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ORIGINAL ARTICLES

EXTRACTION OF 4H-PYRAN-4-ONE, 2,3- DIHYDRO -6-METHYL-, AN ALTERNATIVE ANTIFUNGAL AGENT, FROM *SCHIZOPHYLLUM COMMUNE*: OPTIMIZATION AND KINETIC STUDY

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ABSTRACT. *4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP) was believed as a promising alternative in term antifungal activity towards fungal attack in rubberwood. Solid-liquid extraction is performed from basidiomycetes fungus Schizophyllum commune in methanol-water solvent, in order to obtain valuable antifungal agent. Statistical optimization was employed to optimize the extraction condition for maximal total flavonoid content (TFC) and DDMP productivity. The optimum conditions were 70.75% (v/v) methanol, 29 °C, and 145 rpm. The optimization studies were verified and the experimental data fitted well to the selected models with error percentage less than 1%. The extraction kinetics was then investigated using Parabolic diffusion model, Power law model, Peleg's model, and Elovich's model. All empirical models gave a good fit to the experimental data ($R^2 > 0.9$), in which the Power law model having the highest R^2 and lowest RMSD values.*

Keywords. *Schizophyllum commune*; total flavonoid content (TFC); 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP); optimization; extraction kinetics

INTRODUCTION

The interest in the investigation of bioactive compounds, especially total flavonoid content, from natural resources had greatly increased in recent year. According to Das *et al.* (2010), these secondary metabolites were generally synthesized by plants in response to microorganism infection and thus found *in-vitro* to be effective as antimicrobial substance.

Filamentous fungi have a large number of coding for secondary metabolites (Frisvad *et al.*, 2008). At present, the exploitation, conservation, and utilization of fungi belonging to Basidomycetes had gained much attention, which proved beneficial to human and environment. Slana *et al.* (2011) investigated that there might be a variety of flavonoid present in the fungal hosts of saprophytic fungus *Rhizopus nigiricans*, which could exhibit the fungitoxic effect. Moreover, Teoh *et al.* (2012) demonstrated the presence of 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP), a flavonoid fraction compound, in *Schizophyllum commune* during cultured on defined medium. It was important to note that

this flavonoid fraction compound exhibited effective *in-vitro* antifungal activity to the attack of wood-degrading fungi of rubberwood (Teoh & Mashitah, 2013).

Extraction was a very important stage in isolation, identification, and use of total flavonoid content. Several extraction methods had been widely used in pharmaceutical process, such as homogenization in solvent, serial exhaustive extraction, soxhlet extraction, sonication, and also supercritical fluid extraction (Ncube *et al.*, 2008 & Das *et al.*, 2010). Besides, water, methanol, and ethanol were the preliminary used solvent for the investigation of antimicrobial activity of flavonoid. It had been found that flavonoids and most other bioactive compounds were generally soluble in polar solvents such as methanol (Tiwari *et al.*, 2011).

However, literature data about optimization, modeling and simulation of solid-liquid extraction process were scarce. Therefore, there is a need for mathematical modeling, as a useful engineering tool, which considerably facilitates optimization, simulation, design and control of process and contributes to utilization of energy, time and solvent. On the other hand, numerous researches had been conducted to describe the kinetics and mechanism of solid-liquid extraction process for plant tissues (Sturzoiu *et al.*, 2011). However, there is lack of information on the extraction kinetic for filamentous fungus.

In this work, an investigation was carried out to understand the optimum condition for flavonoid and DDMP extraction by *S. commune* using Response Surface Methodology (RSM) coupled with Box-Behnken design (BBD). Then, the applicability of few empirical equations was examined for the extraction process under optimized condition.

MATERIAL AND METHODS

Fungal strain

The wild species fungal strain, *S. commune* was obtained from the Biocomposite and Protection of Timber Forest Products Laboratory, Forest Research Institute Malaysia (FRIM), Kepong, Malaysia. The stock culture was grown on malt extract agar (MEA) at 30^{o}C and maintained on agar slant before subsequent studies.

Mycelia suspension preparation

Mycelia suspension was prepared by suspending mycelia discs from 7-day-old culture plates in sampling bottles containing sterilized distiller water, and 0.1%(v/v) Tween 80. The disc of 5 mm diameter was cut on the mycelia mats of the agar plate using a sterilized cork borer. A total of 10 discs for every 100 ml sterilized water were vortexed for 5 min in order to homogenize the mycelia suspensions.

Mycelia extract preparation

In order to produce maximum biomass by *S. commune*, the production medium used containing 18.7 g/l yeast extract, 10.0 g/l malt extract, 38.6 g/l glucose, 1.0 g/l KH_2PO_4, 1.0 g/l K_2HPO_4, 0.59 g/l $MgSO4{\cdot}7H_2O$, and 2.0 g/l $(NH_4)_2SO_4$. Ten milliliters (10%v/v) of the mycelia suspension with 0.5 McFarland standard turbidity was added into 90 ml of medium in 250 ml Erlenmeyer flasks. The medium was sterilized at 121°C for 15 min before transferring the mycelia suspension into the culture media. The culture was incubated at 30±2°C, pH 6.5 in an incubator shaker at 200 rpm for 5 d. The culture broth was then harvested and centrifuged at 4,000 *g* for 15 min. The residue was then homogenized for 2 min prior to the extraction process. Meanwhile, the supernatant was evaporated using a rotary evaporator and the residues were maintained in vacuum until the extraction process was carried out.

Mycelia extract preparation

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Experimental design using design of experiment (DoE)

Response surface methodology (RSM)

The RSM used in this study was three-factor and three-level Box-Behnken design (BBD) experimental plan in order to evaluate the effect of the extraction parameters on the extraction efficiency (Table 1). The results were analyzed using Analysis of Variance (ANOVA) by Design Expert 6.0.6 software. The simultaneous interactions of the three factors can be studied based on the three-dimensional plots. The optimum region was also identified based on the main parameters in the overlay plot. The experiment was then repeated for five times and each result obtained was compared with the predicted values in order to determine the validity of the model.

Table 1: Variables and their levels used for Box-Behnken design for optimization of extraction parameters

Independent Variable	Symbol		Level		
	Actual	Coded	-1	0	+1
Methanol concentration (% v/v)	X1	A	60	70	80
Extraction temperature (°C)	X2	B	25	35	45
Mixing rate (rpm)	X3	C	100	150	200

Verification of the model

The optimum conditions for extraction optimization studies were obtained using RSM coupled with BBD. The experiment was repeated five times and result was compared with the predicted values in order to determine the validity of the model.

Selected empirical model on the extraction kinetics

In this work, four two-parametric kinetic models were applied for modeling of recovered solutes and were validated based on the following assumptions:

i. The fungal biomass was isotropic and of equal size.
ii. Distribution of antifungal agent within the fungal biomass was uniform and varied only with time.
iii. Net diffusion occurred only towards the external surface of fungal biomass.
iv. Diffusion coefficient of antifungal agent was a constant.

Parabolic diffusion model

Orthogonal polynomial was a useful empirical equation in solid-liquid extraction. The parabolic diffusion equation was often used to indicate the diffusion-controlled process, and had successfully described the pesticide reactions (Spark, 1999). The equation was generally in the form as shown in Eqn. (1).

$$y = \sum_{i=0}^{n} A_i t^{1/2} \quad (1)$$

where A_i is the parameter to be determined, t is the extraction time in minutes, and y represent the antifungal agent extracted.

This empirical equation was corresponded into two-steps extraction mechanisms, which were washing and diffusion steps (Kitanovic *et al.*, 2008). Hence, the above equation could be simplified into Eqn. (2) where A_0 is the washing coefficient, and A_1 is the diffusion rate constant.

$$y = A_0 + A_1 t^{1/2} \quad (2)$$

Power law model

The power law model, which was similar to Freundlich type, was applied widely in the diffusion process of an active agent through non-swelling devices (Kitanovic *et al.*, 2008 & Sturzoiu *et al.*, 2011). It could be applied as in Eqn. (3).

$$y = Bt^n \quad (3)$$

Where y is the antifungal agent extracted, B refers to the constant incorporating the characteristics of the carrier-active agent system, t is the time in minutes, and n is the diffusion exponent, an indicative of transport mechanism. In literature, n was less than 1 ($N < 1$) for extraction from plant or vegetal materials (Sturzoiu *et al.*, 2011). This value was considered for the fungal biomass in this study. The constants for this model were estimated using a regression analysis. In the linearized form, the equation was transformed into Eqn. (4).

$$\ln(y) = n\ln(t) + \ln(B) \quad (4)$$

Peleg's model

In this work, the extraction curve (concentration of total flavonoid content versus time) had similar shape as the sorption curve (moisture content versus time). Hence, it was possible to describe this study using the hyperbolic model proposed by Peleg (Bucic-Kojic *et al.*, 2007 & Sturzoiu *et al.*, 2011). In the case of extraction, the model was adapted and used in the form of Eqn. (5).

$$y = y_o + \frac{t}{K_1 + K_2 t} \quad (5)$$

Where y_0 is the initial yield of product extracted at $t = 0$, t is the extraction time in minutes, K_1 is the Peleg's rate constant, and K_2 is the Peleg's capacity constant. Assuming that the desired product was not found in any extraction process when $t = 0$, the Eqn. (5) was then be simplified and used in the form of Eqn. (6).

$$y = \frac{t}{K_1 + K_2 t} \quad (6)$$

Elovich's model

The Elovich's model has been studied by Paterson *et al.* (1999) in order to fit the leaching curves such as the extraction of polycyclic aromatic hydrocarbons from coal tar-contaminated soil. The relationship assumed that the rate of adsorption and leaching rate decreased exponentially with increasing extraction yield. It was expressed as in Eqn. (7).

$$y = E_0 + E_1 ln(t) \tag{7}$$

Model validation

The profiles from simulation of the experimental data and models were then evaluated using the linear correlation coefficient (R^2) and the root mean square deviation (RMSD) computed as Eqn. (8) (Bucic-Kojic *et al.*, 2007).

$$RMSD = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(y_{exp} - y_{calc}\right)^2} \tag{8}$$

Another criterion used to evaluate the best fitting equation was the mean relative percentage deviation (P) value. P value was defined as shown in Eqn. (9).

$$P\ value = \frac{100}{N} x \sum \frac{|Y_{exp} - Y_{calc}|}{Y_{exp}} \tag{9}$$

Where Y and Y_p are experimental and predicted yield of antifungal agent, respectively, and N is the number of experimental data. A model was considered acceptable if the P values are below 10% (Kaymak-Ertekin & Gedik, 2004).

