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Artificial neural network (ANN) model for prediction and optimization of bacoside A content in *Bacopa monnieri*: a statistical approach and experimental validation

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ABSTRACT
The current research aims to elucidate the drug environment Interactions and predict a suitable growing condition for *Bacopa monnieri* (L.) Wettst with maximum bacoside A content using an artificial neural network (ANN) model. An experimental dataset was generated by collecting *B. monnieri* wild accessions from 81 locations across different geographical regions of eastern Indian (Odisha and West Bengal). The obtained ANN results specified that a single hidden layer containing 11 neurons namely 13-11-1 structure of multilayer perceptron (MLP) neural network showed the highest prediction accuracy for bacoside A content. The developed ANN model exhibited a better predictive potential for the training dataset with a coefficient of determination (R^2), a root mean square error (RMSE), and a mean absolute percentage error (MAPE) of 0.90, 0.16, and 7.76%, respectively. Further, the results on sensitivity analysis showed nitrogen levels and altitude to have the highest impact on bacoside A content. Additionally, the ANN model exhibited a prediction accuracy of 93.60% for bacoside A content when tested at a new geographical location. The results of this study thus indicates that ANN model can be used for predicting and optimizing bacoside A content in *B. monnieri* (L.) at a specific location.

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Artificial neural network; *Bacopa monnieri*; bacoside A; climatic factor; soil nutrients

Introduction

Bacopa monnieri (L.) Wettst (Scrophulariaceae) commonly known as 'Brahmi' in India is a highly valued medicinal herb used widely as memory enhancer, vitalizer, and nerve tonic (Christopher et al. 2017) in the Indian system of medicine such as Ayurveda, Siddha, and Unani,. It is distributed in the warmer and marshy wetland region of India, East Asia, Australia and United States (Nemetchek et al. 2017). *B. monnieri* possesses significant pharmacological properties such as anti-inflammatory (Channa et al. 2006), neuroprotective (Ramasamy et al. 2015), hepatoprotective (Ghosh et al. 2007), anti-cancer (Ghosh et al. 2020), immunostimulatory (Saraphanchotiwitthaya et al. 2008), anti-depressant (Charoenphon et al. 2016) and antioxidant activity (Jauhari et al. 2019). It is also used in treating skin disorders, epilepsy, diabetes, inflammation and hyperpyrexia (Sivaramakrishna et al. 2005). The therapeutic potential of *B. monnieri* is attributed to the major bioactive compound, i.e., 'bacoside A', which is a mixture of four triglycosidic saponins namely bacoside A3, bacopaside II, jujubogenin isomer of bacopasaponin C and bacopasaponin C (Christopher et al. 2017). Bacoside A retards neuron degeneration associated with aging and also helps in protecting neurons in Alzheimer's disease by preventing the interactions of amyloid beta peptide with neuron cell membrane (Rastogi et al. 2012; Malishev et al. 2017). Moreover, bacoside A inhibits the progression of glioblastoma multiforme, a brain tumor, by balancing the hydrostatic pressure via a mechanism of excessive phosphorylation of calcium/calmodulin dependent protein kinase IIA (CaMKIIA) (John et al. 2017).

The scarcity of *B. monnieri* accessions with high bacoside A concentrations due to overexploitation is a major setback for its industrial applications. The identification of elite *B. monnieri* accessions by simple chemotyping would not be promising since the regulation of secondary metabolite production is strongly affected by edaphic and climatic factors of (Karthikeyan et al. 2011; Dey et al. 2020). Therefore, it is imperative to determine the edaphic and climatic factors exerting the strongest influence on the production of bacoside A in different habitats of eastern India. It would be

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difficult to understand the bacoside A x environmental factors interactions using conventional, mathematical models. Artificial neural network (ANN) models have emerged as powerful tools for the prediction and optimization of drug yield as compared to conventional models (Alam and Naik 2009).

Multilayer perceptron (MLP) is the most accepted feed forward type of ANN model (Emamgholizadeh et al. 2015). The basic architecture of an MLP consists of an input, hidden and the output layer. All the layers of MLP are joined to each other through nodes, and each node communicates with another node by transferring weight through an activation function (Niazian et al. 2018). There are numerous report regarding crop yield prediction using various types of architecture of the ANN model, such as generalized feed forward (GFF), Jordan/Elman (JE) and radial basis function (RBF) (Mohammadi and Siosemarde 2016; Kazem and Yousif 2017; Michelon et al. 2018). ANN models have been extensively used to predict and optimize the yield of many valuable crops (Fieuzal et al. 2017; Abdipour et al. 2019). ANN models have also been used in the prediction and optimization of secondary metabolite production in several plants (Shokri et al. 2011; Khajeh et al. 2012; Pilkington et al. 2014; Akbar et al. 2016; Amdoun et al. 2019; Ramli et al. 2020; Ray et al. 2020; Saffariha et al. 2021). Nevertheless, previous studies have confirmed the unavailability of application of an ANN model for prediction and optimization of bacoside A content in *B. monnieri* from different geographical areas of eastern India. Hence, for the first time the present study was conducted to predict, using an ANN model, a suitable environmental niche that would be best suited for the commercial cultivation of *B. monnieri* with maximum bacoside A content.

Materials and methods

Plant materials and sampling point

Bacopa monnieri accessions were collected in the months of May to July 2018–2020 at the flowering stage from different geographical locations of Odisha and West Bengal. The authentication of samples were performed by the taxonomist Dr. Pratap Chandra Panda, Siksha 'O' Anusandhan (Deemed to be) University, Odisha and are kept in the herbarium of the same institute (Table 1). Roots of the collected samples was removed and the aerial parts were properly washed using tap water. The aerial parts were air-dried and ground using a homogenizer. The geographical locations of each sample were recorded in terms of latitude, longitude and altitudinal data using a Global Positioning System (GPS) device (Garmin 276 C, Garmin, Olathe, KS). Climatic data such as annual rainfall, average relative humidity (ARH), UV index, and temperature (minimum, maximum and average) were taken as average data of the past five years from the Indian Meteorological Department (IMD) near to each sampling site are shown in Table 2.

Quantitative analysis of soil nutrients

Approximately 400 g of the soil samples were taken in triplicate from each location at a depth of 10 to 20 cm from the ground level. Then the samples were dried, cleaned, pulverized and passed through a sieve with a mesh size of 2 µm to analyze various soil parameters such as pH, electrical conductivity (EC), organic carbon (OC), nitrogen (N), phosphorus (P) and potassium (K). The pH of the soil samples were measured with the help of a pH meter (Systronics Model no: 802) by preparing a soil:water (1:2) suspension made by constantly stirring for 30 min. The EC value of soil was measured by taking 1:5 (soil: water) suspension made by continuously stirring for 30 minutes. The organic carbon content of the soils was estimated by Walkey and Black acid mineralization procedure (Walkey and Black 1934; Díaz-Zorita 1999). The nitrogen content of the soil was estimated by the Kjeldahl method (Sáez-Plaza et al. 2013). The K content of soil was analyzed by mixing 5 g of soil in 25 mL of 1 N ammonium acetate in a volumetric flask and vibrated it for 5 min, followed by filtration of the residue and tested by means of a flame photometer (Systronics model no: 126). The estimation of soil P content was carried out using Bray's no-1 extractant method (Bray and Kurtz 1945). Briefly, 2 g of soil sample was mixed with 40 mL of Bray's-1 solution (0.03 N NH_4F and 0.025 N HCL) and agitated up to 5 min. followed by filtration using Whatman filter paper. Then 0.5 mL of filtrated soil solution was mixed with 0.5 mL solution of ammonium molybdate in a volumetric flask and the volume was adjusted to 25 mL by adding distilled water and $SnCl_2$. Finally, P content was determined from a standard curve with different concentration of phosphorus by measuring the absorbance at 660 nm using a UV/Vis spectrophotometer (Thermo scientific evolution 220, Thermo Fisher Scientific). Results of soil analyses are shown in Table 3.

