Current Trends in Biotechnology and Pharmacy
Vol. 17(Supplementary issue - 3B) 1303-1315,September 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.3s.65

Adaptability Assessment of *Bruguiera gymnorrhiza* from the Four Southern Districts of Kerala: Analytical and Biochemical Approach

Sreeram. S, V. Sanakara Vel , and A. Arunprasath*

Department of Botany, PSG College of Arts & Science, Coimbatore-14, Tamil Nadu, India
Corresponding Author: arunprasath194@gmail.com

Abstract

The comparative adaptability of the species *Bruguiera gymnorrhiza* from the southern districts of Kerala *viz*., Kollam, Alappuzha, Cochin and Thrissur was assessed *in-vitro*. *B. gymnorrhiza* being a salt-sensitive plant must be carefully analyzed before restoration practices. Without efficient ROS scavenging, the plant will not be able to survive the salinity fluctuation, there should be an equilibrium between both. The study reveals the adaptability of mangroves to salt tolerance assessed through secondary metabolites and enzymatic activity for the promotion of antioxidant activity with respect to salinity fluctuation from the four districts. The presence of secondary metabolites was further confirmed by GC-MS analysis. The plant thrived better in optimum salinity, and the highest phenolic content was obtained from Kollam. The antioxidant activity of DPPH and Fe^{2+} was higher in the Alappuzha sample and the $ABTS^{+}$ was higher in the leaf sample collected from Thrissur. Methyl Commate B was reported for the first time in mangroves and from the species *B. gymnorrhiza*. Being a novel identification, it would pave the way for the medicinal use of the species and the SEM-EDX results reveal the high sequestration capacity of the plant.

Keywords: Mangroves, Plant-physiology, Adaptability, Methyl Commate B, Biochemical analysis

Introduction

Mangroves are productive coastal ecosystems that house numerous protective halophytic plant communities around the tropical and sub-tropical regions of the world. Located in inter-tidal zones, it is prone to cyclones, floods, typhoons, Tsunamis, and storms (1). In India, mangroves are used as a folk remedy and suffice the religious significance of the people living in its vicinity. Their unique property of carbon sequestration makes them excellent natural filtrates (2), nonetheless, they provide some excellent breeding grounds for fishes and migratory birds. The rise in sea levels is also a serious concern for the degradation of mangrove vegetation (3). Mangroves are mainly found in coastal and estuarine wetlands of tropical and subtropical regions of the world. The biomass and the extent of the mangroves are determined by variations in rainfall, tidal influence, wave energy, and salinity levels. In 1980, a group of scientists discovered the influence of salinity among other physicochemical determi-

Vol. 17(Supplementary issue - 3B) 1303-1315,September 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.3s.65

nants on species competition and growth which ascertain the forest structure (4). Rainfall is considered an important factor as it lowers the water salinity and stimulates the optimal growth of the mangroves.

Kerala is situated between the Arabian sea and the western ghats to the east with a spread over 38,863 km2. Therefore, Kerala experiences an equatorial tropic climate. According to FSI (5), the state has a mangrove cover of 9km^2 survey reveals that the southern zone has a total mangrove cover of 5.91km^2 (6). In Kerala, the mangrove forests are being cleared for the development of roads, bridges, and other projects like the development of container terminals, and fishing harbours (7-9). *Bruguiera gymnorrhiza,* a mangrove species from the Rhizophoraceae family is facing serious threats among the others in the state. The salinity ranges from 125-250 mM NaCl for the optimum growth of the plant, but at 500 mM NaCl, the growth is inhibited (10). *B. gymnorrhiza* is found predominantly in the districts of Cochin and Thrissur while it is sparsely distributed among the districts of Kollam and Alappuzha. The occurrence of the plant in the districts seems to be disappearing at a faster rate. The northern parts of Kerala are devoid of this species(11).

The mangroves are said to possess numerous antibacterial, anticancer, anti-diabetics, antifungal and insecticidal properties (12). Mangroves are believed to be excellent models for tolerance of salt and it is very vital to study their physiological adaptations before the initiation of any mangrove restoration project. Changes in osmoregulation can be seen in different species, which can be evaluated through the deposition of excessive amounts of solutes such as free sugars, free amino acids, and low molecular weight proteins (13). Unlike *Avicennia marina* which can thrive in high saline conditions by excreting the accumulated salts via its leaves, *B. gymnorrhiza* finds it hard to thrive in high saline conditions. It states that salinity stress has implications on growth, photosynthesis, ion regulation, biomass yield, and water relations. The absorbed inorganic ions are cloistered into the vacuoles (in the form of Na^+ and Cl^-) to avoid ionic toxicity (14). The increase in the number of inorganic ions in the vacuole leads to bi-phasic stress in the cytoplasm that is; internal and external stress (15). To reduce these stresses, plants accumulate amino acids like proline, alanine, and tyrosine; soluble sugars such as sucrose; polyols mainly mannitol; and betaines such as glycine betaine to adjust the osmotic potential and besides the organic solutes that don't hinder the normal metabolic process (16-17).

Scientists suggest that to maintain biological homeostasis, the generation of reactive oxygen species (ROS) and the ratio between oxidation and anti-oxidation is a crucial step under oxidative stress (18). In the *Bruguiera* genus, quercetin displayed a high antioxidant activity when compared to other isolated compounds like kochioside and oleanolic acid. The phytochemical compounds such as alkaloids, tannins, and saponins have efficient anti-inflammatory and anti-microbial activities, making them excellent wound healers (19). Most of the secondary metabolites are having bioactivities toward efficient salt management. Polyphenols are the major group attributed to their antioxidant property (20).

Polyphenols like flavonoids and phenolic acids are the group of metabolites in plants that aid in plant defence by dissuading the biotic stressors and assuaging the abiotic stressors. GC-MS (Gas Chromatography-Mass Spectrophotometry) helps in the identification of these compounds. There are some commonly employed methods to determine the antioxidant properties like ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] and DPPH (1, 1-diphenyl-2-picrylhydrazyl) assays. Prevention of chain reaction, chelating metals, radical scavenging ability, and reducing capacity are the factors that are attributed to the antioxidant capacity of compounds (21). ROS generation can be avoided by the chelation of metal ions which is related to the catalysis of redox-active metals.

