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QUANTITATIVE ESTIMATION OF TOTAL CHLOROPHYLL AND CAROTENOID CONTENT IN *OREOPANAX XALEPENSIS*

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ABSTRACT

Manipuri people have the traditional habit of eating raw leaves and other parts of plants by preparing traditional delicacies such as eromba, kangshu and shingju. The present work is based on the medicinally important plant Oreopanax xalepensis, locally called chom which is consumed as boiled leaves in kangshu preparation. We quantified the total chlorophyll and carotenoid content in both the septate and aseptate leaves of O. xalepensis using five different solvents (80% acetone, acetone absolute, 95% ethanol, methanol or water). Here, we report the higher content of both chlorophyll and carotenoid in septate leaves compared to the aseptate leaves. Our results showed that methanol and ethanol are the best extraction solvents for septate and aseptate leaves of O. xalepensis respectively.

KEY WORDS

Oreopanax xalepensis, Manipur, cholorophyll, carotenoid, spectrophotometry

INTRODUCTION

Manipur located in the Indo-Burma biodiversity hotspot region is famous for its rich flora and fauna. With a wide range in temperature and altitude, it is a natural habitat to different species of endemic plants and animals. Many of these plants and their derived products are used in the traditional medicine system to treat various ailments. The chlorophyll and carotenoid content of these medicinal plants are linked to their therapeutic values in earlier studies [1,2,3,4]. Chlorophyll has an important role in plant physiology and have a long history in traditional medicine [5,6].

Chlorophyll is a photo pigment and absorbs light mainly in the red (650-700nm) and blue violet (400-500nm) regions of the visible spectrum [7]. Chlorophyll a/b ratio is also considered as a sensitive biomarker for environmental stress [8,9,10]. Chlorophyll a is the primary pigment and chlorophyll b is the accessory pigment [11]. The total chlorophyll content (a+b) and their ratio (chl a/b) influence the photosynthetic capacity of a plant [12]. Variation in chlorophyll content indicates about it physiological condition of the leaf or plant. It is imperative to study the chlorophyll content of the plants in order to check the photosynthetic activity of physiological changes on the particular leaf/plant during their development, senescence, adaptation to different environmental conditions and stress and its ability to tolerate it [8,9,10]. The difference between the structure of chlorophyll a and chlorophyll b is that chlorophyll a has a methyl group on carbon (C-7), whereas chlorophyll b has an aldehyde group. On the other hand, carotenoids are bioactive compounds present in green leafy vegetables which plays major role in the prevention of cancers, cardiovascular disease etc [13]. Carotenoids absorbs light mainly 400-500nm of visible spectrum [14]. The

carotenoid level of a plant depends on several factors including species, variety, maturity and abiotic factors like light, temperature and soil properties [15]. Carotenoids contribute to the photosynthesis by transmitting the light energy they absorb to chlorophyll [16]. Chlorophyll and carotenoid concentration correlate to the photosynthetic potential of a plant [17]. Chlorophyll and carotenoid content vary with microclimatic condition as reported in *Adiantum* species [18]. Photosynthetic pigment concentration of a plant gives information about the plant productivity and health status and produce accurate estimates of plant vigor and environmental quality [19].

In the present study, we aimed to quantify the total chlorophyll and carotenoid content of the medicinally important plant of Manipur *Oreopanax xalepensis* [20] using various extraction solvents (acetone 80%, acetone absolute, methanol, ethanol and water) for both septate and asepate leaves and compared the results.

MATERIALS AND METHODS

Sample collection: Leaf samples were collected from *Oreopanax xalepensis* grown in different places of Lamlongei, Langjing, Lamsang and Phumlou in Imphal West District, Manipur. The healthy leaves with no mechanical injuries were selected and further classified as septate and aseptate. The leaves were washed with distilled water and then allowed to dry at room temperature before the extraction process.