HPLC analysis of bacoside A content

Extract preparation

The dried aerial parts of *B. monnieri* samples were homogenized and passed through a 60 µm sieve to obtain a fine powder. The powdered sample (0.5 g) was taken and mixed with 20 mL of methanol in a volumetric flask. The mixture solution was placed in a water bath at 60 °C for 20 min followed by sonication using the Ultrasonicator (Phoenix, Model: LS-DK-240HTD) over 10 min. Then, using a 0.22-µm membrane filter the extracted solution sample was filtered prior to their HPLC analysis.

Apparatus and chromatographic condition

HPLC analysis was carried out using a modular HPLC instrument (Shimadzu, Kyoto, Japan) assembled with a binary pump (LC-20 AD), an injector (Rheodyne 8125), a photodiode array detector (SPD-20) and a CTO-20AC column oven. Separation of the chemical compounds in the sample extract was accomplished in Restek C18 Reverse-phase column (Shimadzu, Kyoto, Japan, 250 × 4.6 mm, 5 µm) in a binary gradient mode, composed of 0.001 M potassium dihydrogen phosphate buffer (pH was adjusted to 2.4 with ortho-phosphoric acid) as solvent A and acetonitrile as solvent B. The gradient program was set as follows: 0–0.01 min, 0–30% B; 0.01–25 min, 30–40% B; 25–26 min, 40–30% B; 26–30 min, 30–30% B. Column temperature was set at 27 °C. The flow rate and injection volume of each sample and

Table 1. Geographical location of collected *Bacopa monnieri* populations.

Acc. No	District and State	Latitude and Longitude	Altitude (m)	Voucher no.	Acc. No	District and State	Latitude and Longitude	Altitude (m)	Voucher no.
BM1	Balasore, OD	21° 36′ 59.76′′ N, 87° 00′ 59.76′′ E	15	CBT/1041	BM42	Gajapati, OD	19° 26′ 48.35" N, 84°15′ 02.10" E	15	CBT/1071
BM2	Mayurbhanj, OD	21° 50′ 00.48′′ N, 86° 56′ 23.28" E	41	CBT/1042	BM43	Khurdha, OD	20° 19′ 59.23" N, 85° 54′ 13.97" E	55	CBT/1072
BM3	Mayurbhanj, OD	21° 39′ 52.64" N, 87° 02′ 1.44" E	31	CBT/1043	BM44	Dhenkanal, OD	20° 38′ 19.86" N, 85° 38′ 9.708" E	81	CBT/1073
BM4	Khurdha, OD	20° 17′ 53.88" N, 85° 48′ 11.52" E	44	CBT/1044	BM45	Ganjam, OD	19° 22′ 39.86" N, 84° 34′ 0.37" E	65	CBT/1074
BM5	Nayagarh, OD	20° 18′ 51.84" N, 85° 18′ 5.4" E	120	CBT/1045	BM46	South 24 Parganas, WB	21° 52′ 33.6" N, 88° 11′ 07.79" E	6	CBT/1075
BM6	Nayagarh, OD	20° 07′ 42.81" N, 85° 5′ 44.16" E	115	RPRC/12522	BM47	South 24 Parganas, WB	21° 54′ 4.68" N, 88° 15′ 03. 6" E	5	CBT/1076
BM7	Puri, OD	19° 47′ 35.44" N, 85° 38′ 28.77" E	12	RPRC/12512	BM48	South 24 Parganas, WB	22° 11′ 55.32" N, 88° 12′ 08.28" E	8	CBT/1077
BM8	Puri, OD	19° 47′ 35.45" N, 85° 38′ 28.77" E	12	RPRC/12528	BM49	PaschimBardhaman, WB	23° 40′ 26.04" N, 86° 57′ 08.64" E	110	CBT/1078
BM9	Khurdha, OD	19° 47′ 57.40" N, 85° 30′ 42.27" E	10	CBT/1046	BM50	North 24 Parganas, WB	22° 43′ 33.24" N, 88° 23′ 33.00" E	480	CBT/1079
BM10	Phulbani, OD	20° 10′ 33.27′′ N, 83° 55′ 25.07′′ E	10	CBT/1047	BM51	North 24 Parganas, WB	22° 42′ 0.72" N, 88° 22′ 31.79" E	14	CBT/1080
BM11	Khurdha, OD	19° 43′ 08.00" N, 85° 11′ 22.19" E	16	CBT/1048	BM52	North 24 Parganas, WB	22° 46′ 2.64" N, 88° 23′ 17.88" E	15	CBT/1081
BM12	Cuttack, OD	20° 32′ 40.56" N, 85° 42′ 48.23" E	31	CBT/1049	BM53	North 24 Parganas, WB	22° 53′ 34.08" N, 88° 25′ 19.20" E	13	CBT/1082
BM13	Dhenkanal, OD	20° 39′ 28" N, 85° 35′ 40" E	82	RPRC/12519	BM54	Nadia, WB	23° 0 32.04" N, 88° 29′ 28.32" E	11	RPRC/12531
BM14	Balasore, OD	21° 29′ 18" N, 85° 35′ 40" E	14	CBT/1050	BM55	Howrah, WB	22° 28′ 27.84" N, 88° 5′ 60.00" E	2	CBT/1083
BM15	Balasore, OD	21° 33′ 25" N, 86° 47′ 48" E	34	CBT/1051	BM56	Howrah, WB	22° 34′ 54.12" N, 88° 19′ 57.36" E	11	CBT/1084
BM16	Balasore, OD	21° 42′ 40" N, 87° 13′ 12" E	9	CBT/1052	BM57	Kolkata, WB	22° 27′ 41.76" N, 88° 18′ 21.96" E	8	CBT/1085
BM17	Jajpur, OD	21° 07′ 26.76" N, 85° 55′ 37.56" E	319	CBT/1053	BM58	East Medinipur, WB	21° 46′ 51.96" N, 87° 44′ 42.00" E	6	RPRC/12524
BM18	Kendrapada, OD	20° 36′ 11.16" N, 86° 19′ 28.2" E	12	CBT/1054	BM59	Hooghly, WB	22° 44′ 53.88" N, 88° 20′ 18.60" E	19	RPRC/12525
BM19	Boudh, OD	20° 39′ 45.36" N, 84° 24′ 37.08" E	94	CBT/1055	BM60	Birbhum, WB	23° 41′ 8.683" N, 87° 40′ 52.60′′ E	31	RPRC/12526
BM20	Phulbani, OD	20° 09′ 33.42" N, 84° 24′ 30.43′′ E	94	CBT/1056	BM61	South 24 Parganas, WB	22° 29′ 53.16" N, 88° 18′ 38.88" E	12	RPRC/12527
BM21	Boudh, OD	20° 39′ 55.08" N, 84° 24′ 16.56" E	94	CBT/1057	BM62	South 24 Parganas, WB	22° 26′ 36.10" N, 87° 07′ 36.02" E	6	RPRC/12554
BM22	Boudh, OD	20° 41′ 12.12" N, 84° 22′ 50.52" E	94	CBT/1058	BM63	North 24 Parganas, WB	23° 16′ 18.50" N, 87° 31′ 25.03" E	5	CBT/1086
BM23	Boudh, OD	20° 50′ 13.92" N, 84° 19′ 18.12" E	94	CBT/1059	BM64	South 24 Parganas, WB	22° 21′ 34.92" N, 88° 25′ 54.48" E	10	CBT/1087
BM24	Kalahandi, OD	19° 48′ 6.48" N, 83° 10′ 54.839" E	229	CBT/1060	BM65	South 24 Parganas, WB	22° 26′ 56.76" N, 88° 23′ 29.40" E	9	RPRC/12552
BM25	Kalahandi, OD	19° 51′ 51.12" N, 83° 10′ 10.92" E	255	CBT/1061	BM66	South 24 Parganas, WB	22° 01′ 22.44" N, 88° 18′ 1.199" E	7	RPRC/12530
BM26	Mayurbhanj, OD	21° 55′ 18.44" N, 86° 44′ 4.146" E	46	CBT/1062	BM67	South 24 Parganas, WB	22° 07′ 13.08" N, 88° 23′ 39.48" E	7	RPRC/12553
BM27	Ganjam, OD	19° 36′ 43.56" N, 85° 05′ 34.8" E	10	CBT/1063	BM68	North 24 Parganas, WB	22° 51′ 24.70" N, 88° 44′ 51.05" E	9	RPRC/12555
BM28	Ganjam, OD	19° 28′ 23.754" N, 84° 33′ 31.93" E	10	CBT/1064	BM69	Nadia, WB	23° 41′ 18.96" N, 88° 18′ 33.84" E	21	RPRC/12523
BM29	Keonjhar, OD	21° 38′ 46.68" N, 85° 36′ 26.64" E	420	CBT/1065	BM70	South 24 Parganas, WB	22° 04′ 53.04" N, 88° 14′ 41.64" E	6	CBT/1088
BM30	Mayurbhanj OD	21° 50′ 24.72" N, 86° 55′ 43.32" E	35	CBT/1066	BM71	South 24 Parganas, WB	22° 12′ 14.40" N, 88° 25′ 41.88" E	9	RPRC/12540
BM31	Ganjam, OD	19° 15′ 20.16" N, 84° 45′ 51.12" E	27	RPRC/12539	BM72	North 24 Parganas, WB	22° 41′ 06.1" N, 88° 22′ 17.04" E	16	RPRC/12541
BM32	Dhenkanal, OD	20° 35′ 23.64" N, 85° 22′ 12.72" E	120	RPRC/12518	BM73	South 24 Parganas, WB	22° 27′ 46.44" N, 88° 23′ 48.12" E	9	CBT/1089
BM33	Angul, OD	20° 46′ 4.8" N, 84° 58′ 49.079" E	212	RPRC/12517	BM74	South 24 Parganas, WB	22° 00′ 4.32" N, 88° 26′ 07.79" E	7	CBT/1090
BM34	Bargarh, OD	20° 55′ 6.6" N, 82° 48′ 49.104" E	213	CBT/1067	BM75	East Medinipur, WB	21° 51′ 1.8" N, 87° 44′ 39.48" E	4	CBT/1091
BM35	Balangir, OD	20° 41′ 21.12" N, 83° 29′ 39.48" E	175	CBT/1068	BM76	South 24 Parganas, WB	21° 47′ 31.2" N, 88° 21′ 19.08" E	4	CBT/1092
BM36	Balasore, OD	21° 36′ 51.3" N, 87° 10′ 04.07" E	9	RPRC/12520	BM77	South 24 Parganas, WB	22° 21′ 01.2" N, 88° 25′ 20.07" E	42	CBT/1093
BM37	Rayagada, OD	19° 10′ 16.32" N, 83° 24′ 58.68" E	220	RPRC/12544	BM78	South 24 Parganas, WB	22° 06′ 35.28" N, 88° 19′ 14.88" E	7	CBT/1094
BM38	Rayagada, OD	19° 11′ 19.32" N, 83° 17′ 12.12" E	220	CBT/1069	BM89	South 24 Parganas, WB	22° 24′ 03.3" N, 88° 03′ 00.09" E	9	CBT/1095
BM39	Rayagada, OD	19° 06′ 49.68" N, 83° 14′ 17.88" E	567	RPRC/12543	BM80	Birbhum, WB	23° 49′ 44.27′′ N, 87° 25′ 09.26′′ E	70	CBT/1096
BM40	Rayagada, OD	19° 21′ 52.2" N, 83° 2′6 43.08" E	268	CBT/1070	BM81	Birbhum, WB	23° 56′ 38.65′′ N, 87° 43′ 32.00′′ E	44	CBT/1097
BM41	Rayagada, OD	19° 40′ 17.04" N, 83° 28′ 24.6" E	384	RPRC/12542					

