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Leaf Pigments and Epicuticular Wax Content Variation of Selected Mangrove Species in Andaman Islands, India

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Abstract

The mangrove intertidal zone is described by bounty botanical and faunal variety. The mangroves give significant territory to a wide scope of animal varieties like assorted networks of benthic organic entities and capacity as nursery living spaces for various sorts of crab, prawn and fish species and backing seaward fish populaces and fisheries. Three regions were taken; each study region was separated into three zones viz. close to settlement (Corbyn's cove), undisturbed region (Sipighat) and Inter- Island of Andaman Sea (Havelock) which were dissected by the Abundance of mangrove species. The comparative extraction of chlorophylls (Chlorophyll-a, Chlorophyll-b and total chlorophyll), Xanthophyll & Carotenoids and Epicuticular wax from selective mangrove species in South Andaman region were studied. Among this highest level of chlorophylls (Chlorophyll-a, Chlorophyll-b and total chlorophyll), Xanthophyll, Carotenoids and Epicuticular wax value observed in Corbyn's cove and lowest value noticed in Sipighat region and average value recorded at Havelock.

Key words: Carotenoids, Chlorophylls, Spectrophotometric analysis, Epicuticular wax, Mangrove, South Andaman

Introduction

The mangrove plant species are containing facultative halophytes, characterized by the regular tidal inundation and fluctuating salinity (Chauhan, 2006). They are highly adapted to the coastal environment, thriving in intertidal zones of tropical and sub-tropical regions (Duke, 1992) and exhibit several unique morphological and eco physiological adaptations to the coastal environment. The aerial root system, succulent sclerophyllus leaves and viviparous seedling together constitute one of the most convincing cases for convergent evolution among diverse taxa in response to similar environment constraints (Farnsworth and Ellison, 1991). Mangroves are physiologically interesting as potential models for stress tolerance. Gas exchange and chlorophyll fluorescence studies (Ball, 1981) have revealed that often mangroves have low photosynthetic rates and typically became light saturated at 30 – 50 % of the full sunlight when compared with tropical moist rainforest. They not only survive but also dominate a harsh ecosystem. Even in areas with pronounced seasonality, most of the mangrove species maintain active leaves under conditions, which might be expected to severely reduce the photosynthetic capacity through photoinhibition and raises a question as how the mangroves sustain their productivity throughout the year (Cheeseman et al, 1991).

The chlorophyll pigments played major role in photosynthesis and drought causes reduction in net photosynthetic rate (Thorati et al., 2016). Chlorophyll (Chl) 'a' and (Chl) 'b' content decreased with increasing severity of water stress (Gadallah, 1999). Carotenoids are essential components in the photosynthetic apparatus in plants where they protect against photo oxidative damage and contribute to the light harvesting in photosynthesis (Goodwin, 1980). Leaf surface features such as the wax layer, cuticular rethickening and trichomes play an important role in the variability of optical properties (Vogelmann, 1994). Epicuticular wax during moisture stresses varies in different crops and depends on environmental factors like high radiant energy and low humidity.

Certain studies revealed that there is no photoinhibition when *Rhizophora stylosa* was directly illuminated in the field (Bjorkman et al, *1988*). While Cheeseman *et al,* (1991) reported no evidence of photoinhibition in exposed leaves of *Bruguiera parviflora* under natural illumination and in *Rhizophora mangle* under greenhouse, water-stressed conditions (Cheeseman, 1994). There are several numerical and empirical simulation models involving various environmental factors with a single physiological process viz., transpiration, stomatal conductance, photosynthesis, or respiration (Barr et al, 2009). Systematic efforts to test selected mangrove species under different zones for estimation of its pigmentation are meager. This study was, therefore, undertaken to study the presence of plant pigmentation and epicuticular wax of these species to assess the mangrove species health, population, and its productivity.