Vol. 17(Supplementary issue - 3B) 1303-1315,September 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.3s.65

In the purview of the above discussion, this work aims at the salt adaptability of *B. gymnorrhiza* from the southern districts of Kerala (which is under threat of habitat loss) by assessing the leaf water potential, and regulation of two antioxidant enzymes (PRX and SOD) and also confirming the polyphenols present in the leaf extract through GC-MS studies. The SEM (Scanning Electron Microscope) studies also confirm the species' carbon sequestration capacity and the accumulation of hazardous elements in the leaf sample.

Materials and Methods

The leaf sample of the plant *B. gymnorrhiza* was collected from the four districts of Kerala *viz*., Kollam, Alappuzha, Cochin and Thrissur. The methanol leaf extract was prepared by following the maceration method. The steps are illustrated.

Amino acids

Six free amino acids like Proline, Alanine, Aspartic acid, Tryptophan, Phenylalanine, and Leucine were analysed from mangrove species. About 2 grams of freeze-dried leaves from 4 different districts of Kerala were homogenized in acetone and centrifuged at 6000 rpm for 10 min. Thin layer chromatography of supernatants was performed and the spots were allowed to run in a running buffer in a mixture of 4:1:1 n-butanol, acetic acid and distilled water up to an equal distance. In the chromatogram, different coloured spots appeared for different amino acids, which could be identified from their respective standard R_{mf} values. The spots were eluted with 80% ethanol and optical densities were measured in specific wavelengths mainly 550nm for proline and 400nm for other amino acids. Estimations were done based on the standard curves plotted for the individual amino acid. The R_{mf} values were very close for tryptophan and tyrosine, therefore the confirmatory tests (Hopkins -Cole test for tryptophan) were performed. The value of each amino acid is the average of nine observations.

Antioxidant secondary metabolites

Total Phenols

0.1 ml of methanolic extract of the sample was mixed with 2 ml of diluted Folin-Ciocalteu reagent and 1.6 ml of 7.5% of Sodium carbonate (Na_2CO_3). The samples were mixed thoroughly and allowed to stand at room temperature for 30 minutes for colour development. The optical density of the samples was taken at 765 nm using Spectrophotometer. The same concentration of Gallic acid was used as a positive control (Folin – Ciocalteu method). The total phenolic content was expressed as Gallic acid equivalents (GAE) in milligrams per gram of dry material using the calibration curve, with X-axis as the absorbance and Y axis as the Gallic acid equivalent ($mg \cdot g^{-1}$) (22).

Total flavonoids

In 0.1 ml of methanolic leaf extract mixed with 1.2 ml of distilled water, 0.120 ml of 5% Sodium nitrite ($NaNO_2$) was added and mixed vigorously. The mixture was kept at 25˚C for 5 mins. Later 0.120 ml of 10%, $AlCl_3$ solution was added to the mixture and mixed thoroughly. The tubes are allowed to stand at room temperature for 5 minutes. 0.8 ml of 1 mM Sodium hydroxide (NaOH) solution and 1.16 ml of distilled water were added and absorbances were measured at 510 nm. The same concentration of Quercetin was used as a positive control. Total flavonoid content was calculated as Quercetin ($mg \cdot g^{-1}$) using the calibration curve, where X was the absorbance and Y was the Quercetin equivalent ($mg \cdot g^{-1}$) (23).

ROS scavenging ability assay

DPPH quenching assay

Freshly prepared DPPH solution (25 $mg \cdot L^{-1}$) in methanol was prepared and 3.9 ml of this solution was mixed with 0.1 ml of methanolic leaf extract. After 30 minutes, the absorbances of the samples were measured at 517 nm using a Spectrophotometer (Helios γ, Thermo Electron Corporation). The same concentration

Vol. 17(Supplementary issue - 3B) 1303-1315,September 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.3s.65

of Butylated Hydroxy Toluene (BHT) was used as a positive control (24). The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH radical scavenging activity}(\%) = \left\{\frac{Ac - At}{Ac}\right\} \times 100$$

Where,

A_c = absorbance of the blank reaction

A_t = absorbance in presence of the sample of the extracts

$$\text{ABTS radical scavenging activity}(\%) = \left\{\frac{Ac - At}{Ac}\right\} \times 100$$

ABTS⁺ scavenging assay

The ABTS⁺ scavenging assay was performed according to the protocol laid down by [25]. Butylated Hydroxy Toluene (BHT) in the same concentration as the sample was used as a positive control. The ABTS scavenging capacity of the extract was calculated as;

where A_c is the absorbance of the blank reaction and A_t is the absorbance in presence of the sample of the extracts.

Fe^{2+} chelation activity

The chelating capacity of plant extract was assessed through ferrous ions (24). EDTA in the same concentration as the sample was used as a positive control. The Fe2+ Chelation capacity of the extract was calculated as;

$$Fe^{2+}\ \text{Chelationability}(\%) = \left\{\frac{Ac - At}{Ac}\right\} \times 100$$

where A_c is the absorbance of the blank reaction and A_t is the absorbance in presence of the sample of the extracts.

All scavenging/chelating abilities are expressed in terms of IC_{50}. It is defined as the concentration of the plant extract that's needed to scavenge/chelate 50% of the scavengers/chelating ions present were calculated by the following equation;

$$IC_{50} = \left\{\frac{\text{Percent inhibition}}{\text{Concentration of the Sample}}\right\} \times 50$$

Enzyme assay

Peroxidase

From 200 mg fresh leaf, extraction was prepared in 1 - 1.5 ml of 0.9% KCl and centrifuged at 12,000 rpm for 15 min at 4˚C; the supernatants used as enzyme sample. Absorbance was taken spectrophotometrically (Shimadzu, Japan) at 460 nm to the standard curve (26) with minute modification.