Analytical method

Calculation of extraction yield

The extraction yield was calculated by the percentage ratio between the dry extract residues to the mycelia extract masses, according to Eqn. (10).

$$Extraction\ yield\ (\%) = \frac{g(dried\ biomass\ extract + filter\ paper) - g(filter\ paper)}{g(biomass)} X\ 100\% \tag{10}$$

Determination of total flavonoid content (TFC)

Total flavonoid content (TFC) was measured spectrophotometrically by the aluminium chloride colorimetric assay, as presented by Ordonez *et al.* (2006) with slight modification. 100 mg sample (extract biomass) was diluted with 85% ethanol, and 0.5 ml

of the diluted sample was then pipetted into 0.5 ml of 2 %(v/v) $AlCl_3$ ethanol solution (2 g $AlCl_3$ in 100 ml ethanol). Ethanol was used as blank in this study. The absorbance was then measured at 420 nm after 30 min at room temperature. A yellow color indicated the presence of flavonoids. The flavonoid content was expressed as micrograms of quercetin per milligram of sample (µg QE/mg sample). A standard TFC calibration was performed within a range of quercetin concentration from 1 - 20 µg/ml.

Analysis using UV-visible spectrophotometer for determination of DDMP

In this analysis, the DDMP was determined using the method of Cechovska *et al.* (2011). Since the commercially DDMP was not available in the market, the concentration of this compound was determined using a spectrophotometer with a UV-Vis (Model: Evolution 201). Norfuraneol, a pentose-derived analogue of DDMP, which had similar electrochemical properties-half-wave potential at range 0.30 – 0.33 V, was used as a calibration standard for the quantitation of DDMP. A standard DDMP calibration was performed within a range of DDMP concentration from 0 – 10 µg/ml.

RESULTS AND DISCUSSION

Optimization of extraction process using Response Surface Methodology (RSM)

In this work, the box-behnken design (BBD) was used to develop a correlation between the three variables in order to improve the extraction yield and the production of TFC and DDMP from *S. commune* biomass. The complete design matrixes of the variables in coded units together with the values of the corresponding response were obtained from the experimental work (Table 2).

Table 2: Experimental design for three-level, three variables box-behnken design, and the extraction yield, TFC, and DDMP represented as responses

Run	Independent variables			Responses		
	A, Methanol conc.	B, Temp.	C, Mixing rate	Extraction yield (%)	TFC (µg QE/ mg sample)	DDMP (µg/mg sample)
1	1 (80)	0 (35)	1 (200)	2.9101±0.05	1.076±0.03	0.789±0.03
2	-1 (60)	1 (45)	0 (150)	2.7297±0.05	1.092±0.01	0.828±0.03
3	0 (70)	1 (45)	-1 (100)	2.8992±0.06	1.051±0.03	0.922±0.03
4	0 (70)	0 (35)	0 (150)	3.0742±0.07	1.301±0.01	1.296±0.03
5	-1 (60)	0 (35)	-1 (100)	2.7393±0.03	1.235±0.03	0.792±0.04
6	-1 (60)	0 (35)	1 (200)	2.8506±0.03	1.227±0.03	0.781±0.04
7	-1 (60)	-1 (25)	0 (150)	2.7019±0.03	1.282±0.01	0.882±0.03
8	0 (70)	-1 (25)	1 (200)	2.9193±0.03	1.191±0.01	0.871±0.03
9	1 (80)	1 (45)	0 (150)	2.8426±0.05	1.018±0.05	0.852±0.04
10	0 (70)	0 (35)	0 (150)	3.0835±0.03	1.317±0.03	1.268±0.02

11	0 (70)	0 (35)	0 (150)	3.0729±0.03	1.321±0.03	1.266±0.03
12	1 (80)	0 (35)	-1 (100)	2.9498±0.05	1.238±0.01	0.878±0.04
13	1 (80)	-1 (25)	0 (150)	2.8611±0.05	1.214±0.03	0.801±0.04
14	0 (70)	1 (45)	1 (200)	2.9829±0.03	0.999±0.05	0.818±0.02
15	0 (70)	-1 (25)	-1 (100)	2.8795±0.03	1.301±0.03	0.872±0.02
16	0 (70)	0 (35)	0 (150)	3.0638±0.03	1.321±0.04	1.257±0.03
17	0 (70)	0 (35)	0 (150)	3.0645±0.03	1.315±0.04	1.286±0.03

*Values in parentheses are the actual factors for each independent variable in A: % (v/v), B: ^{o}C, and C: rpm, respectively. The extraction yield, total flavonoid content, and DDMP results are as mean±SD.

By employing the ANOVA with Design Expert 6.0.6 software, the predicted responses Y for extraction yield (Eqn. (11)), TFC (Eqn. (12)), and DDMP (Eqn. (13)) in terms of coded values were obtained. The statistical significance of Eqn. (11), (12), and (13) were confirmed by an F-test and the ANOVA for response surface quadratic model was summarized in Table 3, 4, and 5, respectively.

Table 3: Analysis of variances (ANOVA) for quadratic regression model of extraction yield of *Schizophyllum commune*

Source	Sum of squares	Degree of freedom	Mean square	F value	$(P) > F$
Model	0.25	9	0.027	494.42	<0.0001
A	0.065	1	0.065	1179.18	<0.0001
B	7.633E-05	1	7.633E-05	1.28	0.2781
C	1.026E-03	1	1.026E-03	18.58	0.0035
A^2	0.12	1	0.12	2093.99	<0.0001
B^2	0.061	1	0.061	1106.09	<0.0001
C^2	8.010E-03	1	8.010E-03	145.05	<0.0001
AB	3.822E-04	1	3.822E-04	6.92	0.0339
AC	5.700E-03	1	5.700E-03	103.23	<0.0001
BC	9.303E-06	1	9.303E-06	0.17	0.6938
Residual	3.865E-04	7	5.522E-05		
Lack of fit	1.254E-04	3	4.179E-05	0.64	0.6280
Pure error	2.611E-04	4	6.529E-05		
Cor total	0.25	16			
Std. dev.	0.007			Adj. R^2	0.996
R^2	0.997			Pred. R^2	0.991

$$Y_{\text{extraction yield}} = 2.97 - 0.36A - 0.005B - 0.02C - 0.17A^2 - 0.12B^2 - 0.044C^2 - 0.01AB - 0.038AC - 0.002BC \quad (11)$$

$$Y_{TFC} = 1.23 - 0.14A - 0.11B - 0.08C - 0.052A^2 - 0.11B^2 - 0.068C^2 - 0.002AB - 0.038AC + 0.015BC \quad (12)$$

Table 4: Analysis of variances (ANOVA) for quadratic regression model of TFC of *Schizophyllum commune*

Source	Sum of squares	Degree of freedom	Mean square	*F* value	(*P*) > *F*
Model	0.21	9	0.023	239.73	<0.0001
A	0.019	1	0.019	192.04	<0.0001
B	0.029	1	0.029	304.21	<0.0001
C	0.017	1	0.017	176.60	<0.0001
A^2	0.012	1	0.012	120.08	<0.0001
B^2	0.052	1	0.052	536.80	<0.0001
C^2	0.020	1	0.020	204.43	<0.0001
AB	9.000E-06	1	9.000E-06	0.093	0.7691
AC	5.929E-03	1	5.929E-03	61.35	0.0001
BC	8.410E-04	1	8.410E-04	8.70	0.0214
Residual	6.765E-04	7	9.664E-05		
Lack of fit	4.045E-04	3	1.348E-04	1.98	0.2588
Pure error	2.720E-04	4	6.800E-05		
Cor total	0.21	16			
Std. dev.	0.010			Adj. R^2	0.995
R^2	0.997			Pred. R^2	0.986

Table 5: Analysis of variances (ANOVA) for quadratic regression model of DDMP of *Schizophyllum commune*

Source	Sum of squares	Degree of freedom	Mean square	*F* value	(*P*) > *F*
Model	0.68	9	0.076	137.69	<0.0001
A	0.22	1	0.22	404.11	<0.0001
B	1.734E-03	1	1.734E-03	3.14	0.1198
C	5.430E-03	1	5.430E-03	9.83	0.0165
A^2	0.26	1	0.26	465.96	<0.0001

B^2	0.15	1	0.15	265.15	<0.0001
C^2	0.20	1	0.20	359.76	<0.0001
AB	2.756E-03	1	2.756E-03	4.99	0.0607
AC	1.521E-03	1	1.521E-03	2.75	0.1411
BC	2.652E-03	1	2.652E-03	4.80	0.0646
Residual	3.868E-03	7	5.526E-04		
Lack of fit	2.853E-03	3	9.511E-04	3.75	0.1172
Pure error	1.015E-03	4	2.538E-04		
Cor total	0.69	16			
Std. dev.	0.024		Adj. R^2	0.988	
R^2	0.994		Pred. R^2	0.979	

$$Y_{DDMP} = 1.03 - 0.49A + 0.026B - 0.045C - 0.25A^2 - 0.19B^2 - 0.22C^2 + 0.026AB - 0.019AC - 0.026BC \quad (13)$$

The *P* value was used as a tool to check the significance of the model and each coefficient; the smaller the value of *P*, the more significant was the corresponding coefficient. As shown in Table 3 until 5, the ANOVA of the quadratic regression model demonstrated that the model was significant with the *F*-test of a very low probability value ($P > F$) < 0.0001. For example, the model *F*-value of 494.42 for extraction yield implied that the model used was significant. In the study of TFC response, the value of R^2 (0.997) suggested that the sample variation of 99.7% were attributed to the independent variable, and only about 0.3% of the total variation could not be explained by the model. Furthermore, an adequate precision was used to measure the ratio of signal to noise, which is generally desired to be greater than 4. In this study, the value of this ratio for all responses (extraction yield = 64.432, TFC = 41.115, DDMP = 28.016) suggested that the polynomial quadratic model was of an adequate signal, and it could be used to navigate the design space.

Figures 1-3 are three-dimensional surface plots of extraction yield, TFC, DDMP of *S. commune* biomass by extraction based on the effect of methanol-water concentration, temperature and mixing rate, respectively. Figure 1 presents that the maximal extraction yield was attained when methanol concentration was between 70 and 75 %(v/v) and extraction temperature between 28 to 30 ^{o}C. Uma *et al.* (2010) reported that as the solvent concentration was greater than 60 %(v/v), it gave higher total phenolic yield. According to Guo *et al.* (2013), higher temperature could increase the extraction efficiency which it accelerated the active diffusion. In this present study, the maximal extraction yield was obtained within between 28 to 30 ^{o}C, in which beyond this level the extraction yield was decreased. This could be due according to Guo *et al.* (2013) the occurrence of oxidation and hydrolysis process at higher temperature, and hence reduced the extraction yield.