Materials and methods

Study area

The Andaman and Nicobar Islands are situated in the Bay of Bengal between 6° to 14° N and 92° to 94° E longitudes. The total area of these islands is 8249 km^2 consisting of 836 Islands/Islets/Rocky Outcrops, 38 inhabited Islands and 206 rocks and rocky outcrops with a coastal line of about 1962 km (https://www.andaman.gov.in/). Total area of mangrove forest is 616 km^2 in the year 2021 exclusively mangrove patches in South Andaman is 189.72 km^2 (FSI, 2021). About 39 mangrove species were reported from India (Kathiresan, 2000), of which 34 species belonging to 15 genera and 12 families are documented (Dam Roy et al, 2009) in Andaman & Nicobar Islands. Despite extensive studies of biogeography, ecology, and forestry of the mangrove ecosystems of these Islands since the 1870, considerable disparities in species composition still exist, and there is a lack of comprehensive locality data. The present study evaluates the biochemical estimation such as Chlorophyll a, b, Total chlorophyll, Xanthophyll, Carotenoids and Epicuticular wax content for selected mangrove species in South Andaman region. Three sample locations were selected based on the human habitat *viz.,* near settlement (Corbyn's cove), undisturbed patch (Sipighat) and Inter Island in Andaman Sea (Havelock).

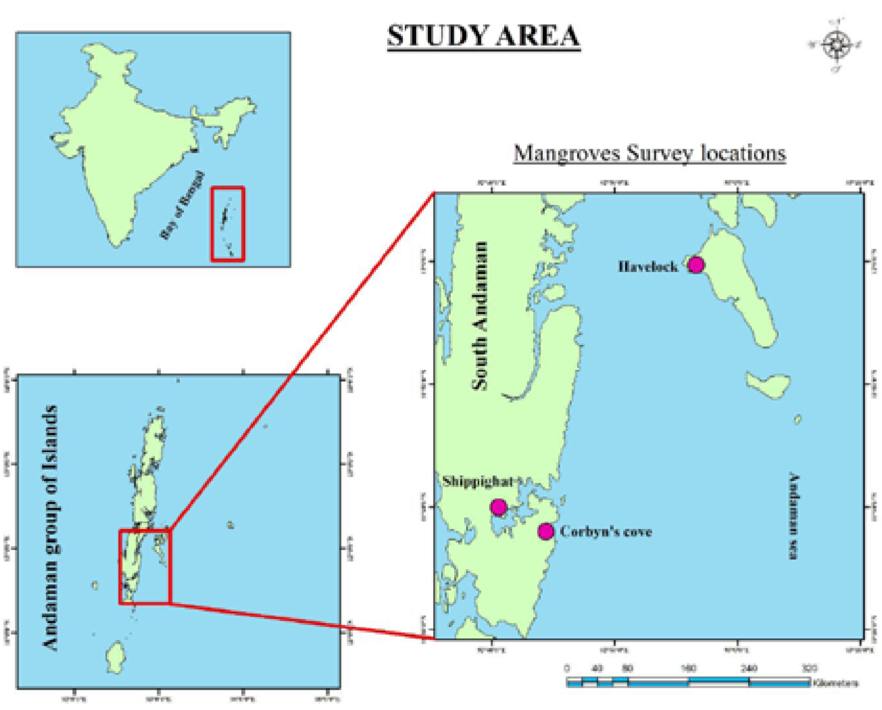

Fig 1. Selective mangrove species location of South Andaman

Preparation of plant Extract

Acetone Extracts: Fresh leaves of *Acrostichum aureum, Avicennia marina, Avicennia officinalis, Rhizophora apiculata, Rhizophora mucronata, Excoecaria agallocha* and *Nypa fruticans* were collected and rinsed in running tap water to remove the dust and grinded using clean dried motor and pestle separately. The extraction of these selected mangrove specieswere carried out by soaking 1g of sample in Acetone at the ratio of 1:20 (1g of sample in 20ml of acetone). The mixture was left undisturbed for 24 hours and then filtered using Whatman filter paper no. 1. The mixture was stored in falcon tubes at refrigerator until further used.