BM: *Bacopa monnieri*; Acc. No: Accession number; WB: West Bengal; OD: Odisha

Table 2. Climatic data of 81 *Bacopa monnieri* accessions from two provinces of eastern India.

Acc. No.	Prec (mm)	Max. Temp. (°c)	Min. Temp. (°c)	Avg. Temp. (°c)	Avg. Rel. Hum. (%)	Altitude (m)	UV index	Acc. No.	Prec (mm)	Max. Temp. (°c)	Min. Temp. (°c)	Avg. Temp. (°c)	Avg. Rel. Hum. (%)	Altitude (m)	UV index
BM1	1576	36.50	14.10	26.80	59.50	15	10.66	BM42	1486	36.20	14.10	26.80	64.25	527	7.08
BM2	1596	37.60	13.40	26.10	59.25	41	7.08	BM43	1428	36.10	15.70	27.20	67.50	55	6.66
BM3	1459	38.30	14.50	27.10	59.25	31	7.08	BM44	1342	38.30	13.70	26.50	62.83	75.7	6.83
BM4	1505	37.00	15.60	27.40	67.25	44	6.66	BM45	1204	33.90	16.00	26.60	69.66	120	6.75
BM5	1460	39.00	19.00	29.33	67.50	120	5.83	BM46	1594	33.40	14.50	26.20	73.00	7	6.66
BM6	1388	37.80	14.40	26.90	62.83	115	6.83	BM47	1594	33.40	14.50	26.20	54.83	7	7.16
BM7	1337	32.50	17.10	26.90	73.75	12	7.25	BM48	1399	34.60	13.40	26..3	63.91	9	7.00
BM8	1337	32.50	17.10	26.90	73.75	12	7.25	BM49	1298	38.90	12.10	26.50	51.91	122	7.25
BM9	1337	32.50	17.10	26.90	67.50	10	6.66	BM50	1533	36.40	12.30	26.40	60.00	12	5.00
BM10	1337	32.50	17.10	26.90	67.50	546	6.66	BM51	1627	36.30	12.40	26.40	60.08	7	7.08
BM11	1337	32.50	17.00	26.90	67.50	19	6.66	BM52	1533	36.40	12.30	26.40	60.00	7	6.41
BM12	1515	38.30	15.50	27.60	65.91	54.8	6.83	BM53	1512	36.20	12.50	26.40	60.25	15	7.00
BM13	1472	37.90	14.60	27.00	65.86	82.2	6.73	BM54	1345	36.80	11.90	26.30	51.16	16	7.16
BM14	1601	36.50	14.10	26.70	64.25	40.7	7.08	BM55	1744	36.00	12.70	26.30	59.75	12	7.08
BM15	1598	36.80	13.90	26.70	64.23	30.3	7.08	BM56	1721	36.20	12.70	26.40	58.50	15	7.00
BM16	1512	35.40	14.40	26.70	64.25	21.3	6.91	BM57	1384	34.50	13.60	26.40	59.00	19	7.10
BM17	1519	37.20	14.50	27.00	62.41	108	7.08	BM58	1667	33.90	14.50	26.60	56.50	12	6.32
BM18	1498	36.30	15.40	27.10	71.00	16.5	6.81	BM59	1542	37.10	8.10	26.00	77.00	20	7.00
BM19	1430	40.80	13.40	27.20	57.58	123.9	6.75	BM60	1287	38.00	12.00	26.30	56.25	59	7.25
BM20	1430	40.80	13.40	27.20	57.58	590	6.75	BM61	1735	35.90	12.60	26.20	59.08	20	7.08
BM21	1430	40.80	13.40	27.20	57.58	129.6	6.75	BM62	1472	34.10	14.00	26.30	62.08	5	6.58
BM22	1430	40.80	13.40	27.20	57.58	126	6.75	BM63	1657	33.20	14.70	26.30	73.25	6	6.66
BM23	1430	40.80	13.40	27.20	57.58	106	6.75	BM64	1609	35.10	13.10	26.30	61.25	10	6.91
BM24	1320	38.00	13.40	26.70	59.25	280	5.98	BM65	1645	35.40	13.00	26.30	59.08	11	7.08
BM25	1253	39.60	13.40	26.70	60.66	255	6.83	BM66	1472	34.10	14.00	26.30	62.08	5	6.58
BM26	1596	38.00	13.40	26.80	59.25	46	7.08	BM67	1529	34.00	14.00	26.30	61.16	4	6.91
BM27	1194	32.50	16.60	26.80	69.66	39.5	6.75	BM68	1527	36.20	12.30	26.30	60.08	14	7.08
BM28	1194	32.50	16.60	26.80	69.66	35	6.75	BM69	1350	37.80	11.60	26.40	58.33	24	7.08
BM29	1295	37.70	11.20	24.80	63.66	427.5	6.33	BM70	1472	34.10	14.00	26.30	62.08	6	6.58
BM30	1610	37.10	13.70	26.70	59.25	32.6	7.08	BM71	1609	35.10	13.10	26.30	61.25	10	6.91
BM31	1185	32.70	16.50	26.70	69.66	18	6.75	BM72	1627	36.30	12.40	26.40	60.08	7	7.08
BM32	1342	38.30	13.70	26.50	62.83	114	6.83	BM73	1645	35.40	13.00	26.30	59.08	11	7.08
BM33	1280	42.90	12.40	27.20	52.41	212	6.91	BM74	1529	34.00	14.00	26.30	61.16	4	6.91
BM34	1470	41.80	13.20	26.90	55.50	213	6.00	BM75	1436	38.10	13.10	26.80	59.50	36	7.16
BM35	1376	41.30	13.40	27.10	55.50	175	6.83	BM76	1594	33.10	14.50	26.20	68.41	8	6.66
BM36	1512	35.40	14.40	26.70	64.25	9	6.91	BM77	1609	35.10	13.10	26.30	61.25	10	6.91
BM37	1312	36.70	14.90	26.50	68.53	220	6.58	BM78	1399	34.60	13.70	26.30	63.91	9	6.50
