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RESEARCH ARTICLE

Morphological and phytochemical variability among *Vitex negundo* L. accessions from South Gujarat, India

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Abstract

Vitex negundo L. is a potential medicinal and aromatic plant that is important for herbal medicine production. In this study, to evaluate the morphological and phytochemical variability among 50 accessions of *V. negundo* from South Gujarat of India. The result revealed that among the 50 accessions, VN 47 (Waghai, Dang) observed the highest plant height (5.50 m), plant girth (52 cm), number of primary branches (28) and crown spread (4.80 m^2). And VN 20 (Mahuva, Surat) was identified as a promising accession for phytochemical parameters, exhibiting the highest total phenol content (22.19 ± 0.04 mg GAE/100 g DW), total flavonoid content (102.80 ± 1.47 mg QE/100 g DW), antioxidant activity (88.55 %) and essential oil content (0.193 %). Differences at the 5 % level ($p < 0.05$) were statistically significant. The principal component analysis of *V. negundo* accessions which revealed nine principal components that contributed to 76.6 % of the total variation in morphological and phytochemical parameters such as TFC, TPC, AA and EO have strong positive loading on PC1 and morphological plant height, canopy spread and number of primary branches were negatively correlated with PC1 and grouped in three main clusters, indicating that the observed diversity was largely driven by the influence of diverse genetic variability within accessions. This suggests superior accessions like VN 20 and VN 47 can be selected as chemotypes for further breeding programs, improvement and industrial application of *V. negundo.*

Keywords: antioxidant activity; aromatic plant; flavonoid; phenol; *Vitex negundo* L.

Introduction

Recently, interest in plant research has increased globally because of the potential use of plants in traditional medicine systems for treating a wide array of diseases. Numerous medicinal and aromatic plants (MAPs) have been identified and modern scientific approaches have been employed to study their authenticity, safety and therapeutic efficacy. These results highlight the significant potential of medicinal and aromatic plants in pharmacology (1). Medicinal and aromatic plants have gained considerable popularity worldwide. Various plant parts, including seeds, flowers, stems, roots, leaves and bark, are used as medicinal agents and sources of natural bioactive compounds for treating different ailments and as natural antioxidants. These plants contain high concentrations of non-nutritive, nutritive and bioactive compounds such as flavonoids, phenolics, anthocyanins and phenolic acids, as well as essential oils and minerals. MAPs are known for their distinct flavour, taste, excellent medicinal value and healthcare functions (2). Plants in nature possess unique phytochemicals and serve as valuable genetic resources for community-based medicines. Studying the morphological and phytochemical diversity among wild MAP populations is crucial for their domestication, cultivation, crop improvement, commercial production technology and the development of value-added products in agricultural systems. Such investigations have been conducted for several MAPs, including *Vitex negundo* L., *Syzygium Cumini* L., *Polygonatum verticillatum* L. and *Vitex trifolia* (3–9). *Vitex* is the largest genus in the subfamily Vitioicoideae of the family Verbenaceae and comprises 250 deciduous shrub species distributed worldwide. Recently, Lamiaceae has been placed on the basis of DNA sequence data (10). It has been classified under Lamiaceae. The genus is widely distributed in the tropical and subtropical regions of Australia, Asia and Africa, with a few South American species (11). *V. negundo* native to India and the Philippines, is an underexploited plant adaptable to various soil textures and climatic conditions. It grows in almost all the parts up to an elevation of 1500 m and naturally grows abundantly in wastelands, along roadsides, near water canals and river basins and is often used as a hedge plant around farms and homes.

The morphological variability in the *V. negundo* plant heights of 2–5 m with quadrangular branchlets, the leaves are palmately compound, petiole 2.5–3.8 cm long, 2–5 foliate and the middle leaflet is petiolate; the middle leaflet is petiolate; the trifoliate leaf is lanceolate or narrowly lanceolate, acute, entire or rarely crenate; the middle leaf is 5–10 cm long and 1.6–3.2 cm

broad, with a 1–1.3 cm petiolule; the remaining two subsessiles; the inner three leaflets have petiolules; the odor is agreeably aromatically glabrous above and tomentose beneath; and the texture is leathery (12). In Indian traditional medicine, *V. negundo* L. is known as "sarvaroganivarani," meaning "the remedy for all diseases." It is believed to cure many ailments, hence its Sanskrit and Hindi name "Nirgundi," which translates to "that which protects the body from diseases." The *Vitex* species found in India include *V. negundo*, *V. glabrata*, *V. leucoxylon*, *V. penduncularis*, *V. pinnata* and *V. trifolia* (13). In India, the plant has numerous uses, including basketry, dyeing, fuel, food, grain protection, field pesticide, growth promotion, manure and medicine for poultry, livestock and humans. It is utilised in all treatment systems—Ayurveda, Unani, Siddha and Homoeopathy (AYUSH).

Many studies have documented the antioxidant activity of *V. negundo* in both *in vivo* and *in vitro* models (14, 15). Antioxidants play crucial roles in protecting biological systems from oxidative stress. Plants possess a wide array of free radical-scavenging molecules, including terpenoids, phenolic acids, quinones, coumarins, lignans, tannins, nitrogen-containing compounds (such as alkaloids, amines and betalains), vitamins and other endogenous metabolites (16). Among these compounds, phenolic compounds, particularly polyphenolics, are frequently identified as allelopathic agents. Polyphenolics are known for their dietary role, medicinal importance, herbicidal potential and phytotoxicity. Owing to the pesticidal properties of plant extracts, their formulations with pesticides are recommended to be beneficial for agricultural practices. With respect to chemical antioxidants, the search for alternative sources is safer. Essential oils from a wider number of plant sources and *Vitex* spp. have been evaluated and reported to have excellent antioxidant properties (17).

Studying the morphological and phytochemical variability of plants is crucial for any crop improvement program. Phytochemical analysis facilitates the identification of potential chemotypes and assessing the biochemical composition of MAPs growing at different elevations can help determine the best genotype and optimal altitude for commercial cultivation (18). There is an urgent need to conserve MAPs through cultivation, making the standardisation of propagation methodologies a top priority. Cultivating these plants ensures a controlled and consistent supply of valuable products and protects species from extinction due to overexploitation of wild populations in Gujarat. Successful cultivation requires a focus on species improvement, with the first step being the assessment of genetic diversity. This allows breeders to select genotypes with desirable traits (19). Despite the extensive medicinal applications of *V. negundo*, there is a lack of comprehensive studies examining the combined morphological and phytochemical variability among its accessions, particularly in South Gujarat. The absence of standardised protocols for selecting high-yielding chemotypes and optimising cultivation practices further highlights the need for systematic evaluation. Valuable insights into genetic diversity will help in selecting superior accessions of *V. negundo* and developing superior clones thereafter for medicinal and industrial applications and contribute to the conservation and sustainable utilisation of this important medicinal plant. The present investigation was carried out to evaluate the morphological and phytochemical variability among 50 accessions of *V. negundo* L. collected from South Gujarat, India.

Materials and Methods

Study area

The South Gujarat Region of India is located between latitudes 21° 14' and 22°49' N and longitudes 72°22' and 74°15' E comprises seven districts, namely, Surat, Navsari, Narmada, Dangs, Tapi, Valsad and Dangs covering a total geographical area of 31,495 km^2 Fig. 1. To the north and northeast, it is bordered by Anand and

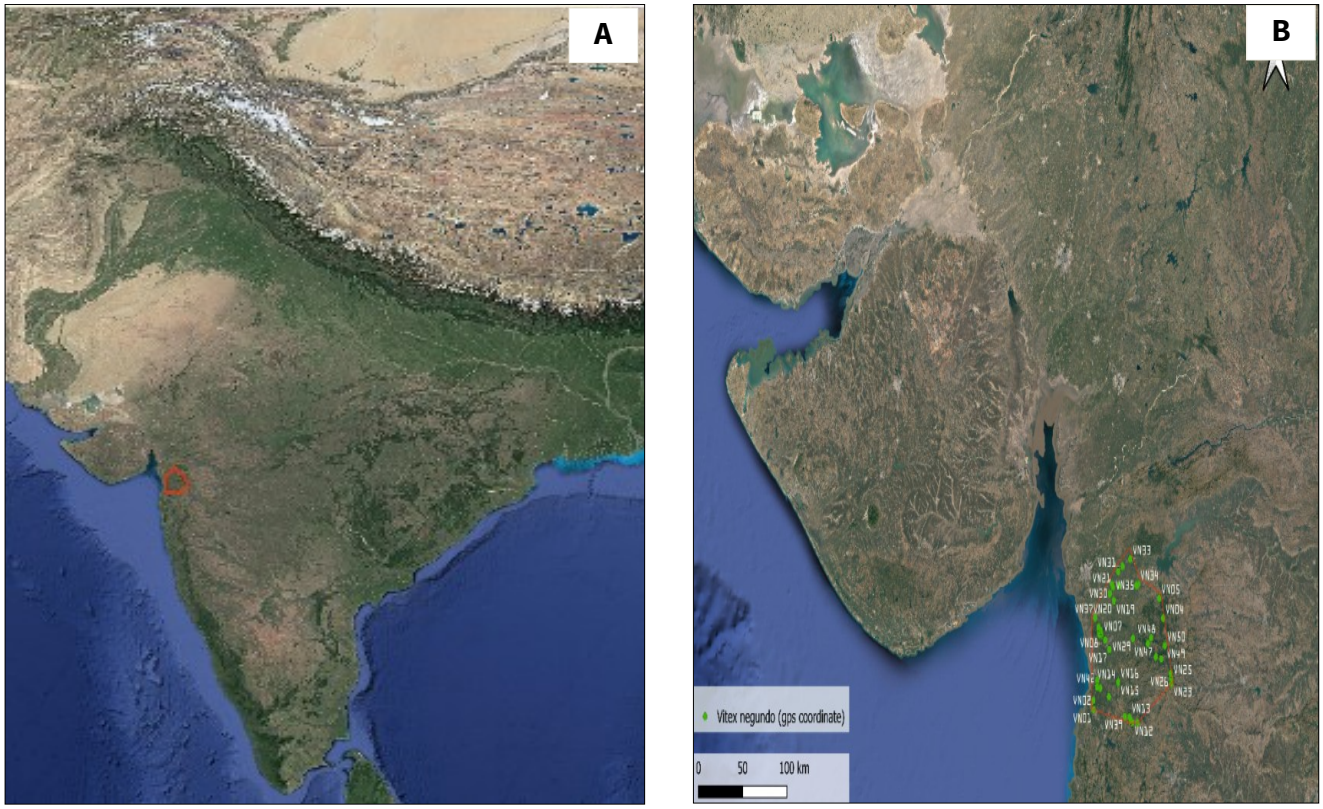

Fig. 1. (A) Map of India and (B) map of Gujarat state.