Biochemical analysis

The chlorophyll was estimated adopting the method of Yoshida et al, (1971) and expressed as mg per gram of fresh weight. The optical density of the content was measured at 663 nm, 652 nm, and 645 nm. The chlorophyll a, chlorophyll b and total chlorophyll content were calculated using the following formula.

$$\text{Chlorophyll a (mg g} - 1) = \frac{12.7 \text{ x OD at } 663 - 6.29 \text{ x OD at } 645 \text{ x V}}{1000 \times \text{W}}$$

$$\text{Chlorophyll b (mg g} - 1) = \frac{22.7 \text{ x OD at } 645 - 6.29 \text{ x OD at } 663 \text{ x V}}{1000 \times \text{W}}$$

Where,

V = volume made (25 ml)

W = weight of fresh sample taken (250 mg).

Epicuticular leaf wax

The colorimetric methods in based on the color change produced due to the reaction of wax with the acidic Potassium dichromate ($K_2Cr_2O_7$) reagent (Vogelmann, 1994).

$Y = 0.007x + 0.030$ Value of x is equal to O.D.

Carotenoid

Carotenoids were determined as per the methods of Kirk and Allen (1965) methods whereas like the sample taken in chlorophyll.

Carotenoid (μg/g.fr. wt) = ΔA480 + (0.114 × ΔA663) (0.638 × ΔA645)

Results and Discussion

Estimation of mangrove leaf pigments

Table.1. Phytochemical content of mangroves trees for different locations of South Andaman.

S. No	Species/Pigments	Sipighat				Havelock				Corbyn's Cove			
		Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Xanthophyll & Carotenoids (mg/g)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Xanthophyll & Carotenoids (mg/g)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Xanthophyll & Carotenoids (mg/g)
1	*Acrostichum aureum*	0.7	0.5	0.42	1.3	0.34	0.1	0.41	5.48	0.14	0.57	0.72	3.44
2	*Avicenna marina*	0.1	0.42	0.1	1.08	0.34	0.4	0.2	1.36	0.22	0.4	0.27	4.27
3	*Avicennia officinalis*	0.27	0.34	0.31	3.59	0.36	0.37	0.41	1.68	0.62	0.19	0.82	5.22
4	*Rhizophora apiculata*	0.8	0.8	0.5	0.8	0.31	0.2	0.14	2.57	0.7	0.7	0.92	5.79
5	*Rhizophora mucronata*	0.23	0.6	0.25	4.55	0.54	0.65	0.44	4.9	0.54	0.23	0.77	4.62
6	*Excoecaria agallocha*	0.14	0.7	0.15	3.07	0.23	0.4	0.14	3.1	0.47	0.14	0.61	4.45
7	*Nypa fruticans*	0.55	0.26	0.46	1.38	0.1	0.6	0.3	2.45	0.2	0.22	0.26	4.11
	Mean	0.4	0.52	0.3	2.25	0.32	0.39	0.29	3.08	0.41	0.35	0.62	4.56

The Mangrove leaf pigments viz., Cholorphyll, Carotenoides and Xanthophylls were estimated for the selected 7 mangrove species (Table 1).

The Chlorophyll content was estimated for seven mangrove species for tree different location in South Andaman. The chlorophyll results revealed the Chlorophyll a & b content was ranged from 0.10 mg/g to 0.80 mg/g and the Total Chlorophyll content range from 0.10 mg/g to 0.92 mg/g for all the locations. Among the selected mangrove species highest Chlorophyll a & b, Total Chlorophyll recorded in *Rhizophora apiculata* (0.80 mg/g, 0.80 mg/g and 0.50 mg/g respectively) at samples from Sipighat followed by samples collected from Corbyn's cove (0.70 mg/g, 0.70 mg/g and 0.92 mg/g respectively). The lowest value of the Chlorophyll a (0.31 mg/g), Chlorophyll b (0.20 mg/g) and the Total Chlorophyll (0.14 mg/g) was recorded at Havelock samples in *Rhizophora apiculata*. Overall average of Chlorophyll a was estimated at Corbyn's Cove (0.41 mg/m) followed by Sipighat (0.40 mg/g) and Havelock (0.32 mg/g). Similar studies conducted by Murthy and Rao (2014) reported a wide variation in the ranges of chlorophyll-a and b.