BM38	1312	36.70	14.90	26.50	68.53	220	6.58	BM79	1157	38.40	12.70	26.60	59.10	9	7.08
BM39	1312	36.70	14.90	26.50	68.53	567.5	6.58	BM80	1289	38.30	11.80	26.30	54.25	70	6.14
BM40	1312	36.70	14.90	26.50	68.53	268	6.58	BM81	1328	38.10	11.60	26.30	56.28	44	6.06
BM41	1312	36.70	14.90	26.50	68.53	384	6.58								

BM: *Bacopa monnieri*; Acc. No: Accession number; Prec: Precipitation; Max. Temp: Maximum temperature; Min. Temp: Minimum temperature; Avg. Temp.: Average temperature; Avg. Rel. Hum.: Average relative humidity; UV index: Ultra violet index

Table 3. Soil parameter analysis of studied collection sites.

Acc. No.	pH	E.C. (ds/m)	O.C. (%)	N_2 (kg/ha)	P_2O_5 (kg/ha)	K_2O (kg/ha)	Acc. No.	pH	E.C. (ds/m)	O.C. (%)	N_2 (kg/ha)	P_2O_5 (kg/ha)	K_2O (kg/ha)
BM1	5.70	0.32	0.38	212.5	26.9	0.32	BM42	6.80	0.28	0.25	134	17.9	0.28
BM2	4.90	0.47	0.57	225	7.7	0.47	BM43	6.07	0.27	0.34	112.5	138.3	0.27
BM3	4.70	0.02	0.32	121	4.4	0.02	BM44	5.36	0.26	0.31	180	27	0.26
BM4	4.50	0.29	0.38	88.9	14.3	0.29	BM45	5.90	0.12	0.20	129.5	19.5	0.12
BM5	5.37	0.19	0.43	156.9	9.8	0.19	BM46	6.14	1.60	0.62	356	187	1.60
BM6	5.86	0.20	0.57	75	9	0.20	BM47	6.27	1.70	0.45	382	135	1.70
BM7	4.66	0.04	0.29	140	22	0.04	BM48	6.11	0.97	0.32	193	23.8	0.97
BM8	4.66	0.04	0.29	130	22	0.04	BM49	5.97	0.26	0.65	376	67	0.26
BM9	4.80	0.13	1.19	125	17	0.13	BM50	7.52	0.21	0.37	182	315	0.21
BM10	4.80	0.13	1.19	25	17	0.13	BM51	5.23	0.23	0.32	177	326	0.23
BM11	5.12	0.24	0.39	180.2	8.3	0.24	BM52	7.00	0.41	0.69	350	420	0.41
BM12	5.36	0.16	0.32	101	19.2	0.16	BM53	6.90	0.29	0.47	296	362	0.29
BM13	4.75	0.11	0.34	167	34	0.11	BM54	6.65	0.23	0.51	454	69	0.23
BM14	6.10	0.31	0.25	115.9	17.4	0.31	BM55	7.20	0.68	0.86	540	25	0.68
BM15	5.90	0.20	0.33	165	23.9	0.20	BM56	7.31	0.45	0.52	371	67	0.45
BM16	5.70	0.28	0.20	29.9	5.9	0.28	BM57	6.59	0.28	0.68	290	26	0.28
BM17	5.07	0.13	0.29	66.4	9	0.13	BM58	6.75	0.58	0.34	325	130	0.58
BM18	5.70	0.34	0.23	102.8	45	0.34	BM59	5.90	0.53	0.66	292	86	0.53
BM19	5.98	0.07	0.81	127.6	24	0.07	BM60	6.40	0.35	0.70	290	134	0.35
BM20	5.67	0.19	0.23	22.5	313.8	0.19	BM61	5.21	0.56	0.69	305	119	0.56
BM21	5.47	0.41	0.11	69.4	19	0.41	BM62	6.32	0.67	0.24	376	192	0.67
BM22	6.08	0.15	0.65	78.5	35	0.15	BM63	5.61	0.22	0.37	220	360	0.22
BM23	5.96	0.21	0.53	110.5	29	0.21	BM64	6.10	0.45	0.88	420	84	0.45
BM24	6.20	0.70	0.55	156.8	19	0.70	BM65	6.90	0.43	0.93	412	60	0.43
BM25	6.20	0.67	0.48	156.4	17	0.67	BM66	6.20	0.66	0.59	335	53	0.66
BM26	4.50	0.11	0.47	120.5	7.8	0.11	BM67	5.80	0.90	0.75	284	116	0.90
BM27	5.40	0.39	1.00	45.8	55	0.39	BM68	7.20	0.54	0.34	287	320	0.54
BM28	5.70	0.23	1.20	51	41.6	0.23	BM69	6.10	0.23	0.28	360	62	0.23
BM29	6.20	0.39	0.80	25	10	0.39	BM70	6.32	1.05	0.34	235	46.3	1.05
BM30	5.90	0.03	0.23	252	7.7	0.03	BM71	7.28	0.91	0.63	451	65	0.91
BM31	5.80	0.59	1.35	127.5	16.2	0.59	BM72	5.81	0.52	0.72	393	117	0.52
BM32	4.93	0.10	0.47	170.5	12	0.10	BM73	6.56	0.71	0.69	362	58.5	0.71
BM33	6.12	0.28	0.71	29	8	0.28	BM74	6.15	0.89	0.78	450	43.8	0.89
BM34	5.60	0.10	1.49	12.5	121.3	0.10	BM75	6.30	0.22	0.45	319	45	0.22
BM35	6.60	0.19	0.62	18.4	20.4	0.19	BM76	7.20	1.10	0.53	294	38	1.10
BM36	5.00	0.18	0.33	240	8.9	0.18	BM77	5.90	1.70	0.59	401	70	1.70
BM37	6.18	0.17	1.03	12.5	7.7	0.17	BM78	5.10	0.98	0.65	254	81	0.98
BM38	5.30	0.26	0.34	12.5	7.7	0.26	BM79	6.30	0.59	0.98	410	96	0.59
BM39	5.66	0.14	1.60	12.5	2.3	0.14	BM80	5.87	0.45	0.61	285	58	0.45
BM40	5.42	0.12	0.34	37.5	6.9	0.12	BM81	5.92	0.51	0.39	415	42	0.51
BM41	5.95	0.19	0.25	25	7.7	0.19							