Dahod, while the state of Madhya Pradesh lies to the east and Maharashtra, Dadra and Nagar Haveli and Daman are to the south and southeast. The Arabian Sea and the Gulf of Khambhat are located northwest. South Gujarat covers an area of 17500 km^2. The region has a sub-humid climate with temperature variations ranging from 6 to 45 °C and annual rainfall varying from 250 mm in the North West to more than 1500 mm in South Gujarat. Twelve types of vegetation and nine land use classes were mapped in this region. Vegetation covers 20.40 % of the total geographical area, whereas forested areas account for 17.43 %. The predominant vegetation type is teak mixed dry deciduous forest, which covers 14.94 % of the region. Other key vegetation types include teak mixed dry and moist deciduous forests, mangrove scrub, riverine forests, rain-thorn forests, forest plantations, degraded forests, scrublands, *Prosopis juliflora* stands, grasslands and orchards (20).

Selection of accessions

For this study, accessions of *V. negundo* were selected from the naturally growing regions of the South Gujarat Region, which had been previously surveyed for this purpose. A voucher specimen 2023-VN 01 to VN 50 of the plant material was deposited in our departments' herbarium. A detailed survey was conducted between June and July 2023 to locate and identify the trees. The collection was performed via a selective sampling strategy; each collection was allotted an accession number and detailed passport information was recorded. During this survey, 50 accessions of *V. negundo* exhibiting favourable phenotypes were identified and marked via a global positioning system (GPS) device (Make and Model: e-Trax Vista, Garmin). The geographic information of the selected accessions of *V. negundo* is presented in Fig. 2 and Table 1.

Recording the morphological data of the plant

The morphological data of these trees were recorded. Plant height was measured from ground level to the tip of the plant with the help of a graduated survey rod. The height of each plant was measured in metres. The girth was measured at breast height from the base of the plant with the use of a measuring tape. Girth was measured in centimetres. The number of primary branches was recorded through visual observation. Measuring the spread of a

Table 1. Geospatial distribution of *V. negundo* accessions: GPS coordinates of sampling locations across South Gujarat

Accessions number	Latitude	Longitude	Elevation (MSL) m	Village	Tehsil (Block)	District
VN 01	20°8'08".3	72°91'43".9	24.74	Vapi	Vapi	Valsad
VN 02	20°43'54".5	72°91'23".9	28.21	Bagwada	Vapi	Valsad
VN 03	20°55'32".9	72°95 '48".3	38.83	Parnera	Valsad	Valsad
VN 04	20°91'74".0	73°66 '67".6	229.74	Mahal	Subir	The Dangs
VN 05	21°03'40".2	73°62'36".3	157.30	Umarada	Tapi	Tapi
VN 06	20°84'45".9	72°99'06".3	16.78	Khakhwada	Navsari	Navsari
VN 07	20°81'77".6	72°99'86".9	15.69	Gandevi	Navsari	Navsari
VN 08	20°82'07".8	72°98'92".5	11.19	Ajarai	Navsari	Navsari
VN 09	20°82'17".2	72°98'39".3	13.87	Hathiyawadi	Navsari	Navsari
VN 10	20°82'14".8	72°97'55".5	14.54	Dhamdachha	Navsari	Navsari
VN 11	20°30'65".7	73°39'01".5	550.11	Barpuda	Kaprada	Valsad
VN 12	20°30'44".2	73°33'25".2	452.83	Ghotan	Kaprada	Valsad
VN 13	20°33'65".5	73°30'22".6	484.04	Hatty	Kaprada	Valsad
VN 14	20°53'77".4	73°17'74".0	86.87	Dharampur	Dharampur	Valsad
VN 15	20°54'19".9	73°18'13".4	83.96	Dharampur	Dharampur	Valsad
VN 16	20°54'29".1	73°18'13".9	77.36	Dharampur	Dharampur	Valsad
VN 17	20°73'37".7	73°08'28".2	36.77	Khundha	Chikhali	Navsari
VN 18	20°84'25".0	72°99'25".4	23.60	Khakhwada	Navsari	Navsari
VN 19	21°01'74".6	73°13'54".1	32.62	Mahuva	Bardoli	Surat
VN 20	21°02'05".8	73°13'44".1	32.55	Mahuva	Bardoli	Surat
VN 21	21°10'02".1	73°12'34".1	39.74	Isroli	Bardoli	Surat
VN 22	21°11'61".2	73°11'45".8	31.97	Talawadi	Bardoli	Surat
VN 23	20°53'43".4	73°75'49".9	873.89	Saputara	Waghai	The Dangs
VN 24	20°56'14".8	73°75'46".2	880.52	Saputara	Waghai	Dang
VN 25	20°56'31".5	73°75'47".3	874.61	Saputara	Waghai	Dang
VN 26	20°59'40".3	73°74'88".8	669.94	Malegaon	Waghai	Dang
VN 27	20°67'90".8	73°64'72".0	322.44	Shivarimal	Waghai	The Dangs
VN 28	20°69'01".3	73°58'77".6	227.97	Sakarpatal	Waghai	The Dangs
VN 29	20°79'92".1	73°34'01".1	87.52	Nani Bhmati	Vansda	The Dangs
VN 30	21°06'19".3	73°08'91".4	49.28	Sarbhon	Bardoli	Surat
VN 31	21°19'19".7	73°18'04".8	51.47	Kantali	Bardoli	Surat
VN 32	21°22'24".5	73°22'70".1	49.65	Kadod	Bardoli	Surat
VN 33	21°26'42".9	73°31'13".0	56.47	Vaghnera	Mandvi	Surat
VN 34	21°11'69".2	73°39'59".8	109.80	Vyara	Vyara	Tapi
VN 35	21°11'01".0	73°37'63".0	91.12	Vyara	Vyara	Tapi
VN 36	21°10'74".6	73°18'90".0	51.53	Hindolia	Bardoli	Surat
VN 37	20°91'97".1	72°93'21".3	29.60	Dantej	Navsari	Navsari
VN 38	20°31'72".9	73°31'56".3	486.92	Chavshala	Kaprada	Valsad
VN 39	20°34'10".0	73°25'34".2	408.19	Dinbari	Kaprada	Valsad
VN 40	20°45'65".1	73°08'04".7	84.98	Nevri	Valsad	Valsad
VN 41	20°50'83".5	72°98'62".7	36.95	Kumbhariya	Valsad	Valsad
VN 42	20°51'26".3	72°95'16".2	33.11	killapardi	Valsad	Valsad
VN 43	20°79'04".0	73°04'01".0	39.92	Ambheta	Bilimora	Navsari
VN 44	20°83'93".9	72°97'20".3	30.35	Kachholi	Bilimora	Navsari
VN 45	20°86'35".2	72°96'80".8	22.51	Kolva	Navsari	Navsari
VN 46	20°76'80".1	73°50'01".2	138.10	Waghai	Waghai	The Dangs
VN 47	20°76'89".0	73°50'20".0	152.80	Waghai	Waghai	The Dangs
VN 48	20°80'24".2	73°53'69".0	180.14	Kudkas	Waghai	The Dangs
VN 49	20°75'72".1	73°68'11".1	478.79	Ahwa	Ahwa	The Dangs
VN 50	20°75'63".3	73°68'46".4	482.98	Ahwa	Ahwa	The Dangs

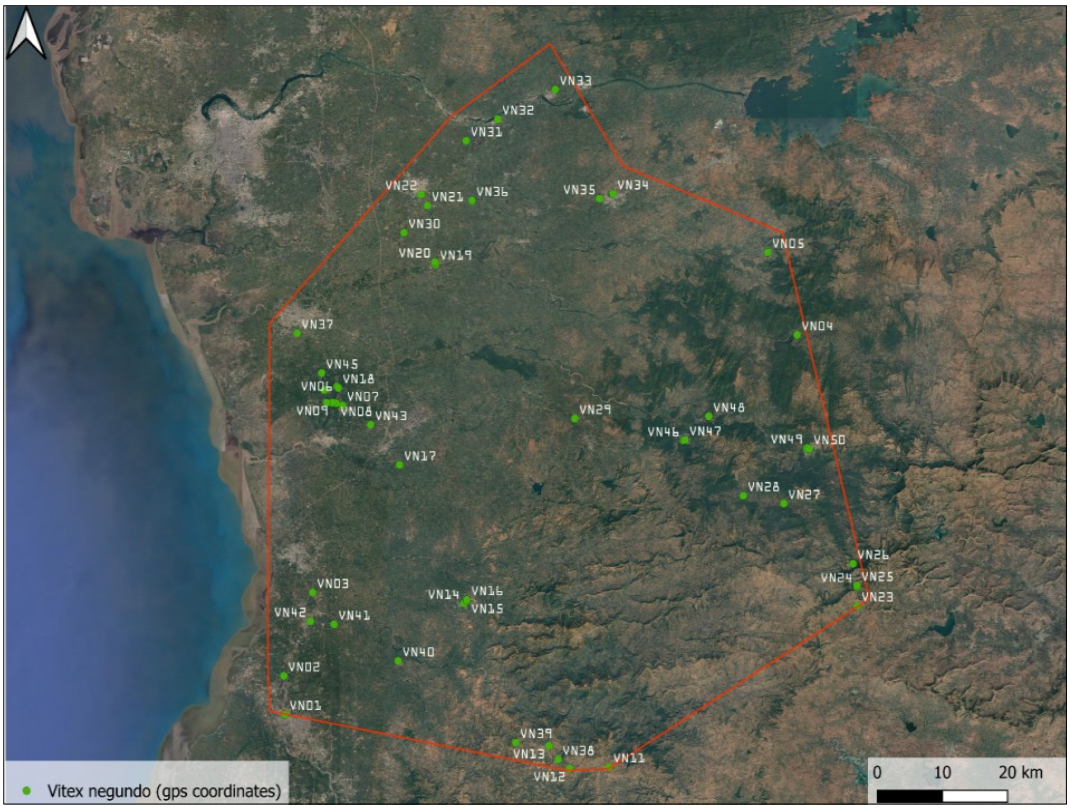

Fig. 2. Location map of *V. negundo* accessions in South Gujarat.

plant crown in square metres (m^2) involves determining the area covered by the plants' crown, which includes the outermost layer of leaves and branches. Leaves were randomly collected from each selected accession at the time of the survey. The leaf area of these samples was measured via Biovis PSM Leaf v4.60 computer software.

