Whole plant elicitation: Anew approach toward enhanced production of plant secondary metabolites harvest index

Hawa Z. E. Jaafar, PhD
Director, University Community Transformation Centre (UCTC)
Department of Crop Science, Faculty of Agriculture
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor
MALAYSIA
Tel .: +06-89471801 /4821 Email: hawazej@upm.edu.my; hawazej@gmail.com

INTRODUCTION

- Medicinal plants ~ most exclusive source of lifesaving drugs ~ used in > one country
- Up to 80% ~ people in developing countries ⇨ rely primarily on Traditional Medicine (TM) for their healthcare (WHO 2010) ~ mostly plants (herbs)
- Plants ~ tremendous source for drug discovery of new products with medicinal importance
- Plant secondary metabolites (SM) ~ rich source of bioactive constituents (phyto-pharmaceuticals: alkaloids, glycosides, flavonoids, volatile oils, tannins, resins) ~ fast gaining commercial interest for use in pharmaceutical industry, food additives, flavors, and other industrial materials (fragrances, dye, pigments, pesticides)

- Currently, most of these SM isolated from wild/ cultivated plants: chemical synthesis~extremely difficult or economically not feasible (Namdeo, 2007)

- Evolving commercial importance of SM ~ in recent years resulted in a great interest in secondary metabolism & production particularly the possibility of altering production of bioactive plant metabolites

- Constraints of plant SM production:

i) recovery rate ~ low (<1% dry weight) (Oksman-Caldentey & Inze, 2004; Dixon, 2001)

ii) greatly responsive to biotic/abiotic factors (≅ elicitors) altering physiological, biochemical, morphological & growth properties (Dornenburg & Knorr, 1995; Balandrin & Klocke, 1988; Rates, 2001; Dixon, 2001)

- Elicitation ~ a process of induced or enhanced synthesis of plants SM ~ ensure spp. survival, persistence and competitiveness

- Elicitors ~ usually capable to induce various modes of plant defense including production of ROS, hypersensitive response and production of phytoalexins

(Dornenburg & Knorr, 1995; Balandrin & Klocke, 1988; Rates, 2001; Dixon, 2001)

- Phytoalexin biosynthesis Induction ~ gained special importance in biotechnological approaches as enhancers of plant-SM synthesis, and could play an important role in biosynthetic pathways to enhance production of commercially important compounds

(Murthy et al., 2008)

Examples:

- Stuhlfauth et al. (1987): CO_2 & water stress ☛ increased foxglove SM ☛ cardiac glycoside *digoxin*
- Curtis et al., 1994: Calcium alginate immobilization of *Hyoscyamus muticus* plant tissue culture ☛ enhanced rapid formation of sesquiterpenes
- Marcia et al. (2006): excess of carbon influenced race-specific accumulation of phytoalexins in soybean~modify plant responses to herbivores & pathogens
- Jeong & Park, 2007: Phanax ginseng (hairy roots) treated with selenium ☛ enhanced ginseng saponin
- Ghasemzadeh et al. (2010) and Ibrahim et al. (2011) *Zingiber officinale* & *Labisia pumila,* respectively ~ impacts of CO_2 elicitation enhanced SM & anti-oxidative properties ☛ increased concentrations of several therapeutic compounds

Elicitation of whole plants/seedlings grown in the field or raised under controlled environment ~ Result in increased and speeded up growth and development along with improved secondary metabolites production

(Stuhlfauth et al., 1987; Jaafar; 2006; Amdoun et al., 2009; Ghazemzadeh & Jaafar, 2011; Ibrahim & Jaafar, 2011; Jaafar et al., 2012; Ibrahim and Jaafar, 2013)

Impact of CO_2 enrichment on secondary metabolites production and profiling

Halia Bentong

Halia Bara

CO_2 on Lp growth @ 8 m (4 m enrichment)

Biomass, leaf area & nos., basal diameter, plant height

Total phenolics & flavonoids contents in different parts of *Labisia pumila* under different CO2 concentration.

CO_2 levels (µmol/mol)	Plant parts	Total phenolics (mg/g gallic acid dry weight)	Total flavonoid (mg/g rutin dry weight)
400	Leaf	0.835 ± 0.017b	0.111 ± 0.018c
	Stem	0.531 ± 0.022d	0.071 ± 0.022d
	Root	0.311 ± 0.018e	0.052 ± 0.032d
800	Leaf	1.167 ± 0.023a	0.247 ± 0.017a
	Stem	0.678 ± 0.021c	0.143 ± 0.023b
	Root	0.343 ± 0.011c	0.067 ± 0.024d
1200	Leaf	1.259 ± 0.032a	0.276 ± 0.021a
	Stem	0.862 ± 0.027b	0.165 ± 0.032b
	Root	0.554 ± 0.041d	0.085 ± 0.031d

Means not sharing similar alphabets (in column) are significantly different at $p<0.05$

Ibrahim and Jaafar, 2011

Total phenolics and flavonoids contents in different parts of *Labisia pumila* under different CO2 concentration.

CO_2 levels (µmol/mol)	Plant parts	Total phenolics (mg/g gallic acid dry weight)	Total flavonoid (mg/g rutin dry weight)
400	Leaf	0.835 ± 0.017b	0.111 ± 0.018c
	Stem	0.531 ± 0.022d	0.071 ± 0.022d
	Root	0.311 ± 0.018e	0.052 ± 0.032d
800	Leaf	1.167 ± 0.023a	0.247 ± 0.017a
	Stem	0.678 ± 0.021c	0.143 ± 0.023b
	Root	0.343 ± 0.011c	0.067 ± 0.024d
1200	Leaf	1.259 ± 0.032a	0.276 ± 0.021a
	Stem	0.862 ± 0.027b	0.165 ± 0.032b
	Root	0.554 ± 0.041d	0.085 ± 0.031d

Means not sharing similar alphabets (in column) are significantly different at $p<0.05$

Ibrahim and Jaafar, 2011

CO_2 on compositions of phenolics in different varieties & parts of *Labisia pumila*

CO_2 levels ($\mu mol \cdot mol^{-1}$)	Varieties	Gallic acid *	Pyragallol	Caffeic acid
400	*Alata*	448.12 ± 2.44^{d}	810.03 ± 2.44	47.83 ± 3.22^{f}
	Pumila	215.48 ± 4.32^{g}	ND	43.92 ± 2.11^{f}
	Lanceolata	406.03 ± 3.22^{f}	ND	115.21 ± 1.14^{e}
800	*Alata*	837.434 ± 0.87^{b}	ND	215.51 ± 2.54^{c}
	Pumila	282.17 ± 0.43^{g}	ND	177.35 ± 2.56^{d}
	Lanceolata	474.33 ± 3.67^{c}	ND	ND
1200	*Alata*	948.28 ± 6.77^{a}	ND	543.88 ± 3.44^{a}
	Pumila	435.69 ± 9.87^{e}	ND	237.86 ± 5.66^{b}
	Lanceolata	935.91 ± 4.34^{a}	ND	ND