Figure 2(a) depicts a higher amount of flavonoid fraction yielded in the region of methanol concentration between 65 and 75% (v/v) and temperature between 25 and 30 ^{o}C. Both methanol concentration and temperature showed significant negative quadratic effects on TFC at $P < 0.0001$ (Table 4). Chan *et al.* (2009) stated that more polar phenolic compounds such as flavonoids fraction could be extracted in higher concentration of methanol and water mixture, following the "like dissolve like" principle. Figure 2(b) denotes the effect of methanol concentration and mixing rate on the TFC produced at fixed temperature of 30 ^{o}C. Methanol concentration demonstrated a pronounced negative influence on the TFC production in a linear and a quadratic manner with $P < 0.0001$ (Table 4). The relationship between the extraction temperatures and mixing rate with TFC production was shown in Figure 2(c). Both factors displayed a significant linear and quadratic effect ($P < 0.0001$) on TFC response (Table 4). Chan *et al.* (2009) reported that moderate heating and mixing rate might weaken the mycelia wall integrity, hydrolyzed the bonds of bound flavonoid compounds, and enhanced the flavonoid fraction solubility. Hence, more flavonoid would be distributed to the solvent.

Figure 3(a) shows that the maximal extraction of *S. commune*'s DDMP occurred within methanol concentration of 65 to 75 % (v/v) and temperature range between 28 to 32 ^{o}C. Result showed that a small increment in temperature caused the DDMP solubility increased, by which according to Guo *et al.* (2013), the solvent viscosity and surface tension decreased as increased the temperature and hence contributed to the sample wetting and matrix penetration. On the other hand, since DDMP had low polarity and was hydrophilic, the mixture of methanol and water had high affinity to extract the compound. Figure 3(b) shows that the optimal mixing rate ranged between 125 to 150 rpm. Beyond to that point, the production of DDMP decreased due to denaturation of internal structure of biomass and resulted in lower product formation. As can be seen in Figure 3(c), once the extraction temperature and mixing rate exceeded 32 ^{o}C and 150 rpm, the extraction of DDMP decreased slightly.

The Design-Expert plot also illustrated the interaction between methanol-water concentrations, extraction temperature and mixing rate corresponding to the extraction yield, TFC and DDMP. The optimum values of the tested variables in uncoded units were 70.75% (v/v) methanol concentration, extraction temperature of 29 ^{o}C, and mixing rate at 145 rpm. In this study, the maximum predicted extraction yield was 3.0741 %, while the maximum predicted production of TF and DDMP was 1.321 µg QE/mg sample, and 1.273 µg/mg sample, respectively. The desirability value for this situation was 0.997, in which supports the application of this model.

Model verification

In order to verify the model adequacy, five sets of experiments were repeated randomly at optimum condition to obtain a maximum extraction yield, and the production of TF and DDMP by *S. commune* experimentally. As shown in Table 6, the percentage error

differences between the experimental and predicted values were in the range of 0.010-0.393 %. Since the differences between actual and predicted responses were always less than 1%, thus providing its validity.

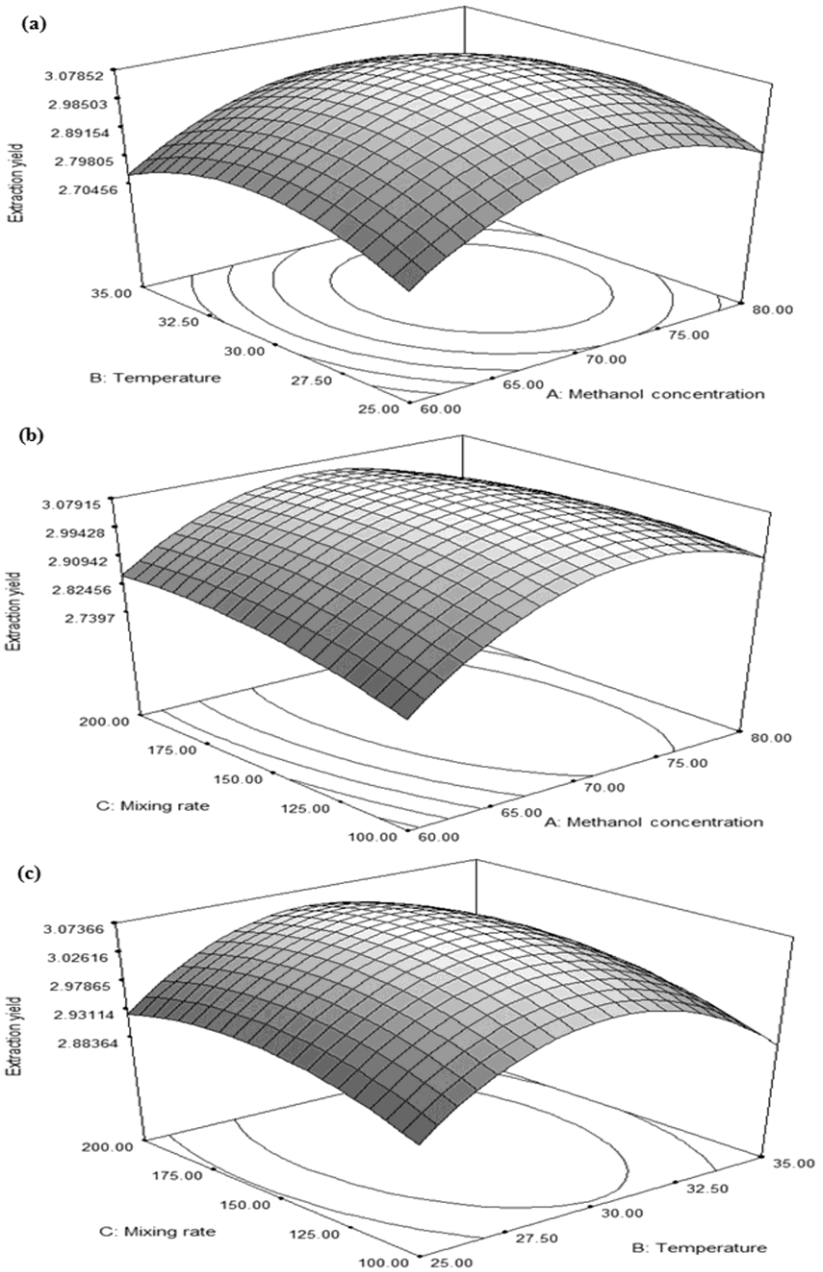

Figure 1: (a) A three-dimensional surface plot of extraction yield as a function of methanol concentration and extraction temperature. (b) A three-dimensional surface plot of extraction yield as a function of methanol concentration and mixing rate. (c) A three-dimensional surface plot of extraction yield as a function of extraction temperature and mixing rate

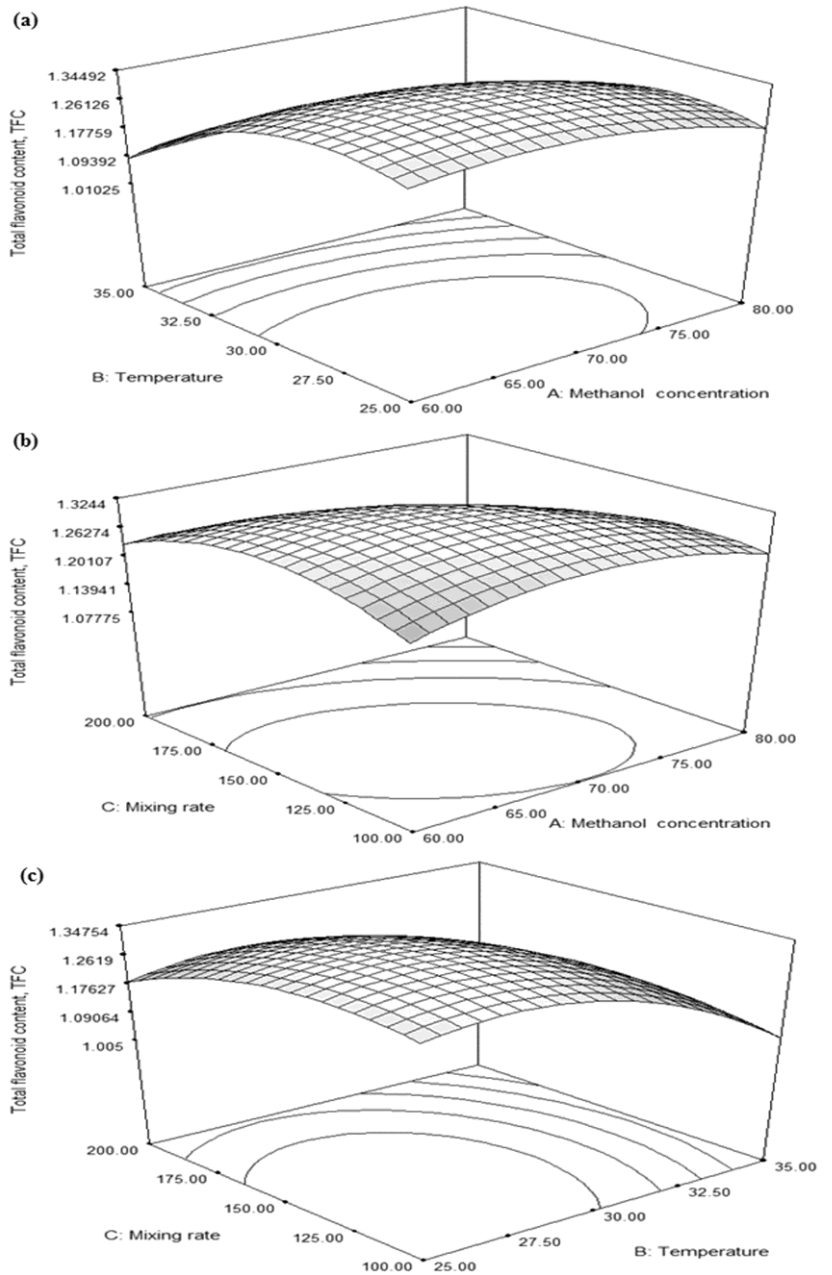

Figure 2: (a) A three-dimensional surface plot of TFC as a function of methanol concentration and extraction temperature. (b) A three-dimensional surface plot of TFC as a function of methanol concentration and mixing rate. (c) A three-dimensional surface plot of TFC as a function of extraction temperature and mixing rate