Superoxide dismutase

Cell sap extracted by macerating 200 mg of leaf in 1 - 1.5 ml of 50 mM Phosphate buffer, pH adjusted to 7.0 and centrifuged at 12,000 rpm for 15 min at 4˚C. Absorbances were measured at 550 nm following the protocol described (26) with slight modification.

GC-MS analysis

The methanolic leaf extracts were quantified for GC-MS analysis to know the secondary metabolic compounds present in the sample. Derivatization was done before GC-MS. Add 40 µl of methoxyamination reagent to the sample. Incubate for 2hrs at 37°C. After that 20µl MSTFA reagent was added to the sample and shaken for 30min at 37°C. The aliquots were then transferred to a GC-MS glass vial and taken to be analysed. Shimadzu GCMS-TQ8040 NX, Japan with a column Rtx 5 MS (5% diphenyl /95% dimethyl polysiloxane) and flow rate of 2ml/min was used for the purpose (27). The injector temperature was 260°C and the temperature program was made at 50°C initially and then raised to 250 by 10°C and increased to 280°C with an increase of 5°C/min which is held at 280°C for 9 min. The ion source temperature was 240°C,

Vol. 17(Supplementary issue - 3B) 1303-1315,September 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.3s.65

the interface temperature was 270°C and the mode of analysis was Scan mode with splitless injection.

SEM-EDX Analysis

The leaf samples were prepared according to the protocol (28). EDX was combined to know the elements present in the leaf sample.

Results and Discussion

Amino acids

All the six amino acids investigated were not present in all the samples of mangrove species collected from different locations. The leaf extracts collected from the Kollam region predict the presence of Proline (9.02µg g^{-1}), Alanine (2.47 µg g^{-1}), and Aspartic acid (8.63 µg g^{-1}). Whereas, the samples collected from the Alappuzha region results in presence of Alanine (7.12 µg g^{-1}), Tryptophan (6.46 µg g^{-1}), and Phenylalanine (7.31 µg g^{-1}). Amino acids such as Aspartic acid (10.11 µg g^{-1}), tryptophan (9.28 µg g^{-1}) and phenylalanine (8.02 µg g^{-1}) are found in the leaf samples collected from Cochin. The leaf samples collected from Thrissur display the presence of amino acids such as Leucine (4.21 µg g^{-1}), Tryptophan (5.03 µg g^{-1}) and Phenylalanine (4.82 µg g^{-1}). Amino acids like Tryptophan and Phenylalanine are found in three out of four samples. Whereas Aspartic acid is found only in two samples (Fig. 1). Leucine and Proline are found exclusively in Thrissur and Kollam samples alone.

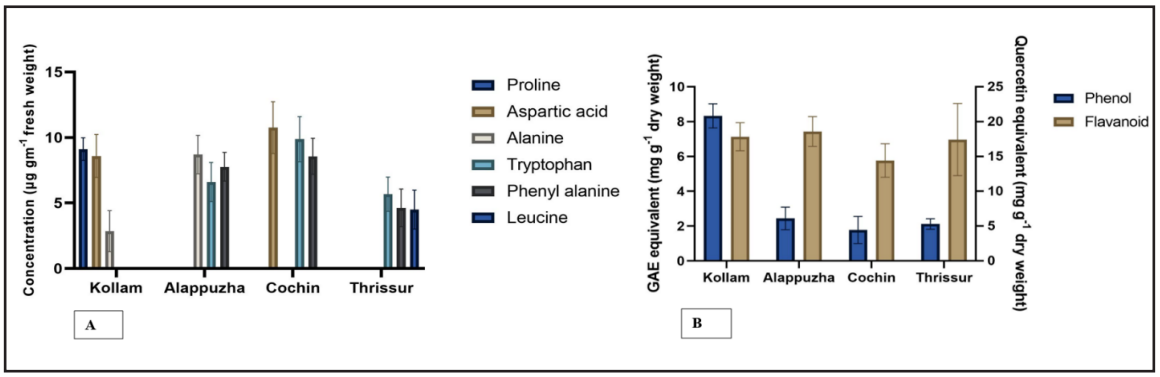

Fig 1: A- The amino acid contents of the leaf extract from four southern districts of Kerala; B- total phenol and flavonoid content of the leaf extract of *B. gymnorrhiza.*

Antioxidant activity

Total phenol

The total phenol activity from the four districts of Kerala was assessed. From the four districts, the Kollam sample exhibited high amounts of phenolic content. The phenolic content was found to be incremental with the variation in salinity. The *Bruguiera* species will not be able to withstand high salinity, and after a point of 200mM salinity, the plant seems to die. The Kollam site had a salinity of 142mM which proves to be a good thriving condition better for phenolic regulation of the plant and phenolic content of 19. 6mg.g^{-1} was reported. Whereas in the Cochin (2. 2mg.g^{-1}) the phenolic content was found to be low when compared with that of Alappuzha (5. 6mg.g^{-1}) and Thrissur (4. 6mg.g^{-1}) (Fig. 1)

Total flavonoids

The total flavonoids also seem to be fluctuating with the salinity. The Alappuzha sample (7. 6mg.g^{-1}) exhibited higher amounts of flavonoid content than the Kollam leaf sample (7. 2mg.g^{-1}). Thrissur sample was reported at 6. 6mg.g^{-1}). Again, the least incidence was reported in the Cochin sample (5. 6mg.g^{-1}) out of the four districts. (Fig. 1).

Vol. 17(Supplementary issue - 3B) 1303-1315,September 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.3s.65

ROS scavenging ability assay

DPPH scavenging assay

The DPPH scavenging assay from the four districts of different salinity were studied. As salinity increased there was a continuous percentage reduction in the evolved ROS. The maximum DPPH value was found in the sample from Kollam (45.80%) followed by Alappuzha (40.44%), Cochin (23.62%), and Thrissur (25.44%). The positive control BHT was kept as the reference value was found to be 12.18%. The highest IC_{50} value was recorded in Alappuzha (11.78%) and the least at Cochin (1.04%) (Fig. 2).