BM: *Bacopa monnieri*; Acc. No: Accession number; E.C: Electrical conductivity; O.C: Organic carbon standard solution were set to 1.5 mL/min and 20 µL, respectively. The detector wavelength was set at 205 nm for acquiring the chromatograms. Before starting the analysis, the freshly prepared mobile phase was filtrated using a 0.45-µm membrane filter, followed by degassing using an ultra-sonicator.

Quantification of bacoside A

Quantitative determination of bacoside A in all the accessions of *B. monnieri* was calculated from the calibration curve prepared by using peak areas and concentrations of reference standard solutions of four individual components of bacoside A (purity > 99%, Natural Remedies Private Limited, Bangalore, India). Five points linear calibration curves were constructed by serially diluting the stock solution of bacopaside II (0.14 mg/mL), bacoside A3 (0.14 mg/mL), jujubogenin isomer of bacopasaponin C (0.1 mg/mL), and bacopasaponin C (0.3 mg/mL) in methanol.

Development of an artificial neural network model

Neural network models have been commonly used as a functional tool to identify, classify and recognize pattern in different set of experimental data (Agatonovic-Kustrin and Beresford 2000). In this study, the most commonly used supervised MLP feed-forward ANN model was developed by Statistica software (Tibco Stat Inc., version 13.5.07) using back propagation learning algorithm for the prediction of bacoside A content. The model was trained by taking the input (climatic and edaphic factors) and output variable (bacoside A content). The topological study of ANN structure consisted of 13 input variables: precipitation, altitude, maximum temperature, average annual temperature, minimum temperature, average relative humidity (ARH), ultra-violet index, pH, electrical conductivity (EC), organic carbon (OC), N, P and K as the input layers and one dependent variable (bacoside A) as the output layer. In this study, the 81 samples dataset were randomly partitioned consisting of 70% (57 samples) for training, 15% (12 samples) for testing and remaining 15% (12 samples) for validation dataset. The connection weight between the neurons and biases were adjusted during the training process to reduce the variation between the experimental and predicted value. The nodes or neurons in the hidden and output layer act as synaptic junction of biological neurons that connect and rearrange the inputs using the mathematical formula given below.

$$Y_s = \left[\sum_{t=1}^{p} \mathrm{v}_s u_{st} + x_t \right]$$

in which Ys, s, v_s, u_{st} and x_t is the input to node t in hidden and output layer, the number of nodes, the output of the previous layer, linkage of weight in between the s and t node and the biased linked with node, t respectively.

The ANN model was developed to estimate the nonlinear relationship using a sigmoid transfer function by the following equation:

$$Z_t = \left[\frac{1}{1 + w^{ys}} \right]$$

where Z_t represents the output of node t.

The normalization of input and output data was carried out within a uniform range (0–1) to prevent over fitting and was performed according to the following formula.

$$A_{norm} = \left[\frac{(A - A_{min})}{(A_{max} - A_{min})} \right]$$

where A_{norm}, A, A_{max} and A_{min} are the normalized value, real value, maximum and minimum value, respectively.

Additionally, the prediction potential of the achieved neural network model was determined by analyzing the statistical quality parameters, such as R^2, RMSE, mean absolute error (MAE) and MAPE as given below,

$$R^2 = \left[1 - \frac{\sum_{i=1}^{n} (X_i - X_{ik})^2}{\sum_{i=1}^{n} (X_{ik} - X_z)^2} \right]$$

$$\mathrm{RMSE} = \left[\frac{1}{n} \sum_{i=1}^{n} (X_i - X_{ik})^2 \right]$$

$$\mathrm{MAE} = \left[\frac{1}{n} \sum_{i=1}^{n} |X_i - X_{ik}| \right]$$

$$\mathrm{MAPE} = \left[\frac{1}{n} \sum_{i=1}^{n} \left| \frac{X_i - X_{ik}}{X_i} \right| \times 100\% \right]$$

in which n, X_i, X_{ik} and X_z denote the number of observations, predicted value, experimental value and the mean experimental value, respectively.

Additionally, sensitivity analysis was carried out to test the individual effect of independent input variables on bacoside A content.

Results

ANN model development for the optimization of bacoside A content

The present ANN model was trained using back propagation algorithm by weights adjustment in order to reduce the mean square error between the experimental and predicted output values. The developed ANN architecture consisted of an input layer with 13 neurons, single hidden layer with 11 neurons and an output layer with one neuron as shown in Figure 1. The determination of the best number of neurons of the hidden layer was carried out by trial and error method. The model was tested with neurons ranging from 2 to 12 in the hidden layer and on the basis of the coefficient of determination (R^2) and least error value. Finally, a good fit ANN

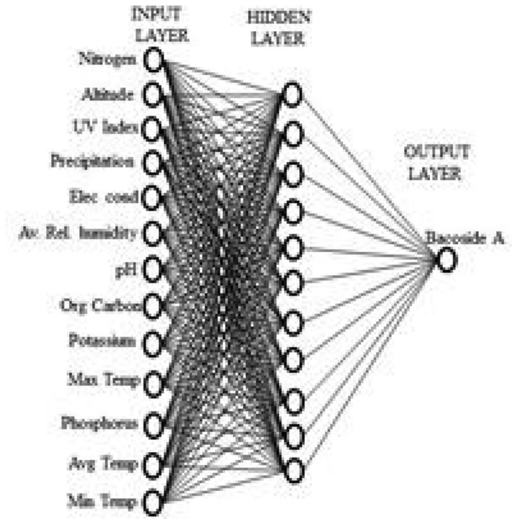

Figure 1. Architecture of multilayer perception feed forward neural network used in the study.

model with a single hidden layer containing 11 neurons was chosen on the basis of least error and maximum R^2 value. The neural network analysis revealed that MLP neural network has a strong nonlinear relationship between input and output variables. Probably, for an appropriate network model its RMSE and R^2 values should be closed to 0 and 1, respectively. The RMSE value of training (0.16), testing (0.14) and validation (0.22) dataset revealed that the developed 13-11-1 network model is accurate and precise (Tables 4–6). A linear regression plot with R^2 value of 0.90, 0.95 and 0.87 for train, test and validation, respectively was developed by plotting experimental against predicted value of bacoside A (Figure 2).

