

Chlorophyll Content Assessment in Black Ginger (*Kaempferia parviflora*) Across Different Leaf Development Stages

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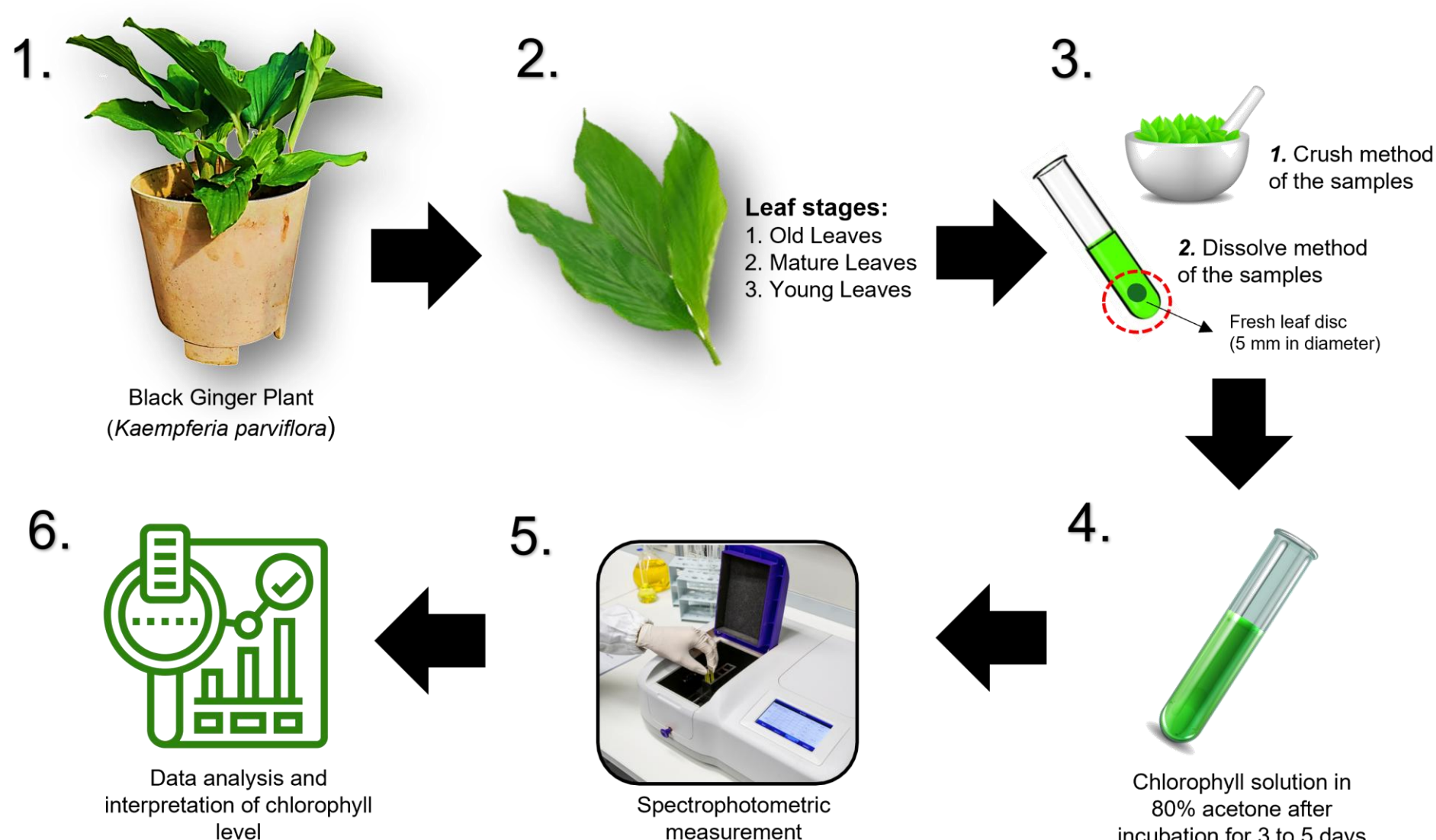
Abstract