ND = not detected. All analyses are the mean of nine measurements ± standard error of mean. Results expressed in $\mu g \cdot g^{-1}$ of dry plant material. Means not sharing a common letter were significantly different at $p \leq 0.05$.*

CO_2 on composition of flavonoids in different plant parts of *Labisia pumila*

CO_2 levels ($\mu mol \cdot mol^{-1}$)	Varieties	Flavonoid content ($\mu g \cdot g^{-1}$ dry weight)				
		Kaempferol	Quercetin *	Myricetin	Rutin	Naringenin
400	*Alata*	186.71 ± 0.34 ^{b}	57.61 ± 1.22 ^{g}	87.81 ± 0.34 ^{c}	ND	139.20 ± 2.56 ^{c}
	Pumila	221.91 ± 0.21 ^{a}	105.66 ± 2.11 ^{f}	30.41 ± 2.33 ^{e}	24.51 ± 0.45 ^{c}	80.44 ± 0.98 ^{d}
	Lanceolata	162.71 ± 0.31 ^{c}	56.90 ± 2.34 ^{g}	27.45 ± 3.11 ^{f}	ND	87.11 ± 1.78 ^{e}
800	*Alata*	ND	160.88 ± 3.44 ^{c}	273.84 ± 7.44 ^{b}	ND	ND
	Pumila	ND	117.42 ± 4.11 ^{e}	ND	41.8 ± 3.22 ^{b}	619.59 ± 9.78 ^{b}
	Lanceolata	ND	103.13 ± 2.78 ^{f}	49.73 ± 0.54 ^{d}	ND	ND
1200	*Alata*	ND	183.32 ± 5.43 ^{b}	287.77 ± 0.21 ^{a}	ND	ND
	Pumila	ND	127.52 ± 0.45 ^{d}	ND	87.45 ± 2.54 ^{a}	947.85 ± 9.76 ^{a}
	Lanceolata	ND	205.91 ± 0.21 ^{a}	85.76 ± 1.45 ^{c}	ND	ND

ND = not detected. All analyses are the mean of nine measurements ± standard error of mean. Results expressed in $\mu g \cdot g^{-1}$ of dry plant material. Means not sharing a common letter were significantly different at * $p \leq 0.05$.

Effect of CO2 enrichment on total phenolics and flavonoids contents of two ginger varieties.

Varieties	Plant parts	TF (mg /g dry weight)		TP (mg /g dry weight)	
		400	800	400	800
Halia Bentong	Leaves	5.44 ± 0.45^{de}	6.04 ± 0.79^{d}	31.22 ± 2.41^{d}	39.68 ± 5.61^{c}
	Stems	1.61 ± 0.22^{g}	1.96 ± 0.17^{g}	6.14 ± 0.8^{f}	7.6 ± 0.66^{ef}
	Rhizomes	4.03 ± 0.081^{f}	7.35 ± 1.99^{c}	11.33 ± 0.27^{e}	28.76 ± 7.74^{d}
Halia Bara	Leaves	8.66 ± 0.42^{bc}	9.23 ± 0.36^{ab}	43.22 ± 2.15^{b}	60.69 ± 2.6^{a}
	Stems	1.74 ± 0.37^{g}	2.04 ± 0.31^{g}	7.1 ± 1.04^{ef}	7.89 ± 1.17^{ef}
	Rhizomes	4.48 ± 0.08^{ef}	9.78 ± 0.77^{a}	13.5 ± 0.26^{e}	38.16 ± 1.55^{c}

Means not sharing similar alphabets (in column & row) are significantly different at $p<0.05$

Ghasemzadeh and Jaafar, 2010

Flavonoids compounds profiling in two varieties of *Zingiber officinale* (Halia Bentong (A); Halia Bara (B)) grown under different CO_2 concentrations (400 and 800 µmol/mol).

Flavonoid compounds	Halia Bentong				Halia Bara			
	400		800		400		800	
	Leaves	Rhizomes	Leaves	Rhizomes	Leaves	Rhizomes	Leaves	Rhizomes
Quercetin	0.972 ± 0.013^{c}	0.895± 0.03^{c}	1.22± 0.07^{b}	1.138± 0.023^{b}	1.19±0.122^{ab}	0.986±0. 032^{c}	**1.33± 0.134^{a}**	1.27 ± 0.01^{a}
Rutin	0.171± 0.002^{de}	**0.452±0.004^{a}**	0.141±0.03^{e}	0.388± 0.026^{b}	0.174±0.007^{d}	0.334±0.009^{c}	0.151±0.025^{de}	0.404± 0.016^{b}
Epicatechin	**0.122 ± .018^{a}**	0.083± .007^{bc}	0.073±0.08^{c}	0.048± 0.018^{d}	0.12±0.004^{a}	0.103± 0.0035^{ab}	0.096±0.022^{bc}	0.037± 0.009^{d}
Catechin	0.409 ± 0.027^{d}	0.491±0.019^{cd}	0.673±0.04^{ab}	0.637± 0.034^{b}	0.668±0.079^{ab}	0.533±0.034^{c}	**0.733± .014^{a}**	0.682± 0.05^{ab}
Kaempferol	0.042 ± 0.002^{e}	0.053± .003de	0.118±0.01^{c}	0.148± 0.023^{b}	0.051±0.002^{de}	0.068±0.005^{d}	0.163±0.011^{ab}	**0.181± 0.009^{a}**
Naringenin	0.089 ± 0.0052^{c}	0.047± 0.003^{d}	0.127±0.02^{b}	0.083± 0.004^{c}	0.061±0.004^{d}	0.028±0.003^{e}	**0.155±0.027^{a}**	0.121± 0.011^{b}
Fisetin	0.986 ± 0.012^{e}	0.633± 0.033^{f}	2.05± 0.27^{c}	**2.82 ± 0.19^{a}**	1.53± 0.121^{d}	1.32±0.12^{d}	2.38± 0.395^{b}	**3.11± 0.185^{a}**
Morin	0.514 ± 0.027^{e}	0.463± 0.014^{e}	0.49± 0.052^{e}	**0.875± 0.036^{a}**	0.765±0.024^{b}	0.606±0.006^{d}	0.661±0.029^{c}	0.515± 0.025^{e}

Means not sharing similar alphabets (in column & row) are significantly different at $p<0.05$

Ghasemzadeh and Jaafar, 2010

Phenolics compounds profiling in two varieties of *Zingiber officinale* (Halia Bentong (A); Halia Bara (B)) grown under different CO_2 concentrations (400 and 800 µmol/mol).