Preparation of leaf samples for phytochemical analysis

Fresh leaves were collected in triplicate from these marked trees. These leaf samples were allowed to dry in the shade, ensuring that they retained their natural properties without exposure to direct sunlight. Once the leaves were completely dry, they were ground into a fine powder via a heavy-duty grinder. To maintain uniformity, the resulting powder was sieved and then stored in zip-lock bags at room temperature in a dry place.

Extraction of plants

For the phytochemical analysis, 0.5 g of the stored leaf powder from each sample was used. The extraction process involved the addition of 10 mL of 98 % methanol, which was added to the powder. The mixture was vortexed for 1 min to ensure thorough mixing and then allowed to rest for 24 hr so that phytochemicals were extracted into methanol from the leaf powder. After this, the homogenate was centrifuged at 20000 rpm for 20 min to separate the components and the supernatant was collected. The collected supernatant was then filtered through 42 mm filter paper to remove any remaining particulate matter. The collected 5 ml of filtrate was evaporated in a boiling water bath at 100 °C to remove the methanol, leaving behind the concentrated extract. This residue was then dissolved in 3 mL of distilled water to ensure that all the phytochemicals were present in the solution. After this dissolution, the solution was filtered again through 42 mm filter paper to ensure purity. The final filtered extract was stored in airtight containers at 4 °C to preserve its integrity for analysis. Total phenol content, total flavonoid content and antioxidant activity were then measured (21, 22).

Total phenol content (TPC)

The total phenol content was estimated via the Folin-Ciocalteu method with modifications as needed (22). *V. negundo* L. plant extract or gallic acid standard (0.1 mL) was mixed with 0.5 mL of Folin-Ciocalteu 2 N (Normality) grade reagent and allowed to stand at room temperature for 3 min, after which 2 mL of Na_2CO_3 (20 %) was added to the mixture. After mixing thoroughly and placing in boiling water for exactly 1 min, the absorbance was measured using a spectrophotometer (Labman) UV-vis at 650 nm after cooling. Aqueous solutions with known gallic acid concentrations in the range of 0.1 mg/mL were used for calibration. To prepare the standard series, varying volumes (0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mL) of the working standard solution were pipetted into separate test tubes. The results are expressed as mg of gallic acid equivalent (GAE/g) DW sample.

Total flavonoid content (TFC)

The total flavonoid content was determined via the modified Aluminium chloride ($AlCl_3$) method (22). Distilled water (4.9 mL) was added to 0.1 mL of the extract in a test tube. Then, 0.3 mL of 5 % $NaNO_3$ solution was added after 5 min, followed by 0.3 mL of 10 % $AlCl_3$ solution. The test tubes were incubated at ambient temperature for 6 min, after which 2 mL of 1 M NaOH was added to the mixture. The volume of the reaction mixture was immediately adjusted to 10 ml with distilled water. The mixture was thoroughly mixed via a test tube shaker and the absorbance of the resulting pink colour was determined at 510 nm using a UV-Vis spectrophotometer (Labman). Aqueous solutions with known quercetin concentrations in the range of 1 mg/mL were used for calibration and the results are expressed in mg quercetin equivalents (QE/g) DW per sample. This stock solution was then used to perform serial dilutions (0.1, 0.2, 0.3, 0.4 and 0.5 mL) to create solutions of varying concentrations. Different concentrations were prepared to generate a standard curve, which is a graphical representation used to determine the

concentration of quercetin in unknown samples by comparing their absorbance values to those of standard solutions.

Antioxidant activity

The antioxidant activity (free radical scavenging ability) was determined via a modified DPPH method based on the stable 1,1-diphenyl-2-picryl hydrazyl radical (22). A 50 mg/L solution of 95 % purity DPPH in 98 % methanol was prepared and 3.9 mL of this solution was added to 0.1 mL of the extract solution in methanol. The resulting mixtures were incubated at room temperature in the dark for 30 min. After incubation, the absorbance was measured using a spectrophotometer (Labman) UV-vis at 517 nm. The same procedure was followed for the standard solution (ascorbic acid). DPPH free radical scavenging activity was calculated via the following formula.

$$\text{Antiradical activity (\%)} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100 \quad \text{(Eqn. 1)}$$

Where, $A_{control}$ is the control absorbance (DPPH + methanol), A_{sample} is the sample absorbance.

The antioxidant activity (AA) value was estimated via a standard curve prepared with different concentrations of ascorbic acid.

Essential oil extraction

The essential oil from the leaves of *V. negundo* L. was extracted via hydro-distillation via a closed type Clevenger apparatus for the extraction of oils lighter than water. One hundred grams of 24 hr shade-dried leaves were cut into small pieces and placed into 2000 mL glass round flasks along with distilled water. The content of the flask was heated to boiling. Heating was continued for 4 hr and the mixture was allowed to stand for 1 hr. The stopper of the Clevenger apparatus was opened. The water was drawn out slowly until the surface of the oil layer corresponded to the preparation line and was allowed to stand for some time. The surface of the layer was lowered to the zero line and the volume of the oil was measured under the same conditions. The process was repeated in triplicate. The leaf essential oil average yield per plant was expressed as a percentage per plant and was determined via the following formula:

$$\text{Leaf essential oil content (\%)} = \frac{\text{Essential oil extracted (ml)}}{\text{Weight of shade dried leaves used for extraction (g)}} \times 100 \quad \text{(Eqn. 2)}$$

Statistical analysis

The data collected for all parameters in the study underwent descriptive statistical analysis. Phytochemical observations were analysed using the standard statistical procedure of a completely randomised design (CRD) and correlation analysis was done using OPSTAT online software. Additionally, multivariate analyses, including Cluster Analysis and Principal Component Analysis (PCA), were performed by JMP statistical analysis software. The results of these analyses are presented in tables and complemented with appropriate graphical representations, which are included in the results.

Results and Discussion

Morphological parameters

Plant height

The results for plant height (m) in various accessions of *V. negundo* are presented in Table 2. The variability was found in *V. negundo* L accessions collected from the South Gujarat region. The plant height ranged from 3.10 m to 5.50 m. Among the 50 accessions, the maximum plant height (5.50 m) was recorded in accession VN 47 (Waghai, Dangs), while the minimum (3.10 m) was observed in accession VN 17 (Khundha, Navsari). Across all regions, the average plant height was recorded as 4.09 m. The top five accessions with the maximum plant height were VN 47 (5.50 m), followed by VN 01 (4.90 m), VN 20 (4.90 m), VN 10 (4.80 m) and VN 09 (4.70 m) respectively. The environmental and genetic factors influencing plant height are relatively stable across accessions (23). However, the presence of outliers, such as accessions with a height of 5.50 m, points to some degree of genetic or environmental uniqueness. Similarly, Significant differences in the frequency of morphological traits in *Vitex agnus-castus*, both overall and within individual origin sites (24). In another study, substantial morphological variability in *Vitex doniana* was attributed this diversity partly to climatic influences (25).

Plant girth

The results for plant girth (cm) in various accessions of *V. negundo* L. are presented in Table 2. The variability was found in *V. negundo* L accessions plant girth varied between 20 cm to 52 cm. Among the 50 accessions, the maximum plant girth (52 cm) was reported in VN 47 (Waghai, Dangs), whereas the minimum (20 cm) was observed in VN 17 (Khundha, Navsari) and VN 43 (Ambheta, Navsari) accessions, respectively, with an overall mean plant girth of 29.88 cm. Among the 50 accessions, the top five accessions with the maximum plant girth in descending order were VN 47 (52 cm), followed by VN 35 (38 cm), VN 42 (38 cm), VN 49 (38 cm) and VN 07 (36 cm), respectively. The observed variability is shown by a high standard deviation, suggesting that some accessions present plant girths that deviate substantially from the mean. This variation could be attributed to genetic differences, environmental influences or a combination of both. Similar findings were reported by other researchers in *V. negundo* L. and *Vitex doniana* Sweet (6, 25, 26).

Number of primary branches

The number of primary branches per plant in various accessions of *V. negundo* L. is presented in Table 2. The variability in the number of primary branches per plantvaried between 8 to 28. Among the 50 accessions, the maximum number of primary branches per plant was reported as 28 in VN 47 (Waghai, Dangs), whereas the minimum was 08 observed in VN 17 (Khundha, Navsari) accession. Irrespective of the region, the highest average number of primary branches per plant (13.44) was recorded. Among the 50 accessions, the top five accessions with the maximum number of primary branches per plant were reported in VN 47 (28), followed by VN 49 (25), VN 48 (24), VN 15 (20) and VN 09 (18), respectively. The observed morphological variation is likely influenced by factors such as altitude, latitude and microclimatic conditions at the collection sites, as in *Salvia fruticose* Mill. (27). This diversity could result from genetic factors, environmental influences, or a combination of both, as similarly noted in *Vitex doniana* Sweet (26).