Chlorophyll content is an important indicator of photosynthetic activity and plant health. Black ginger (*Kaempferia parviflora*) is a promising medicinal plant increasingly cultivated in Malaysia. This study investigated how different extraction methods and leaf maturity stages affect chlorophyll levels in Black ginger. Chlorophyll content was measured using a spectrophotometric method after grinding 5 mm leaf discs with acetone and centrifuging the mixture. Two extraction methods (grinder and dissolved) and three leaf stages (young, mature, and old) were tested to determine chlorophyll a, b, and total chlorophyll (a+b). Absorbance readings were taken at 647 and 664 nm. Results showed that the extraction method significantly affected chlorophyll a content ($P<0.0001$), with the grinder method yielding higher values. However, chlorophyll b was not significantly influenced ($P=0.46$). Leaf developmental stage strongly affected both chlorophyll a and total chlorophyll ($P<0.0001$ and $P=0.0002$), with young leaves showing the highest chlorophyll content. There were also significant interactions between extraction method and leaf stage for chlorophyll a ($P<0.0001$) and total chlorophyll ($P=0.01$). In conclusion, the grinder extraction method and young leaf stage produced the most reliable chlorophyll measurements. These results emphasize the need to optimize extraction techniques and leaf selection for accurate physiological assessments and future studies on Black ginger growth and bioactive compounds.

INTRODUCTION & AIM OF STUDY

- Chlorophyll content is widely used as a physiological indicator of photosynthetic efficiency and plant vigour.
- Black ginger (*Kaempferia parviflora*) is increasingly recognized as a valuable medicinal crop in Malaysia (Hairunisa et al., 2024), and reliable chlorophyll assessment is essential to support research on its growth and bioactive potential.
- However, extraction techniques and leaf maturity may influence chlorophyll measurement accuracy (Ašimović et al., 2016).
- This study investigated how different extraction methods and leaf maturity stages affect chlorophyll levels in Black Ginger plants.

MATERIALS & METHOD



- Plant material: Leaves categorized into young, mature, and old stages.
- Two extraction methods which were 1. Grinder method and 2. Dissolved method.
- Sample: Leaf disc of 5 mm diameter.
- Pigment extraction using acetone, followed by centrifugation (Ašimović et al., 2016).
- Spectrophotometric determination of chlorophyll a and b at 647 nm and 664 nm.
- Data analysis included statistical comparison of extraction × leaf stage effects (Two-Way ANOVA).

RESULTS & DISCUSSION

Table 1. Main effects of extraction methods and different leaf stages on the chlorophyll content of Black Ginger.

Main effects	Chlorophyll a	Chlorophyll b	Total chlorophyll (a+b)
Extraction method (EM)			
Dissolved	1.1288 (±0.0454) ^b	1.4154 (±0.0536) ^a	2.8336 (±0.1164) ^b
Grinder	1.5786 (±0.0309) ^a	1.4772 (±0.0346) ^a	3.1194 (±0.0805) ^a
LSD _{0.05}	0.1118	0.1277	0.2855
P-value	$P<0.0001$	$P=0.46$	$P=0.06$
Leaf stages (LS)			
Old leaves	1.2806 (±0.0481) ^b	1.3395 (±0.0482) ^b	2.6801 (±0.1208) ^b
Mature leaves	1.4630 (±0.0472) ^a	1.5230 (±0.0585) ^a	3.0624 (±0.1281) ^a
Young leaves	1.5541 (±0.0472) ^a	1.5448 (±0.0585) ^a	3.3158 (±0.1186) ^a
LSD _{0.05}	0.1274	0.1277	0.3279
P-value	$P<0.0001$	$P=0.03$	$P=0.0002$
Interactions (EM x LS)	$P<0.0001$	$P=0.78$	$P=0.01$

Means sharing same letters within a column are not significantly different at $P\leq 0.05$ according to LSD_{0.05} test. Numbers in the parentheses are standard error of means (±).

- Chlorophyll accumulation increases in younger, more photosynthetically active leaves.
- Grinder method improves pigment release, leading to more accurate and higher readings.
- Both methodological and physiological factors must be optimized for reliable chlorophyll assessment.

CONCLUSION & FUTURE WORK

- Grinder method is recommended for chlorophyll extraction in Black Ginger.
- Young leaves provide the most representative chlorophyll measurements.
- Experimental conditions play a crucial role in physiological assessments.
- This study will be expanded to evaluate the correlations between chlorophyll content and bioactive compounds.
- This study can be served as a guidelines for crop quality monitoring and agronomic optimization in Black Ginger.

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