Phenolic compounds	Halia Bentong				Halia Bara			
	400		800		400		800	
	Leaves	Rhizomes	Leaves	Rhizomes	Leaves	Rhizomes	Leaves	Rhizomes
Gallic acid	0.173±0.009^{d}	0.141±0.031^{d}	0.576±0.049^{b}	0.489±0.043^{c}	0.191±0.008^{d}	0.152±0.0081^{d}	**0.645±0.066^{a}**	0.537±0.034^{bc}
Vanillic acid	ND	ND	0.229± 0.058^{b}	**0.335±0.028^{a}**	0.082±0.016^{c}	ND	0.24±0.052^{b}	**0.357±0.038^{a}**
Ferulic acid	0.081±0.022^{f}	0.116±0.016^{ef}	0.117±0.026^{de}	0.21±0.022^{b}	0.071±0.017^{f}	0.148±0.017^{cd}	0.162±0.014^{c}	**0.285±0.038^{a}**
Tannic acid	0.388±0.072^{a}	n.d	ND	ND	0.224±0.041^{b}	ND	ND	ND
Cinnamic acid	ND	ND	**0.134±0.027^{a}**	0.0336±0.25^{b}	ND	ND	**0.125±0.027^{a}**	0.0457±0.01^{b}
Salicylic acid	ND	ND	0.22±0.021^{b}	0.037±0.012^{c}	ND	ND	**0.269±0.027^{a}**	0.0417±0.04^{c}

ND: Not Detected. Means not sharing similar alphabets (in column & row) are significantly different at $p<0.05$

Ghasemzadeh and Jaafar, 2010

Anticancer activities (cell viability) of ginger extracts towards MCF–7 and MDA–MB–231 cell lines as determined by the MTT assay

CO_2 (µmol/mol)	Varieties	Plant parts	MCF–7	MDA–MB–231	Normal cell
400	H.Bentong	Leaves	59.55±2.55^{a}	63.36±1.85^{b}	96.75±1.18^{a}
		Rhizomes	57.66±1.68^{a}	69.41±2.3^{a}	94.28±1.04^{ab}
	H.Bara	Leaves	50.65±0.56^{b}	58.22±1.09^{c}	95.15±0.46^{ab}
		Rhizomes	57.14±1.74^{a}	66.60±2.31^{ab}	92.38±1.86^{bcd}
800	H.Bentong	Leaves	44.83±1.53^{c}	48.16±1.03^{d}	93.25±1.94^{bc}
		Rhizomes	49.07±1.04^{b}	44.35±1.86^{e}	90.15±2.02^{de}
	H.Bara	Leaves	40.47±1.46^{d}	43.12±1.99^{e}	91.07±0.67^{cde}
		Rhizomes	38.98±2.2^{d}	39.61±2.43^{f}	88.47±1.24^{e}
Positive control	Tamoxifen		24.6±1.7	26.29±2.1	---

Means not sharing similar alphabets (in column) are significantly different at $p<0.05$

Ghasemzadeh and Jaafar, 2010

• Composition changes ~ mostly **source-sink hypotheses** (CNBH) and **growth-differentiation balance hypothesis** assume that elevated CO_2 concentration supports a comparative increase in **carbon accessibility** that is accumulated in **total non-structurable carbohydrate** (TNC) and **carbon based secondary metabolites** (CBSM) when the provided carbon amounts exceed growth requirements (Panuelas et al., 1998).

• High atmospheric CO_2 concentrations often increase **total non-structurable carbohydrate** concentrations in plants and possibly **stimulate** the **secondary metabolism** and antioxidant activity in plants (Hogy et al., 2009)

Salicylic Acid (SA) non secondary metabolites production, HPLC profiling and antioxidant activity

Halia Bentong

Halia Bara

Effect of foliar SA on total soluble carbohydrate (TSC), total flavonoids (TF) and total phenolics (TP) in ginger varieties

phytochemicals	Halia Bentong			Halia Bara		
	Control	SA 10^{-5}	SA 10^{-3}	Control	SA 10^{-5}	SA 10^{-3}
TSC	5.95 ± 0.46 ^{b}	7.59 ± 0.69 ^{ab}	7.41 ± 0.69 ^{ab}	6.3 ± 0.97 ^{ab}	7.98±0.97 ^{a}	7.72 ± 1.32 ^{a}
TF	9.3 ± 0.88 ^{ab}	7.98±0.76 ^{b}	8.21 ± 0.92 ^{b}	10.87 ± 1.04 ^{a}	8.97 ± 0.78^{b}	9.35 ± 0.28 ^{ab}
TP	39.6 ± 2.91 ^{c}	49.5 ± 0.72 ^{ab}	46.9 ± 3.01 ^{ab}	44.06 ± 3.85 ^{bc}	53.23±5.4 ^{a}	50.1 ± 2.78 ^{ab}

All analyses are the mean of triplicate measurements ± standard deviation; All of results expressed in mg/g dry weight; Means not sharing a common single letter were significantly different at $P < 0.05$.

HPLC analysis of ginger (*Zingiber officinale*) varieties treated with salicylic acid (SA).

Compounds	Halia Bentong			Halia Bara		
	Control	SA 10^{-5}	SA 10^{-3}	Control	SA 10^{-5}	SA 10^{-3}
Rutin	$0.893 \pm 0.03^{b,c}$	0.736 ± 0.09^{c}	0.79 ± 0.06^{c}	1.13 ± 0.12^{a}	$0.883 \pm 0.07^{b,c}$	$0.993 \pm 0.07^{a,b}$
Apigenin	0.384 ± 0.049^{c}	0.276 ± 0.08^{d}	0.305 ± 0.09^{d}	0.553 ± 0.06^{a}	0.45 ± 0.06^{b}	0.55 ± 0.04^{a}
Myricetin	0.04 ± 0.009^{b}	$0.088 \pm 0.009^{a,b}$	0.059 ± 0.018^{b}	0.06 ± 0.001^{b}	0.112 ± 0.004^{a}	$0.074 \pm 0.006^{a,b}$
Naringenin	0.227 ± 0.049^{a}	0.29 ± 0.1^{a}	0.259 ± 0.045^{a}	0.259 ± 0.033^{a}	0.303 ± 0.097^{a}	0.304 ± 0.02^{a}
Fisetin	ND	0.237 ± 0.017^{c}	0.228 ± 0.03^{c}	ND	0.359 ± 0.046^{a}	0.304 ± 0.01^{b}
Morien	$0.117 \pm 0.02^{a,b}$	$0.173 \pm 0.055^{a,b}$	$0.158 \pm 0.042^{a,b}$	0.102 ± 0.042^{b}	0.193 ± 0.03^{a}	0.182 ± 0.017^{a}
Anthocyanin	ND	0.381 ± 0.05^{b}	0.369 ± 0.053^{b}	ND	0.442 ± 0.041^{a}	0.426 ± 0.122^{a}