Table 2. Variability in morphological parameters among *V. negundo* accessions

Accessions number	Plant height (m)	Girth (cm)	Number of primary branches per plant	Crown spread (m^2)	Leaf area (cm^2)
VN 01	4.90	32	10	4.20	56.01
VN 02	4.50	30	14	4.10	65.02
VN 03	4.40	28	10	3.60	55.25
VN 04	3.40	22	09	3.30	53.22
VN 05	3.80	30	14	4.30	50.29
VN 06	3.50	34	10	4.20	50.95
VN 07	3.30	36	09	3.20	57.89
VN 08	4.20	28	10	3.50	57.69
VN 09	4.70	32	18	4.20	52.89
VN 10	4.80	36	12	4.20	55.87
VN 11	3.20	30	11	3.50	54.07
VN 12	4.30	32	12	3.30	56.58
VN 13	4.10	30	10	3.50	50.49
VN 14	4.20	28	16	4.40	48.93
VN 15	4.00	30	20	4.20	54.48
VN 16	3.50	22	12	3.40	47.63
VN 17	3.10	20	08	2.40	52.42
VN 18	4.20	30	12	3.10	52.81
VN 19	4.10	22	14	3.50	49.75
VN 20	4.90	30	12	3.20	58.12
VN 21	4.60	26	12	4.20	53.79
VN 22	4.20	30	14	3.50	53.33
VN 23	4.10	28	12	3.40	63.55
VN 24	3.20	34	14	3.50	58.93
VN 25	4.20	32	12	4.10	58.12
VN 26	3.20	26	10	2.70	59.49
VN 27	4.40	22	09	3.50	52.92
VN 28	4.20	32	12	3.50	58.16
VN 29	4.10	30	14	3.20	64.20
VN 30	4.50	22	14	4.10	55.51
VN 31	3.50	28	12	3.20	58.54
VN 32	3.60	36	17	3.50	57.36
VN 33	4.30	28	18	3.80	60.77
VN 34	4.60	28	16	4.20	54.28
VN 35	4.20	38	12	4.20	57.22
VN 36	4.30	36	15	3.40	58.16
VN 37	3.80	30	13	2.80	53.05
VN 38	4.20	28	14	3.40	54.45
VN 39	4.50	34	12	3.60	56.08
VN 40	3.50	24	10	3.20	55.98
VN 41	3.50	26	12	3.40	50.90
VN 42	4.20	38	12	3.20	46.19
VN 43	4.00	20	10	3.50	50.83
VN 44	3.50	26	12	3.80	57.70
VN 45	4.30	28	16	4.80	57.34
VN 46	3.80	26	12	3.50	58.72
VN 47	5.50	52	28	4.60	62.55
VN 48	4.70	36	24	4.20	49.52
VN 49	4.60	38	25	4.20	59.23
VN 50	4.10	30	16	3.60	54.12
Mean	4.09	29.88	13.44	3.66	55.43
Maximum	5.50	52	28	4.80	65.02
Minimum	3.10	20	08	2.40	46.19
SD	0.52	5.70	4.06	0.50	4.18

Crown spread

The variability was found in *V. negundo* L accessions collected from the South Gujarat region, presented in Table 2. Crown spread (m^2) varied between 2.40 to 4.80 m^2. Among the 50 accessions, the maximum crown spread (4.80 m^2) was reported in VN 47 (Waghai, Dangs), whereas the minimum (2.40 m^2) was observed in VN 17 (Khundha, Navsari) accession. Irrespective of the region, the average crown spread (3.66 m^2) was recorded. Among the 50 accessions, the top five accessions having maximum crown spread (m^2) were reported in VN 47 (4.80 m^2) followed by VN 47 (4.60 m^2), VN 14 (4.40 m^2), VN 05 (4.30 m^2) and VN 01 (4.20 m^2) respectively.

The analysis data of crown spread (m^2) among the 50 accessions (VN 01 to VN 50) of *V. negundo* L. revealed a notable range of variation. Crown spread is a crucial parameter for assessing a plants' overall size and vigour, as it directly influences light capture, photosynthetic efficiency and the ability to compete for space and resources. The observed mean crown spread of 3.66 m^2 provides a central estimate of lateral growth among the accessions. The range, spanning from 2.40 m^2 to 4.80 m^2, highlights significant variability in the horizontal expansion of the plant canopy across accessions. This variation may result from genetic differences, environmental conditions during growth or interactions between the two in *Helichrysum oocephalum* Boiss. (28). Similar variations in crown spread (m^2) among accessions have been reported in *Vitex doniana* Sweet (25, 26).

Leaf area

The results for the leaf area (cm^2) of various accessions of *V. negundo* L. are presented in Table 2. Leaf area (cm^2) varied between 46.19 to 65.02 cm^2. Among the 50 accessions, maximum leaf area (65.02 cm^2) was reported in VN 02 (Bagwada, Valsad), whereas the minimum (46.19 cm^2) was observed in VN 42 accession (Killapardi, Valsad). Irrespective of the region, the

average leaf area (55.43 cm^2) was reported. Among the 50 accessions first top five accessions having maximum leaf area (cm^2) were reported in VN 02 (65.02 cm^2), followed by VN 29 (64.20 cm^2), VN 23 (63.55 cm^2), VN 47 (62.55 cm^2) and VN 33 (60.77 cm^2), respectively. The analysis of leaf area across 50 accessions (VN 01 to VN 50) of *V. negundo* L. reveals variation in leaf size. The analysis of leaf area across 50 accessions (VN 01 to VN 50) of *V. negundo* L. revealed variation in leaf size. Leaf area is a critical trait in plants, as it directly influences photosynthetic efficiency, transpiration rates and overall growth. Larger leaf areas generally increase the photosynthetic capacity, promote biomass production and improve a plants' ability to capture sunlight (29). The analysis of the 50 *V. negundo* L. accessions revealed significant variability in leaf area, with a mean value of 55.43 cm^2 representing the average leaf size within the population. The wide range of leaf sizes observed reflects both genetic diversity and potential environmental influences among the accessions. Other researchers highlighted the impact of light and soil moisture, two crucial abiotic factors, on plant growth (30). Their study revealed that *V. negundo* L. exhibited minimal variation in leaf morphology under reduced soil moisture, emphasising the influence of environmental conditions on this trait. Similar variations in leaf area have been reported in several studies in *Vitex doniana* Sweet., *V. negundo* L. and *V. negundo* L. and *Ziziphus jujube* and similar variability in other *Vitex* species, including *V. gamosepala*, *V. glabrata*, *V. longisepala*, *V. millsii*, *V. negundo*, *V. pinnata*, *V. quinata*, *V. trifolia* and *V. vestita* (6, 26, 30, 31).

Phytochemical parameters

Total phenol content (TPC)

The total phenol content (TPC) of various accessions of *V. negundo* L. is presented in Table S_1 and Fig. 3. The results ($p < 0.05$) were statistically significant, which were collected from the South Gujarat region. Total phenol content varied between 4.31 ± 0.17 to 22.19 ± 0.04 (mg GAE /100 g) DW. Among the 50 accessions, the maximum TPC was reported in VN 20 Mahuva, Surat (22.19 ± 0.04 mg GAE /100 g) DW, whereas the minimum was (4.31 ± 0.17 mg GAE /100 g) DW observed in VN 42 (Killapardi, Valsad) accession. Irrespective of the region, the highest average mean TPC (13.75 mg GAE /100 g) DW was reported. Among the 50 accession, first top five accessions having maximum TPC was reported in VN 20 (22.19 ± 0.04 mg GAE /100 g) DW followed by VN 27 (21.46 ± 0.09 mg GAE /100 g) DW, VN 13 (20.49 ± 0.07 mg GAE /100 g) DW, VN 47 (19.39 ± 0.16 mg GAE /100 g) DW and VN 02 (18.97 ± 0.22 mg GAE /100 g) DW respectively.

Phenolic compounds represent one of the most significant groups of bioactive compounds found across a wide variety of plants (32). The biosynthesis and accumulation of these phytochemicals, including phenolic compounds, are profoundly influenced by internal factors such as the plants' genotype and physiological conditions (33, 34). In this study, TPC was determined via the Folin-Ciocalteu method. Methanolic extracts of the leaves of *V. negundo* L. plants were analysed and the results are expressed as gallic acid equivalents per gram of dry weight (DW) of the extract. Variability in TPC was observed in the leaves of *V. negundo* L. across 50 accessions from the South Gujarat region. The TPC ranged from 4.31 ± 0.17 to 22.19 ± 0.04 mg GAE/100 g dry weight (DW). Our findings align with those of similar studies reported a TPC of 27.72 mg GAE/100 g in leaves of *V. negundo* L. and TPC in the leaves of *V. negundo* L. at 52.56 ± 0.25 mg GAE/g DW (35). Comparable variations were reported in the leaves of *V. negundo* L. and *V. trifolia* L. (36).

Total flavonoid content (TFC)

The total flavonoid contents of various accessions of *V. negundo* L. are presented in Table S_1 and Fig. 4. The results ($p < 0.05$) were statistically significant. Total flavonoid content varied between 18.56 ± 0.41 to 102.80 ± 1.47 (mg QE /100 g) DW. Among the 50 accessions, the maximum TFC was reported in VN 20 Mahuva, Surat (102.80 ± 1.47 mg QE /100 g) DW, whereas the minimum was (18.56 ± 0.41 mg QE /100 g) DW observed in VN 43 (Ambheta, Navsari) accession. Irrespective of the region, the highest average total flavonoid content (46.59 mg QE /100 g) DW was reported. Among the 50 accessions, the first top five accessions having maximum TFC was reported in VN 20 (102.80 ± 1.47 mg QE /100 g) DW followed by VN 13 (101.85 ± 0.54 mg QE /100 g) DW, VN 47 (91.48 ± 2.30 mg QE /100 g) DW, VN 29 (90.77 ± 1.41 mg QE /100 g) DW and VN 02 (90.05 ± 0.41 mg QE /100 g) DW, respectively.

