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ORGANIC CONSTITUENTS IN LEAVES OF 9 MANGROVE SPECIES OF ORISSA COAST, INDIA.

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Abstract

Photosynthetic pigments, TAN (Titrable Acid Number), proteins, polyphenols and tannin content in leaves of 9 species of mangroves viz., *Acanthus ilicifolius, A. ebracteatus, A. volubilis, Brownlowia tersa, Bruguiera cylindrica, B. gymnorrhiza, Ceriops tagal, Rhizophora apiculata* and *Xylocarpus mekongensis* found in the mangrove forests of Bhitarkanika and Mahanadi delta of Orissa were estimated. Total chlorophyll content varied from 0.16% in *B. tersa* to 1.05% in *A. volubilis*. Calculated chlorophyll a:b ratio was minimum (1.23) in *C. tagal* and maximum (3.85) in *A. ilicifolius*. The carotenoids as accessory pigments also varied from 0.08% in *B. tersa* to 0.76% in *A. ilicifolius*. Analysis of variance showed intra- and interspecific variations in photosynthetic activities. TAN values revealed appreciable variations from 20.61 to 32.86 in *B. gymnorrhiza* and *A. ilicifolius*, respectively. TAN values showed negative correlation with chlorophyll b and carotenoids but highly significant positive correlation was noted with chlorophyll a:b ratio among the species. Total buffer soluble protein content in leaf varied significantly from 13.26% in *B. cylindrica* to 21.05% in *X. mekongensis*. The quantitative analysis of tannin and polyphenols from the leaves of mangroves showed significant variation ranging from 14.56% to 40.11% in *X. mekongensis* and *C. tagal*, and 15.65% to 38.64% in *A. volubilis* and *C. tagal*, respectively. Statistical analysis of the endogenous level of polyphenols and tannins showed no interdependence with leaf proteins.

Introduction

Mangroves, like other higher plants, depend on the photosynthetic reduction of carbon dioxide to form carbohydrates and other organic constituents necessary for growth and maintenance. Because of the physiologically xeric nature of the habitat the physiology of the mangrove plants is of considerable interest. An understanding of their photosynthetic characteristics and their correlation with organic constituents is thus necessary for the assessment of growth and productivity of mangroves. Unlike other plants, mangroves commonly grow on saline, waterlogged soils, inundated with tidal water. Despite being waterlogged, the high salinity and low osmotic potential of mangrove soils, the mangroves experience moderate to severe physiological stress. Mangroves are known to synthesize more polyphenols and tannins in response to salinity and their organic acid metabolism which vary with different species (Basak *et al.*, 1996). The present report describes the organic constituents and their correlations in 9 different species of mangroves of the Orissa coast, India.

