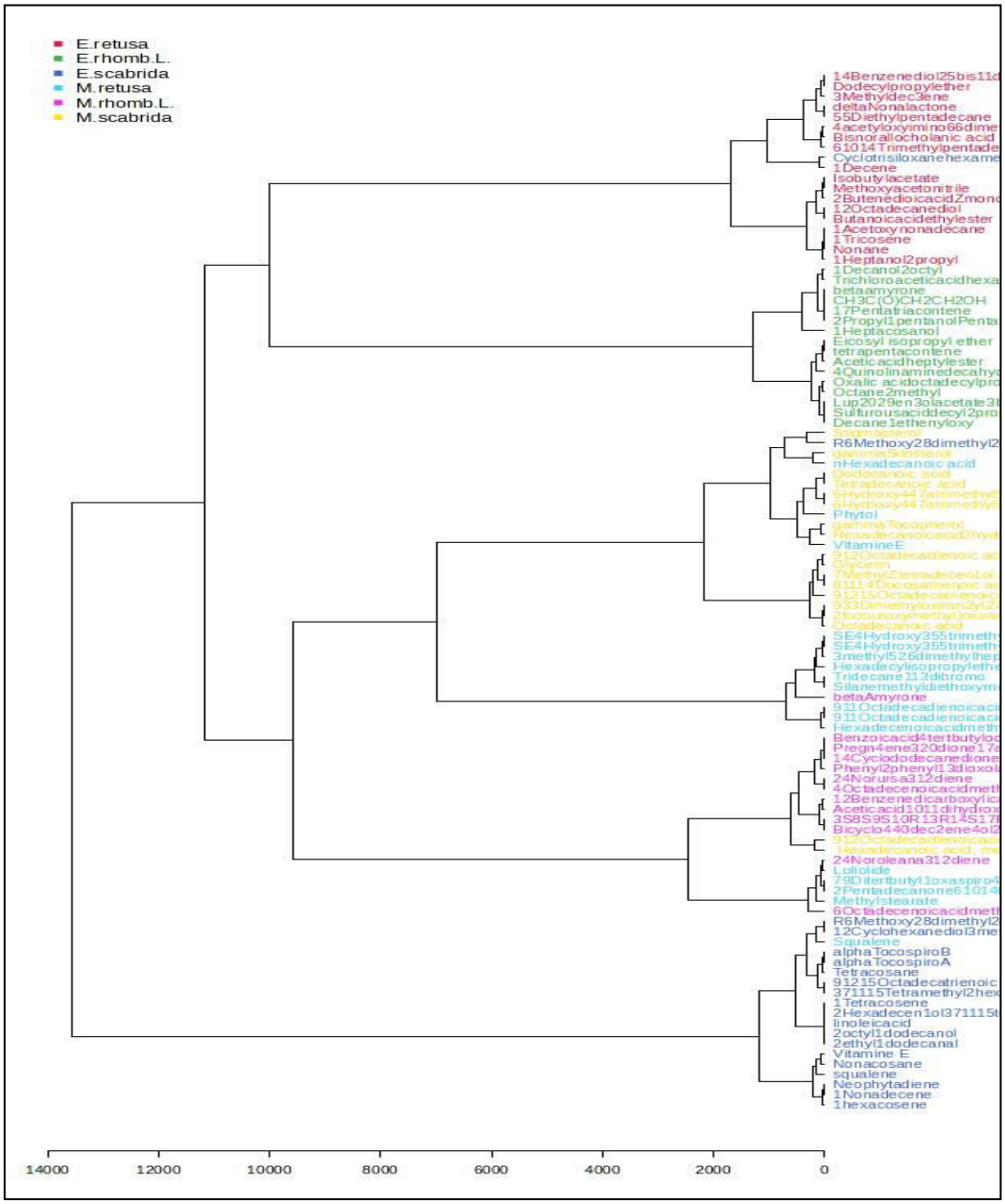


Fig.7 Phytochemical clustering of S. rhombifolia complex by PLSDA depicted by HCA Dendrogram

4.5 Random Forest: It explains the presence of those phytochemicals that are found outside their cluster (Fig.8). Cyclotrisiloxane, hexamethyl- is present in highest amount followed by (R)-6-Methoxy-2,8-dimethyl-2-(4R,8R)-4,8,12).