Flavonoids are phytochemicals characterised by their polyphenolic structure (37). They constitute a major subclass of dietary polyphenols known for their potent antioxidant activity (38). Flavonoids play crucial roles in the antioxidant defence system of plants. In this study, the total flavonoid content was determined via the aluminium chloride colourimetric method. Methanolic extracts of the leaves of *V. negundo* L. plants were analysed and the results are expressed as quercetin equivalents

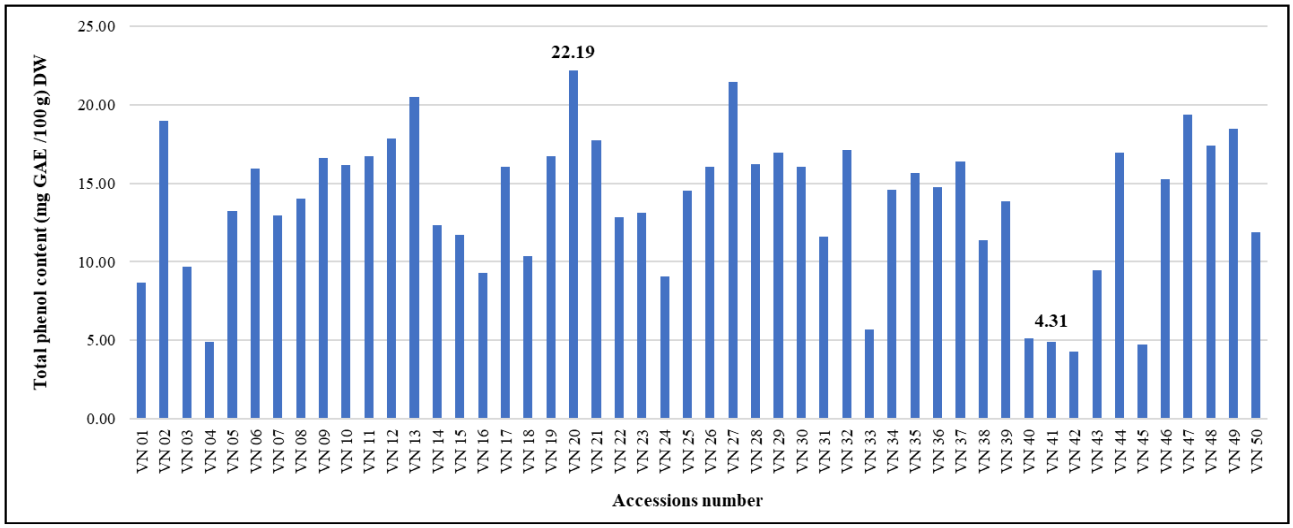

Fig. 3. Variability in total phenol content among *V. negundo* accessions.

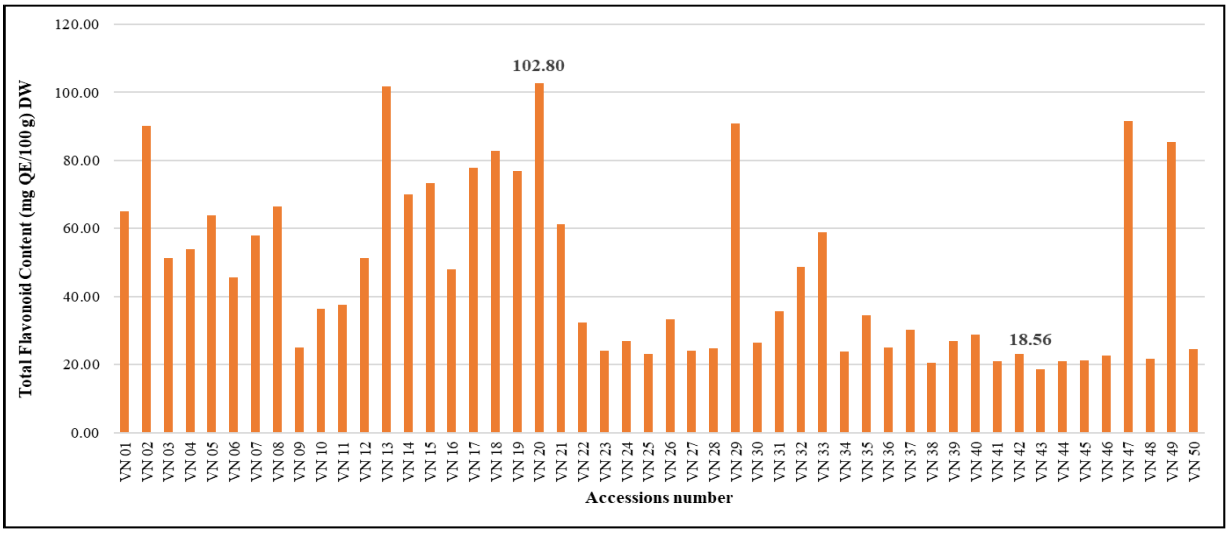

Fig. 4. Variability in total flavonoid content among *V. negundo* accessions.

per gram of dry weight (DW) of the extract. Our findings align with similar studies conducted on the TFC in the leaves of *V. negundo*. L. A TFC of 196.04 ± 0.8 mg QE/100 g and 27.32 ± 0.205 QE mg/g) DW in the leaves of *V. negundo* L. (35, 39) and TFC in two species of 63.11 mg QE/g DW in *V. negundo* L. compared with 77.20 mg QE/g DW and 57.41 mg QE/g DW in *V. trifolia* L. (40).

Antioxidant activity

The results of the antioxidant activity of various accessions of *V. negundo* L. are presented in Table $S_2$and Fig. 5. The results ($p < 0.05$) were statistically significant. Antioxidant activity varied between 61.24 % to 88.55 %. Among the 50 accessions, the highest antioxidant activity was observed in VN 20 Mahuva, Surat (85.55 %), while the lowest (61.24 %) was recorded in VN 43 (Ambheta, Navsari) accession. Regardless of regional variation, the mean antioxidant activity was 73.89 %. Among all accessions, the top five with the highest antioxidant activity were VN 20 (85.55 %), VN 13 (83.32 %), VN 47 (82.38 %), VN 02 (81.33 %) and VN 05 (81.00 %), respectively.

The evaluation of antioxidant activity has gained significant importance in the field of nutrition, as it provides valuable insights into the health-promoting and functional qualities of raw materials, including fruits, vegetables and medicinal plants (41). The antioxidant actions of medicinal plants are often attributed to the presence of bioactive phytochemicals, such as phenolic compounds and alkaloids (42). The production of these phytochemicals and their associated antioxidant activities are closely linked to plant defence mechanisms, particularly in response to the environmental stresses experienced during different seasons (37). This adaptive response highlights the dynamic relationship between environmental factors and the biosynthesis of antioxidants in plants. In this study, antioxidant activity was assessed via simple, efficient and rapid methods, specifically the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay (43, 44). These methods provide reliable measures of the antioxidant potential of plant extracts, reflecting their capacity to mitigate oxidative stress and promote health benefits. Our findings are consistent with a free radical scavenging activity of 79.82 % via the DPPH assay at a concentration of 500 µg/mL in *V. negundo* L (35). Similar studies by other workers demonstrated that ethanolic extracts of *V. negundo* L. leaves exhibit a dose-dependent antioxidant effect in the DPPH assay (45–47).

Essential oil content

The essential oil contents of various accessions of *V. negundo* L. are presented in Table $S_2$and Fig. 6. The results ($p < 0.05$) were statistically significant. The essential oil content ranged from 0.063 % to 0.193 %, with the highest yield recorded in VN 20 Mahuva, Surat (0.193 %) and the lowest in VN 45 (0.063 %) Kolva, Navsari accession. The mean essential oil content across all accessions was 0.127 %. The top five accessions with the highest essential oil content were VN 20 (0.193 %), VN 47 (0.187 %), VN 13 (0.183 %), VN 02 (0.180 %) and VN 49 (0.180%), respectively.

The variability in essential oil content extracted from the

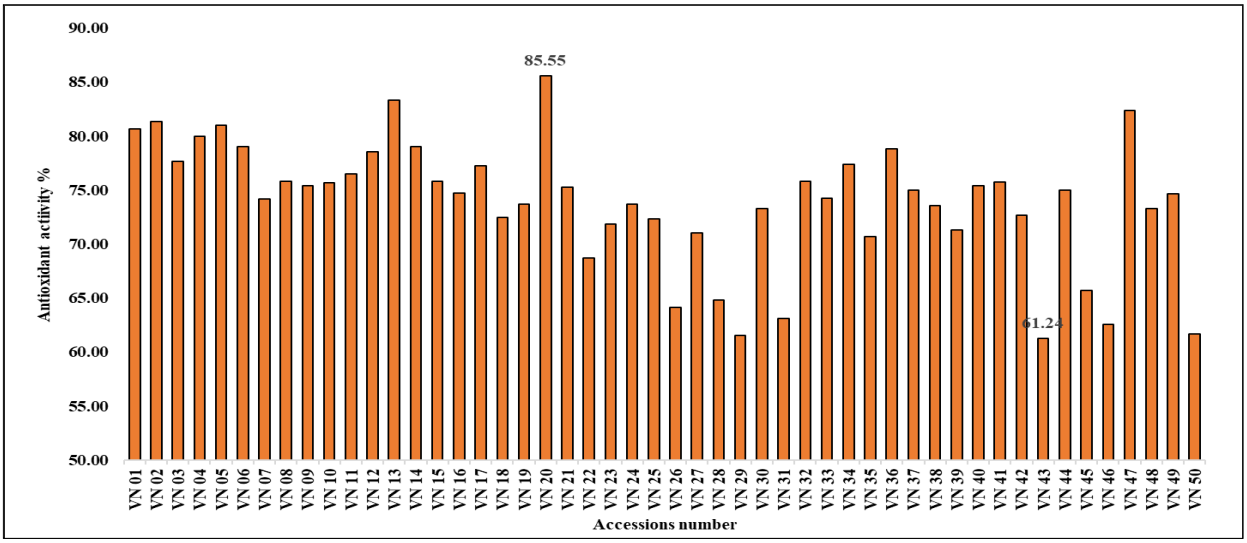

Fig. 5. Variability in antioxidant activity (%) among *V. negundo* accessions.