Materials and Methods

The propagating materials like seeds and propagules hypocotyl of 9 mangrove species viz., *Acanthus ilicifolius, A. ebracteatus, A. volubilis* (Acanthaceae), *Brownlowia tersa* (Tiliaceae), *Bruguiera cylindrica, B. gymnorrhiza, Ceriops tagal, Rhizophora apiculata* (Rhizophoraceae) and *Xylocarpus mekongensis* (Meliaceae) were collected from the mangrove forests of the Bhitarkanika and the Mahanadi delta of Orissa lying between 20°4' and 20°8' N latitude and 86° 454' E longitude. The seedlings were raised at Mangrove Research Centre of the Regional Plant Resource Centre, Bhubaneswar, Orissa with non-saline and non-brackish water under lath house conditions having the light intensity of 30,000-35,000 lux. Young, semi-mature and mature leaves were collected from one and half year old seedlings for extraction of chlorophyll, carotenoids, TAN, polyphenols, tannin and protein. Fresh and clean leaves were extracted for determination of chlorophylls and carotenoids (Arnon, 1949). Extraction of polyphenols was done by the use of 80% ethanol followed by centrifugation at 5000 rpm for 30 min. Quantitative estimation of the polyphenols were carried out by the modified method of Swain & Hills (1959). Commercial gallic acid was used as standard. Spectrophotometric observations were taken at 515 nm using Jasco UVIDEC-650 double beam spectrophotometer. Tannin was estimated following the method of Ravi & Kathiresan (1990). For the study of total protein content, leaves were homogenised in chilled 0.05 M Tris glycine buffer at pH 8.3 using cold mortar and pestle at 4°C. The homogenate were centrifuged in Kubota-2000C centrifuge at 10000 rpm for 60 min., at 4°C. The supernatant was treated with trichloroacetic acid to precipitate proteins and centrifuged for 30 min., to obtain the protein pellet. The protein pellet was dissolved in 1N Sodium hydroxide (NaOH). The extracts were then used for the estimation of total protein following the method of Lowry *et al.*, (1951). To estimate titrable acid number (TAN), fresh leaves were extracted in 80% ethanol and the extract treated with activated charcoal (Sigma). The clear supernatant after filtration was titrated against 0.1N NaOH following the method of Thomas & Beevers (1949). Each treatment was replicated 10 times and the experiments were repeated twice. Statistical analysis of different data following ANOVA (Sokal & Rohlf 1980), Duncan multiple range test (Harter, 1960) and correlation coefficient analysis were carried out. All the graphs were plotted following the computation of data in Harvard Graphics.

Results and Discussions

Chlorophylls, Carotenoids and TAN value: Significant variation in different constituents was observed at intergeneric as well as interspecific level. *Acanthus ilicifolius* recorded the highest chlorophyll-a content (0.81% on dry wt. basis) whereas the minimum (0.11%) was found in *Brownlowia tersa* (Table 1). However, no remarkable variation was noted in chlorophyll-b content in the leaves of different mangrove species (Table 1). Total chlorophyll varied considerably from 0.16% to 1.05% in *Brownlowia tersa* and *Acanthus volubilis*, respectively. The calculated a:b

Table 1. Variation in organic constituents (% dry wt.) in leaves of 9 mangrove species.

No. Species	Chloro-phyll a	Chloro-phyll b	Chloro-phyll a+b	Chloro-phyll a:b	Carot-enoid	Poly-phenol	Tannin	Protein	TAN*
1. *Acanthus ilicifolius*	0.81	0.21	1.02	3.85	0.76	17.57	21.65	19.08	32.86
2. *A. ebracteatus*	0.55	0.21	0.86	2.61	0.38	16.86	20.45	17.66	26.25
3. *A. volubili*	0.76	0.29	1.05	2.62	0.59	15.65	19.88	16.25	30.16
4. *Brugaria cylindrica*	0.14	0.07	0.21	2.00	0.12	22.38	33.05	13.26	23.80
5. *B. gymnorrhiza*	0.28	0.09	0.37	3.11	0.17	26.11	38.56	15.55	20.61
6. *Brownlowia tersa*	0.11	0.05	0.16	2.20	0.08	16.32	18.95	18.80	28.66
7. *Ceriops tagal*	0.16	0.13	0.29	1.23	0.09	38.64	40.11	18.56	18.05
8. *Rhizophora apiculata*	0.31	0.12	0.43	2.58	0.11	19.78	36.85	16.87	28.56
9. *Xylocarpus mekongensis*	0.37	0.10	0.47	3.70	0.11	23.62	14.56	21.05	32.50

*TAN values expressed as ml of decinormal NaOH required to netralize the acid in the extract of 100 g fresh weight.

Table 2. ANOVA of different parameters showing significant variation among the species.