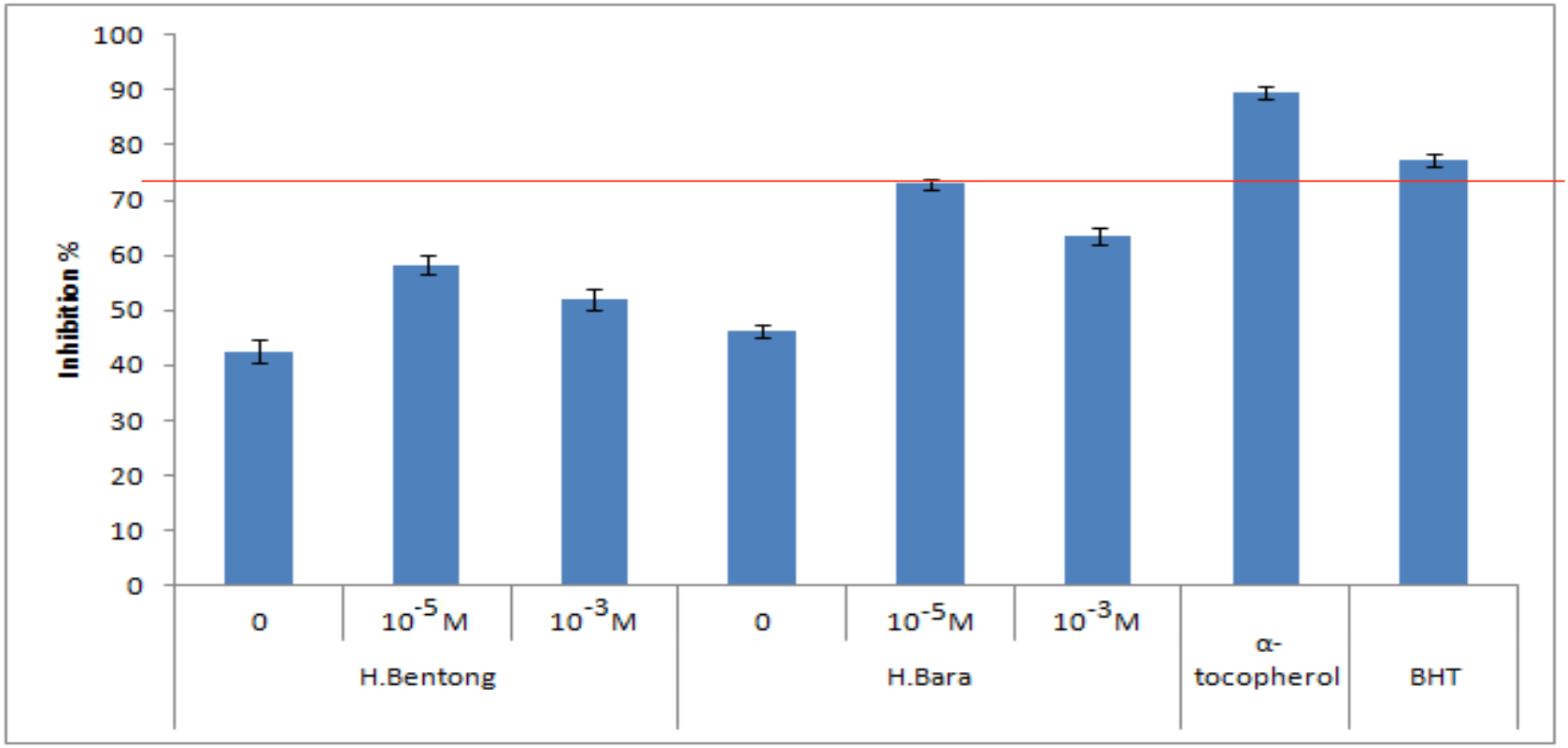
ND: Not detected. All analyses are the mean of triplicate measurements ± standard deviation; All of results expressed in mg/g dry weight; Means not sharing a common single letter in a row were significantly different at $P < 0.05$.

Foliar SA on ferric reducing antioxidant potential of Malaysian young ginger varieties

Variety	SA (M)	FRAP (µmol Fe (II)/g)
H. Bentong	control	522.4 ± 14.7 ^{d}
	10^{-5}	739.2 ± 30.1 ^{b}
	10^{-3}	621.5 ± 24.5 ^{c}
H. Bara	control	540.3 ± 12.44 ^{d}
	10^{-5}	**862.6 ± 29.4 ^{a}**
	10^{-3}	772.1 ± 28.5 ^{b}
Positive controls	BHT	607.8 ± 18.4^{c}
	α-tocopherol	966.0 ± 22.1^{a}

Means not sharing similar alphabets (in column) are significantly different at $p<0.05$

Foliar application of SA on free radical scavenging activity of Malaysian young ginger varieties



Anticancer activity

Foliar application of SA on anticancer activity of Malaysian young ginger on breast cancer cell lines

Variety	SA (M)	MCF-7 (cell viability)	MDA-MB-231 (cell viability)	Inhibition % (MCF-7)	Inhibition % (MDA-MB-231)
H. Bentong	0	50.40 ± 2.25 ^{a}	55.25 ± 2.46 ^{a}	44.1	41.5
	10^{-5}	**40.22** ± 2.16 ^{c}	**42.17** ± 2.50 ^{c}	60.3	56.2
	10^{-3}	45.60 ± 1.72 ^{b}	46.30 ± 2.62 ^{b}	55.7	52.7
H. Bara	0	49.20 ± 2.49 ^{a}	55.6 ± 2.34 ^{a}	50.2	45.1
	10^{-5}	**35.21** ± 1.47 ^{d}	**37.19** ± 1.66 ^{d}	**64.8**	**61.6**
	10^{-3}	40.55 ± 2.14 ^{c}	43.30 ± 2.11 ^{c}	60.5	55.3
Positive control	Tamoxifen	22.56 ± 1.07	26.18 ± 1.27	77.4	73.8

Low cell viability shows high activity of plant extract. Means not sharing similar alphabets (in column) are significantly different at $p<0.05$

C50 values

Foliar application of SA on half maximal inhibitory concentration (IC_{50}) of Malaysian young ginger varieties

Variety	SA (M)	MCF-7 µg/mL	MDA-MB-231 µg/mL
H. Bentong	0	50.6 ± 1.45 [a]	54.7 ± 1.74 [a]
	10^{-5}	43.5 ± 1.33 [b]	48.8 ± 1.3b [c]
	10^{-3}	44.4 ± 1.72 [b]	50.6 ± 1.28 [b]
H. Bara	0	39.1 ± 1.18 [c]	45.2 ± 1.14 [d]
	10^{-5}	**30.5 ± 1.66 [d]**	**38.6 ± 1.06 [f]**
	10^{-3}	35.6 ± 1.2 [e]	42.5 ± 1.66 [e]
Tamoxifen		17.4 ± 2.16	19.5 ± 1.88

Low cell viability shows high activity of plant extract. Means not sharing similar alphabets (in column) are significantly different at p<0.05

- Foliar application of SA ~ promote the production of SM ☛ improved antioxidant and anticancer properties
- Treatment of H. Bentong and H. Bara with SA improved production of fisetin and anthocyanin ☛ potent antioxidant activity.
- MTT assay indicated that enriched Halia Bara leaf with 10^{-5} M of SA ☛ a potential source of anticancer therapeutic compounds
- SA could be used to enhance phytochemical production and the pharmaceutical quality of ginger.