Extraction: The extraction and subsequent analysis was done as described earlier [21]. Briefly, the leaves were cut into small pieces (20*50 mm^2) and 0.5 g of the leaves was grounded in mortar and pestle after adding 10 ml of the respective solvent (80% acetone, acetone absolute, 95% ethanol, methanol or water). The grounding was continued after freezing the sample for two hours. The homogenized sample was centrifuged at 10,000 rpm for 20 minutes at 4˚C and 0.5 ml of the supernatant was mixed with 4.5ml of the respective solvents. This solution was then used for estimating chlorophyll -a, chlorophyll-b and carotenoid content in a spectrophotometer. All measurements were done in triplicates.

Quality control: During the extraction process, analytical chemicals of AR Grade (Merck) were used. During the spectrophotometric observation, quartz cuvette (1cm^2) was used and respective solvent was taken as reference. During the entire investigation, double distilled water was used. The equations used with each of the five solvents to calculate chlorophyll and total carotenoid concentrations are given in Table 1.

Table 1: The equations to quantify concentrations (µg/ml) of chlorophyll a, chlorophyll b and total carotenoid by different extraction solvents [22,23]

Extraction solvent	Equation
80% acetone	Chlorophyll a=12.7A663 – 2.69A645
	Chlorophyll b=22.9A646.8 – 4.68A663
	Carotene=(1000A470 – 1.82Ca –85.02Cb)/198
Acetone	Chlorophyll a = 11.75 A662 – 2.350 A645
	Chlorophyll b = 18.61 A645 – 3.960 A662
	Carotene = 1000 A470 – 2.270 Chl a – 81.4 Chl b/227
Methanol	Chlorophyll a = 15.65 A666 – 7.340 A653
	Chlorophyll b = 27.05 A653 – 11.21 A666
	Carotene = 1000 A470 – 2.860 Chl a – 129.2 Chl b/245
95% ethanol	Chlorophyll a=13.36A664 – 5.19 A649
	Chlorophyll b=27.43A649 – 8.12 A664
	Carotene=(1000A470 –2.13Ca- – 97.63Cb)/209
Water[a]	Chlorophyll a=12.7A663 – 2.69A645
	Chlorophyll b=22.9A646.8 – 4.68A663
	Carotene=(1000A470 – 1.82Ca –85.02Cb)/198

[a]**Formula for water is taken as same as that of 80% acetone**

RESULTS AND DISCUSSION

The spectrophotometric absorbance data of chlorophyll a, chlorophyll b and carotenoid were determined separately for septate and aseptate leaf (Table 2).

Table 2 Spectrophotometric absorbance data for chlorophyll a, chlorophyll b and carotenoid of *O. xalepensis* with various solvents

Solvents used	Septate			Aseptate		
	A663 nm	A645nm	A450nm	A663 nm	A645nm	A450nm
80% acetone	0.277	0.387	Not determined	0.525	0.508	0.538
Acetone	0.368	0.93	1.089	0.246	0.475	1.047
Methanol	0.42	1.036	1.236	0.212	0.146	0.574
95% ethanol	0.288	0.769	0.944	0.359	0.676	1.168
Water	0.177	0.197	0.75	0.095	0.124	0.561

Quantitative estimation of chlorophyll

Estimation of chlorophyll and carotene content using the formula in Table 1. showed large variation in their content among the different solvents for both leaf types. The cholorophyll content was generally high in septate leaf than aseptate leaf. It may be due to that reason that septate leaves were mature while the aseptate leaves were not fully matured [24]. In the case of septate leaves, chlorophyll a (12.03 µg/ ml) and b content (4.72 µg/ ml) was the highest when extracted with methanol [figure 2]. On the contrary, 95% ethanol extraction gave the highest yield of both chlorophyll a (7.62 µg/ ml) and b (5.03 µg/ ml) in the aseptate leaves [figure 3] while its corresponding content by methanol extraction were low (5.42 µg/ ml). Generally, chlorophyll extraction by water was the lowest in both septate (5.15 µg/ ml) and aseptate (2.94 µg/ ml) leaves [figure 2,3]. Overall, septate leaves (5.15 to 16.75 µg/ ml) have higher content of chlorophyll compared to aseptate leaves (2.94 to 12.65 µg/ ml) [figure 2,3]. Among the five solvents used, methanol and ethanol were the most effective in extracting chlorophyll from the septate and aseptate leaves respectively.