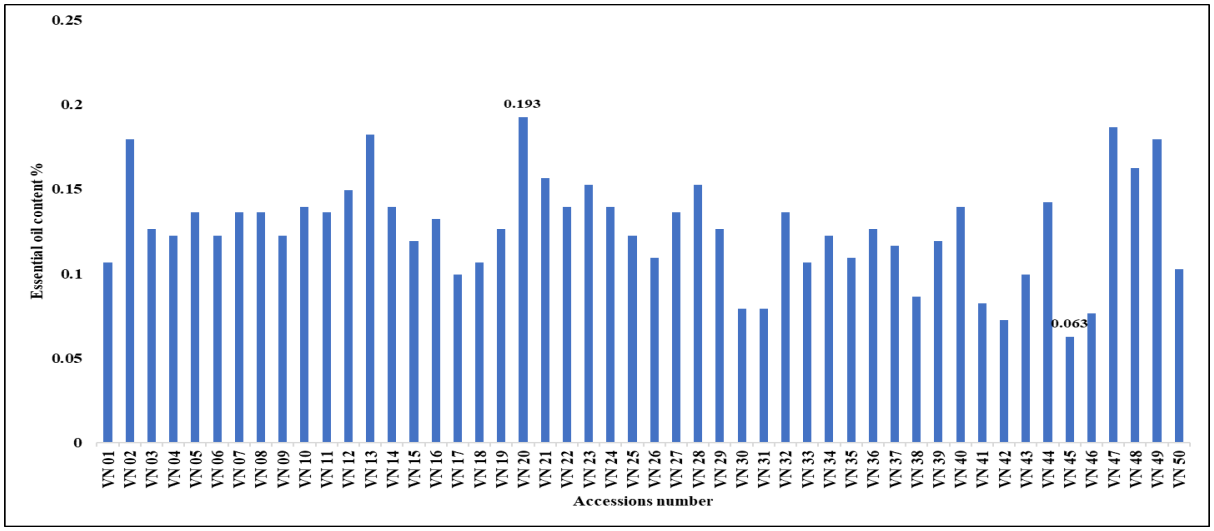

Fig 6. Variability in essential oil content (%) among *V. negundo* accessions.

leaves of *V. negundo* L. on a dry weight basis via a clevenger apparatus was studied across 50 accessions collected from the South Gujarat region. The essential oil yield ranged from 0.063 % to 0.193 %. Accessions VN 20 (0.193 %), VN 13 (0.183 %) and VN 47 (0.187 %) demonstrated the highest oil content, indicating their potential for high-yield essential oil production. Conversely, accessions VN 45 (0.063 %) and VN 42 (0.073 %) presented lower oil yields. These findings align with the results of the essential oil yields ranging from 0.06 % to 0.10 % across various populations of *V. negundo* L. Their study highlights significant variability in leaf volatile oils, reflecting the diversity within the species. Notably, both the yield and composition of essential oils vary considerably depending on the geographic origin of the populations (3). October is the optimal month for harvesting leaves to achieve the maximum essential oil yield (0.28 %) in *V. negundo* L. (48). In contrast, other researchers reported a significantly greater yield (0.5 % v/w) and (1.6 % v/w) of essential oil from *V. negundo* L. leaves (49, 50). These findings collectively emphasise the considerable variability in essential oil yield, which is influenced by factors such as genetic diversity, environmental conditions and harvesting time.

Correlation between different morphological and phytochemical parameters

The correlations between different morphological traits and phytochemical and essential oil content parameters among accessions of *V. negundo* L. are depicted in Table 3. Plant height (PH) was moderately correlated with girth (G) (r = 0.424) and crown spread (CS) (r = 0.563). A weaker positive correlation with the number of primary branches (NPB) (r = 0.5) and essential oil content (EO) (r = 0.31) was detected. The correlations with other traits, such as leaf area (LA), total phenol content (TPC) and total flavonoid content (TFC), are relatively low, indicating that plant height does not strongly influence these traits. Girth was moderately correlated with NPB (r = 0.562) and EO (r = 0.345), suggesting that thicker stems might be associated with more branches and a higher essential oil content. The correlation with crown spread (CS) is lower (r = 0.364), implying that a larger girth does not necessarily correspond to a wider crown. The correlations with LA, TPC and TFC are weaker, suggesting that girth is not strongly influenced by these phytochemical traits. The number of primary branches (NPB) was moderately correlated with both PH (r = 0.5) and G (r = 0.562), indicating that taller and thicker plants tend to have more branches. It also had moderate correlations with CS (r = 0.543) and EO (r = 0.271), suggesting that more branched plants might have wider crowns and potentially higher essential oil contents. The low correlations with LA, TPC and TFC indicate that the number of branches is not strongly linked to these phytochemical traits. Crown spread (CS) was moderately correlated with PH (r = 0.563) and NPB (r = 0.543), indicating that taller plants with more branches tend to have wider crown spreads. It has a low correlation with EO (r = 0.154) and very weak correlations with other traits, implying that crown spread is not a significant indicator of phytochemical traits or essential oil content. The leaf area (LA) was weakly correlated with all the other traits, with the greatest correlation with EO (r = 0.232), suggesting that leaf size is relatively independent of most morphological and phytochemical traits. The negative correlation with AA (r = -0.141) is notable but weak, indicating that larger leaves might slightly reduce the antioxidant potential. The significant positive correlations in *Erythrina sepium* include between plant height and crown height (r = 0.86, p < 0.001), between plant height and collar diameter (r = 0.57, p < 0.001) and between plant height and the

Table 3. Correlation between different morphological and phytochemical parameters among *V. negundo* accessions

Variable	PH	G	NPB	CS	LA	TPC	TFC	AA	EO
PH	1.000	0.424*	0.500*	0.563*	0.159**	0.298*	0.208**	0.215**	0.310*
G		1.000	0.562*	0.363**	0.278**	0.219**	0.141**	0.220**	0.345*
NPB			1.000	0.543	0.167**	0.165**	0.150**	0.083**	0.271**
CS				1.000	0.036**	0.062**	0.017**	0.203**	0.154*
LA					1.000	0.224**	0.147**	-0.141	0.232*
TPC						1.000	0.326*	0.187**	0.602**
TFC							1.000	0.487*	0.517**
AA								1.000	0.520**
EO									1.000

significant at * 5 % and ** 1 %. PH: Plant height (m); G: Girth (cm); NPB: Number of primary branches; CS: Crown spread (m^2); LA: Leaf area cm^2; TPC: Total phenol content (mg GAE/100 g) DW; TFC: Total flavonoid content (mg QE /100 g) DW; AA: Antioxidant activity (%); EO: Essential oil (%).

number of stems ($r = 0.49$, $p < 0.001$).

The total phenol content (TPC) was weakly positively correlated with most traits, with the highest being with EO ($r = 0.602$), indicating that a relatively high phenol content may be associated with a relatively high essential oil content. The correlations with pH, G, NPB and CS are relatively low, suggesting that TPC is more influenced by phytochemical rather than morphological traits. The total flavonoid content (TFC) was moderately correlated with EO ($r = 0.517$) and AP ($r = 0.487$), indicating that a relatively high flavonoid content was associated with increased essential oil content and antioxidant potential. The correlations with other traits are weak, implying that flavonoid content is not strongly linked to morphological traits. The antioxidant activity (AA) was moderately correlated with the TFC ($r = 0.487$) and EO ($r = 0.52$), suggesting that plants with relatively high antioxidant potential also tended to have relatively high flavonoid and essential oil contents. The correlations with the morphological traits were weak, indicating that the antioxidant potential is closely related to the phytochemical traits. The essential oil content (EO) was most strongly correlated with the TPC ($r = 0.602$), TFC ($r = 0.517$) and AA ($r = 0.52$), indicating that a relatively high essential oil content is associated with relatively high levels of phenols, flavonoids and antioxidant potential. Plants grown under high light intensity often have smaller leaves, but the concentration of certain phytochemicals such as phenolic and flavonoids can be higher. This is a protective response to intense sunlight. Conversely plants in partial shade may grow larger, thinner leaves to maximize light capture, but the concentration of some compounds may be lower. Our studies consistently demonstrate a positive correlation between the TPC, TFC and AA of *V. negundo* extracts. This means that as the concentration of these compounds increases the antioxidant potential also increases. The correlations with morphological traits such as pH, G and NPB are moderate, suggesting that while plant size and structure can influence EO content, phytochemical traits are more decisive.

The correlation matrix reveals important insights into the relationships between morphological traits and phytochemical and essential oil content parameters among *V. negundo* accessions. Moderate correlations between EO content and traits such as pH, G and NPB suggest that larger and more structurally sturdy and strong plants tend to produce more essential oils. However, the influence of morphological traits on EO content is less pronounced than that of phytochemical traits. The strong correlations between EO content and phytochemical traits (TPC, TFC and AA) highlight the critical role of these compounds in determining essential oil yield and quality. Significant correlation was found in TPC, TFC and AA in *Vitex* species. The favourable properties resulting from the presence of TPC in *Vitex* species have been ascribed to their antioxidant activity. TPC may contribute directly to antioxidative action mainly due to their redox properties, which can play an important role in absorbing and neutralising free radicals. Flavonoids are the most important natural phenolic compounds and have a large number of biological and chemical properties, including radical scavenging. The leaf area (LA) appears to be relatively independent of most other traits, indicating that it may not be a significant determinant of phytochemical composition or EO content. This could imply that leaf area is more influenced by environmental conditions or genetic factors unrelated to the other traits measured. The TPC, TFC, AA and oil content can vary depending on the leaves harvested, dried samples and the extraction method, essential oil and phytochemical employed. Accessions VN 20 and VN 47 were found superior among 50 accessions due to the variation in altitude, environmental and genetic factors, are also affect the morphology and content of phytochemicals (18). Our findings align with other studies' significant positive correlations among the morphological characteristics of *Vitex agnus-castus* genotypes, as indicated by Pearsons' correlation coefficients (24). Similarly, a strong correlation between antioxidant activity and the total phenolic and flavonoid contents in *V. negundo* and *Vitex trifolia* L. plants (40). In contrast, low correlations could arise due to the influence of factors such as mutation, genetic drift and gene flow on molecular differentiation. However, research emphasised that differences in morphological traits are more strongly influenced by natural selection and environmental factors (23). Since morphological characteristics are shaped by environmental conditions and the developmental stage of the plant, they do not fully account for the genetic diversity observed among individuals or populations. Similarly, a significant correlation between essential oil composition and genetic diversity in populations of *Salvia fruticosa* Mill. and a significant correlation among morphological, phytochemical, antioxidant and genetic variations in *Polygonatum verticillatum* (7, 27). These findings collectively underscore the importance of studying trait correlations for understanding plant diversity and growth dynamics.