Abscisic acid on secondary metabolites production and antioxidant enzymes

Abscisic acid on total phenolics, flavonoids and soluble sugars produced in different parts of *Orthosiphon stamineus*

ABA (µM)	Parts	Total Phenolics (mg g^{-1} gallic acid dry weight)	Total Flavonoids (mg g^{-1} rutin dry weight)	Soluble sugar (mg g^{-1} sucrose dry weight)
0	Leaves	3.11 ± 0.27 ^{c}	1.47 ± 0.21 ^{c}	79.12 ± 11.21 ^{d}
	Stems	1.32 ± 0.02 ^{l}	0.52 ± 0.02 ^{g}	40.23 ± 8.98 ^{l}
	Roots	2.71 ± 1.24 ^{e}	1.21 ± 0.34 ^{k}	62.18 ± 12.12 ^{h}
2	Leaves	3.98 ± 0.34 ^{b}	1.72 ± 0.56 ^{b}	88.21 ± 9.76 ^{c}
	Stems	1.50 ± 0.04 ^{h}	0.76 ± 0.34 ^{f}	47.21 ± 11.21 ^{k}
	Roots	2.87 ± 0.45 ^{d}	1.18 ± 0.12 ^{j}	68.21 ± 12.12 ^{g}
4	Leaves	4.10 ± 0.21 ^{ab}	1.98 ± 0.32 ^{ab}	90.17 ± 10.76 ^{bc}
	Stems	1.57 ± 0.05 ^{g}	0.86 ± 0.12 ^{e}	50.11 ± 5.67 ^{j}
	Roots	2.92 ± 0.03 ^{d}	1.27 ± 0.32 ^{i}	70.82 ± 5.88 ^{f}
6	Leaves	4.21 ± 0.02 ^{a}	2.12 ± 0.04 ^{a}	98.12 ± 7.98 ^{a}
	Stems	1.92 ± 0.21 ^{f}	0.97 ± 0.08 ^{d}	57.12 ± 12.12 ^{l}
	Roots	2.97 ± 0.11 ^{de}	1.46 ± 0.12 ^{h}	76.21 ± 10.12 ^{e}

Ibrahim and Jaafar, 2013

Abscisic acid on antioxidant enzyme activity in different parts of *Orthosiphon stamineus*

BA (µM)	Parts	Ascorbate peroxidase activity (APX; mg protein^{-1} min^{-1})	Superoxide dismutase activity (SOD mg protein^{-1} min^{-1})	Catalase activity (CAT; µmol mg protein^{-1} min^{-1})
0	Leaves	15.23 ± 2.34 ^{d}	4.62 ± 0.11 ^{d}	19.21 ± 1.27 ^{d}
	Stems	6.12 ± 0.81 ^{k}	1.34 ± 0.01 ^{l}	6.66 ± 2.11 ^{l}
	Roots	10.11 ± 0.03 ^{h}	2.98 ± 0.41 ^{h}	12.17 ± 0.97 ^{h}
2	Leaves	17.11 ± 0.51 ^{c}	4.82 ± 0.21 ^{c}	20.12 ± 0.82 ^{c}
	Stems	6.11 ± 0.53 ^{k}	1.52 ± 0.36 ^{k}	8.27 ± 0.78 ^{k}
	Roots	11.27 ± 0.14 ^{g}	3.62 ± 0.15 ^{g}	13.24 ± 0.11 ^{g}
4	Leaves	19.71 ± 0.16 ^{b}	5.01 ± 0.17 ^{b}	23.17 ± 0.78 ^{b}
	Stems	7.23 ± 0.42 ^{j}	1.71 ± 2.11 ^{j}	9.23 ± 1.19 ^{j}
	Roots	13.22 ± 0.31 ^{f}	3.89 ± 1.02 ^{f}	16.59 ± 0.89 ^{f}
6	Leaves	21.62 ± 0.26 ^{a}	5.27 ± 0.81 ^{a}	25.12 ± 1.21 ^{a}
	Stems	9.12 ± 0.98 ^{i}	1.76 ± 0.92 ^{i}	10.24 ± 2.17 ^{i}
	Roots	14.21 ± 1.32 ^{e}	4.02 ± 1.24 ^{e}	17.21 ± 0.98 ^{e}

Ibrahim and Jaafar, 2013

Light intensity on secondary metabolites production, HPLC profiling, antioxidant activities

Total flavonoid & total phenolics content in different ginger parts grown under different light intensities

Light intensities (µmol/m²/s)	Plant part	TF (mg/g dry weight)		TP (mg /g dry weight)	
		Halia Bentong	Halia Bara	Halia Bentong	Halia Bara
310	Leaves	**5.95±0.2^{c}**	**8.45±0.38^{a}**	27.43±2.34^{e}	31.73±2.10^{cd}
	Stems	1.83±0.22^{hi}	1.96±0.28^{h}	6.38±1.25^{h}	7.11±1.58^{gh}
	Rhizomes	3.91±0.083^{efg}	4.34±0.08^{e}	8.9±0.23^{fgh}	9.48±0.21^{fgh}
460	Leaves	5.04±0.27^{d}	5.7±0.09^{cd}	28.96±1.55^{de}	34.16±4.8^{bc}
	Stems	1.27±0.2^{i}	1.47±0.21^{hi}	7.33±1.13^{fgh}	8.432±1.19^{fgh}
	Rhizomes	3.47±0.14^{fg}	4.03±0.061^{efg}	9.69±0.38^{fgh}	11.22±0.16^{fg}
630	Leaves	4.14±0.18^{ef}	6.12±0.015^{c}	31.1±0.98^{cde}	37.33±4.45^{ab}
	Stems	1.3±0.24^{hi}	1.55±0.33^{hi}	7.47±1.37^{fgh}	8.83±1.82^{fgh}
	Rhizomes	3.37±0.079^{g}	3.97±0.28^{efg}	9.81±0.21^{fgh}	11.05±0.77^{fg}
790	Leaves	5.71±0.54^{cd}	7.05±1.67^{b}	**33±1.13^{cd}**	**39.06±9.23^{a}**
	Stems	1.26±0.12^{hi}	1.5±0.14^{hi}	7.8±0.68^{fgh}	8.56±0.81^{fgh}
	Rhizomes	3.66±0.125^{fg}	4.14±0.13^{ef}	10.22±0.33^{fgh}	11.53±0.36^{f}

Ghasemzadeh et al., 2011

HPLC analysis of flavonoid and phenolic compounds extracted from different parts of ginger varieties grown under different light intensities.