Figure 1 Comparision between septate (left) and aseptate (right) leaves of *Oreopanax xalepensis*

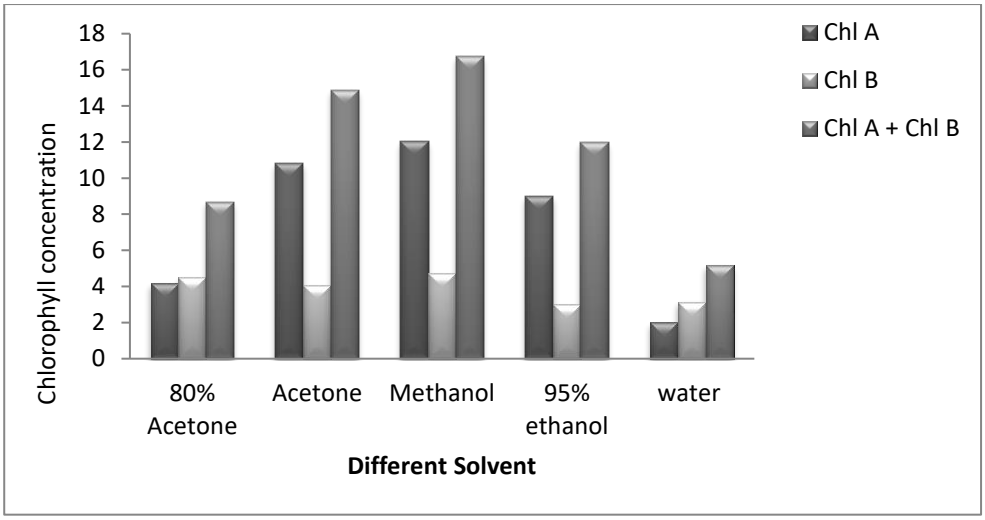

Figure 2 The average concentration (µg/ ml) of Chlorophyll a, Chlorophyll b and Total chlorophyll in *Oreopanax xalepensis* (septate)

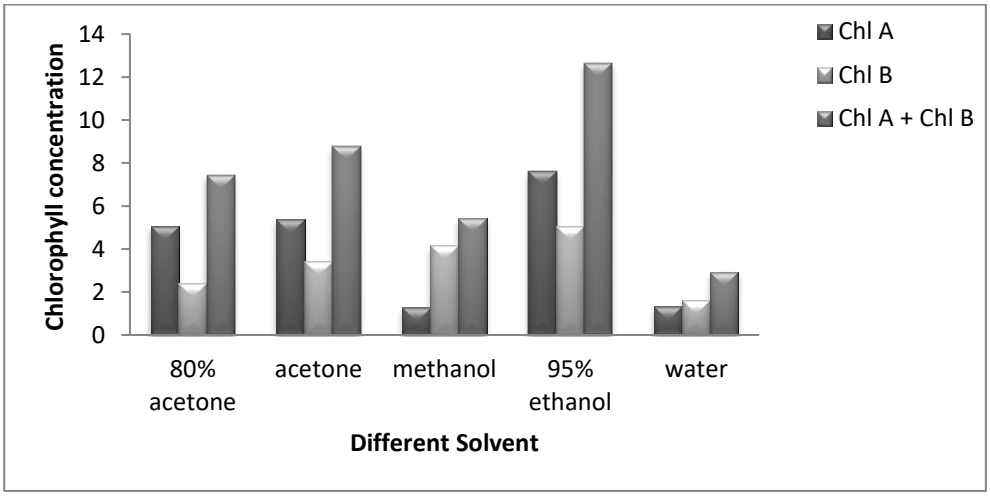

Figure 3 The average concentration (µg/ ml) of Chlorophyll a, Chlorophyll b and total chlrophyll in *Oreopanax xalepensis* (Aseptate)