Principal component analysis

Principal component analysis of the morphological, phytochemical, antioxidant activity and essential oil content parameters of the *V. negundo* L. accessions revealed nine principal components, four of which were reported to have eigenvalues of 3.27, 1.67, 1.20 and 0.73, respectively (Fig. 7), which contributed to 76.6 % of the total variation Table 4. The loading matrix is given in Table 4 and the result in a significant positive association of the first principal component with essential oils, plant height, girth, number of primary branches, crown spread, total phenol content, total flavonoid content and antioxidant activity. Similarly, the second component was associated with total flavonoid content, essential oil content, antioxidant activity and total phenol content, whereas the third principal component was positively associated with leaf area, total phenol content, girth and essential oil, whereas the fourth component was associated with girth, leaf area and total flavonoid content Table 4.

The scatter plot revealed minor variations in grouping compared to the cluster analysis, as illustrated in Fig. 8. PC1 explain 36.4 % of the variance, while PC2 explains 18.6 % of the variance. Most of the accessions are clustered around the centre but some, like VN 17, VN 13, VN 20, VN 47 and VN 45, were positioned far away, indicating variability among the accessions. Because of higher genetic variability in these accessions for morphological and phytochemical parameters within the population. A bioplot can visually represent how these morphological parameters contribute to the within-variation in accessions. The phytochemical parameters such as TFC, TPC, AA and EO have strong positive loading on PC1. And plant height, canopy spread and number of primary branches were negatively correlated with PC1.

Table 4. PCA and loadings matrix of parameters in the first four PC of *V. negundo* accessions based on morphological and phytochemical parameters

Variables	PC1	PC2	PC3	PC4
Plant height	0.710	-0.335	-0.094	-0.252
Girth	0.679	-0.312	0.125	0.312
Number of primary branches	0.667	-0.501	-0.006	0.091
Crown spread	0.565	-0.558	-0.319	-0.123
Leaf area	0.325	-0.0415	0.802	0.301
Total phenol content	0.571	0.3847	0.333	-0.570
Total flavonoid content	0.538	0.571	-0.086	0.282
Antioxidant activity	0.519	0.453	-0.558	0.220
Essential oil	0.743	0.473	0.070	-0.083
Eigen value	3.273	1.676	1.204	0.736
%	36.736	18.324	13.386	8.179
Cumulative %	36.376	55.000	68.386	40.333

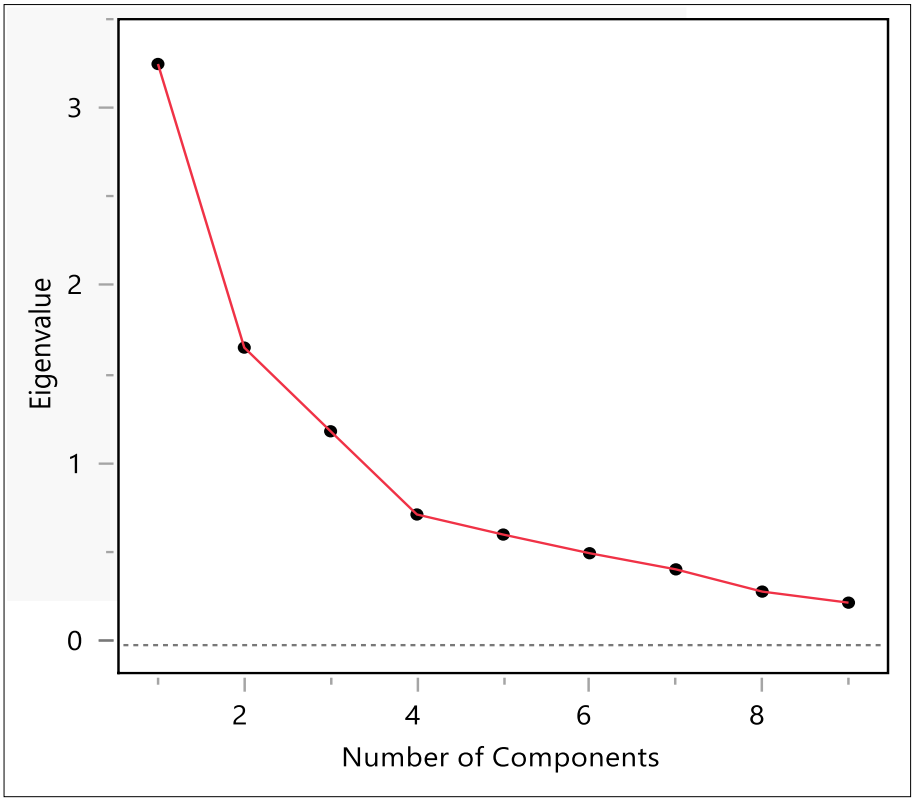

Fig. 7. Scree plot based on various morphological and phytochemical parameters among *V. negundo* accessions.

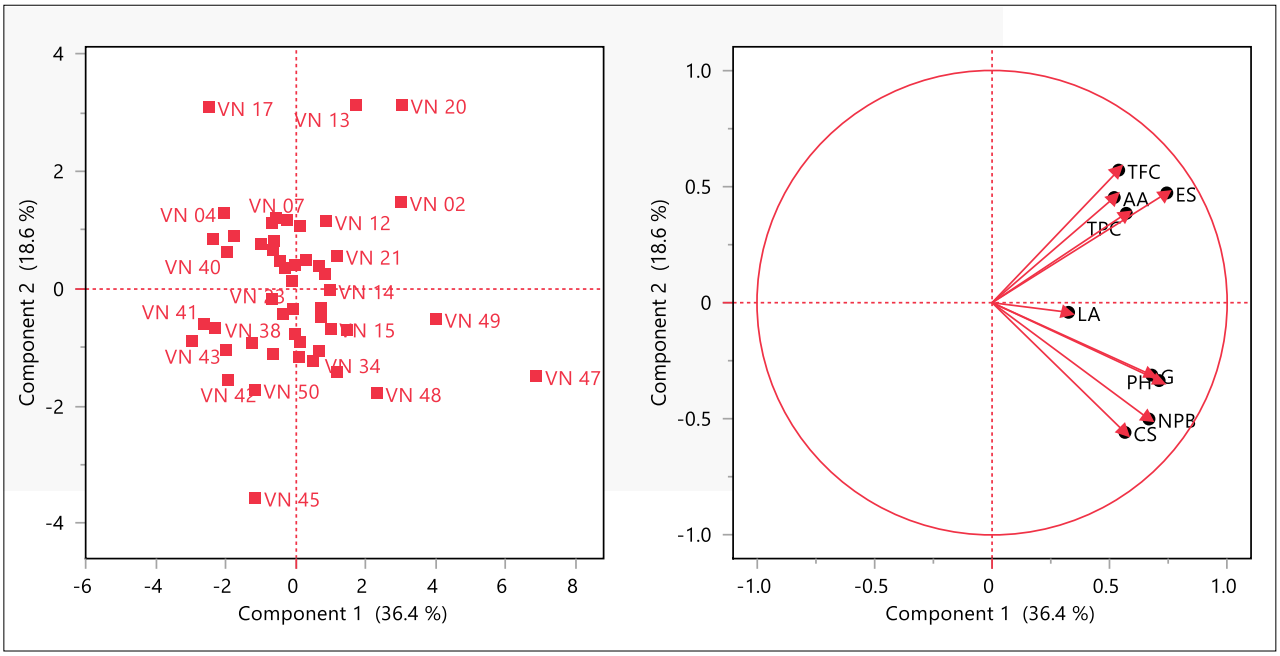

Fig. 8. Scatter plot of 50 accessions based on two principal components for different morphological and phytochemical parameters.

PH: Plant height (m); G: Girth (cm); NPB: Number of primary branches; CS: Crown spread (m^2); LA: Leaf area cm^2; TPC: Total phenol content (mg GAE/100 g) DW; TFC: Total flavonoid content (mg QE /100 g) DW; AA: Antioxidant activity (%); EO: Essential oil (%).

Principal component analysis (PCA) is a statistical technique that reduces large datasets with numerous correlated variables into a smaller set of new variables while retaining most of the variation present in the original data. This simplification makes the interpretation of results more practical and meaningful. Principal component analysis focuses on retaining only a few key components that account for the majority of the variation while discarding less informative components. Principal component analysis revealed multiple multivariate directions of variation in the morphological and phytochemical parameters of the investigated *V. negundo* L. accessions. The variation in all the input variables was effectively captured by four principal components (PCs). Additionally, significant relationships were observed among most of the studied variables, suggesting that similar environmental or genetic factors may govern the morphological traits of *V. negundo* L.

Our findings align with those of previous studies (24) reported that the first two principal components explained 97.3 % and 1.9 % of the total variation, respectively, with a biplot confirming the cluster analysis results on the basis of the morphological traits of *Vitex agnus-castus* L. genotypes.