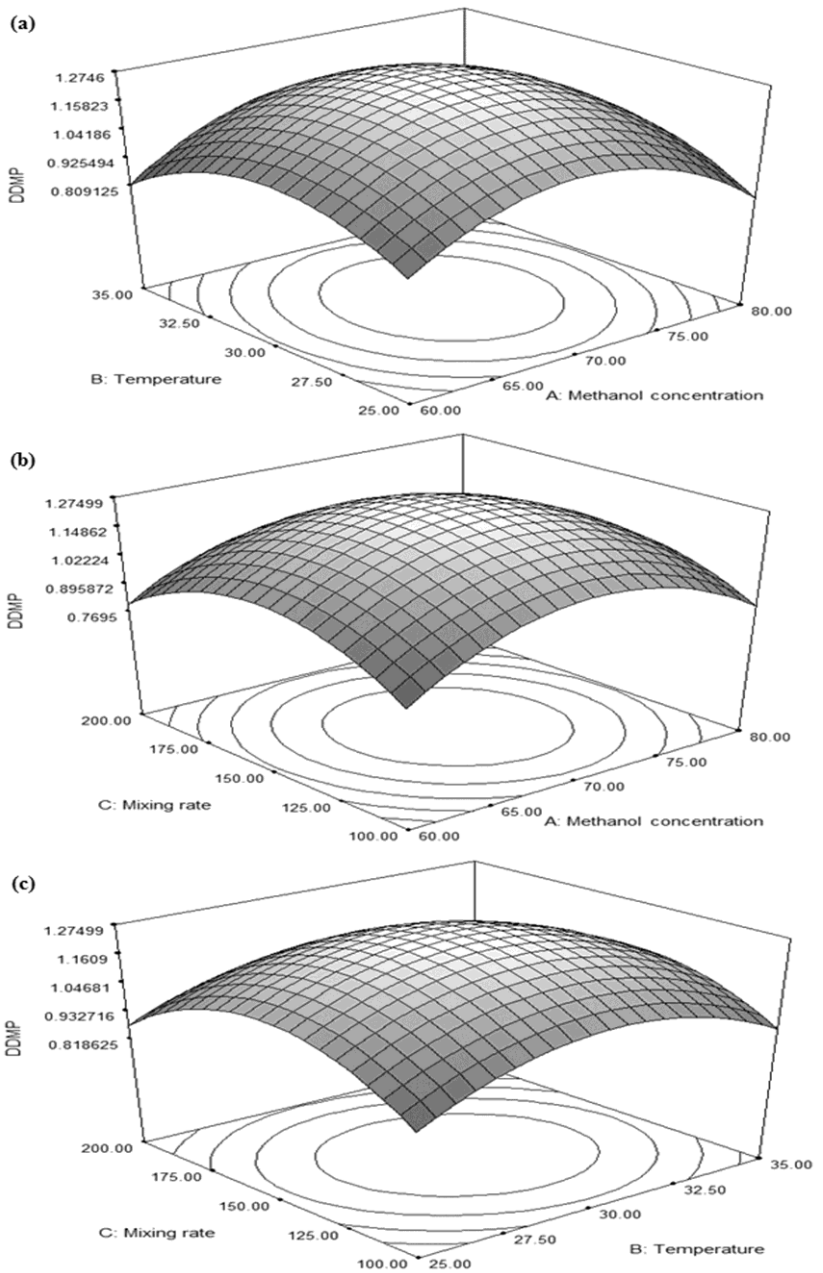

Figure 3: (a) A three-dimensional surface plot of DDMP as a function of methanol concentration and extraction temperature. (b) A three-dimensional surface plot of DDMP as a function of methanol concentration and mixing rate. (c) A three-dimensional surface plot of DDMP as a function of extraction temperature and mixing rate

Table 6: Validation of the data and model constructed

Run	Extraction yield (%)			TFC (μg QE/mg sample)			DDMP (μg/mg sample)		
	Exp.	Pred.	Error (%)	Exp.	Pred.	Error (%)	Exp.	Pred.	Error (%)
1	3.0712	3.0741	0.094	1.318	1.321	0.227	1.273	1.273	0.393
2	3.0745	3.0741	-0.013	1.323	1.321	-0.151	1.269	1.273	0.314
3	3.0738	3.0741	0.010	1.318	1.321	0.227	1.275	1.273	-0.157
4	3.0750	3.0741	-0.029	1.322	1.321	-0.076	1.278	1.273	-0.393
5	3.0734	3.0741	0.017	1.317	1.321	0.303	1.270	1.273	0.236

Selected empirical kinetic models for the extraction of antifungal agent from *Schizophyllum commune* biomass

The common extraction kinetic models consisted of two periods: a fast washing action in the very beginning, and a slow-diffusion-controlled extraction in the last period. Under certain operating conditions, the extraction of easily accessible extractive substance was so fast that it was difficult to observe the first period of washing (Kitanovic *et al.*, 2008). Figure 4(a) represents the profile of extraction yield versus time for the *S. commune* extract obtained under the optimized condition (70.75 %(v/v) methanol, extraction temperature of 29 ^{o}C, and mixing rate at 145 rpm) as described in above section. A typical trend was obtained in this study which was similar as those reported in the literature on the solid-liquid extraction profile. Similar trend was also observed for the TFC and DDMP profile, as shown in Figure 4(b) and 4(c), respectively. Due to that, four empirical kinetic models were selected from the literature, which were usually used to fit the experimental data of extraction curves, such as parabolic diffusion model, power law model, Peleg's model, and Elovich's model.

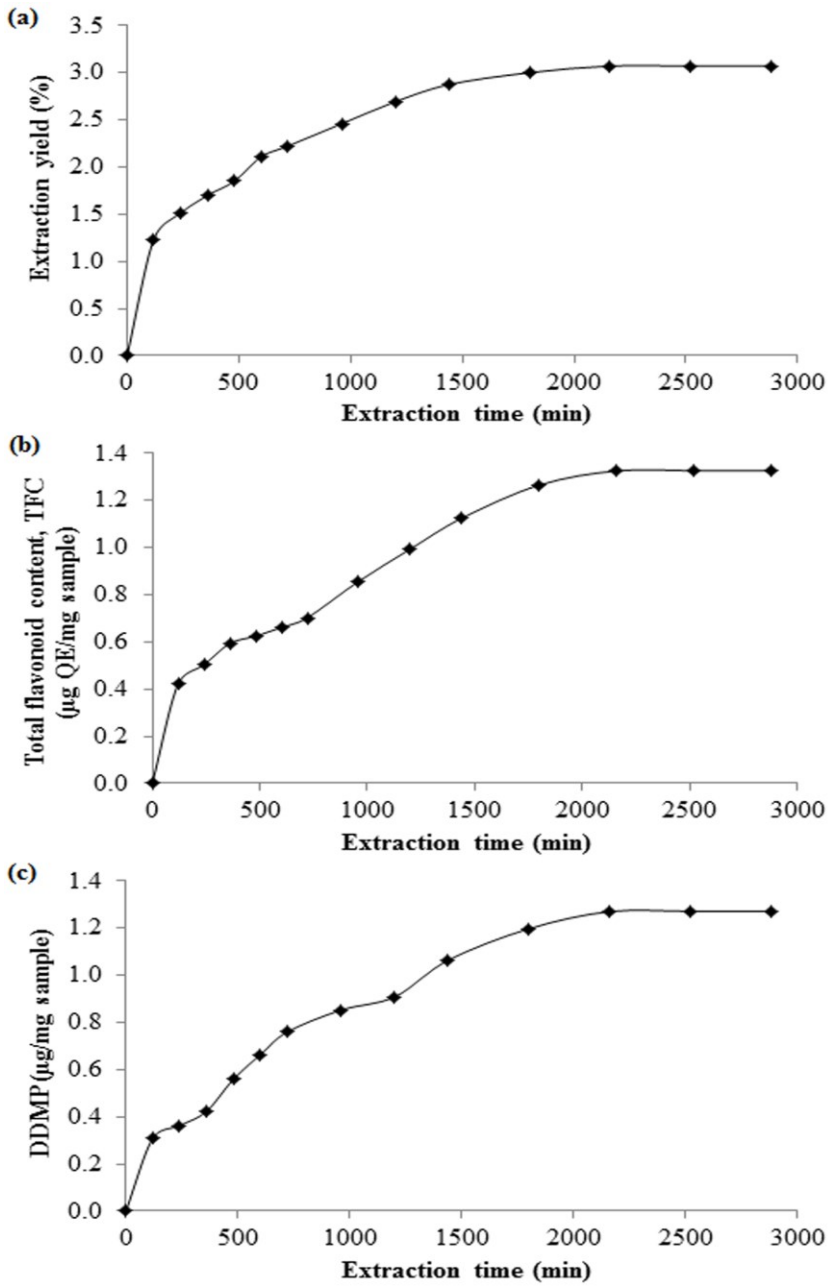

Figure 4: Extraction profile for (a) extraction yield, (b) TFC, and (c) DDMP from *Schizophyllum commune* extract under the optimized condition (70.75 %(v/v) methanol, temperature of 29 ^{o}C, mixing rate 145 rpm)

The experimental results of antifungal agent extraction were then analyzed using the linearized equations of the selected empirical kinetic models as summarized in Table 7. Model parameters were then calculated by linear regression using Microsoft Excel software.