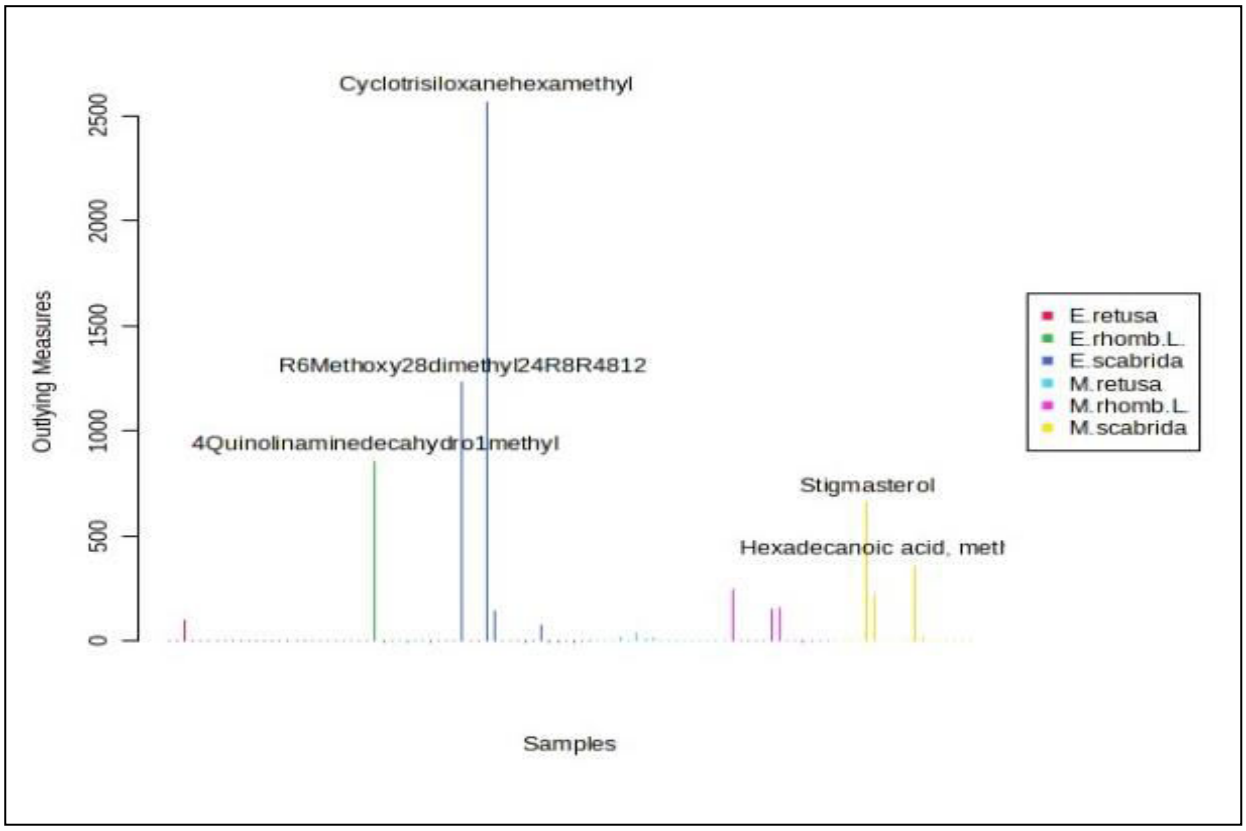


Fig. 8 Top 5 Outliners of dendrogram of S. rhombifolia complex depicted by Random Forest after sum normalization.