Hierarchical clustering analysis by the Ward method

The *V. negundo* L. accessions were separated into three clusters in the cluster analysis on the basis of their morphological, phytochemical, antioxidant and essential oil content parameters Fig. 9 and Table 5. Hierarchical cluster analysis (HCA) groups similar observations into to nested hierarchy of clusters, which can be visualised in a dendrogram. It shows that accessions are most alike, forming the basis for correlating similar morphological and phytochemical parameters within clusters. Cluster I comprised twenty-five accessions, namely, VN 01, VN 03, VN 08, VN 18, VN 33, VN 05, VN 14, VN 15, VN 09, VN 34, VN 10, VN 25, VN 35, VN 21, VN 27, VN 06, VN 11, VN 44, VN 37, VN 07, VN 24, VN 23, VN 12, VN 36 and VN 32, with a cluster mean plant height (4.09 m), girth (30.80 cm), number of primary branches (13.04), crown spread (3.77 m^2), leaf area (55.58) and total phenol content (14.13 mg GAE/100 g). DW, total flavonoid content (45.15 mg QE/100 g), DW, antioxidant activity (75.71 %) and essential oil content (0.130 %) were measured. Cluster II included nineteen accessions, namely, VN 04, VN 40, VN 41, VN 16, VN 19, VN 17, VN 22, VN 39, VN 28, VN 50, VN 30, VN 38, VN 43, VN 45, VN 42, VN 26, VN 31, VN 46 and VN 29, which presented a cluster mean plant height (3.88 m), girth (26.73 cm), number of primary branches (12.26), crown spread (3.42 m^2), leaf area (54.57), total phenol content (11.45 mg GAE/100 g) DW, total flavonoid content (37.23 mg QE/100 g) DW, antioxidant activity (69.53 %) and essential oil content (0.100 %). Cluster III included six accessions, namely, VN 02, VN 13, VN 20, VN 47, VN 48 and VN 49, which presented relatively high mean cluster values for plant height (4.71 m), girth (36.00 cm), number of primary branches (18.83), crown spread (3.96 m^2), leaf area (57.48) and total phenol content (19.47 mg GAE/100 g). DW, total flavonoid content (82.21 mg QE/100 g), antioxidant activity (80.09 %) and essential oil content (0.180 %) were measured. Because of higher genetic variability in these accessions for morphological and phytochemical parameters within the population.

Hierarchical cluster analysis (HCA) is a statistical method used to simplify complex multivariate data by grouping it into smaller, more manageable subsets. It classifies data into distinct groups, with the data within each group being considered similar. In addition to HCA, principal component analysis (PCA) is one of the most widely used multivariate exploratory techniques. Both methods provide a straightforward way to represent similarities between samples on the basis of complex analytical data. The cluster analysis of *V. negundo* L. accessions on the basis of morphological traits, phytochemicals, antioxidant activity and essential oil content categorised the accessions into three distinct clusters. Morphological parameter clustering serves as a proxy for genetic similarity, which in turn influences phytochemical production. Research indicates that hierarchical clustering divided *Vitex agnus-castus* genotypes into two clusters based on variations in morphological characteristics (24). Similarly, applied cluster analysis to fifteen traits, creating a dendrogram that classified thirteen *Thymus* accessions into two major clusters based on morphological and chemical variation (23).

Conclusion

The variability was observed among the 50 *V. negundo* accessions collected from South Gujarat across all morphological and phytochemical parameters, indicating strong potential for selection and improvement of this species. Among the 50 accessions evaluated, VN 47 (Waghai, Dangs) was found as the best-performing accession for morphological parameters. However, accession VN 20 (Mahuva, Surat) was best for phytochemical parameters with the maximum total phenol content, total flavonoid content, antioxidant activity and essential oil content. Principal component analysis of the morphological, phytochemical, antioxidant and essential oil parameters of the *V. negundo* L. accessions revealed nine principal components that contributed to 76.6 % of the total variation. On the basis of the morphological and phytochemical traits of the accessions, three main clusters were identified, indicating that the observed diversity was largely driven by the influence of diverse genetic variability within the population. This suggests these superior accessions (VN 20, VN 47) can be selected as chemotypes for further breeding programs, crop improvement and industrial applications of *V. negundo* L.

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Table 5. Cluster and mean values of *V. negundo* accessions based on morphological and phytochemical parameters

Cluster	Number accessions	PH	G	NPB	CS	LA	TPC	TFC	AA	EO
1	25	4.09	30.80	13.04	3.77	55.58	14.13	45.15	75.71	0.13
2	19	3.88	26.73	12.26	3.42	54.57	11.45	37.23	69.53	0.10
3	6	4.71	36.00	18.83	3.96	57.48	19.47	82.21	80.09	0.18

PH: Plant height (m); G: Girth (cm); NPB: Number of primary branches; CS: Crown spread (m^2); LA: Leaf area cm^2; TPC: Total phenol content (mg GAE/100 g) DW; TFC: Total flavonoid content (mg QE /100 g) DW; AA: Antioxidant activity (%); EO: Essential oil (%).

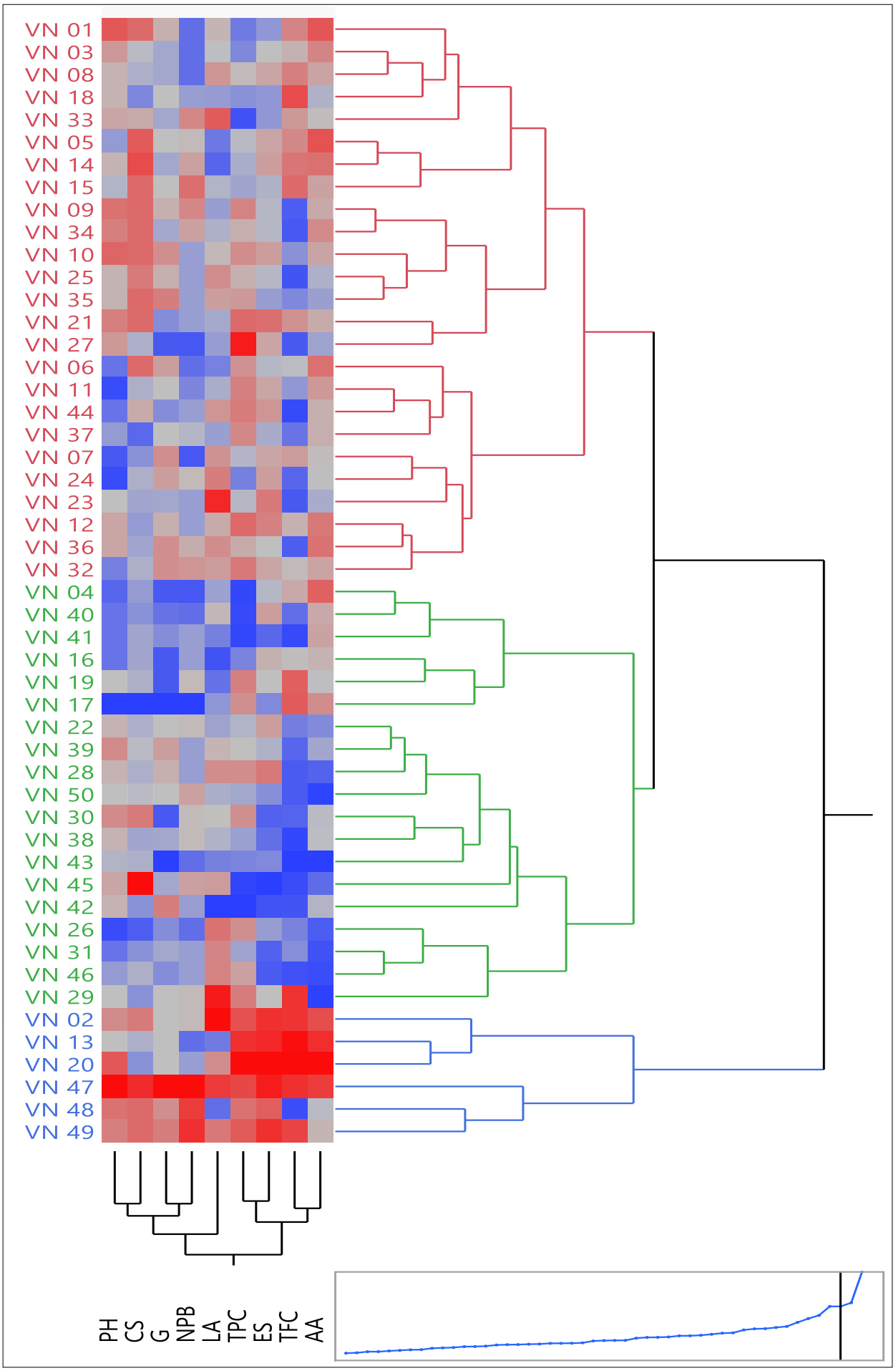

Fig. 9. Hierarchical cluster analysis (Ward method) based on morphological and phytochemical parameters of 50 accessions of *V. negundo*.

PH: Plant height (m); G: Girth (cm); NPB: Number of primary branches; CS: Crown spread (m^2); LA: Leaf area cm^2; TPC: Total phenol content (mg GAE/100 g) DW; TFC: Total flavonoid content (mg QE /100 g) DW; AA: Antioxidant activity (%).

Authors' contributions

ASG contributed to research design and methodology, conducted field surveys, carried out data and sample collection from various locations, performed laboratory analysis, prepared graphs and tables and wrote the manuscript for publication. BSD contributed to research methodology, supervision, provision of laboratory facilities, formal data analysis and reviewing and editing of the manuscript. SSJ contributed to research methodology, supervision, provision of laboratory facilities, formal data analysis and reviewing and editing of the manuscript. SKS contributed to research methodology, supervision, provision of laboratory facilities, formal data analysis and reviewing and editing of the manuscript. DPP contributed to research methodology, supervision, provision of laboratory facilities, formal data analysis and reviewing and editing of the manuscript. YAG contributed to research methodology, supervision, provision of laboratory facilities, formal data analysis and reviewing and editing of the manuscript. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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