Table 7: Selected empirical extraction kinetic models in linearized forms

Model	Model equation	Linearized form
Parabolic diffusion model	$y = A_o + A_1 t^{1/2}$	-
Power law model	$y = Bt^n$	$ln(y) = nln(t) + ln(B)$
Peleg's model	$y = t/(K_1 + K_2 t)$	$(t/y) = K_1 + K_2 t$
Elovich's model	$y = E_0 + E_1 ln(t)$	-

Table 8 summarizes the calculated parameters for the selected empirical models, and the corresponding statistical correlation values. For parabolic diffusion model, it was found that the washing coefficient (A_0) in term of TFC and DDMP production was lower compared to the extraction yield, in which indicated the lower solubility of the TFC and DDMP (called as extractive substance) in the 70.75 %(v/v) methanol concentration at time t=0. During the extraction process, the diffusion rate (A_1) of extraction yield was 0.0462 $1/min^{0.5}$, while the diffusion rate of TFC (0.0246 $1/min^{0.5}$) was almost a similar rate as in DDMP (0.0259 $1/min^{0.5}$). This indicated that TFC and DDMP had lower diffusion coefficient in the extraction process. Similar trend was also observed for the kinetic parameters obtained using power law and Elovich's model, in which the extraction yield always provide a higher diffusion rate during solid-liquid extraction of *S. commune* biomass compared to other two extractive substances (e.g., TFC and DDMP). On the other hand, the K_1 and K_2 value in Peleg's model was related to the extraction rate and the equilibrium concentration, respectively. In this study, the extraction of DDMP showed the highest K_1 and K_2 values with 524.28 min.g/mg and 0.5851 g/mg, respectively. As mentioned by Karacabey *et al.* (2013), as K_1 and K_2 values increased, the extraction of extractive substances were also accelerated, thus achieving higher equilibrium concentration.

Table 8: Summary of model parameters and statistical correlation values for each empirical extraction kinetic model

Model parameters	Statistical correlation values	Extraction data		
		Extraction yield	TFC	DDMP
Parabolic diffusion model				
A_0 (1/min$^{0.5}$)		0.9002	0.1176	0.0132
A_1 (1/min$^{0.5}$)		0.0462	0.0246	0.0259
	R^2	0.941	0.967	0.969
	RMSD	0.146	0.057	0.059
	P value (%)	5.839	5.404	6.619
Power law model				
n		0.312	0.411	0.510
B (1/min^{n})		0.2802	0.0528	0.0244
	R^2	0.980	0.968	0.975
	RMSD	0.080	0.058	0.046
	P value (%)	2.650	5.362	5.778
Peleg's model				
K_1 (g.min/mg)		99.98	428.55	524.28
K_2 (g/mg)		0.2865	0.5735	0.5851
	R^2	0.976	0.963	0.973
	RMSD	0.128	0.086	0.050
	P value (%)	5.048	7.200	6.651
Elovich's model				
E_0		2.1021	1.3775	1.6021
E_1		0.6651	0.3386	0.3627
	R^2	0.975	0.919	0.952
	RMSD	0.099	0.082	0.062
	P value (%)	4.094	7.937	7.349

Figure 5 illustrates the profiles of experimental and simulated data for the extraction of antifungal agent from *S. commune* biomass under the optimized condition of 70.75 %(v/v) methanol concentration, at temperature of 29 ºC, and mixing rate 145 rpm using the parabolic diffusion model, power law model, Peleg's model, and Elovich's model, respectively. The predicted results gave a relatively good agreement to the experimental data, with the linear correlation coefficient (R^2) values above 0.9. In fact, the R^2 values were higher for all the empirical models tested, ranging between $0.919 < R^2 < 0.980$, which showed that the selected empirical models were sufficient to describe both the

fast-washing action and slow-diffusion of the extraction process for the antifungal agent extraction process of this study. Generally, the R^2 value was frequently used to judge whether the model correctly represented the data, implying that if R^2 closer to 1, then the regression model was correct. However, there are many examples exist where the R^2 value is close enough to 1 but the model is still not appropriate. Thus, the root mean square deviation (RMSD) was used with the R^2 value for the comparison of various empirical models. As reported by Kitanovic *et al.* (2008), the higher the value of R^2 and the lower the value of RMSD, the better the goodness of fit. In this study, the RMSD results ranged within 0.046 – 0.146, which implied a good agreement between the experimental and simulated data.

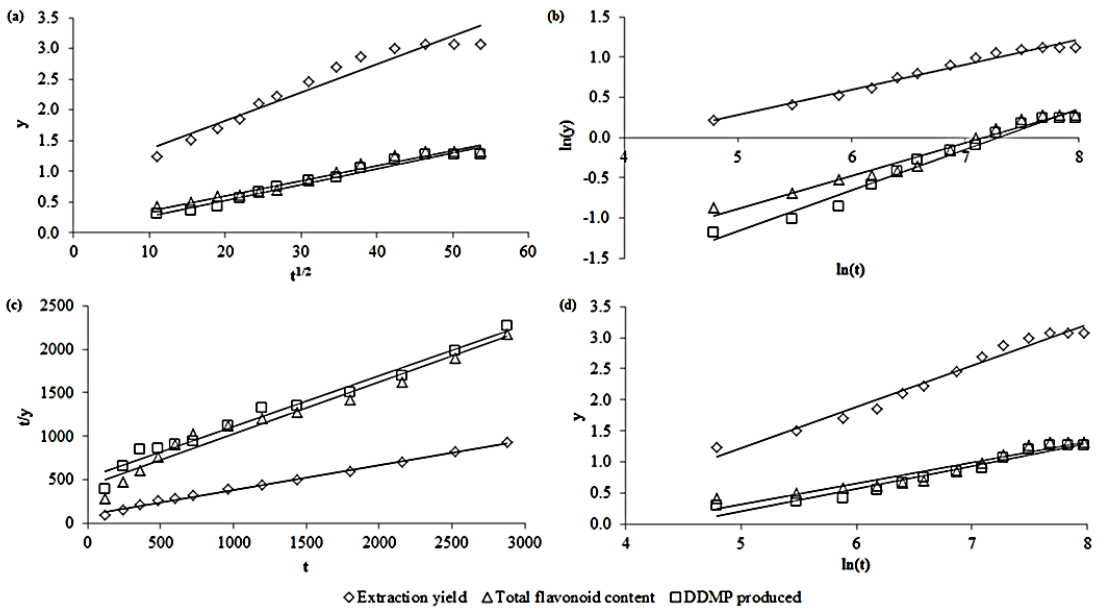

Figure 5: Comparison between the experimental (symbol) and simulated (line) data for the extraction of antifungal agent under optimized condition based on the linearized form of kinetic equations: (a) Parabolic diffusion model, (b) Power law model, (c) Peleg's model, and (d) Elovich's model.

In order to further evaluate the best fitting models for predicting the antifungal agent extraction process, *P* values corresponding to each selected empirical model were calculated. Kaymak-Ertekin & Gedik (2004) suggested that a model was acceptable if the *P* value was less than 10%. Observing the data shown in Table 8 of the present study, it was found that all selected models used in this study were adequate in describing the kinetics or behavior of antifungal agent extraction with the calculated *P* values ranged from 2.650 to 7.937%. In fact, as shown in Table 8, the Power law having the highest value of R^2, the lowest value of RMSD, and the smallest *P* value in all extraction process

either for the extraction yield, TF or DDMP production. Hence, in this study, the Power law was selected as the best empirical model for antifungal agent extraction from *S. commune* biomass.

CONCLUSION

In order to obtain the highest bioactive compounds from *S. commune* biomass, the extraction parameters that affected the extraction process was optimized using RSM coupled with BBD. The optimum values of the tested variables were 70.75% (v/v) methanol concentration, extraction temperature of 29 ºC, and mixing rate at 145 rpm. The results showed that the maximum predicted extraction yield and the production of TFC and DDMP were 3.0741 %, 1.321 µg QE/mg sample, and 1.273 µg/mg sample, respectively. For the extraction kinetics study, Power law described the extraction process well with the highest R^2, the lowest RMSD, and also the smallest *P* values for all tested variables. Hence, the Power law was selected as the best empirical model for antifungal agent extraction from *S. commune* biomass.

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LIST OF SYMBOLS

A_i	Extraction kinetic parameter related to parabolic diffusion model ($1/min^{0.5}$)
A_0	Washing coefficient related to parabolic diffusion model ($1/min^{0.5}$)
A_1	Diffusion rate constant related to parabolic diffusion model ($1/min^{0.5}$)
B	Parameter of the power law model incorporating the characteristics of the extraction system ($1/min^n$)
E_0, E_1	Extraction kinetic parameters of Elovich's model
K_1	Peleg's rate constant (min.g/mg)
K_2	Peleg's capacity constant (g/mg)
n	Diffusion exponent of the power law model
P value	Mean relative percentage deviation (%)
R^2	Linear correlation coefficient
t	Time (min)

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BIOMONITORING OF STREAMS: USING EPHEMEROPTERA, PLECOPTERA AND TRICHOPTERA (EPT) IN RESPONSES TO THE DIFFERENT TYPES OF LAND USE AT TABIN WILDLIFE RESERVE (TWR), LAHAD DATU, SABAH, MALAYSIA

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ABSTRACT. *A preliminary study on three aquatic insect orders, namely Ephemeroptera (mayfly), Plecoptera (stonefly), and Trichoptera (caddisfly) (EPT) was conducted at Tabin Wildlife Reserve (TWR), Lahad Datu, Sabah in January and February 2015. The objectives of this study were to determine (i) the composition of EPT along a stream at TWR, (ii) the distribution of EPT in each different land use at TWR, and (iii) the relationship between EPT communities and the water quality of the stream at TWR. Kick net sampling technique was used for collecting the EPT communities along Sg. Lipad of TWR. The most dominant order was Ephemeroptera consisting of 11 families with 1,354 individuals out of the total of 1,724 individuals and 26 families of EPT communities sampled. Trichoptera was the second most abundant order with nine families and 258 individuals, and lastly, Plecoptera with only six families and 112 individuals. There were more families of EPT communities distributed in secondary forest as compared to the oil palm plantation. Several exclusive families were found in secondary forest, while only one family was found in oil palm plantation. The habitat run showed the highest in abundance of EPT, while pool recorded the least in abundance. Four biotic indices (BMWP, ASPT, FBI, and INWQS) and a few physico-chemical parameters (pH, temperature, conductivity, and DO) were used in this study to determine the water quality of the sampling location. Based on the biotic indices and physico-chemical parameters, the status of water in Sg. Lipad was in excellent condition. The two water quality tests showed profound consistency. This serves as a confirmation that the EPT communities are effective to be used as a biomonitoring tool at TWR.*

KEYWORDS. Aquatic insects EPT physico-chemical parameters biomonitoring Sg. Lipad Tabin

INTRODUCTION

There is a total of 6-10% of all species found on Earth exists in the freshwater ecosystems, and among the 126,000 freshwater animal species, about 60.4% of them are composed of insects (Dudgeon *et al.*, 2006; Balian *et al.* 2008). This makes freshwater ecosystems to be considered as biodiversity hotspots which are heavily threatened by habitat loss and other anthropogenic activities (Conservation International, 2014). According to the World Wide Fund for Nature, WWF (2012), the organization pointed out that the 'Heart of Borneo' is listed as a biodiversity hotspot region which is inclusive of Malaysia, Brunei, and Indonesia.