4.6 Venn diagram: Venn diagram (Fig.9) was created using a bioinformatics tool called TB [20]. Through overlapping regions, this Venn diagram illustrates the similarity between biological compounds, while non- overlapping portions display the percentage of specificity between various categories. Out of all the species, M. rhombifolia has the highest specificity (23%). Thus, this states that S. rhombifolia has least variability as their almost all chemical found in its cluster (Fig. 7). So, highest specificity exists in Sida rhombifolia, but highest variability exists in Sida rhombifolia var. scabrida.

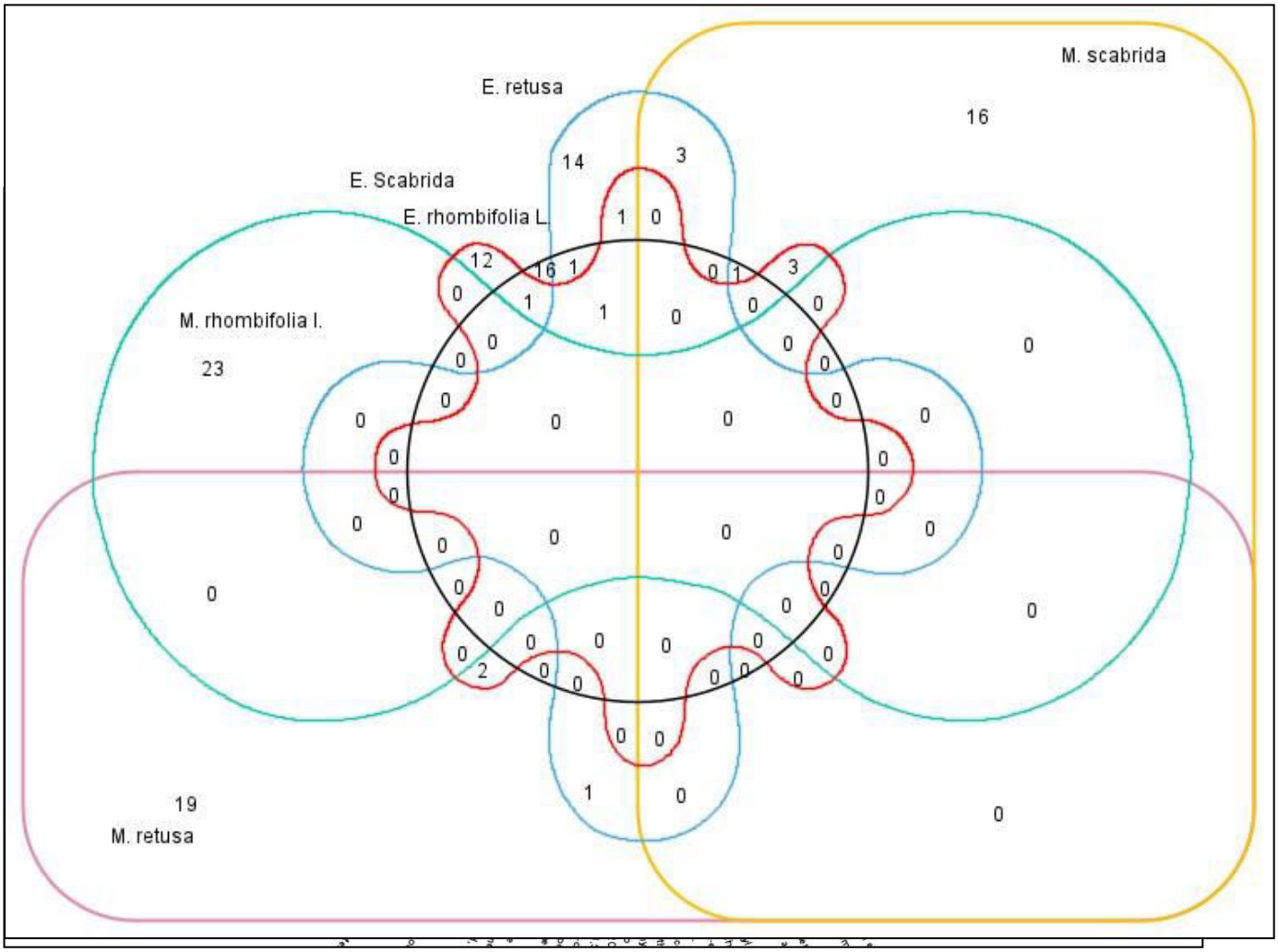


Fig.9 Illustrates the similarity between biological compounds.