Analysis of variables

Bacoside A content

The analyses of bacoside A content by adding the amount of its four components in 81 accessions of *B. monnieri* from different geographical locations of eastern India were carried out using the validated HPLC method. Various mobile phases with different run times were tested, finally, the mobile phase consisting orthophosphate buffer with pH 2.4 and acetonitrile with run time of 30 min were preferred for the establishment of large number of peaks in the chromatograms of the sample solution. A representative HPLC chromatogram of bacoside A standard, which is a combination of four components, and the sample are shown in Figure 3A and B, respectively. The calibration curves were developed by plotting the peak area (y) against the concentration (x, µg/mL) of bacoside A3, bacopaside II, jujubogenin isomer of bacopasaponin C, and bacopasaponin C. The calibration curve showed good linearity (R^2 > 0.989), and within the tested concentration range (Table 7). The method validation was performed based on repeatability, stability, recovery, precision, limit of detection and quantification (LOD and LOQ) parameters and the results were given in Table 7. The LOD and LOQ are defined as the least concentrations at which the signal-to-noise ratios (S/N) were three and ten times, respectively. The LOD and LOQ value of four components were found to be 6.7 – 8.9 µg/mL and 20.5 – 27.1 µg/mL, respectively. The detection limit was sufficiently low to estimate these analytes in *B. monnieri* samples. The determination of precision test was performed by injecting the replicate solution of bacoside A3, bacopaside II, jujubogenin isomer of bacopasaponin C, and bacopasaponin C standard for three times within a day and the RSD value was found to be 0.75% – 1.84%. The stability of the sample was carried out by injecting the four standard

Table 4. Experimental and predicted bacoside A content of training dataset.

Acc. No.	Predicted Bacoside A content	Actual Bacoside A content	Absolute error	Absolute percentage error
BM1	1.54	1.64	0.10	6.22
BM4	1.63	1.67	0.03	1.92
BM6	1.18	1.56	0.39	24.78
BM7	1.88	1.98	0.10	5.16
BM8	1.84	1.84	0.01	0.33
BM9	1.72	1.99	0.27	13.73
BM11	2.00	2.06	0.06	3.10
BM12	1.57	1.31	0.26	19.82
BM13	1.54	1.60	0.06	3.51
BM14	1.59	1.52	0.07	4.28
BM15	1.75	1.99	0.24	12.26
BM18	1.75	1.97	0.22	10.94
BM19	1.22	1.43	0.21	14.73
BM20	0.67	0.67	0.01	0.90
BM21	1.17	1.21	0.04	2.98
BM22	1.09	1.20	0.12	9.64
BM23	1.25	1.11	0.15	13.48
BM24	1.04	1.02	0.02	2.17
BM25	1.00	0.96	0.05	4.81
BM28	1.37	1.16	0.21	17.80
BM30	1.92	1.87	0.05	2.62
BM31	1.58	1.40	0.18	12.83
BM32	1.26	1.18	0.08	6.60
BM33	0.79	0.71	0.08	11.27
BM35	0.87	0.87	0.00	0.23
BM36	2.10	2.14	0.03	1.50
BM37	0.88	0.85	0.03	3.16
BM40	0.86	0.98	0.12	12.30
BM41	0.73	0.89	0.15	17.40
BM43	1.60	1.46	0.15	10.17
BM45	1.35	1.15	0.20	17.23
BM46	2.38	2.53	0.16	6.12
BM47	2.26	2.25	0.01	0.31
BM49	1.79	1.61	0.19	11.51
BM51	1.83	2.00	0.17	8.55
BM52	2.29	2.14	0.15	7.00
BM53	2.06	2.31	0.26	11.04
BM54	2.29	2.26	0.04	1.68
BM56	2.10	2.14	0.04	1.91
BM57	1.85	1.85	0.00	0.05
BM58	2.10	1.83	0.28	15.10
BM59	2.16	1.98	0.19	9.47
BM60	1.81	1.82	0.01	0.71
BM61	2.06	1.94	0.12	6.18
BM64	2.27	1.93	0.35	17.97
BM65	2.16	2.18	0.02	0.78
BM66	2.26	2.19	0.07	3.19
BM68	2.00	2.18	0.17	7.95
BM70	1.94	2.00	0.06	3.14
BM72	2.34	2.40	0.06	2.46
BM73	2.14	2.07	0.07	3.28
BM74	2.36	2.55	0.19	7.38
BM75	2.09	1.79	0.30	16.99
BM76	2.11	1.94	0.18	9.14
BM78	2.22	1.96	0.26	13.05
BM79	2.35	2.41	0.06	2.45
BM81	2.67	2.87	0.20	7.04
Mean			0.13	7.76

BM: *Bacopa monnieri*; Acc. No: Accession number

Table 5. Experimental and predicted bacoside A content of testing dataset.

Acc. No	Predicted bacoside A content	Actual bacoside A content	Absolute error	Absolute percentage error
BM3	1.43	1.67	0.24	14.28
BM5	1.65	1.67	0.01	0.86
BM17	1.34	1.26	0.08	6.64
BM27	1.10	1.38	0.28	20.22
BM34	0.76	0.84	0.09	10.16
BM38	0.92	0.92	0.00	0.16
BM39	0.68	0.67	0.01	1.55
BM44	1.59	1.49	0.10	6.64
BM55	2.59	2.38	0.21	8.95
BM63	2.20	2.09	0.11	5.28
BM69	2.29	2.28	0.01	0.30
BM80	2.20	2.04	0.16	7.91
Mean			0.11	6.91

BM: *Bacopa monnieri*; Acc. No: Accession number

Table 6. Experimental and predicted bacoside A content of validation dataset.

Acc. No.	Predicted bacoside A content	Actual bacoside A content	Absolute error	Absolute percentage error
BM2	1.75	1.86	0.11	5.65
BM10	0.79	0.68	0.11	15.80
BM16	1.81	1.45	0.35	24.11
BM26	1.19	1.51	0.33	21.67
BM29	0.86	0.73	0.13	18.27
BM42	0.89	0.72	0.17	23.98
BM48	1.91	1.78	0.13	7.34
BM50	1.98	2.05	0.07	3.48
BM62	2.19	2.35	0.16	6.60
BM67	2.49	2.05	0.45	21.79
BM71	2.54	2.33	0.21	9.04
BM77	2.21	2.42	0.20	8.45
Mean			0.06	6.21

BM: *Bacopa monnieri*; Acc. No: Accession number

solutions for three consecutive days (0, 24, 48, and 72 h) and observed that the compounds were stable in solution (RSD = 0.61% − 1.59%). Repeatability was tested by injecting three separately prepared replicates of four standard solutions and the obtained RSD value (0.59% − 1.37%) of the compounds specified that the method of repeatability is suitable. The recovery potential of four standards was evaluated by adding known amount of standard reference compound to the analyzed samples. Then, analysis was performed and the result of average recovery percentage of the four compounds was found to be in the range 98.46% − 99.56% (RSD = 0.22% − 1.11%). The quantification results revealed that the content of bacoside A in 81 *B. monnieri* accessions varied from 0.67% (BM20) to 2.87% (BM81), respectively. Screening of bacoside A estimation result revealed that among the eighty one accessions, three accessions (BM 46, BM 74 and BM 81) of *B. monnieri* were found to contain > 2.5% of bacoside A content. These three high-yielding accessions of *B. monnieri* were considered useful for commercial cultivation purpose.