	Halia Bentong				Halia Bara			
	790 (µmol/m²/s)		310(µmol/m²/s)		790(µmol/m²/s)		310(µmol/m²/s)	
Compounds	**Leaves**	**Rhizomes**	**Leaves**	**Rhizomes**	**Leaves**	**Rhizomes**	**Leaves**	**Rhizomes**
Quercetin	0.871±0.031^{cd}	0.803±0.028^{d}	0.98±0.015^{b}	0.902±0.042^{bc}	0.978±0.024^{b}	0.865±0.027^{cd}	**1.123±0.11^{a}**	0.986±0.032^{b}
Rutin	0.35±0.0015^{c}	0.311±0.002^{e}	0.36±0.003^{b}	0.451±0.0045^{a}	0.205±0.003^{b}	0.324±0.002^{d}	0.17±0.0075^{b}	0.331±0.009^{d}
Epicatechin	0.092±0.068^{a}	0.078±0.012^{a}	0.118±0.014^{a}	0.083±0.007^{a}	0.111±0.017^{a}	0.091±0.009^{a}	0.117±0.004^{a}	0.103±0.003^{a}
Catechin	0.328±0.04^{e}	0.362±0.021^{e}	0.413±0.02^{d}	0.491±0.019^{bc}	0.455±0.037^{cd}	0.459±0.026^{cd}	0.671±0.079^{a}	0.533±0.034^{b}
Kaempferol	0.044±0.012^{cd}	0.045±0.005^{cd}	0.04±0.003^{d}	0.05±0.004^{bcd}	0.048±0.004^{cd}	0.061±0.004^{ab}	0.053±0.003^{bc}	0.068±0.006^{a}
Naringenin	0.049±0.003^{c}	0.046±0.001^{c}	0.09±0.006^{a}	0.047±0.003^{c}	0.039±0.004^{d}	0.02±0.002^{f}	0.061±0.004^{b}	0.028±0.003^{e}

Means not sharing similar alphabets (in row) are significantly different at $p<0.05$

Ghasemzadeh et al., 2011

DPPH scavenging activities of the methanol extracts (45 µg/ml) from different plant parts of two varieties of *Zingiber officinale*

Light intensities (µmol/m²/s)	Extraction source	Halia Bentong	Halia Bara
310	Leaves	**59.02±0.87^{b}**	**65.26±0.9^{a}**
	Stems	30.31±1.84^{hi}	29.59±0.59^{i}
	Rhizomes	41.36±0.63^{f}	47.26±0.92^{e}
790	Leaves	51.12±1.65^{d}	56.36±0.97^{c}
	Stems	32.85±0.57^{g}	31.45±1.49^{gh}
	Rhizomes	51.41±0.51^{d}	58.22±1.19^{b}

Ghasemzadeh et al., 2011

FRAP activity in different parts of two varieties of *Zingiber officinale* grown under different light intensities.

Light intensities (µmol/m²/s)	Extraction source	Halia Bentong	Halia Bara
310	Leaves	552.24 ± 32.4^{f}	587.31 ± 25.6^{e}
	Stems	378.4 ± 48.2^{h}	372.33 ± 32.33^{hi}
	Rhizomes	692.71 ± 16.48^{c}	788.57 ± 22.6^{a}
790	Leaves	541.55 ± 34.1^{g}	574.9 ± 58.14^{e}
	Stems	381.11 ± 48.7^{h}	363.1 ± 21.43^{i}
	Rhizomes	677.2 ± 18.38^{d}	770.4 ± 43.11^{b}

Ghasemzadeh et al., 2011

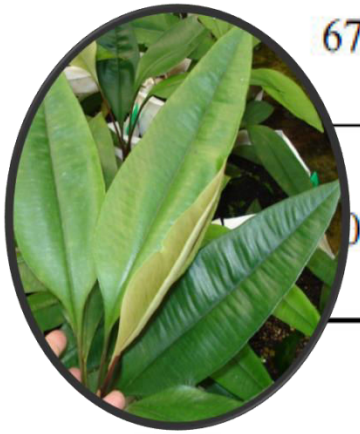
Accumulation and partitioning of total flavonoids (TF) and total phenolics (TP) in different plant parts of *Orthosiphon stimaneus* under different irradiance

Irradiance ($\mu mol/m^2/s$)	Plant Parts	Total flavonoids, TF (mg rutin/g dry weight)	Total phenolics, TP (mg gallic acid/g dry weight)
225	Leaf	2.111 ± 0.013 [a]	5.211 ± 0.028 [a]
	Stem	1.991 ± 0.022 [a]	4.811 ± 0.029 [a]
	Root	1.671 ± 0.013 [b]	4.671 ± 0.039 [b]
500	Leaf	1.567 ± 0.022 [b]	4.211 ± 0.032 [b]
	Stem	1.321 ± 0.030 [b]	3.981 ± 0.037 [b]
	Root	1.231 ± 0.022 [c]	3.761 ± 0.051 [c]
675	Leaf	1.234 ± 0.013 [c]	3.111± 0.021 [c]
	Stem	1.001 ± 0.010 [c]	2.981 ± 0.025 [c]
	Root	0.987 ± 0.015 [d]	2.761 ± 0.040 [c]
900	Leaf	0.913 ± 0.025 [d]	2.345± 0.008 [d]
	Stem	0.813 ± 0.023 [d]	1.981 ± 0.011 [d]
	Root	0.723 ± 0.026 [d]	1.721 ± 0.028 [d]

(Ibrahim and Jaafar , 2012)

Accumulation and partitioning of total flavonoids and total phenolics in different plant parts of *Labisia pumila* under different irradiance levels

Irradiance ($\mu mol/m^2/s$)	Plant Parts	Total Flavonoids (mg quercetin/g Dry Weight)	Total Phenolics (mg gallic acid/g Dry Weight)	Anthocyanin (mg/g Fresh Weight)
225	Leaf	2.211 ± 0.013^{a}	5.511 ± 0.028^{a}	0.74 ± 0.01^{a}
	Stem	1.991 ± 0.022^{a}	4.811 ± 0.029^{a}	0.67 ± 0.02^{a}
	Root	1.571 ± 0.013^{b}	4.571 ± 0.039^{b}	0.63 ± 0.03^{a}
500	Leaf	1.547 ± 0.022^{b}	4.311 ± 0.032^{b}	0.57 ± 0.12^{b}
	Stem	1.301 ± 0.030^{b}	3.971 ± 0.037^{b}	0.50 ± 0.23^{b}
	Root	1.241 ± 0.022^{b}	3.781 ± 0.051^{c}	0.48 ± 0.12^{b}
675	Leaf	1.214 ± 0.013^{c}	3.171 ± 0.021^{c}	0.39 ± 0.03^{c}
	Stem	1.021 ± 0.010^{c}	2.991 ± 0.025^{c}	0.35 ± 0.03^{c}
	Root	0.957 ± 0.015^{c}	2.771 ± 0.040^{c}	0.30 ± 0.02^{c}
0	Leaf	0.903 ± 0.025^{c}	2.395 ± 0.008^{d}	0.19 ± 0.04^{d}
	Stem	0.803 ± 0.023^{d}	1.991 ± 0.011^{d}	0.15 ± 0.04^{d}
	Root	0.713 ± 0.026^{d}	1.711 ± 0.028^{e}	0.10 ± 0.02^{d}