In which, three different location highest value of Chlorophyll a was recorded in Sipighat (0.80 mg/g) in *Rhizophora apiculata* followed by Corbyn's Cove (0.70 mg/g) in *Rhizophora apiculata* and Havelock (0.54 mg/g) in *Rhizophora mucronata*. The lowest value of Chlorophyll a was recorded the species of *Avicenna marina* and *Nypa fruticans* species (0.10 mg/g) in Sipighat and Havelock respectively. High Chlorophyll a content than Chlorophyll b in leaf sample of selected mangrove species of South Andaman region. The climatic factor of light intensity directly influenced by mangrove species. These mangrove species are highly dense and dominated in South Andaman region. Carotenoids are water-repelling pigments that are synthesized in the plastids of plant cells and have both photosynthetic and photoprotective roles in leaves (Demmig-Adams and Adams, 1996).

The highest range of Chlorophyll b was recorded in Sipighat (0.80 mg/g) in *Rhizophora apiculata*, followed by Havelock (0.65 mg/g) in *Rhizophora mucronata* and Corbyn's Cove (0.70 mg/g) in *Rhizophora apiculata*. The lowest range of Chlorophyll b was recorded in Havelock (0.10 mg/g) in the species of *Acrostichum aureum*. Whereas, the highest amount of Total chlorophyll was recorded at Corbyn's cove (0.92 mg/g) in *Rhizophora apiculata* followed by Sipighat (0.50 mg/g) in *Rhizophora apiculata* and Havelock (0.44 mg/g) in *Rhizophora mucronata*. The lowest amount of Total chlorophyll was recorded in Sipighat (0.10 mg/g) in *Avicenna marina.* Studies conducted by Basak et al. (1996) reported a variable range of chlorophyll a/b ratio in the mangrove trees.

During night time the photosynthesis was reacting with the help of Xanthophylls and Carotenoids was recorded range between 0.80 mg/g to 5.79 mg/g. Among the three different locations of South Andaman the average Xanthophylls and Carotenoids was estimated higher range in Corbyn's cove, followed by Havelock and Sipighat (5.79 mg/g, 5.48 mg/g and 4.55 mg/g respectively). The lowest range at Corbyn's Cove (3.44 mg/g), followed by Havelock (1.36 mg/g) and Sipighat (0.80 mg/g). However, the highest range of Xanthophylls and Carotenoids was recorded in the species of *Rhizophora apiculate* (5.79 mg/g) at Corbyn's cove followed by *Rhizophora mucronate* (4.55 mg/g) in Sipighat and *Rhizophora mucronata* (0.44 mg/g) in Havelock. The lowest range of Xanthophylls and Carotenoids content was recorded in *Acrostichum aureum* (3.44 mg/g) in Corbyn's cove followed by *Avicennia officinalis* (0.41 mg/g) in Havelock and *Rhizophora apiculate* (0.8 mg/g) in Sipighat. Xanthophyll & Carotenoids (mg/g) are very high pigment compared to Chlorophyll a, Chlorophyll b and Total Chlorophyll in selected mangrove species. Under stress conditions, chlorophyll a is more susceptible for degradation than chlorophyll b which results in a reduction in Chlorophyll a/b ratio (Young, 1992). The concentrations of leaf pigments were liable to changes depending upon their prevailing conditions. Thus, seasonal monitoring of mangrove ecosystems has emerged as a vital necessity for the management and conservation of coast lines (Kripa et al., 2018).