4.7 Heatmap: A total of 96 metabolites from the S. rhombifolia complex based on Euclidean distances and Ward clustering have been identified. The metabolites show their relative amount on height percent basis. Orange color represent highest amount, while Grey color represents least concentration. Squalene shows largest concentration in methanolic extract of Sida rhombifolia var. scabrida, while vitamin E shows largest concentration in ethyl acetate extract. Squalene a saturated fatty acid found in all profile, while vitamin E being the 2nd most abundant followed by hexadecanoic acid and others (Fig.10).

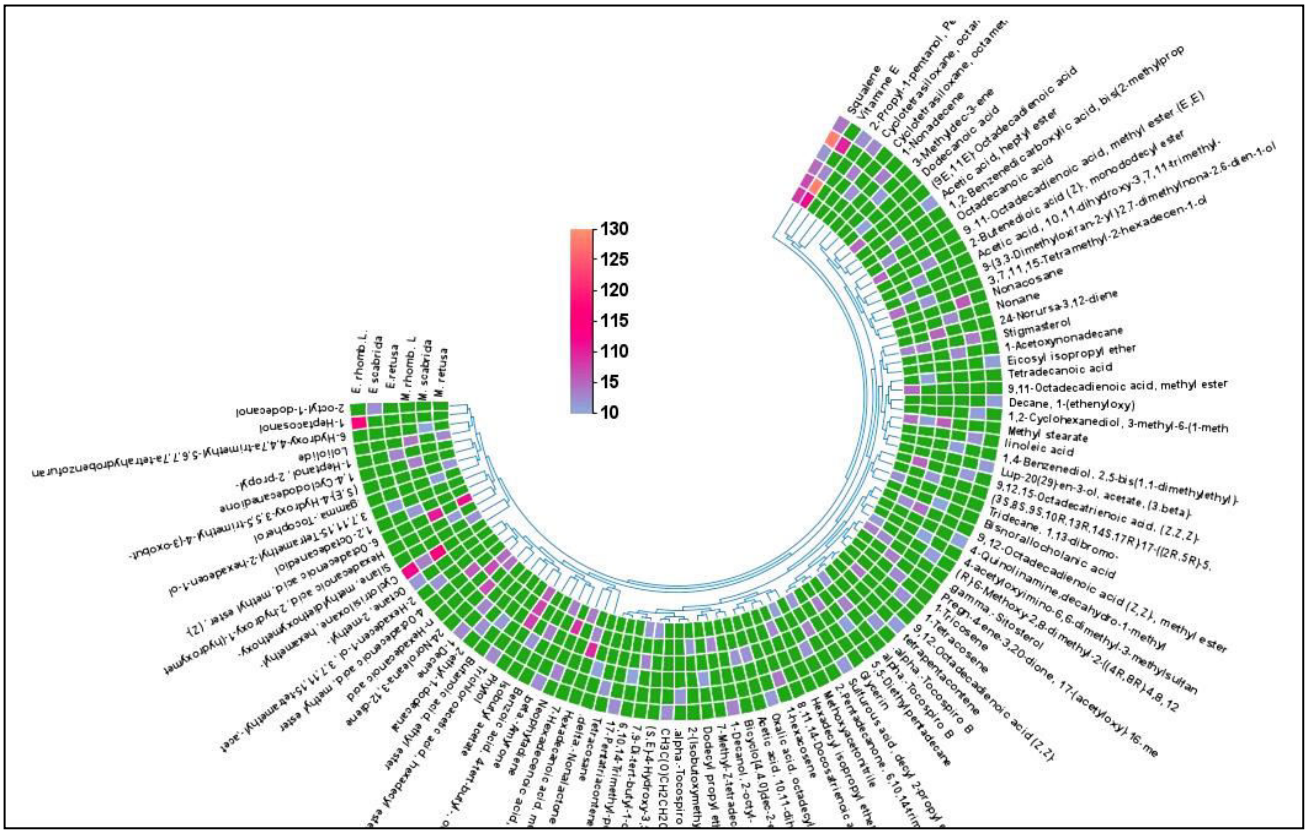


Fig.10 Heat map of 96 different phytochemicals of S. rhombifolia complex based on Euclidean distance and Ward clustering [20].