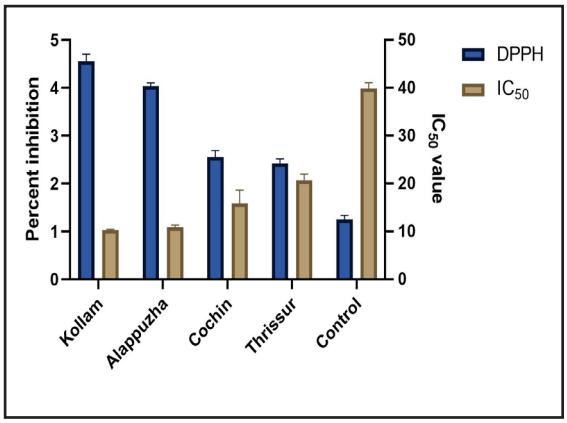

Fig 2: The DPPH free radical scavenging assay from the four southern districts of Kerala and the positive control.

ABTS⁺ scavenging assay

Both ABTS and DPPH assays portrayed a similar trend. The sample from Kollam had the highest percentage of ROS reduction of 54.52% and the lowest was reported in Thrissur (23.25%). Alappuzha (42.76%) and Cochin (30.67%) displayed almost the same result. The reference value of BHT was observed at 25.85%. The IC_{50} value was found to be highest in Thrissur (2.85%) and the least in Kollam (1.003%). The IC_{50} value is inversely proportional to the percentage of inhibition (Fig. 3).

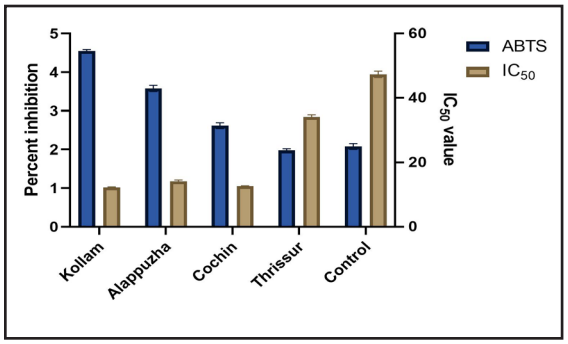

Fig 3: The $ABTS^{+}$ scavenging assay of the leaf samples from four southern districts of Kerala with IC_{50} value

Fe^{2+} chelation activity

The Fe^{2+} chelation is found to be higher in the leaf sample collected from the Thrissur district (109.52%) and the least was reported from Kollam (8.87%). Alappuzha (12.29%) and Cochin (24.83%) were reported intermediately. The positive control EDTA was falling in the range of 40.07%, this was lower when compared to that of Thrissur. The highest IC_{50} value was reported from Alappuzha (10.31%) and the least from Thrissur (1.04%) (Fig. 4).

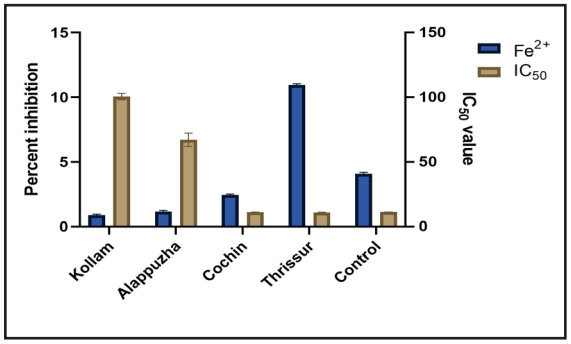

Fig 4: The ferric chelation activity and IC_{50} values of the leaf samples from four districts of Kerala.

Quantitative assay of PRX and SOD

Peroxidase

In the present work the quantity of PRX enzyme was found to be higher in the sample

Vol. 17(Supplementary issue - 3B) 1303-1315,September 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.3s.65

collected from the Kollam district (178. 14µg.g^{-1}). The salinity at Kollam was found to favouring the isoenzyme activity. Whereas the sample was collected from Alappuzha (165.32 µg. g^{-1}), Cochin (176. 23µg.g^{-1}) and Thrissur (148.55 µg. g^{-1}) (Fig. 5).

Superoxide dismutase

The SOD level seems to increase in the sample, where the salinity was found to be 110mM. But it eventually dropped at the Kollam sample where the salinity was higher (111.32 µg. g^{-1}). The sample was collected from Alappuzha (138.76 µg. g^{-1}) which was higher than that of Kollam. The sample from Cochin (142.12 µg. g^{-1}) and Thrissur (150.86 µg. g^{-1}) displayed better responses to the enzyme (Fig. 5).

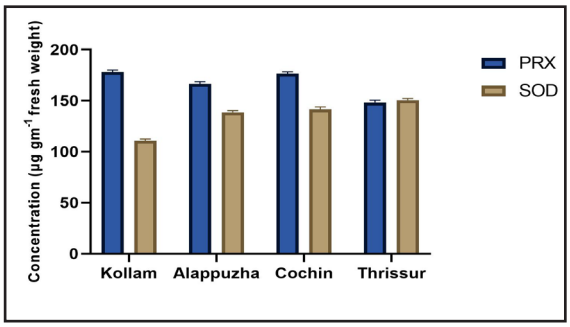

Fig 5: The enzymatic assay of PRX & SOD of the leaf samples from four districts of Kerala.

GC-MS analysis

The GC-MS analysis of the methanol extract revealed the presence of a variety of compounds. The compounds were identified to their peak retention time, peak area (%), and mass spectral fragmentation patterns to that of known compounds described by the National Institute of Standards and Technology (NIST) library.