Sensitivity analysis

The influence of different independent variables (input) on the dependent variable (output) in the ANN model was evaluated using sensitivity analysis (Table 8). The sensitivity analysis of the developed ANN model revealed that N has the maximum impact on bacoside A content with an error coefficient of 4.45, followed by altitude, rainfall, UV index, EC, average relative humidity, pH, OC, K, maximum temperature, P, average temperature and minimum temperature with an error coefficient of 3.13, 1.92, 1.10, 1.07, 1.07, 1.06, 1.05, 1.04, 1.04, 1.03, 1.05 and 1.01, respectively.

Optimization of bacoside A content

Optimization of bacoside A yield was established (Figure 4) by changing influential parameters like N and altitude. The N value was changed from 125 to 140 and altitude values changed from 15 to 10 and bacoside A content measured. It was observed that bacoside A content increased from 1.72% to 1.89% in the developed neural network model. This study demonstrated that the content of bacoside A could be optimized in the developed MLP neural network model by regulating the most influential factors, namely, altitude and N content.

Prediction of bacoside A content at the desired location using developed ANN model

In this study, the developed ANN model was successfully used to predict the bacoside A content at a new geographical location. The prediction performance was carried out by incorporating the soil and climatic factors data of a new location (Mangalajodi, Odisha) in the ANN model. The ANN model displayed a prediction accuracy of 93.60% in bacoside A yield between the experimental (1.61%) and predicted (1.72%) value.

Discussion

In the last few decades, scientists are using artificial neural network tools for better prediction and optimization in several disciplines such as crop production, soil science, pharmaceutical research and bioactive compound yield in medicinal plant (Agatonovic-Kustrin and Beresford 2000; Akbar et al. 2018; Ray et al. 2020). The ANN model is more impressive for its good deduction of data structure and interesting interpretation of puzzle interaction among nodes of different layers in comparison to other statistical models. In ANN models, the information was passed from input layer through the middle hidden layer to output layer by the process of weight adjustment. Many researchers have reported that the back propagation algorithm method used for the learning process in the multilayer perceptron network with sigmoid axon transfers function to predict crop productivity (Pilkington et al. 2014; Astray et al. 2016; Abdipour et al. 2018, 2019).

The estimation of performance of the ANN model was carried out using R^2, RMSE, MAE and MAPE. The R^2, RMSE, MAE, and MAPE of the training data set were 0.90, 0.159, 0.127, and 7.760, respectively. The higher value of the training dataset with a R^2, and lower RMSE value specified that there was minimum deviation between the experimental and predicted value. The estimated ANN model performance

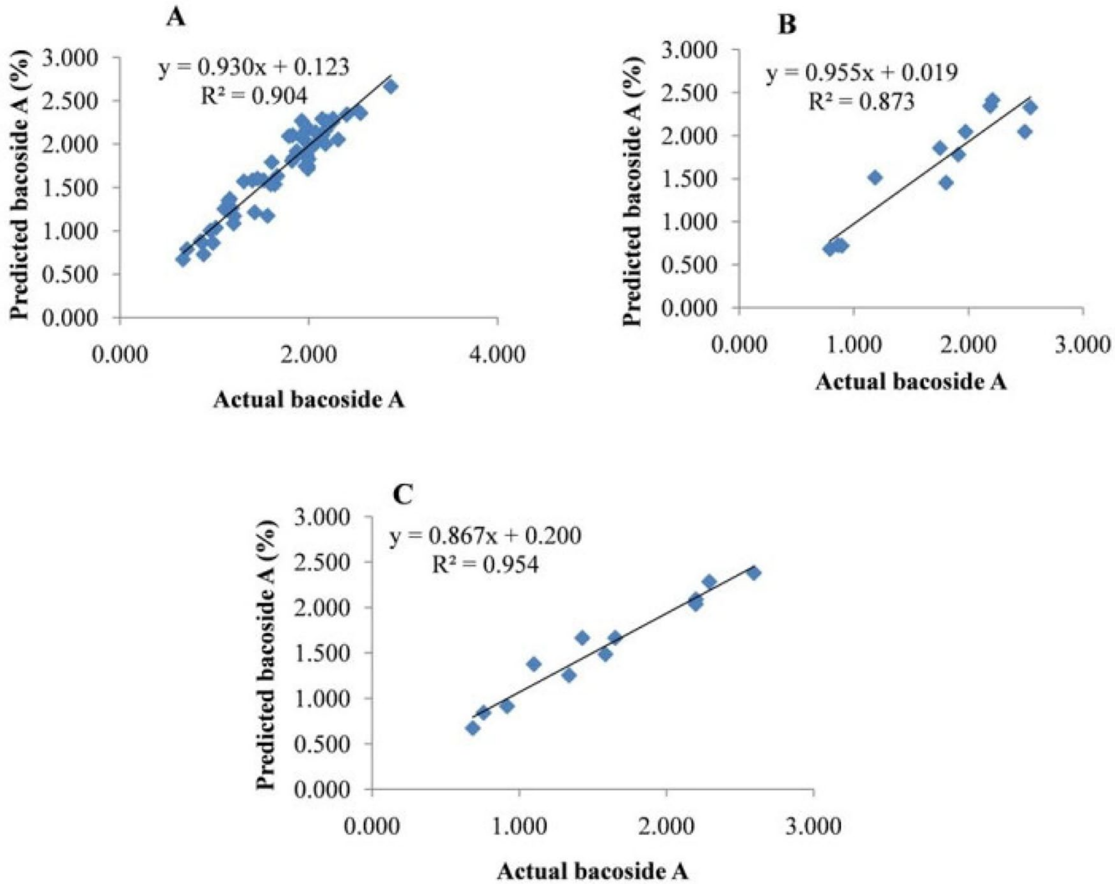

Figure 2. Scatter plot showing experimental and predicted value of bacoside A content of (A) training (B) validation and (C) testing data set.

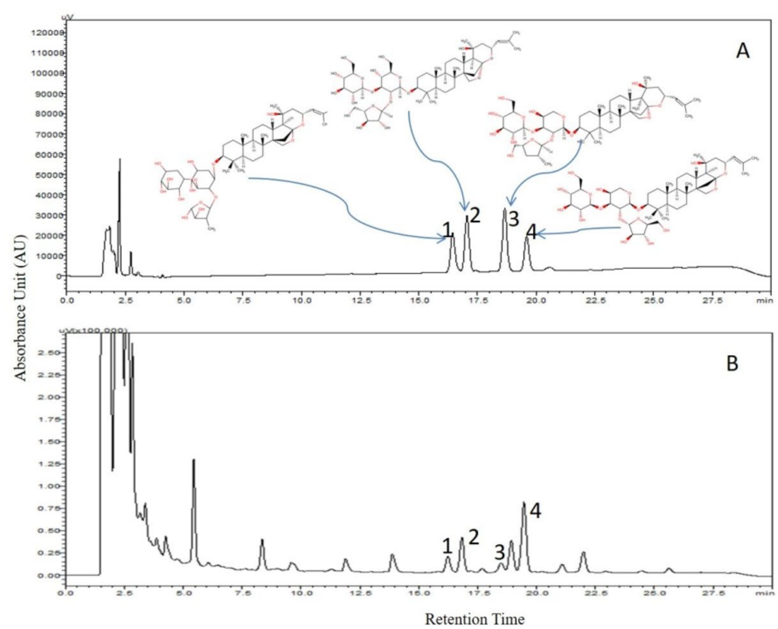

Figure 3. HPLC chromatogram of (A) bacoside A that consist of four components (bacoside A3 (peak 1), bacopaside II (peak 2), jujubogenin isomer of bacopasaponin C (peak 3), and bacopasaponin C (peak 4) and (B) Bacopa monnieri extract.

results revealed that MLP network of 13-11-1 structure with axon transfer function and 11 neurons in hidden layer is a better model because of its high R^2 value with least error values. Thus, the ANN model with four statistical parameters developed by us exhibited better predictability of bacoside A yield.