Source	DF	SS	MS	F
Chlorophyll	8	4.74	0.59	812.00**
Chlorophyll a:b	8	27.20	34.00	2800.00**
TAN	8	986.00	123.00	44.20**
Total protein	8	202.00	25.20	121.00**
Polyphenol	8	2100.00	262.00	5010.00**
Tannin	8	3920.00	490.00	217.00**

** = Highly significant at 0.01% level.

ratio showed significant variation at inter-generic and interspecific level (Tables 1 & 2). The maximum photosynthetic efficiency (3.85) was recorded in *Acanthus ilicifolius* and the minimum (1.23) in *Ceriops tagal*. The amount of total chlorophyll in different species had direct correlation with carotenoids. The values for total chlorophyll vs. carotenoids, chlorophyll-a vs. carotenoids and chlorophyll-b vs. carotenoids were 0.928, 0.947 and 0.842, respectively (Table 3). Evidently, carotenoids played an important role in the process of photosynthesis as accessory pigments besides Photosystem I and II. The TAN values varied significantly from 20.61 in *Bruguiera gymnorrhiza* to 32.86 in *Acanthus ilicifolius*. The correlation coefficient values of TAN with chlorophyll a,b,a:b and carotenoids were -0.360,0.328 and 0.726, respectively (Table 3). The lower TAN value was due to more salt contents in the metabolic environment and salinity inhibited organic acid metabolism (Kotmire, 1983). However, the TAN values proportionally increased with the increase of photosynthetic efficiency which is similar to our earlier findings (Basak *et al.*, 1996) and with the reports of Bhosale *et al.*, (1983) in *Kandelia candel* and *Rhizophora mucronata*. ANOVA of chlorophylls, carotenoids and TAN revealed significant variations between the mangrove species (Table 2, Figs.1&2). Duncan's multiple range test showed distinct group between *A. ebracteatus, A. volubilis, B. gymnorrhiza* and *R. apiculata* with respect to chlorophyll a:b ratio. Moreover, *A. ilicifolius, B. tersa, C.tagal* and *A. ilicifolius, A. ebracteatus, B. tersa, R. apiculata* showed close affinity with regard to total protein content and polyphenol content, respectively (Table 1).

Polyphenols, Tannins and Protein content: The polyphenol content varied from 15.65% to 38.64% in *Acanthus ilicifolius* and *Ceriops tagal*, respectively (Tables 1 & 2) and the polyphenols showed a negative correlation with protein (Table 3). Most of the studies on mangrove polyphenols have been restricted to the samples of bark. The fruits and leaves are also considered good store houses of polyphenols. Earlier, upto 35% of polyphenols were reported in the mangroves and the accumulation of polyphenols was dependent on age of the plant (Karkar & Bhosale, 1986).

Tannin content in the leaf of *Ceriops tagal* was the maximum (40.11%) with minimum (14.56%) observed in *Xylocarpus mekongensis*. Higher tannin content in the