The GC-MS results represented almost 70 compounds from each district (Table 1). We have selected the polyphenols that were present in the result, as phenols play a vital role in the growth and survival of mangroves. This result proves the role of various phenolics present in the mangroves in defence against ultraviolet radiation, and defence against pathogens, parasites, and predators. They also greatly contribute to the plant's colour. The high phenolic content in mangroves makes it an excellent anti-oxidant-rich plant and proves to be highly medicinal. In the present work, in addition, we observed a novel compound Methyl Commate B ($C_{31}H_{50}O_3$) which is a natural compound present in the leaves of *B. gymnorrhiza* collected from the districts of Cochin and Thrissur. This is the first time the presence of such a compound is being reported in mangroves. Other compounds such as catechol, lupeol, and mome inositol

Table 1: Compounds identified through GC-MS analysis from the leaf sample of *B. gymnorrhiza* from four districts of Kerala, KLM- Kollam, ALA-Alappuzha, EKM- Cochin, and TCR- Thrissur.

Sl. No.	Compound Name	*Rt* Value	Chemical Formula	Molecular Weight	Peak Area (%)	Districts
1	Benzyl alcohol	4.246	C_7H_8O	108	0.02	KLM & TCR
2	Cyclotetrasiloxane, octamethyl-	5.808	$C_8H_{24}O_4Si_4$	296	0.03	KLM
3	3-sulfanyl-1-propanol	8.012	C_3H_8OS	92	0.74	KLM, ALA & EKM
4	2,4-Di-tert-butylphenol	10.540	$C_{14}H_{22}O$	206	0.03	KLM, ALA & EKM
6	Norolean-12-ene	28.692	$C_{29}H_{48}$	396	16.16	KLM
7	(Chloromethyl)benzene	3.968	C_7H_7Cl	126	0.07	ALA & EKM
11	1,2,3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester	12.177	$C_{12}H_{20}O_7$	276	31.27	KLM, ALA & TCR
12	Mome inositol	13.310	$C_7H_{14}O_6$	194	21.44	ALA & EKM
13	Lupeol	29.403	$C_{30}H_{50}O$	426	12.03	ALA

Vol. 17(Supplementary issue - 3B) 1303-1315,September 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.3s.65

14	Propanenitrile, 3-(2-methoxy-1-methylethoxy)-	3.105	$C_7H_{13}NO_2$	143	0.04	EKM
15	Phenol	3.610	C_6H_6O	94	0.01	EKM
17	Proceroside	4.326	$C_{29}H_{40}O_{10}$	548	0.26	EKM
18	Propane, 1,1,3-triethoxy-	4.708	$C_9H_{20}O_3$	176	0.15	EKM & TCR
20	Cyclohexasiloxane, dodecamethyl-	8.148	$C_{12}H_{36}O_6Si_6$	444	0.61	EKM
24	Methyl commate b	25.388	$C_{31}H_50O_3$	470	0.14	TCR & EKM
25	Butane, 1,1-diethoxy-3-methyl-	3.223	$C_9H_{20}O_2$	160	0.31	TCR
27	Propane, 1,1,3-triethoxy-	4.797	$C_9H_{20}O_3$	176	0.22	EKM & TCR
28	Ethane, 1,2-bis(methylthio)-	5.351	$C_4H_{10}S_2$	122	0.32	TCR
29	Catechol	6.559	$C_6H_6O_2$	110	0.09	ALA & TCR
30	1-Hexadecanol	8.994	$C_{16}H_{34}O$	242	0.27	TCR
32	2,6,10-trimethyl,14-ethylene-14-pentadecne	14.146	$C_{20}H_{38}$	278	0.51	TCR
33	N-Hexadecanoic acid	15.381	$C_{16}H_{32}O_2$	256	2.55	TCR

were observed at various retention times in the extracts of four districts. These compounds are proven high in antioxidant activity, which plays a major role in plant defence and pathogen protection. The presence of these secondary metabolites makes the leaf rich in antioxidants and suitable for pharmacological activity.

SEM-EDX

The SEM-EDX result is widely used to ascertain the presence of elements in the sample. The surface morphology of the sample can be determined from the scanning electron microscope (SEM) analysis. EDX is a chemical technique which is used in conjunction with SEM. The leaf samples from four districts of Kerala viz., Kollam, Alappuzha, Cochin and Thrissur were subjected to EDX. The leaf's surface area was subjected to SEM visualization at a magnification rate of 20µm (Fig. 6). The leaf samples collected from the Kollam district had high salt deposition on their leaf surface, making a good example of salt exclusion capacity. The EDX analysis reveals the presence of various elements present in the leaf. The presence of carbon in very high amounts reveals the carbon sequestration capacity of mangroves. The evidence of other metals in the mangrove leaf may be due to the presence of elements present in the mangrove sediments. The presence of certain elements like Oxygen (O), Sodium (Na), Chlorine (Cl), Cadmium (Cd), Molybdenum (Mo), Potassium (K), Iron (Fe), Calcium (Ca) was assessed. Only these elements were targeted due to the presence of industries that utilises such elements as raw materials for production purposes nearby the sampling area. The weight and atomic percentage of heavy metals such as iron, cadmium, and molybdenum are presented (Table 2).

Table 2: EDX weight ratio of the leaf sample from 4 districts of Kerala

Sample	Cadmium		Molybdenum		Iron	
	Weight (%)	**Atomic (%)**	**Weight (%)**	**Atomic (%)**	**Weight (%)**	**Atomic (%)**
Kollam	0.07	0.01	0.11	0.01	0.04	0.01
Alappuzha	0	0	0.08	0.01	0.02	0.01
Cochin	0.06	0.01	0.12	0.02	0.02	0.01
Thrissur	0.09	0.01	1.27	0.18	0.04	0.01

Vol. 17(Supplementary issue - 3B) 1303-1315,September 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.3s.65