Freshwater ecosystems are considered as tough habitats for small bodied animals, such as grasshoppers, mantises, or mosquitoes, but yet aquatic insects are successful in colonizing the ecosystems due to their wide range of adaptations (Susheela *et al.*, 2014). By viewing the anthropogenic disturbances globally, aquatic ecosystems can definitely be affected in great extent and intensity (Quist and Schultz, 2014), without any doubt can also bring about effects towards the aquatic organisms which rely on it. Thus, maintaining the integrity of the freshwater ecosystems from different forms of anthropogenic disturbance is of utmost importance, and for this reason, much attention should be given to it.

Aquatic macroinvertebrates or benthic macroinvertebrates are mainly aquatic insects that spend most of their lives in the freshwater ecosystems. The term benthic is used to define aquatic insects as they are found in abundance associated with the bottom or with a substrate (Susheela *et al.*, 2014). Aquatic insects are found ubiquitous in stream ecosystems, and present throughout a wide range of environmental conditions which make them to become a successful and appropriate model group for investigation at different levels of process, including at the individual, populational, and also community level (Heino and Peckarsky, 2014). At multiple space (spatial) and time (temporal) scales, the variation in the structure and organization of the aquatic insect communities are greatly influenced by abiotic environmental conditions, biotic conditions, and dispersal processes (Malmqvist, 2002; Heino *et al.*, 2013).

The physical, chemical and biological conditions of streams have a direct influence towards aquatic insects which make them good indicators for stream water quality (Budin *et al.*, 2007). Ephemeroptera, Plecoptera, and Trichoptera (EPT) are orders for aquatic insects which have the common names of mayflies, stoneflies, and caddisflies respectively. EPT is considered as an essential taxonomic group due to its wide range of distribution with high abundance and species richness (Righi-Cavallaro *et al.*, 2010). EPT is also the three major orders of aquatic insects that can be found abundantly in freshwater systems (Corona, 2010).

According to Bispo and Oliveira (2007), EPT makes up a rich collection of taxa in low and medium order cobble streams which occur primarily in clean and well-oxygenated water. The diversity and composition of EPT which functions as indicator species make them possible to determine the status of aquatic system water quality (Che Salmah *et al.*, 2001). EPT is highly sensitive towards any anthropogenic and environmental disturbances which allow them to become excellent indicators in evaluating and accessing the water quality of streams (Corona, 2010; Myers *et al.* 2011). Thus, EPT can be considered as the key aquatic insect orders as they play vital roles in the aquatic ecosystems.

There is not much in the database of EPT in Malaysia as compared to temperate regions. According to Susheela *et al.* (2014), aquatic insects are usually overlooked and unfamiliar to the public. In view of the fact that EPT has not been widely examined in Malaysian ecological studies (Suhaila *et al.*, 2012), this study could provide baseline data for the records of EPT in Malaysia, especially in Sabah. Previous research done by Arman (2004) at Tabin Wildlife Reserve (TWR) focused on the diversity, composition, and

distribution of aquatic insects at the study site, without focusing on the EPT communities. Therefore, this study was able to concentrate on the EPT communities at the same study site. This study also helped to provide more information for future research in terms of the composition and distribution of EPT communities at Tabin Wildlife Reserve.

Apart from that, EPT has been used to evaluate the water quality of freshwater ecosystems as EPT is highly specific to environmental stressors, such as temperature, anthropogenic disturbances, and pollutions (Corona, 2010). The study could also highlight the effects of anthropogenic disturbances towards Malaysian aquatic insect communities as there is very little information available on the subject matter (Wahizatul *et al.*, 2011).

Hence, the aim of this research was to understand in details the composition and distribution of EPT to provide more ecological information on EPT community in Malaysian streams. Integrated approaches of conservation would be able to be tested in order to determine the success of conservation efforts through the comparison between the EPT community at the study site and the EPT found in previous studies on aquatic insects. The objectives of this study were to determine: (1) the composition of EPT along the stream at TWR, (2) the composition and distribution of EPT in each different land use and habitat, and also (3) the relationship between EPT communities and the water quality of streams.

METHODS

Study Site

Tabin Wildlife Reserve (TWR) is a forest reserve gazetted under the Forest Enactment 1984. Dawson (1992) reported that TWR was initially gazetted with the main purpose of conserving three endangered large mammal species (Asian Elephant, Sumatran Rhinoceros, and Tembadau). TWR is located at 5°10'N, 118°40'E in the middle of the Dent Peninsula in eastern Sabah. It is a protected area situated at the north-east of Lahad Datu Town. The area is made up of approximately 120,521 hectares (WWF Malaysia, 1986).

Sampling

Sampling activities for EPT were carried out in Sg. Lipad of Tabin Wildlife Reserve. Sg. Lipad represented the secondary forest area located inside TWR, and another sampling area where the stream was used for agricultural purpose (oil palm plantations) was situated outside the boundary of TWR. The upstream of Sg. Lipad flowed into the oil palm plantation. The stream flowing into the secondary forest was less succumbed to human activities, while in the oil palm plantation the stream was often exposed to human disturbances.

A total of four stations with 12 sub-stations were selected from Sg. Lipad. A 100m range of the stream was selected for each station. Two of the stations (upstream and downstream) were located in secondary forest area, while the other two were in oil palm plantation area. A 500m difference in distance was made between the stations for each of the two land use types. The samplings for EPT communities from three different habitats (pool, run, and riffle) for each station were replicated three times.

Kick net technique was the standard method used for sampling at all the stations. Kick net has the net frame size of 390mm x 320mm with 210µm as the mesh size. As suggested by Hazelton (2003), some aquatic insects that clung onto larger rocks were removed by brushing them off into the net with hands. For the stream profile, the width was measured using a measuring tape and the depth of the stream was measured using a steel ruler. The measurement of stream profile required three replications at different habitats of pool, run, and riffle for each station. Water quality of the stream was measured at the sampling sites where kick net was carried out. Physico-chemical parameters, such as dissolved oxygen (DO), pH, temperature and conductivity were measured using a HANNA multi-parameter water quality meter (HI9828).

RESULTS AND DISCUSSION

Throughout this study, a total of 1,724 individuals representing 26 families of the three EPT orders were collected (Table 1). The overall diversity index of EPT communities in Sg. Lipad with H'=2.3074 indicated that the EPT communities were relatively diverse, and the habitat structure was rather intact with minimal pollution effects. The same pattern on the abundance of EPT could also be seen in the previous study done by Arman (2004) at the same study site. From all the sampled individuals, Ephemeroptera appeared to have the highest number of individuals with 4,551, followed by Trichoptera with 2,459, and Plecoptera with 1,845 individuals. However, the study done by Che Salmah *et al.* (2001) at Kerian river basin, Perak obtained different results with Ephemeroptera being the most abundant, to be followed by Plecoptera and Trichoptera.

Table 1: List of EPT families distributed across different land uses.

Orders	Families	No. of Individuals	
		Oil Palm Plantation	Secondary Forest
Ephemeroptera	Baetidae	31	46
	Behningiidae	15	18
	Caenidae	40	3
	Ephemerellidae	120	151
	Heptageniidae	19	118
	Leptophlebiidae	218	311
	Neophemeridae	31	0
	Oligoneuriidae	1	7
	Potamanthidae	0	5
	Siphlonuridae	89	102
	Tricorytidae	18	11
Total	**11**	**582**	**772**
Plecoptera	Capniidae	2	5
	Chloroperlidae	0	23
	Leuctridae	4	4
	Peltoperlidae	8	12
	Perlidae	0	52
	Perlodidae	1	1
Total	**6**	**15**	**97**
Trichoptera	Brachycentridae	1	2
	Glossosomatidae	20	3
	Hydrascyhidae	5	1
	Hydropscyhidae	9	175
	Hydroptilidae	11	4
	Leptoceridae	0	2
	Limnephilidae	0	1
	Polycentropodidae	5	5
	Psychomyiidae	4	10
Total	**9**	**55**	**203**
Grand Total	**26**	**652**	**1072**

The difference in diversity and composition in the study was due to the variation in sampling method. Kick net was the only sampling technique used in this study, while Arman (2004) used kick net and dip net method. Apart from that, the number of habitats and microhabitats where samplings were done differed in which only three habitats (pool, run, and riffle) were covered in this study, while for Arman (2004), there were three additional microhabitats studied (leaf litters, stone substrates, and aquatic vegetation). For Che Salmah *et al.* (2001), 16 tributaries were selected as the sampling sites without being further divided into habitats and microhabitats.

Ephemeroptera yielded the highest abundance along Sg. Lipad with 1,354 individuals (78.54%) out of the total 1,724 individuals sampled. The second highest was from the order Trichoptera which consisted of 258 individuals constituting 14.96% of the total individuals sampled. The least number of the individuals captured was from Plecoptera with only 112

individuals (6.50%) (Table 2). A total of 25 families were sampled from the stream flowing through the secondary forest, while for oil palm plantation, only 21 families were collected. A total of 1,072 individuals (62.18%) were sampled in the secondary forest, whereas only 652 individuals with 37.82% were captured during the sampling activities in oil palm plantation.

Table 2: List of families and respective abundance of EPT in different habitats.

Orders	**Families**	**Habitats**			**Total**
		Pool	**Run**	**Riffle**	
Ephemeroptera	Baetidae	19	39	19	77
	Behningiidae	2	10	21	33
	Caenidae	14	29	0	43
	Ephemerellidae	84	140	47	271
	Heptageniidae	51	59	27	137
	Leptophlebiidae	68	168	293	529
	Oligoneuriidae	0	0	8	31
	Neophemeridae	13	11	7	8
	Potamanthidae	0	0	5	5
	Siphlonuridae	35	92	64	191
	Tricorytidae	19	4	6	29
Total	**11**	**305**	**552**	**497**	**1354**
Plecoptera	Capniidae	2	4	1	7
	Chloroperlidae	5	14	4	23
	Leuctridae	0	3	5	8
	Peltoperlidae	9	7	4	20
	Perlidae	11	21	20	52
	Perlodidae	0	2	0	2
Total	**6**	**27**	**51**	**34**	**112**
Trichoptera	Brachycentridae	2	1	0	3
	Glossosomatidae	11	9	3	23
	Hydrascyhidae	3	3	0	6
	Hydropscyhidae	39	87	58	184
	Hydroptilidae	7	4	4	15
	Leptoceridae	0	2	0	2
	Limnephilidae	0	0	1	1
	Polycentropodidae	2	4	4	10
	Psychomyiidae	0	11	3	14
Total	**9**	**64**	**121**	**73**	**258**
Grand Total	**36**	**396**	**724**	**604**	**1724**

Generally, Ephemeroptera are commonly known to be nearly cosmopolitan, which can be found inhabiting a wide range of habitats (Che Salmah *et al.*, 2001). The large quantity of ephemeropterans may be contributed by the suitability of the habitats, behavioral adaptation, and their sensitivity in detecting predators through chemical cues (Che Salmah *et al.*, 2001).