Epicuticular wax

Epicuticular waxes are complex mixtures of long chain aliphatic and cyclic components, including hydrocarbons, alcohols, aldehydes, ketones, esters, fatty acids, terpenoids, and flavonoids, that cover the external side of the leaf epidermis of all higher plants (Kunst and Samuels 2009). Epicuticular wax load per area sampled was examined in seven species of South Andaman that originated from vastly different worldwide environments (Table 2). The observed results indicate that among all the samples collected, comparing the three different locations, *A. aureum* of Corbyn'scove possessed significantly highest concentration (32.71µg/dm^2) followed by *R. apiculata* of Sipighat (32.14 µg/dm^2) and *N. fruticans* of Havelock (29.74 µg/dm^2). The mean value (23.41 µg/dm^2) suggests that plants from Corbyn's cove had comparatively highest epicuticular wax followed by Havelock (23.02 µg/dm^2) and Sipighat (22.45 µg/dm^2). Among the plant species, *A. aureum* of Corbyn's cove (32.71 µg/dm^2) had the highest epicuticular wax followed by *R. mucronata* of Sipighat (32.14 µg/dm^2) ≥*N. fruticans* of Havelock (29.74 µg/dm^2) ≥*R. apiculata* of Havelock (27µg/dm^2) ≥ *A. marina* of Sipighat (23.28 µg/dm^2) ≥ *E. agallocha* of Sipighat (22 µg/dm^2) respectively. The cuticle layer showed differences in thickness among the three mangroves, which may be related to pollution or other environmental condition. This reduction in thickness may cause an increase of cuticle transpiration and, later, damage to the plants since wax + cuticle layer is the major barrier against water and nutrient losses (Cristiane et al., 2021).

On the other hand, *A. officinallis* possessed the lowest concentration while comparing among the species as well as different location (≤16.75 µg/dm^2). The mean value among the species reveals that *R. mucronata* (29.14 µg/dm^2) comparatively highest concentration of epicuticular wax followed by *N. fruticans* (27.34 µg/dm^2) ≥*A. aureum* (24.76 µg/dm^2) ≥E. agallocha (21.57 µg/dm^2) ≥*R. apiculata* (21.19 µg/dm^2) ≥*A. marina* (20.09 µg/dm^2) whereas *A. officinalis* (16.63 µg/dm^2) possessed significantly lowest mean respectively. It has been proposed that water status of the leaf affects the physical structure of leaf cuticle (Kannan, 1986). Epicuticular wax composition is considered a taxonomic tool for species identification. The concentration of triterpenes is correlated with salinity stress, providing a protective role for mangrove species (Basyuni et al. 2012).

Table 2. Epicuticular wax (µg/dm^2) for selected mangrove species in three different locations of South Andaman

S. No	Species/ Sampling sites	Epicuticular wax (µg/dm^2)			
		Sipighat	Havelock	Corbyn's cove	Mean
1	*Acrostichum aureum*	19.14	22.42	32.71	24.76
2	*Avicennia marina*	23.28	15.71	21.28	20.09
3	*Avicennia officinalis*	16.57	16.57	16.75	16.63
4	*Rhizophora apiculata*	18.28	27.00	18.28	21.19
5	*Rhizophora mucronata*	32.14	28.71	26.57	29.14
6	*Excoecaria agallocha*	22.00	21.00	21.71	21.57
7	*Nypa fruticans*	25.71	29.74	26.57	27.34
	Mean	22.45	23.02	23.41	-

Conclusion

Natural regeneration potential is an important indicator for any forest ecosystem. The natural regeneration selected 26 mangrove species were observed and calculated biodiversity indices based on the literature survey during the period of December 2019 to February 2020. Epicuticular wax content majorly role in plant leaf to control the high evaporation of water in mangrove plants. *Avicennia marina* and *Avicennia officinalis* have a special mechanism of salt excreting by the leaf of mangroves. Likewise, these two plants were recorded very low content of Epicuticular wax.

Sipighat, Havelock and Corbyn's Cove are the selected mangrove area from throughout the studies based on Pollution, anthropogenic activities, and water pollution respectively. Based on the survey and results Sipighat and Corbyn's Cove are clearly showing the pollution highly influence with studies pigment values. The natural regeneration also very slow by the polluted water tidal. The detailed information on diversity, distribution, and vegetative structure of all known mangrove species of south Andaman provided here will eventually help in the long-term monitoring of mangrove species.

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