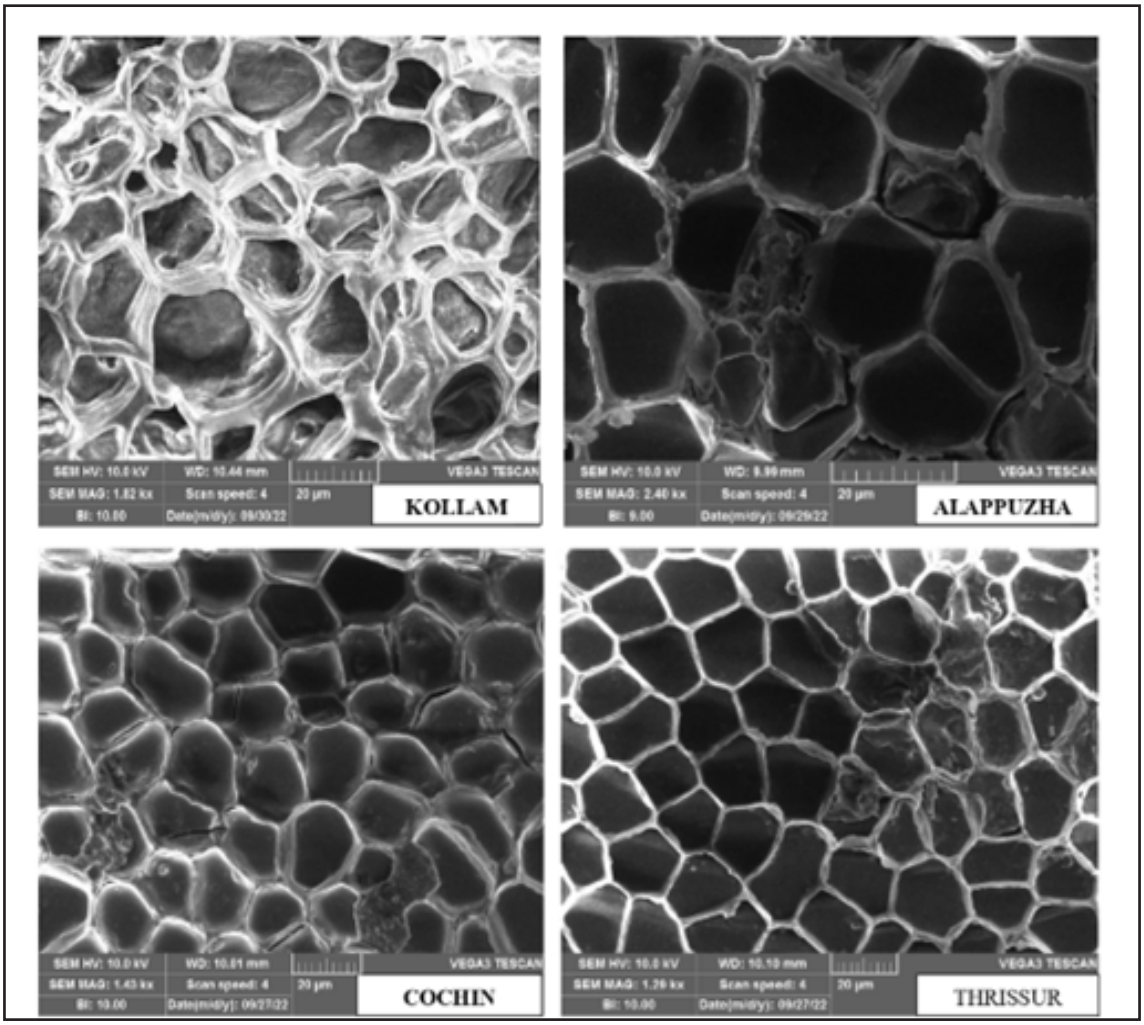

Fig 6: SEM images of leaf sections of *B. gymnorrhiza* with 20µm magnified images of 4 districts of Kerala

The species *Bruguiera gymnorrhiza* from the four southern districts of Kerala had a good affinity towards environmental impairment. The ROS are generally formed from the metabolic bi-product. But nowadays there is a high probability of an environmental disaster triggering the enhanced production of ROS and will kick off the oxidative damage ultimately leading to apoptosis. Equilibrium should be established for ROS assembly and their scavenging as to safeguard the plant cell from premature oxidative destruction. To shield themselves from this drastic effect, mangroves have developed their defence system which encompasses the activation of antioxidant enzymes (AOX) to scavenge the ROS (29). When exposed to salinity stress the free radical level increased exponentially and ultimately led to oxidative damage to cells (30). Certain enzymatic and non-enzymatic activities are happening in the tissues through which competent ROS scavenging occurs during stressful environmental conditions. In this work, we portray a comparative analysis of ROS scavenging activity and secondary

Vol. 17(Supplementary issue - 3B) 1303-1315,September 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.3s.65

line and tryptophan aid in the transamination process of pyruvate and oxaloacetate during photosynthesis through which it is converted to alanine and aspartic acid to serve as the carbon source for other metabolites and eventually increasing the amino acid concentrations in the leaf. It is known that the highest percentage of foliar nitrogen in the majority of the mangroves is integrated with aspartate and alanine (32). The lesser amounts of proline from the leaf sample of four districts make it lesser adaptable to high salinity. High salinity tends to obstruct the physiological process like photosynthesis and nutrient uptake due to the reduction in leaf water potential (33). Proline is said to be the main amino acid responsible for the higher salt adaptation as it helped to maintain the Ψ during the salt stress and is considered a compatible solute that adjusts the osmotic potential (34-35). It was observed that the Kollam sample seems to be more adaptable to salinity than the species from Alappuzha, Cochin and Thrissur as the concentration of amino acids like proline seems to be decreasing with increasing salinity. In an experiment conducted by Parida [36], it was observed that the cell sap of *Bruguiera parviflora* showed the augmentation of osmotic solutes like total sugar and total free amino acids occurred in various degrees under NaCl treatment and these osmoregulatory solutes aid in the restoration of water potential more negatively and thus can be a marker of extended salt tolerance.

There should be an equilibrium between ROS production and efficient scavenging, then only oxidative damage of plant cells occurs(37). Various enzymatic and non-enzymatic antioxidants in the plant cell pave the way for competent scavenging of ROS during various abiotic stress. In the present work, the sample from Kollam and Alappuzha shows a steady increment in phenol and flavonoid along the salinity gradient, but this trend was found to be low in the sample collected from Cochin and Thrissur. Cochin had a very low salinity gradient when compared to other districts. *Bruguiera* being sensitive to higher salinities was differing in its antioxidant activity, the radical scavenging estimation with $ABTS^{+}$, DPPH and Fe^{2+} chelation assays revealed this sequel with differential salinity levels. An experiment conducted by Ebrahimzadeh (38) revealed that the highest chelating activity and phenolic content were observed in *Mellilotus arvensis*.