Apart from that, the population of ephemeropterans was also proven by Suhaila *et al.* (2014) to be in the highest abundance, especially during the wet season, while the population of trichopterans resulted in the highest abundance during the dry season. The sampling was carried out in January, which according to Malaysian Meteorological Department (MMD) (2013), had the maximum rainfall in Sabah. This might resulted in the highest number of individuals recorded for order Ephemeroptera. As for Plecoptera, it showed the lowest in abundance as they are insects that are most sensitive and intolerant to pollution (Galdean *et al.*, 2000). As for the study conducted by Suhaila *et al.* (2014), the population of plecopterans remained low and showed consistency throughout the year, which was similar to Arman (2004), the plecopterans remained the lowest in individuals among the others, probably due to the fact that they are not affected by either the wet or dry season.

Neophemeridae from the order Ephemeroptera was the only family that was found exclusively from the stream in oil palm plantation. On the other hand, there were five families discovered only from the stream in the secondary forest, namely Potamanthidae (Ephemeroptera); Chloroperlidae, Perlidae (Plecoptera), and Leptoceridae, Limnephilidae (Trichoptera). The remaining 20 families out of the total of 26 families were those that had been identified in both land uses.

Apart from being one of the families found only in stream in the secondary forest, Perlidae was also the most abundant among the plecopterans which was similar to the findings by Che Salmah *et al.* (2001) due to their preference towards clean, cool, and well-oxygenated moving water (Fochetti and Tierno de Figueroa, 2008). Their distribution is rather confined to particular type of substrate and stream size (Che Salmah *et al.* 2001).Sg. Lipad was an ideal and suitable habitat for it to live in.

The families Heptageniidae and Hydropscyhidae were found in both oil palm plantation and the secondary forest areas. However, the families were both being highly distributed in the secondary forest, and very low number of individuals in the oil palm plantation. This could be due to the higher canopy coverage at the secondary forest, which was required, enabling them to survive better in the secondary forest (Boonsoong and Braasch, 2013).

There was an increasing trend in terms of the number of families for orders Ephemeroptera and Plecoptera with 11 and six families respectively as compared to the previous study by Arman (2004). From the previous study, only nine families from Ephemeroptera and two families from Plecoptera were recorded. Being of limited mobility, this increase in the number of families sampled over time could be seen as a sign of improvement of the stream condition to be a much healthier stream (Metzeling *et al.*, 2006).

The number of families found in a stream is generally affected by several factors, such as the quality of the habitat, stream size, and nutrient enrichment which in unison help in assessing the health of a stream (Metzeling *et al.*, 2006).

The Shannon-Weiner Diversity Index (H') calculated for EPT communities in stream in the secondary forest area was slightly more diverse as compared to the EPT communities sampled in the stream in oil palm plantation. In contrast, the Evenness Index (E) for the EPT assemblages in oil palm plantation showed a higher evenness (E=0.71) as compared to the secondary forest area (E=0.68).

The stream flowing through the oil palm plantation area which received agricultural runoff, showed greater quantity of suspended solids and sediments, nutrient concentrations, and particulate organic matters which contributed to the lower abundance and diversity of EPT communities (Pliūraitė and Mickėnienė, 2009). With the introduction of anthropogenic influences, it reduced the overall diversity of EPT in the stream as sensitive taxa would eventually decrease to be followed by an increase in the tolerant taxa (Bispo and Oliveira, 2007). In oil palm plantation, it showed a higher evenness than the secondary forest. This could be due to the reason that there were some of the families in the secondary forest which had relatively low number of individuals with some other families that were exceptionally abundant.

The habitat run appeared to be having the highest number of families and number of individuals as well. This was followed by the habitat riffle, which left the pool habitat with the lowest number of families and individuals. The study done by Thambiratnam (2009) also showed the same pattern in the richness of EPT across different habitats with the run recorded the most to be followed by riffle and lastly, the pool.

Pool has been characterized as having fine substrates which resulted in the lowest abundance (Tickner *et al.*, 2000; Fenoglio *et al.* 2004). The low abundance was due to the higher predatory effect attributable to the fine substrate of the pool (Pringle, 1996). As for run and riffle, the depth of the water that was shallow provided more microhabitats on the banks and in leaf litters that could serve as a refuge to protect the EPT communities from predators (Thambiratnam, 2009). Riffle with the highest turbulence and velocity creates aeration, and thus, enables filter-feeders to obtain their food through the help of the water current without the need of spending much energy (Cummins and Meritt, 2008).

However, from the calculation of the Shannon-Weiner Diversity Index (H'), it showed that the pool was more diverse among all three habitats to be followed by run and riffle as the least diverse. The high diversity in the pool in this study could be due to the percentage of canopy coverage which contributed to the huge amount of allochthonous input presence in the form of leaf pack (Subramanian and Sivaramakrishnan, 2005). The input of leaf litters helps to provide nutrient flow into the stream system as they provide energy for the aquatic insect communities, while the emerging aquatic insects provide energy for other organisms, including terrestrial animals, such as birds (Compson *et al.*, 2013).

Allochthonous input was found to be more significant in streams that experience longer flood period, such as in the tropical rainforest than forest in the temperate region (Hein *et al.*, 2003). Apart from that, pool could also have more diverse communities of algae than in riffle habitat that contributes to its greater diversity (Mullner and Schagerl, 2003). These algae also serve as a source of organic matters, and play a role as food sources in the food web (Menninger and Palmer, 2007).

Similarly, the Evenness Index (E) also resulted in the same pattern as for the Diversity Index with even distribution of the EPT communities in habitat pool, while riffle showed uneven distribution. The highest evenness value of 0.82 was calculated for the pool habitat to be followed by run with a value of 0.74. The high diversity in pool was due to the high evenness of the assemblages of EPT living in it (Principe, 2008). A study carried out by Subramanian and Sivaramakrishnan (2005) showed that run and riffle were similar in composition as compared to pool.

The EPT communities are able to reflect the condition of their environment due to their sensitivity toward their surroundings (Che Salmah *et al.*, 2001). Biotic indices were used in this study to evaluate the water quality in the stream at TWR. There were 25 families of EPT found in the stream in the secondary forest, which showed a higher richness for EPT communities as compared to the stream in oil palm plantation with only 21 families, despite that both have been categorized as non-impacted streams. The higher EPT richness in the secondary forest implied that the water at that particular area was much cleaner as it was able to support a much higher number of species of EPT (Phillips *et al.*, 2005).

Both the Family Biotic Index (FBI) calculated showed an excellent water quality of the stream for both land uses with stream in the secondary forest area (2.98) being cleaner, and indicating a better water quality with organic pollution unlikely to occur in comparison to the stream flowing in oil palm plantation (3.12). Family Caenidae with a tolerance value of seven was highly abundant in the stream of oil palm plantation area as compared to the individuals found in the secondary forest. The pollutant intolerant families (Perlidae and Chloroperlidae) with pollution tolerance value of one were only found in the stream in the secondary forest area that resulted in a slightly better water quality as compared to the oil palm plantation area. The FBI has the potential to detect and evaluate the tolerance of EPT communities towards organic toxic pollutants (Mandaville, 2002). The index is used for making rapid assessment of streams, and is advantageous in determining the general status of organic pollution in streams that could help in making decision related to the streams (Hilsenhoff, 1988).

According to the Biological Monitoring Work Party (BMWP) scoring system (Armitage *et al.*, 1983), the score for the oil palm plantation was lesser than the score obtained for the secondary forest area. Stream in the secondary forest was classified as having very high water quality with a value of 158, whereby the stream in oil palm plantation scored a value of 111. The BMWP score was obtained from the families present which were scored respectively in accordance to their tolerance to disturbance in their

habitat regardless of the number of individuals (Azrina *et al.*, 2006). Higher BMWP score indicates much cleaner water, which could be seen from the obtained scores for the two different land uses.

The Average Score Per Taxa (ASPT) was also calculated in this study as it was less sensitive towards the degree of sampling effort and seasonal change as compared to BMWP, by dividing the BMWP score with the number of families (Zamora-Munoz *et al.*, 1995). ASPT for streams in oil palm plantation and the secondary forest denoted the same description of very clean water with values of 8.54 and 8.78 respectively. Despite the large difference in their BMWP scores, the ASPT index values for these two land uses had little difference probably due to immense difference in the abundance of insects obtained from each of the site (Roche *et al.*, 2010).

Physico-chemical water parameters were also measured and analyzed in addition to the biotic indices. Physical water parameters, such as pH, temperature, conductivity, and dissolved oxygen were recorded during sampling (Table 3). By comparing with the Interim National Water Quality Standards for Malaysia (INWQS, 2006), the stream in this study was classified into Class I and Class IIA, whereby Class I stream is important in the conservation of natural water supply with no water treatment required for its water. As for Class IIA stream, it can be used as a water supply provided that conventional water treatment has been done.

Table 3: Range of water quality parameters for streams in different land uses with respective Interim National Water Quality Standards of Malaysia (INWQS).

Parameters	Land Uses	Range	INWQS	Class
DO (mg/l)	Oil Palm Plantation	4.52-6.91	5-7	IIA
	Secondary Forest	5.41-5.93	5-7	IIA
pH	Oil Palm Plantation	7.64-7.79	6.5-8.5	I
	Secondary Forest	7.96-8.01	6.5-8.5	I
Temperature (^{o}C)	Oil Palm Plantation	25.98-26.85	Normal	-
	Secondary Forest	24.85-25.20	Normal	-
Conductivity (µS/cm)	Oil Palm Plantation	80-140	1000	I
	Secondary Forest	151-183.5	1000	I

In general, the physico-chemical water parameters measured indicated a good water quality in which the same could also be seen in the use of biotic indices in this study. There was a strong relationship between the abundance of EPT communities and the good water quality indicated. EPT communities were known to inhabit abundantly, specifically clean water in the stream at TWR. This indicated that the streams at TWR were undisturbed, and had good water quality.