(Ibrahim and Jaafar , 2012)

DPPH scavenging activities in different parts of three varieties of *Labisia pumila* under different irradiance levels

Irradiance (µmol/m^2/s)	Extract Source	Inhibition % [a]
225	Leaf	62.42 ± 1.65 [c]
	Stem	58.14 ± 1.09 [c]
	Root	52.21 ± 1.08 [c]
500	Leaf	51.83 ± 1.05 [d]
	Stem	49.11 ± 0.98 [d]
	Root	47.73 ± 0.43 [d]
675	Leaf	45.43 ± 0.23 [e]
	Stem	44.74 ± 0.98 [e]
	Root	40.31 ± 1.21 [e]
900	Leaf	39.21 ± 2.22 [f]
	Stem	37.16 ± 1.21 [f]
	Root	32.65 ± 3.21 [f]
Controls	BHT	67.81 ± 1.34 [b]
	α-tocopherol	78.41 ± 1.24 [a]

(Ibrahim and Jaafar , 2012)

The effect of irradiance levels on Phenylalanine ammonia-lyase enzyme (PAL) activity in *Labisia pumila*

Irradiance ($\mu mol/m^2/s$)	PAL Activity (nM transcinnamic mg^{-1} protein $hour^{-1}$)
225	33.71 ± 3.22^{a}
500	29.82 ± 1.67^{b}
675	21.71 ± 2.21^{c}
900	12.32 ± 2.31^{d}

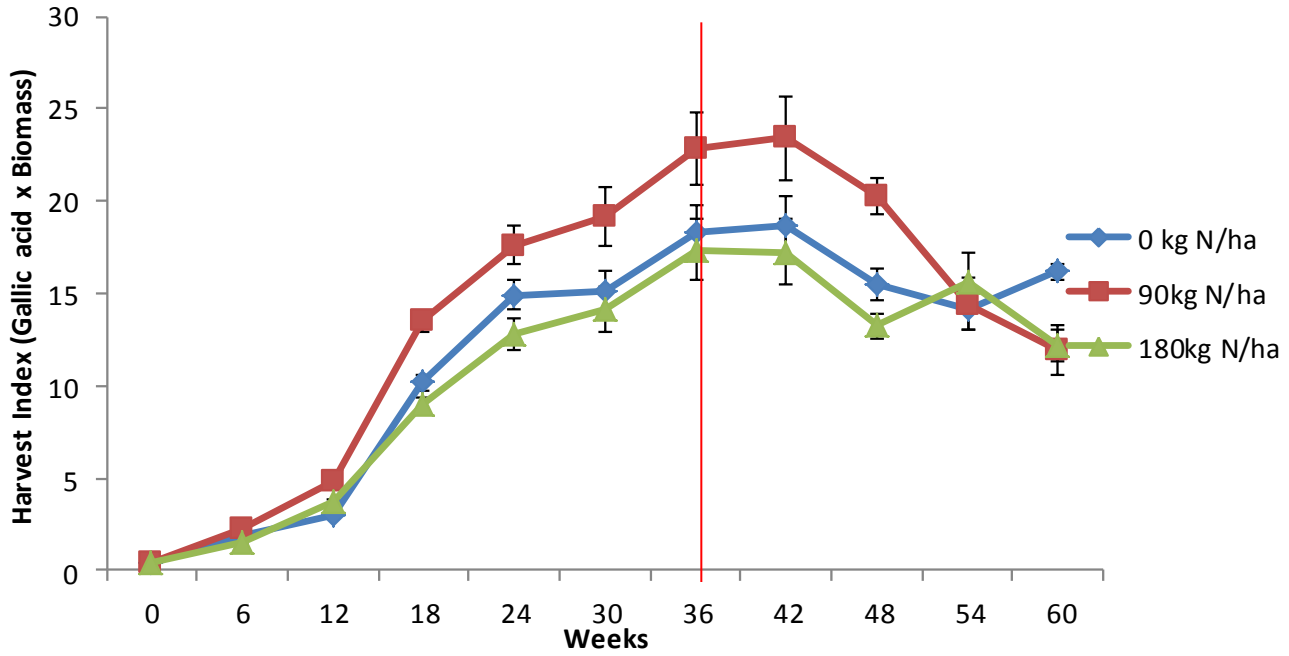
• Increase in carbon based secondary metabolites production (total phenolics and flavonoids) under low irradiance ☛ increased availability of phenyl alanine ☛ precursor for carbon based secondary metabolites (Tsormpatsidis et al., 2008) .

• Increase in Phenylalanine ammonia-lyase enzyme (PAL) activity might stimulate the production of total flavonoids and phenolics, especially under low light conditions for certain plants (Baas, 989)

• Wu et al. (2011): increase carbon-based secondary metabolite production in *Photinia fraseri* under low irradiance ☛ increase activity of PAL ☛ justifies increased production of these compounds under low light conditions

Harvest Index of Secondary Metabolite

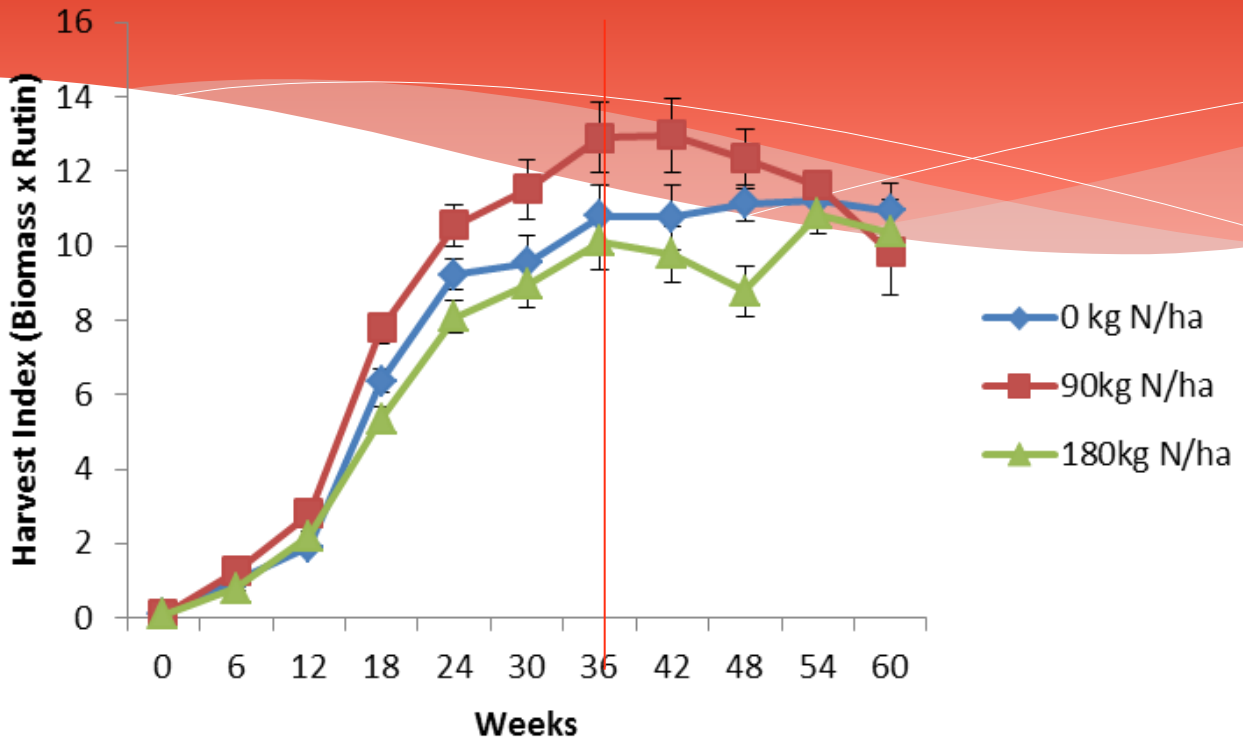
Impact of nitrogen on Harvest index of SM (GAE ~ Total Phenolics) of *L. pumila* Benth.