4.8 Chemotaxonomy relevance: Based on phytochemical analysis, we found that these three taxa viz. Sida rhombifolia, Sida rhombifolia ssp. retusa and Sida rhombifolia var. scabrida had 25, 25, 22 different and 24 similar phytochemicals respectively. Chemotaxonomical depiction of S. rhombifolia complex illustrate in Fig. 11 created by flourish database [21]. It may possible that these specific phytochemical are also responsible for the specific morphological characters because metabolic components encoded by genes. During this study we encountered several classes of secondary metabolite of chemotaxonomic significance (Fig. 5). We observed that 72 show least variability and high specificity for its taxa. Pregn-4-ene-3,20-dione-17-(acetyloxy)-16-methyl, a sterol; 1,4-Cyclododecanedione, a ketonic hydrocarbon; Acetic acid, heptyl ester, Oxalic acid, octadecyl propyl ester, Sulfurous acid, decyl 2-propyl ester, Trichloroacetic acid, hexadecyl ester, 1,2-Benzenedicarboxylic acid, bis(2-methylpropanol), Acetic acid, 10,11-dihydroxy-3,7,11-trimethyl-d, are acidic hydrocarbons; show specificity with S. rhombifolia. While, 1-Tricosene, Nonane, 3-Methyldec-3-ene, are alkenes;

1-Acetoxynonadecane, Methoxyacetonitrile, Butanoic acid, ethyl ester; Dodecyl propyl ether, Hexadecyl isopropyl ether are etheric hydrocarbons; 1,2-Octadecanediol, 1,4-Benzenediol, 2,5-bis(1,1- dimethylethyl), 1-Heptanol, 2-propyl-, 6,10,14-Trimethyl-pentadecan-2-ol, are alcoholic hydrocarbons exhibit specificity for Sida rhombifolia ssp. retusa. Besides these phytochemicals, 8,11,14-Docosatrienoic acid, methyl ester, 9,12-Octadecadienoic acid (Z,Z)- (linoleic acid), linoleic acid, are Unsaturated fatty acids, Dodecanoic acid, Tetradecanoic acid, Hexadecanoic acid, 2-hydroxy-1-(hydroxymet), are saturated fatty acids peculiar for S. rhombifolia var. scabrida.

Futhermore, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, Vitamine E, alpha.-Tocospiro B, Neophytadiene, . beta.-Amyrone, Loliolide, Phytol, .gamma.-Sitosterol, 2-Pentadecanone, 6,10,14-trimethyl-, Stigmasterol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, and 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-, diene-2, are consistently present in each taxa.

Plant phenotype can be affected by different plant metabolites [22]. Production of metabolites in plants is affected by gene interaction and gene expression which ultimately affect the phenotype of plants. Environmental variations in the aforementioned processes may play a key role. Probably, the similarities and differences in secondary metabolite profiles that we have observed are correlated to the morphological traits of these taxa and thus are of chemotaxonomic significance.