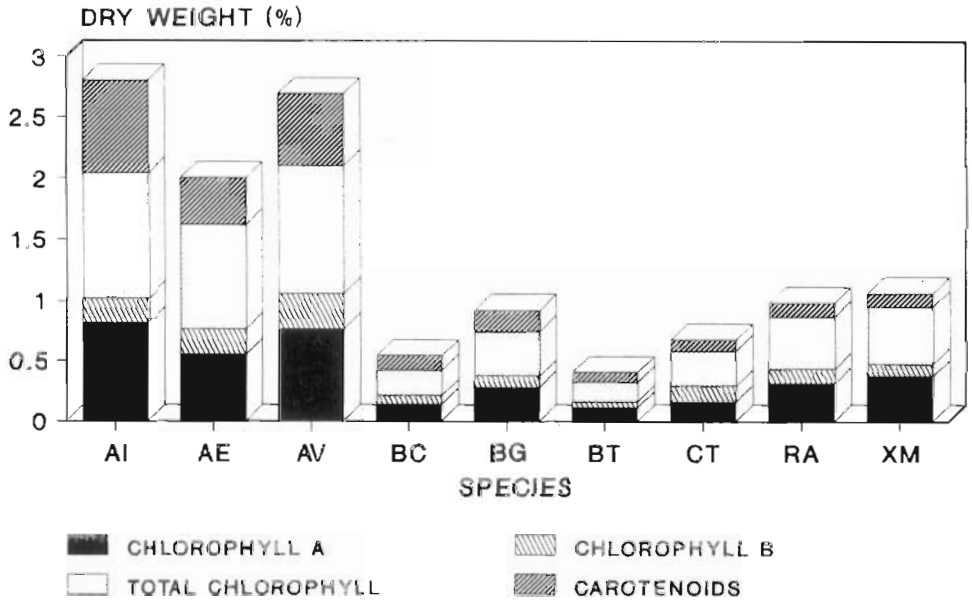

Fig.1. Stack bar-diagram of chlorophyl a, Chlorophyll b, total chlorophyll and carotenoids (%) in 9 species of mangroves.

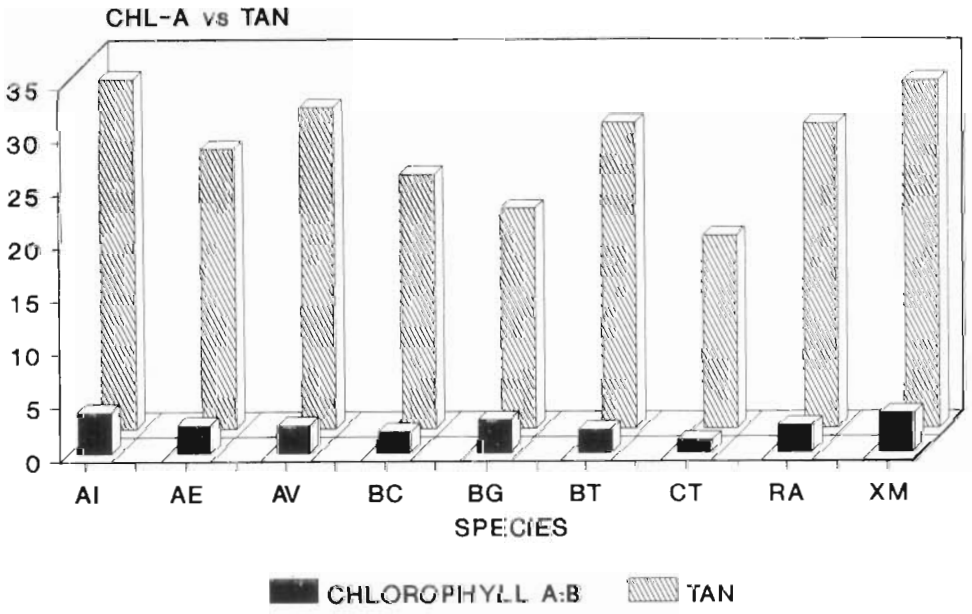

Fig.2. Histograms of chlorophyll a:b ratio and TAN values in 9 species of mangroves.

Table 2a. Duncan's multiple range test (DMRT*) of different parameters on organic constituents among 9 mangrove species.

Species	Total chloro-phyll (%d wt.)	Chlorophyll a:b	Total protein (%d.wt.)	Polyphenol (%d wt.)	Tannin (%d wt.)	TAN
Acanthus ilicifolius	1.02 g	3.85 d	19.08 c	17.57 a	21.65 c	32.86 d
A. chracteatus	0.86 cf	2.61 c	17.66 d	16.86 ab	20.45 d	26.25 bc
A. volubilis	1.05 g	2.62 c	16.25 bc	15.65 a	19.88 c	30.16 cd
Bruguiera cylindrica	0.21 b	2.00 b	13.26 a	22.38 cd	33.05 f	23.80 b
B. gymnorrhiza	0.37 d	3.11 c	15.55 b	26.11 d	38.56 b	20.61 a
Brownlowia tersa	0.16 a	2.20 b	18.80 e	16.32 ab	18.95 b	28.66 c
Ceriops tagal	0.29 c	1.23 a	18.56 e	38.64 e	40.11 l	18.56 c
Rhizophora apiculata	0.43 e	2.58 c	16.87 c	19.78 bc	36.85 g	28.56 c
Xylocarpous mekongensis	0.47 e	3.70 d	21.05 g	23.62 d	14.56 a	32.50 d

* Significant at 1% level.