Similar findings showing the effectiveness of ANN model for accurate prediction of secondary metabolite content have

Table 7. HPLC method validation of four components of bacoside A.

Validation parameter	Bacoside A3	Bacopaside II	Jujubogenin Isomer of Bacopasaponin C	Bacopasaponin C
Linear range(µg/mL)	28-135	25-125	22-110	21-105
Regression equation	y = 33.8x + 0.03	y = 34.4x + 0.01	y = 41.8x-0.01	y = 53.2x + 0.04
R^2	0.991	0.993	0.998	0.989
LOD (µg/mL)	8.9	7.4	6.7	7.2
LOQ µg/mL)	27.1	22.3	20.5	21.8
Precision RSD (%) (n = 3)	7.4	1.39	1.84	1.17
Stability RSD (%)(n = 4)	0.61	1.59	1.92	0.98
Repeatability RSD (%)(n = 3)	1.37	1.03	0.59	0.87
Average Recovery (%)	99.10	98.46	98.73	99.56

Bacoside A is the sum of four components (Bacoside A3, Bacopaside II, Jujubogenin Isomer of Bacopasaponin C; Bacopasaponin C)

Table 8. Sensitivity analysis of neural network.

Parameters	Error quotient	Rank
Nitrogen	4.45	1
Altitude	3.13	2
Precipitation	1.92	3
Ultra-violate index	1.10	4
Electrical conductivity	1.07	5
Average relative humidity	1.07	6
pH	1.06	7
Organic carbon	1.05	8
K_2O	1.04	9
Maximum temperature	1.04	10
P_2O_5	1.03	11
Average annual temperature	1.05	12
Minimum temperature	1.01	13

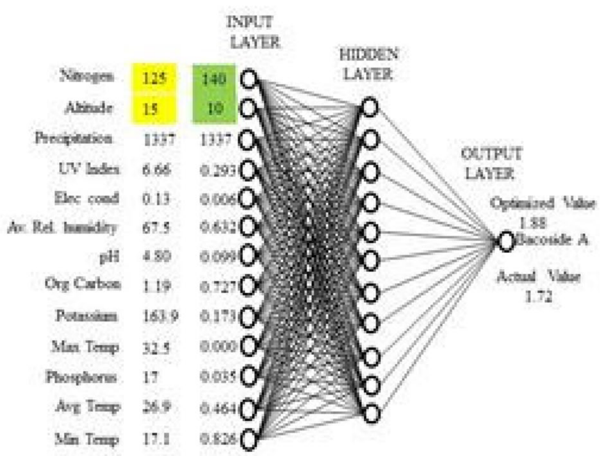

Figure 4. Optimisation of bacoside A content by changing input parameters of ANN model.

been reported earlier by several researchers. Ray et al. (2020) used an ANN model of 18-5-1 architecture by taking 18 edaphic and climatic factors as an input and one dependent variable (coronarin D) as an output parameter to predict the content of coronarin D in *Hedychium coronarium* Koen. Alam and Naik (2009) developed an ANN model with 12-8-1 structure for the prediction of podophyllotoxin yield in *Podophyllum hexandrum* Royle at a new location. Akbar et al. (2018) developed a perfect ANN topology of 11-12-1structure to predict the turmeric oil yield at a particular site.

Sensitivity analysis is the study of influence of different independent variables (input) on the dependent variable (output) in the ANN model. In this analysis it was possible to find out the most and least significant factor responsible for bacoside A yield from *B. monnieri*. Sensitivity analysis was carried out for the evaluation of prediction capacity and validation of the developed neural network model for the yield of bacoside A. It was found out that the input parameter N and altitude with an error quotient value of 4.448 and 3.133, respectively are the most influential parameters, whereas minimum temperature is the least influential parameter with error quotients value of 1.008 for bacoside A production. Our study is in agreement with the report of Naik et al. (2011) who have reported bacoside A content to be higher in biomass of *B. monnieri* cultured in medium containing ammonium nitrate. Ammonium nitrate is a chief source of N, commonly used as fertilizer in agriculture. Another study by Akbar et al. (2018) has also revealed N and altitude to be the highly sensitive parameters for curcumin yield. A study by Salmore and Hunter (2001) reported that the alkaloid content in bloodroot (*Sanguinaria canadensis* L.) rhizome decreases with increase in altitude. In the present study, sensitivity analysis results revealed that N is the most influential parameter for the synthesis of bioactive compound bacoside A. N uptake by the plant in the form of NH_4^+ or NO_3^- upregulates the gene involved in the synthesis of triterpenoid saponins (Wei et al. 2020). Furthermore, N is an important element involved in the synthesis of amino acids, proteins, and enzymes directly involved in plant growth and development. Moreover, the sensitivity analysis studies also suggested that the yield of bacoside A is influenced by more than two factors synergistically in comparison to that of a single factor. Several earlier reports have attributed the variation of secondary metabolites to difference in edaphic, climatic, seasonal, geographic, and factors as well as several other factors (Figueiredo et al. 2008; Liu et al. 2019; Li et al. 2020). Finally, from the present study we have inferred that the variation of bacoside A content exhibited among different, wild accessions of *B. monnieri* sampled from different geographical locations of India (Odisha and West Bengal provinces) was mainly due to varied soil nutrient levels and climatic factors). In general, like all living organisms, medicinal plants also display a maximum potential in production of secondary metabolites while in their natural habitat and any alteration in natural habitat exerts detrimental effects on the drug yield and quality. Thus, for maximizing production of bacoside A in cultivated *B. monnieri* at selected locations, ensuring similar soil types, and climate as in their natural habitat condition is essential.

Secondary metabolite production is a very complex process of metabolism regulated by varying factors such as plant maturity level, growing season, environmental as well as genetic factors. Nevertheless, in the present study it was demonstrated that the content of bacoside A can be optimized employing the ANN model by changing the altitude and N level parameters. The ANN model was used for the prediction of bacoside A yield in *B. monnieri* with a demonstrated 93.60% prediction accuracy of bacoside A yield at a new site (Mangalajudi) of Khordha district of Odisha. This result, therefore opens immense scope for further research in improving bacoside A content through better standardization and management of different soil characteristics as well as environment factors.

Conclusions

In this study, an optimized MLP ANN model having 11 neurons in the hidden layer was developed for the prediction and optimization of bacoside A content in *B. monnieri*. The developed ANN model specified that by changing the most sensitive input parameters (altitude and N) the bacoside A content of *B. monnieri* could be increased from 1.72% to 1.88%. The developed ANN model also summarized the prediction possibility of bacoside A content in *B. monnieri* at a new geographic location.

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Disclosure statement

The authors declare no conflict of interest.

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