The compound Methyl Commate B identified through GC-MS analysis is a natural pigment. Ursolic acid a pentacyclic triterpene acid is having a large array of pharmacological activities. It is proven for its healing effects in rheumatoid arthritis (39). It was evident that the methanolic leaf extract of ginger showed the presence of flavonoids, saponins, phenols, and tannins (40). More light should be put into the isolation and utilization of this compound and its effective isolation. Also, the pharmacological potential of mangroves should be considered and their effective conservation should be carried out at the earliest.

Climate change is the biggest threat to the world. World Wide Fund (WWF) has initiated many tools by reducing carbon pollution and adapting to global warming. One such tool is the mangroves. They have the unique ability to thrive in saltwater environments, their strong roots and complex root system protect the coastal communities and landscapes from extreme weather events like hurricanes. Mangroves pull a massive amount of greenhouse gases from the atmosphere and store them in their soils, four times more than other tropical forests. Studies have reported that mangroves can suck more than 6 billion tons of carbon a year. Thus, mangrove forests help to mitigate climate change.

Conclusion

The present study focuses on the adaptability of *B. gymnorrizha* to varying salt levels from four southern districts of Kerala *viz* Kollam, Alappuzha, Cochin and Thrissur. The presence of amino acids in different samples revealed its ability to thrive in harsh conditions

Vol. 17(Supplementary issue - 3B) 1303-1315,September 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.3s.65

and also secondary metabolites which are responsible for the survival of these plant species. The antioxidant activity was much more in the sample collected from Kollam than from other districts. For the first time, the compound Methyl Commate B was identified through this study in the mangrove species collected from the districts of Cochin and Thrissur. The SEM-EDX activity also confirmed the ability of *B. gymnorrhiza* to accumulate salt which was also predominant in the Kollam sample. This is the first type of study done on this plant on the west coast of Kerala and further study is required to understand the underlying mechanisms and also to utilise the phytochemical compounds as a potential drug candidate.

Declaration of Competing Interest

The authors declare no conflict of interest.

Funding

The work was completed under the UGC-STRIDE IRCECCB Visiting Fellowship- 2022 program at Madurai Kamaraj University.

Acknowledgements

We express our sincere gratitude to the Department of Plant Science, GC-MS facility at the School of Biological Science, and School of Chemistry, Madurai of Kamaraj University, Madurai. We also extend our thanks to Ms Amritha P S, PSGR Krishnammal College for Women and Mr U. Vinoth, PSG College of Arts & Science, Coimbatore for their sincere support and help.

References

1. Tatongjai, S.; Kraichak, E.; Kermanee, P. (2021) Comparative anatomy and salt management of *Sonneratia caseolaris* (L.) Engl. (Lythraceae) grown in saltwater and freshwater. Peer J, 9, e10962.

2. Baishya, S., Banik, S. K., Choudhury, M. D., Talukdar, D. D., & Talukdar, A. D. (2020) Therapeutic potentials of littoral vegetation: an antifungal perspective. In Biotechnological Utilization of Mangrove Resources (pp. 275-292). Academic Press.

3. Anneboina, L.R.; Kumar, K.K. (2017) Economic analysis of mangrove and marine fishery linkages in India. Ecosyst Serv, 24, 114–123.

4. Walters, B.B.; Rönnbäck, P.; Kovacs, J.M.; Crona, B.; Hussain, S.A.; Badola, R.; Primavera, J.H.; Barbier, E.; Dahdouh-Guebas, F. (2008) Ethnobiology, socio-economics and management of mangrove forests: A review. Aquat Bot, 89, 220–236.

5. India State of Forest Report. (2019) Forest Survey of India, Ministry of Environment and Forests, Dehradun.

6. Preethy, C. M. (2019) The systematics, floristics and ecology of selected mangroves of Kerala (Doctoral dissertation, Cochin University of Science and Technology). p.411

7. Sreelekshmi, S., Joseph, P., Varghese, R., Preethy, C. M., & Bijoy Nandan, S. (2020) Structural variability and its relation to edaphic attributes of mangroves in the south-west coast of India. Lakes Reserv, 25(2):143-156.

8. Nandan, S. B., Sreelekshmi, S., Preethy, C. M., & Philomina, J. (2015) Manual on mangroves. Cochin University of Science and Technology, Kochi

9. Hema, M., & Indira Devi, P. (2014) Factors of mangrove destruction and management of mangrove ecosystem of Kerala, India. J Aquat Biol Fish, 2(1), 184-196.

10. Sreelekshmi, S., Preethy, C. M., Varghese, R., Joseph, P., Asha, C. V., Nandan, S. B., & Radhakrishnan, C. K. (2018) Diversity, stand structure, and zonation pattern of mangroves in southwest coast of India. J

Vol. 17(Supplementary issue - 3B) 1303-1315,September 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.3s.65

Asia Pac Biodivers, 11(4), 573-582.

11. Vidyasagaran, K., & Madhusoodanan, V. K. (2014) Distribution and plant diversity of mangroves in the west coast of Kerala, India. J Biodivers Environ Sci, 4(5), 38-45.

12. Habib, M. A., Khatun, F., Ruma, M. K., Chowdhury, A. S. M. H. K., Silve, A. R., Rahman, A., & Hossain, M. I. (2018) A review on phytochemical constituents of pharmaceutically important mangrove plants, their medicinal uses and pharmacological activities. Vedic Res Int Phyto 6(1), 1-9.

13. Joseph, B. and Jini, D. (2011) Development of Salt Stress-Tolerant Plants by Gene Manipulation of Antioxidant Enzymes. Asian J Agric Res, 5, 17-27.

14. Sudhir, S., Arunprasath, A., & Vel, V. S. (2022) A critical review on adaptations, and biological activities of the mangroves. J Nat Pestic Res, 100006.