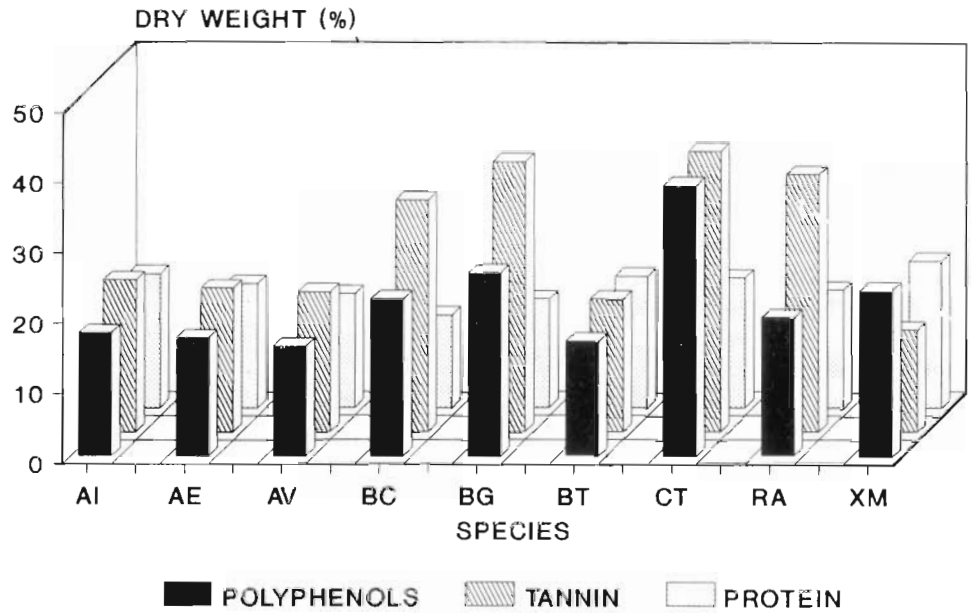

Fig.3. Stack bar-diagram of leaf polyphenols, tannins and proteins in 9 species of mangroves.

bark has been reported in Rhizophoraceae (Watson, 1928). ANOVA showed significant differences in tannin at inter-generic and inter-specific levels (Table 2).

The buffer soluble leaf protein content varied from 13.26% to 21.05% in *Bruguiera cylindrica* and *Xylocarpus mekongensis*, respectively (Table 1). Protein

Table 3. Correlation coefficient values of different parameters in 9 mangrove species.

Source	r
Chlorophyll a vs chlorophyll b	0.898
Total chlorophyll vs carotenoid	0.928
Chlorophyll a:b vs tannin	-0.512
Chlorophyll a vs carotenoid	0.947
Chlorophyll b vs carotenoid	0.842
Chlorophyll a vs TAN	-0.360
Chlorophyll b vs TAN	0.328
Chlorophyll a:b vs TAN	0.726
Carotenoid vs TAN	0.491
Carotenoid vs Tannin	-0.407
Polyphennol vs protein	-0.744
Tannin vs protein	0.451

content in the studied members of the Rhizophoraceae showed significant variation from 13.26% in *Bruguiera cylindrica* to 16.87% in *Rhizophora apiculata*. The correlation coefficient analysis showed negative correlation (-0.744) between polyphenols and proteins. The members of Rhizophoraceae showed moderate to less amount of protein in leaf. Proteins are the translated product of the mRNA and polyphenols and tannins are the secondary end products of different metabolic path ways. The presence of bitter principles, polyphenols or tannin, can be attributed to the supression of protein synthesis at genomic level which act on the synthesis of different metabolic end products for their self protection during macro- and micro-climatic changes during the process of evolution.

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