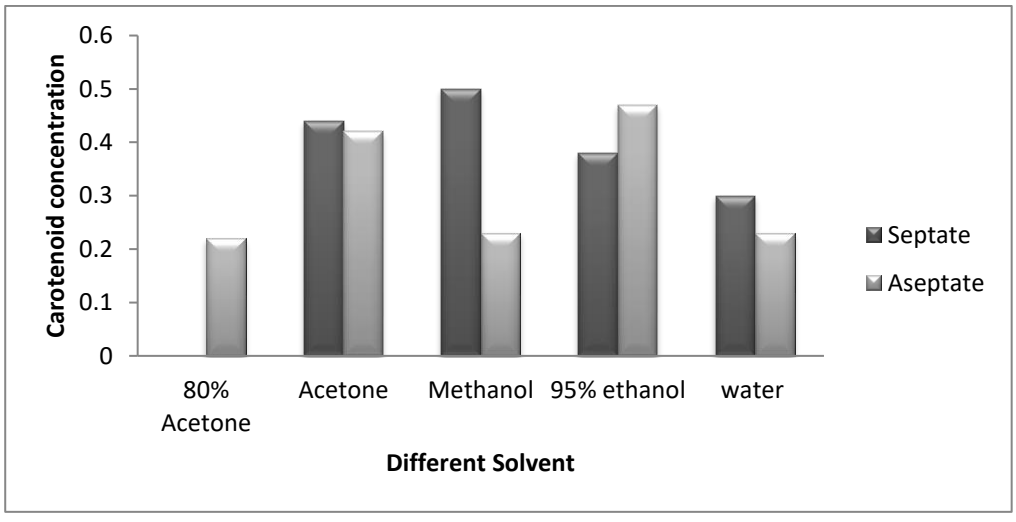

Figure 4 The total concentration (µg/ ml) of carotenoid in septate and aseptate leaves of *Oreopanax xalepensis*

Quantitative estimation of carotenoid

Similar to the chlorophyll content, carotenoid content for both septate and aseptate leaves depended on the extraction solvents used. Generally, higher level of carotenoid content was observed in septate leaves compared to the aseptate leaves except in the case of 80% acetone extraction. Highest carotenoid extraction was observed with methanol for septate (0.5 µg/ ml) and with 95% ethanol (0.47 µg/ ml) for aseptate leaves [figure 4].

The observed changes in the chlorophyll content may be because of changes in the reference system. For example, an increase in chlorophyll content per fresh weight could be solely due to a decrease in fresh weight caused by water loss. The chlorophyll a/b ratio is found to be similar in all cases (0.64 to 2.993) while using different extracting solvents except for methanol in aseptate leaves which shows high contents of chl b compared to chl a. [figure 3,4]. The chlorophyll a/b ratio was higher in septate leaves ranging from 0.6 (water extract) to 2.55 (methanol extract) compared to the aseptate leaves which range from 0.8 (water extract) to 1.51 (ethanol extract) [figure 2,3]. The weight ratio of total chlorophyll to total carotenoid is greater than 16 which indicate the greenness of the plant, low senescence, stress and damage to the plant and its photosynthetic apparatus. Carotenoid was found to be high on septate leaves 0.4 µg/ ml than the aseptate leaves 0.3 µg/ ml [figure 4]. As reported from other studies [25,26], the positive correlation between chlorophyll and carotenoid content is also reported in our study.

CONCLUSIONS

Our results showed that methanol and ethanol are the best extraction solvent for septate and aseptate leaves of *O. xalepensis* respectively. We conclude that septate leaves have more chlorophyll and carotenoid content than the aseptate leaves. Further investigations regarding the seasonal and temporal variation of their content are warranted to have a greater understanding.

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