15. Muns, R. and Tester, M. (2008) Mechanisms of Salt Tolerance. Annu Rev Plant Physiol, 59, 651-681.

16. Kamel, M. (2008) Osmotic Adjustment in Three Succulent Species of Zygophyllaceae. Afr J Ecol, 46, 96-104.

17. Parida, A.K. and Das, A.B. (2005) Salt Tolerance and Salinity Effects on Plants: A Review. Ecotoxicol Environ Saf, 60, 324-349.

18. Flowers, T.J. and Colmer, T.D. (2008) Salinity Tolerance in Halophytes. New Phytol, 179, 945-963.

19. Le Thuy, N. T., & Tung, B. T. (2022) Antioxidant activity of isolated compounds from ethyl acetate extract of *Bruguiera parviflora*. Ho chi minh city open university J Sci Eng Technol. 12(2).

20. Pagarra, H., Rahman, R. A., Hala, Y., & Esivan, S. M. M. (2022) Phytochemical screening, antimicrobial and antioxidant activity from *Sonneratia caseolaris* leaves extract. J Technol, 84(5), 59-66.

21. Stiller A, Garrison K, Gurdyumov K, Kenner J, Yasmin F, Yates P, Song BH. (2021) From Fighting Critters to Saving Lives: Polyphenols in Plant Defense and Human Health. Int J Mol Sci, 22(16), 8995.

22. Singleton, V.L. and Rossi, A. (1965) Colorimetry of Total Phenolics with Phosphomolybdic Phosphotungstic Acid Reagents. Am J Enol Vitic, 16, 144-158.

23. Jia, Z.S., Tang, M.C. and Wu, J.M. (1999) The Determination of Flavonoid Contents in Mulberry and Their Scavenging Effects on Superoxide Radicals. Food Chem, 64, 555-559.

24. Haro-Vicente, J.F., Martínez-Graciá, C. and Ros, G. (2006) Optimization of in Vitro Measurement of Available Iron from Different Fortificants in Citric Fruit Juices. Food Chem, 98, 639-648.

25. Re, R.N., Pellegrini, A., Proteggente, A., Pannala, M., Yang, C. and Evans, R. (1999) Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. Free Radic Biol Med, 26, 1231-1237.

26. Shannon, L.M., Key, E. and Lew, J.Y. (1966) Peroxidase Isozyme from Horseraddish Root. I. Isolation and Physical Properties. J Biol Chem, 249, 2166-2172.

27. Lisec, J., Schauer, N., Kopka, J., Willmitzer, L., & Fernie, A. R. (2006). Gas chromatography mass spectrometry–based metabolite profiling in plants. Nat Protoc, 1(1),

Vol. 17(Supplementary issue - 3B) 1303-1315,September 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.3s.65

387-396.

28. Pathan, A. K., Bond, J., & Gaskin, R. E. (2010) Sample preparation for SEM of plant surfaces. Mater Today, 12, 32-43.

29. Zohmachhuana, A., Malsawmdawngliana, M., Lalrinzuali, K., Lalnunmawia, F., Vabeiryureilai, M., & Kumar, N. S. (2021) The assessment of the free radical scavenging activity and flavonoid contents of selected medicinal plants of Mizoram. Curr Trends Biotechnol Pharm, 15(1), 34-43.

30. Kumarmath, P. S., & Sharada, P. (2021) Antioxidant Assay of *Bryophyllum gastonis* bonnieri at Salt Stress. Curr Trends Biotechnol Pharm, 15(5), 361-364.

31. Chen L, Wang W, Lin P. (2005) Photosynthetic and physiological responses of *Kandelia candel* L. Druce seedlings to duration of tidal immersion in artificial seawater. Environ Exp Bot, 54(3), 256–66.

32. He B, Lai T, Fan H, Wang W, Zheng H. (2007) Comparison of flooding-tolerance in four mangrove species in a diurnal tidal zone in the Beibu Gulf. Estuar. Coast. Shelf Sci, 74(1–2), 254–62.

33. Hamilton, E.W. and Heckathorn, S.A. (2001) Mitochondrial Adaptations to NaCl Complex I Is Protected by AntiOxidants and Small Heat Shock Proteins, Whereas Complex II Is Protected by Proline and Betaine. Plant Physiol, 126, 1266-1274.

34. Munns, R. (2002) Comparative Physiology of Salt and Water Stress. Plant Cell Environ, 25, 239-250.

35. DiMartino, C. and Fuggi, A. (2001) Pattern of Free Amino Acids in Leaves of Salt Stressed Plants of Spinach. In: Proceedings of the 6th International Symposium on Inorganic Nitrogen Assimilation Congress, European Nitrate Ammonium Assimilation Group, Reims, 3-13.

36. Parida, A., Das, A.B. and Das, P. (2002) NaCl Stress Causes Changes in Photosynthetic Pigments, Proteins and Other Metabolic Components in the Leaves of a True Mangrove, *Bruguiera parviflora*, in Hydroponic Cultures. J Plant Biol, 45, 28-36.

37. Dasgupta, N., Chowdhury, P., & Das, S. (2015) Comparative adaptability assessment of two mangroves from Indian Sundarbans: some biochemical appearances. Nat Sci, 7(12), 519.

38. Ebrahimzadeh, M.A., Pourmorad, F. and Bekhradnia, A.R. (2008) Iron Chelating Activity, Phenol and Flavonoid Content of Some Medicinal Plants from Iran. Afr J Biotechnol, 7, 3188-3192

39. Pádua, T. A., de Abreu, B. S., Costa, T. E., et al. (2014) Anti-inflammatory effects of methyl ursolate obtained from a chemically derived crude extract of apple peels: potential use in rheumatoid arthritis. Arch Pharm Res, 37(11), 1487-1495.

40. Ambily, P. G., Mathew, J., & Sudhina, M. (2022) Analysis of Leaf Extract of *Zingiber officinale* by a Hybrid Analytical Technique. Curr Trends Biotechnol Pharm, 16(3), 316-328.