Treatments	R2	Equation
O kg N/ha	0.87	Y=-0.34x2+5.84x-7.5
90 kg N/ha	0.76	Y=-0.60x2+8.84x-11.7
180 kg N/ha	0.89	Y=-0.34x2+5.63x-7.3

Total flavonoid has high correlation coefficient with HI (r2 = 0.813; p≤0.05) compared to total biomass (r2 = 0.615; p≤0.05) this indicate that flavonoid content was important factors in determination of harvest index in *L. pumila* (Jaafar, 2014)

Impact of nitrogen on Harvest index of SM (GAE ~ Total Flavonoids) of *L. pumila* Benth.



Treatments	R2	Equation
O kg N/ha	0.77	Y=-0.27x2+3.84x-6.5
90 kg N/ha	0.86	Y=-0.16x2+3.31x-4.7
180 kg N/ha	0.91	Y=-0.14x2+2.13x-3.7

(Jaafar, 2014)

CONCLUSION

- Elicitation of abiotic factors may be an effective management tool ⇨ enhance the expression of secondary metabolites in herbal / medicinal plants
- ⇨ Secondary Metabolite Harvest Index ⇨ **economic feasibility**
- New tool for establishment of a new, precised factory-line, year-round production system in multi-tiers both for
  - (1) the manufacture of high production of quality, and
  - (2) targeted secondary metabolites for specific functional food, pharmaceutical and cosmaticeutical herbal-based industries in the near future.

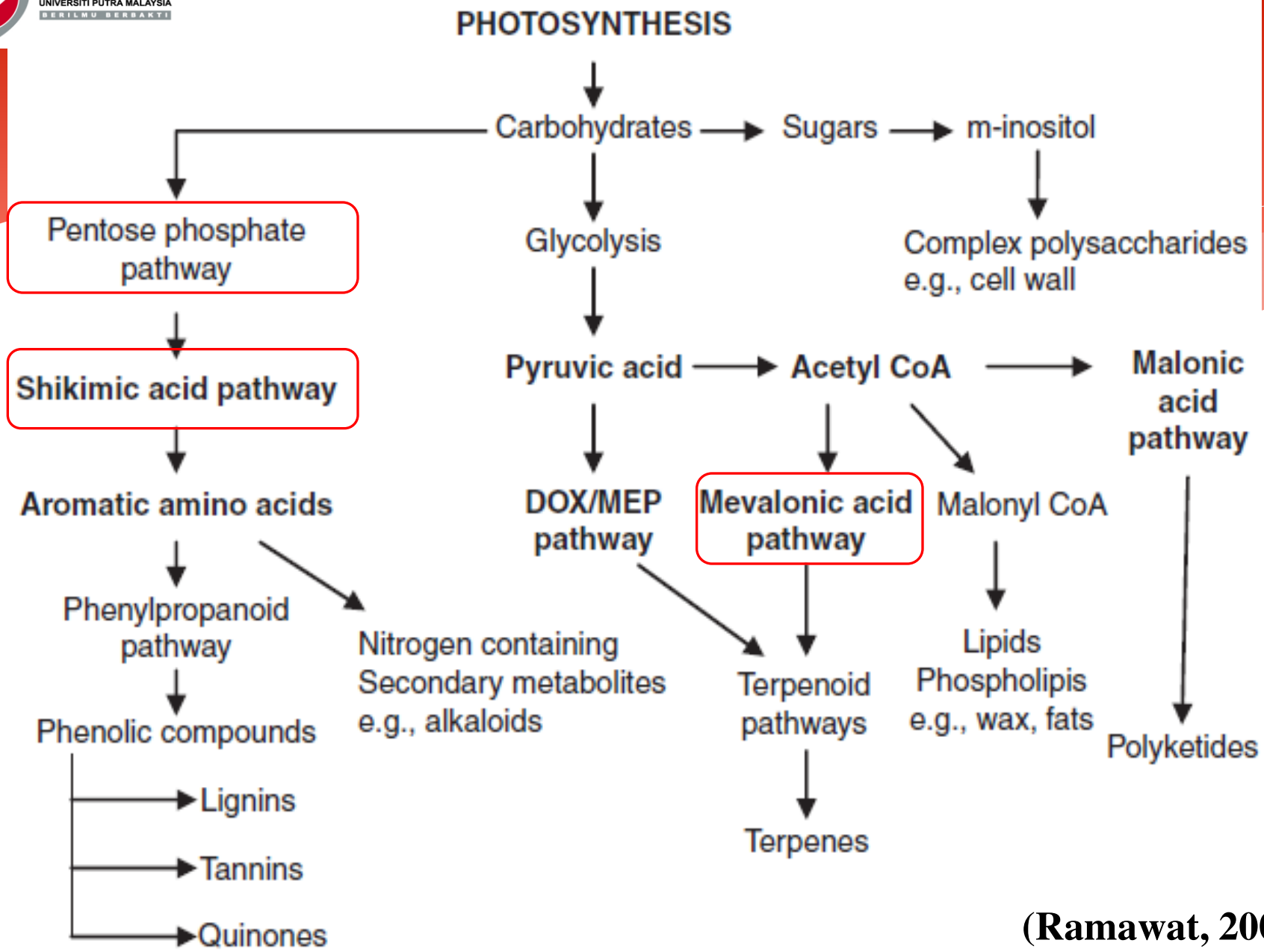
THANK YOU

Contact:

HAWA ZE JAAFAR, PhD
Director
University Community Transformation Centre Centre
UCTC UPM
Universiti Putra Malaysia
43400 UPM Serdang
Selangor, MALAYSIA

Tel: +60-12 372 3585 / +603 8947 4821 / 1801
Email: hawazej@gmail.com; hawazej@upm.edu.my


UPM
UNIVERSITI PUTRA MALAYSIA
BERILMU BERBAKTI
Maca M'x
Take Care of Your Health



(Ramawat, 2009)