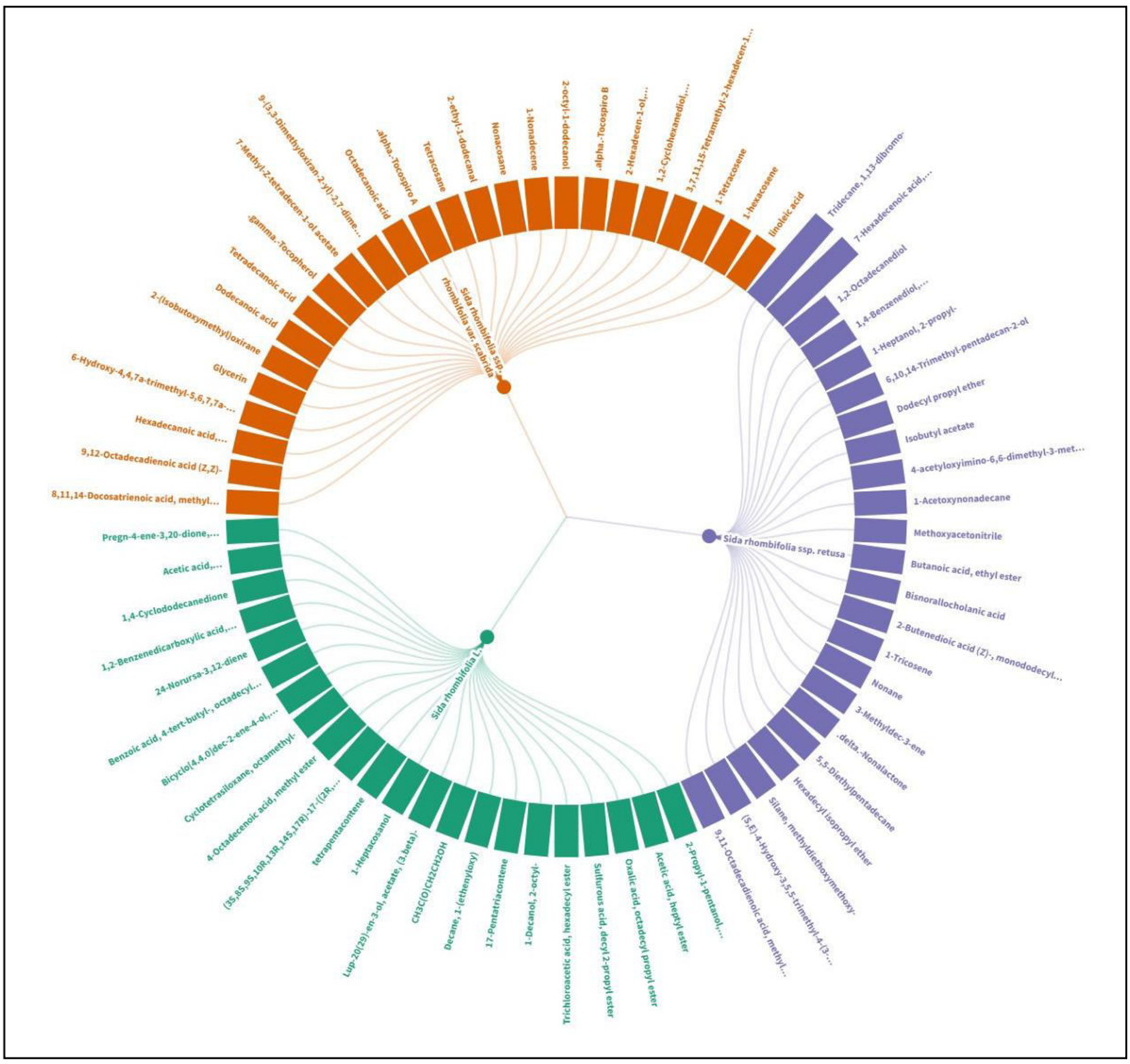


Fig.11 Chemotaxonomical depiction of S. rhombifolia complex.

4.9 Random 2 Way ANOVA: We took random 2 way ANOVA test for our analysis to check whether significant difference in phytochemicals of S. rhombifolia complex [19] (Table 1 & Table 2). Highest skewness reported in E. Scabrida. E. scabrida exhibits skewness and kurtosis statistics of 7.347 and 61.863, respectively, indicating a normally distributed dataset with a leptokurtic bell-shaped graph (Table 1). F critical value is greater than F calculated value (Table 2). This shows significant difference exhibit among the phytochemicals of S. rhombifolia complex. This difference also exists in Plsda graph (Fig. 4) with highest positive value of E. scabrida and its relative dendrogram of HCA (Fig. 7).

Table 1 Descriptive Statistics defines normal skewness. E. scabrida shows highest Kurtosis 61.863 value (157).

Descriptive Statistics										
	N	Minimum	Maximum	Mean	Std. Deviation	Variance	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
Secondary metabolites	99	1	18	9.15	5.509	30.354	.220	.243	-1.095	.481
M. retusa	99	0	12	.99	2.252	5.071	3.093	.243	11.038	.481
M. scabrida	99	0	28	.98	3.239	10.489	6.427	.243	50.308	.481
M. rhomb. L	99	0	10	.99	2.092	4.377	2.436	.243	5.900	.481
E. retusa	99	0	14	.47	1.574	2.476	6.822	.243	56.457	.481
E. scabrida	99	0	28	.81	3.103	9.626	7.347	.243	61.863	.481
E. rhomb.	99	0	16	.59	2.100	4.408	5.872	.243	38.026	.481
Valid N (listwise)	99									

Table 2 ANOVA results depicted through Microsoft Excel data analysis tab. Results significantly reveals F critical value higher than F tabulated value.

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Rows	1221.885	101	12.09787	2.027122	3.39E-07	1.27385
Columns	35.57212	5	7.114425	1.192094	0.311792	2.231864
Error	3013.842	505	5.968005			
Total	4271.3	611				

Discussion:

The Phytochemicals play an important role in Chemotaxonomic Delineation of Taxa. [23]. The presence of monoterpenoids in family Lamiaceae [23], sesquiterpenoids in Asteraceae [24], and furanocoumarins, anthranilic acid derived alkaloids and limonoids presence in Rutaceae are helpful their delimitations [25]. Likewise, presence of fatty acid mainly palmitic acid and oleic acid is helpful in establishing difference in members of family Malvaceae [26]. S. rhombifolia contain oleic and

palmitic acids as major constituents of their fatty acid profiles, in addition to minor amounts of malvalic and sterculic acids [27-28].

In this context we reported palmitic acid, methyl sterate, in all taxa; 9,12-Octadecadienoic acid (Z,Z)-, methyl ester from S. rhombifolia ssp. retusa and S. rhombifolia var. scabrida; 6-Octadecenoic acid, methyl ester, (Z) from S. rhombifolia and S. rhombifolia var. scabrida; 9,11-Octadecadienoic acid, methyl ester; 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-; and 7-Hexadecenoic acid, methyl ester, reported in S. rhombifolia ssp. retusa; 9,12-Octadecadienoic acid (Z,Z)-, methyl ester; 8,11,14-Docosatrienoic acid, methyl ester; Dodecanoic acid; Tetradecanoic acid in S. rhombifolia var. scabrida and 4-Octadecenoic acid, methyl ester from S. rhombifolia (Supplementary Table 2). 4-Octadecenoic acid, methyl ester; 8,11,14-Docosatrienoic acid, methyl ester; 7-Hexadecenoic acid, methyl ester, fatty acids are new to S. rhombifolia complex (26, 28-31]. The presence of secondary metabolites, including flavonoids, alkaloids, and phenolic compounds, in these species is postulated to play a pivotal role in conferring these therapeutic properties [32].

Moreover, tocopherols, terpenoids, sterols, alkaloids, aliphatics, flavonoids and coumarins are also reported in genus Sida beside fatty acids (29, 31-32). Henceforth, application of GC/MS technique used separately on each plant sample by the application of 2 different solvent (ethylacetate and methanol) in order to get different kind of phytochemicals namely fatty acids, tocopherols, terpenoids, sterols, hydrocarbons (Alcoholic, etheric, acidic, aldehydic and silicated) and delta lactones in which, fatty acids and hydrocarbon occupies highest position among all (Fig. 5). Total 96 phytochemicals reported in this study (Supplementary Table 2 & Fig. 3) with 72 shows its specific existence with their relative in different taxa i.e. 25 belongs to S. rhombifolia, 22 belongs to S. rhombifolia ssp. retusa and another 25 encountered in S. rhombifolia var. scabrida (Supplementary Table 2). Besides fatty acids, hydrocarbons, tocopherols and terpenoids evidently proved their presence in this study. Delta lactone and .alpha.-Tocospiro A, reported only in S. rhombifolia var. scabrida having fragrance, flavoring and antituberculosis properties, respectively (Supplementary Table 3). No previous records are reported for delta lactone, .alpha.-Tocospiro A, and beta amyrone for genus Sida [29-33). Nevertheless, beta amyrone in S. rhombifolia and in S. rhombifolia ssp. retusa has been known for their anti-inflammatory property [34].

Eventually, ascribing our taxa with the relative presence of secondary metabolites we reported Squalene, Methyl sterate, Phytol, hexamethyl-Cyclotrisiloxane and Vitamin E in all three taxa. However, Squalene was not reported in genus Sida [29, 32-33]. In addition to this Rodrigues (2020) reported Squalene in Sida cordata.

In this way squalne with delta lactone & 7-Hexadecenoic acid, methyl ester from S. rhombifolia ssp. retusa, .alpha.-Tocospiro A & 8,11,14-Docosatrienoic acid, methyl ester; from S. rhombifolia var. scabrida, beta amyrone & 4-Octadecenoic acid, methyl ester; from S. rhombifolia, can be counted as new phytochemicals for this complex taxa. We also reported several hydrocarbons with different functional groups (Supplementary Table 2). Their presence also illustrated in PLSDA grouping, and HCA dendrogram. Application of visualization tools like 3d sunburst explain relative presence of each phytochemical in each group with respective percent of their occurrence.

Nevertheless, in defining their Chemotaxonomical importance, these phytochemical are responsible for their medicinal efficacy (Supplementary Table 3). In the era of pharmacological importance, plants and its products have been used extensively since ancient time [34]. Thus, the effectiveness of medicinal plant extracts against various diseases has been studied extensively worldwide [34]. These taxa shows Anti-ulcer activity [35], hepatoprotective activity [36], Hypoglycemic and Hypolipidemic [37], antioxidant potential [11], Antivenom [38], Boils or abscesses [39], Abortifacient [40], Antifungal activities [41-44] and many more has been surveyed and enlisted in this study (Supplementary Table 1). All taxa of this complex have pharmacological importance as written and explained by Dinda et al., (2015) and Mishra et al., (2024). In this pharmacological survey we also reported several new phytochemical (Supplementary Table 3) whose biological activities are to be discovered.

Conclusion:

The present study offers valuable insights into the phytochemical composition and chemotaxonomic significance of the Sida rhombifolia complex. It focuses on different taxa collected from distinct regions in northern India. Through meticulous analysis utilizing GC/MS, a total of 96 distinct phytochemicals were identified, including fatty acids, tocopherols, terpenoids, and hydrocarbons, among others. Notably, certain compounds were found to be specific to particular taxa within the complex, highlighting the importance of comprehensive chemical profiling in botanical classification and medicinal research. The identification of novel compounds such as delta lactone and 7-hexadecenoic acid methyl ester underscores the potential pharmacological significance of this complex. Statistical analyses including PLS-DA, HCA, and ANOVA further support the substantial phytochemical diversity exhibited by different taxa. This study emphasizes the need for further exploration and validation of the medicinal properties of these compounds, which could potentially lead to the development of novel drug formulations. Additionally, the comprehensive morphological and chemotaxonomic categorization provided here serves as a robust method for accurate identification and classification of taxa

within the S. rhombifolia complex, emphasizing its importance in both traditional herbal medicine and modern pharmacology.

##Supplementary Material

Supplementary Table 1: Ethanobotanical uses of Sida rhombifolia complex

Supplementary Table 2: Relative Peak percentage of 96 different phytochemical of Sida rhombifolia complex

Supplementary Table 3: Therapeutic uses of all bioactive components decoded thorough GC/MS profile.

Supplementary Table 4: Morphological studies of Sida rhombifolia complex.

Supplementary material is available with first author (poojagoel01995@gmail.com)

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