CONCLUSIONS

This study was the first study at TWR which focused only on the EPT communities, and possibly be the first that was done in Sabah. Among the three orders of insects sampled, Ephemeroptera had the highest abundance among all others, possibly due to it being capable in tolerating more pollutants than the other two orders, making the ephemeropterans to have a much wider range of habitats. Plecoptera, at the opposite end, is more sensitive to their environment, and is intolerant to pollutions. Thus, it explainedthe abundance obtained for Plecoptera was the lowest among the other orders. An increase in the number of families of the EPT communities as compared to the previous study done by Arman (2004) could suggest that the condition of the stream at TWR is improving. However, further study is required as it involves many more factors than what had been assessed in this study, such as the quality of the habitat, nutrient flows, and also stream size.

In addition, the EPT communities were found to be more diverse in the secondary forest than oil palm plantation. There were more species that were exclusively found in the secondary forest as compared to plantation area, hinting that the secondary forest was able to support a much richer community. Some of the families, such as Heptageniidae and Hydropscyhidae, were able to be found in both land uses, but were highly abundant in the secondary forest with only very little individuals in oil palm plantation. This is probably due to the ability of the secondary forest to supply these insects with the requirements to strive well in the environment. Across different habitats of sampling, the EPT communities were found to be abundant in the run habitat, while the least abundant was in the habitat pool. This could be caused by the predatory effect present at pool area, and it had less diverse microhabitats available for the insects.

Biotic indices were used in this study to assess the water quality of the steam at TWR. The indices showed that the water quality in both oil palm plantation and the secondary forest belonged to the same category for EPT Richness, FBI, and ASPT. The indices for the secondary forest indicated that it was of slightly better water quality, despite being classed into the same category. However, the BMWP score for oil palm plantation was in different category as for the secondary forest where it belonged to high water quality, while the secondary forest belonged to very high water quality.

Nonetheless, BMWP value alone cannot be used to determine the quality of the water as it is susceptible to the abundance of insect families obtained from the sampled location. Physico-chemical parameters were measured as well to be cross-checked with the biotic indices. In accordance to INWQS, the water quality in both land uses belonged to Class I water quality for pH and conductivity, while the dissolved oxygen content was categorized into Class IIA. Both biotic indices and physico-chemical water quality parameters implied that the water quality in the stream at TWR were of rather clean water quality. Therefore, it showed that the EPT communities at TWR could be used to establish biological criteria

which serve as bioindicators for stream pollution. The information in this study is important to serve as a baseline study in the area for future studies, and assists in environmental monitoring and management of the area.

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IN VITRO BIOACTIVITIES AND PHYTOCHEMICALS CONTENT OF VEGETABLES FROM SABAH, MALAYSIA

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ABSTRACT. *This study aims to investigate potential of vegetables from Sabah with value-added benefits in nutraceuticals. Fifty-five samples of vegetables were collected from local market and tested for antioxidant activity using DPPH• assay. Four species with high DPPH• scavenging activity (>80%) which are Cosmos caudatus, Eryngium foetidum, Ipomoea batatas and Manihot esculenta Crantz were selected and subjected to different solvents extraction and tested to different scavenging assays (DPPH•, O_2• and NO•), protein kinase-phosphatase assay (GSK-3β, MKK1, and MSG5) and antibacterial tests. Ethanol extract of I. batatas (90.56%), boiled water extract of M. esculenta Crantz (62.77%) and extractable polyphenol extract of E. foetidum (50.93%) exhibits comparable scavenging activities to catechin for DPPH•, O_2• and NO•, respectively. Polyphenols, phenolic acids, flavonoids and proanthocynidins are detected in all extracts at concentration between 0.001 mg/g to 0.52 mg/g. The highest total polyphenols content (0.40±0.01 mg GAE/g), total phenolics content (0.52±0.01 mg GAE/g), total flavonoids content (0.13±0.01 mg CE/g) and total proanthocyanidins content (0.12±0 mg CE/g) were obtained in extractable polyphenols of Cosmos caudatus. No extracts were observed as inhibitor for GSK-3β, MKK1 and MSG5. Inhibition of Pseudomonas aeruginosa (8.0 mm to 12.3 mm) was only obtained in extractable polyphenols and ethanol extracts. Extractable polyphenols of E. foetidum exhibit the largest inhibition of Pseudomonas aeruginosa (12.3 mm).*

KEYWORDS. Antibacterial, Antioxidant, Antikinases, Antiphosphatases, *Cosmos caudatus*, *Eryngium foetidum*, *Ipomoea batatas*, *Manihot esculenta*

INTRODUCTION

Antioxidants are capable of scavenging free radicals that were shown to be linked to age related illnesses and a large number of other illnesses (Amol *et al*., 2013). Restriction of synthetic antioxidants usage which been suspected to promote carcinogenesis has lead research on effective antioxidants originated from natural resources especially that were used as folk medicine or food (Amol *et al*., 2013).

Vegetables are essential to human in providing nutrients and various phytochemicals (Odufuwa *et al*., 2013). Consumption of vegetables could aid the prevention of aging related diseases and cancer (Genkinger *et al*., 2004). Cancer is the leading cause of death in

worldwide and it happen due to misregulation of signal transductions (Lobbezoo *et al*., 2003; Ougolkov and Billadeau, 2006). Protein phosphorylation is a major event in the signal transduction pathway which involves alteration of the downstream proteins conformation namely as protein kinases and phosphatases. Protein kinases serve central regulators of growth, embryogenesis, cell death, differentiation, proliferation, stress responses and apoptosis (Nakagami *et al*., 2005). Protein phosphatases are important regulators in glycogen metabolism, cell signaling, learning and memory, act as positive regulators of many hormonal responses, protein synthesis, muscle contraction, carbohydrate metabolism, transcription and neuronal signaling (Watanabe *et al*., 2001; Bennett *et al*., 2006). Abnormalities in the control of kinases and phosphatases had been detected in various types of cancers (Weinberg, 2007). Antioxidant compounds isolated from plants such as polyphenols and flavonoids are commonly associated as preventive agents against cancer at early stage (Thomas, 2008; El-Sayed *et al*., 2013).

Vegetables also have been long used in folk medicine to treat infectious disease caused by microbial and these have been supported by the discovery of antimicrobial phytochemicals from vegetables (Thomas, 2008). Flavonoid is one of the antioxidant compounds that was suggested to posses antibacterial activities (Thomas, 2008; Ahmad *et al*., 2012). Vegetables especially green leafy vegetables are among the top four common daily diets by Malaysian (Karim *et al*., 2008). To date, even though vegetables offer various health benefits to humans, there is little information on the biological properties of vegetables originating from Sabah. Therefore, the objectives of this study is to determine the *in vitro* bioactivities (antioxidant, antikinases, antiphosphatases and antibacterial) and phytochemicals content of different solvents extraction of 55 vegetable samples from Sabah, Malaysia.

MATERIALS AND METHODS

Plant Materials

A total of 55 samples derived from 33 genera and 42 species were bought from the local market in Sabah and deposited in BORNEENSIS, Institute of Tropical Biology and Conservation (ITBC), Universiti Malaysia Sabah. Plant parts were washed thoroughly with tap water, air dried, powdered, weighed and stored in air-tight containers at room temperature for extraction purposes.

Sample Extraction

Extractable polyphenols extract. Five grams of sample were extracted with acidic methanol:water (50:50, v/v; pH2) as described by Saura-Calixto *et al*. (2007) in the tube and shaken thoroughly for 1 hour at room temperature. The tube was centrifuged at 2500*g* for 10 min and the supernatant was recovered. Twenty-mL of acetone:water (70:30, v/v) then was added to the residues, shacked and centrifuged to recover the supernatant. The combined

methanolic and acetonic extract (extractable polyphenols) was evaporated to remove solvents and then freeze dried. The dried extract was dissolved in methanol at 10 mg/mL and subjected to DPPH (1,1-diphenyl- 2-picryl-hydrazyl) assay. Selected vegetable species with high scavenging activity was subjected to different types of solvent extraction and then tested for their phytochemicals and, antioxidants, antikinase or antiphosphatase and antimicrobial activities at concentration 0.5 mg/mL.

Ethanol extract. Five grams of sample were extracted three times with 95% (v/v) ethanol as described by Jimoh *et al*. (2010). The extract was filtered using Whatman paper No.1 and further evaporated to dryness with a rotary evaporator at 40°C under reduced pressure.

Boiling water extract. Extraction was done as described by Gülçin *et al*. (2004) with some modifications. Hundred milliliters of distilled water were added to 5 g of samples before boiled at 100˚C and stirred (1000 rpm) for 15 minutes. Extract was filtered using Whatman paper No.1 and freeze dried.

Antioxidant Assays

1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH•) scavenging activity. Three-hundred µM of DPPH stock solutions was prepared in methanol. Then, 760 µL of the stock solution was added to the 40 µL of extract/catechin. Final concentration of the samples and the catechin (positive control) in the mixture was 0.5 mg/mL. The mixtures were incubated at 37 ˚C for 30 minutes (Jeong *et al*., 2004) before absorbance was measured at 517 nm (Multiskan^{TM} Go, USA). The scavenging activity was calculated following the equation described by Kumar *et al*. (2008):

Superoxide radical (O_2•) scavenging activity. Superoxide radical scavenging activity was determined as described by Liu *et al*. (1997). About 750 µL of 300 µM NBT solutions and 750 µL of 936 µM NADH solutions were added to 20 µL of 10 mg/mL extracts. Then, the mixture was topped up with 0.1 M Tris-HCl buffer (pH 7.4) to a total volume of 3 mL. 750 µL of 120 µM PMS was added to start the reaction. The mixture was incubated at room temperature (25 ± 2°C) for 5 minutes and the absorbance was measured at 560 nm (Multiskan^{TM} Go, USA). Final concentration of the samples and the positive control (catechin) was 0.053 mg/mL.

Nitric oxide radical (NO•) scavenging activity. Nitric oxide radical scavenging activity was determined as described by Kumar *et al*. (2008). About 500 µL sodium nitroprusside (5 mM in phosphate buffer saline pH 7.4) was mixed with 500 µL of 0.1 mg/mL extracts. The mixtures were incubated at room temperature (25 ± 2°C) for 30 min. Then, Griess reagent (1% (w/v) sulphanilamide, 2% (v/v) phosphoric acid and 0.1% (w/v) *N*- 1-naphthylenediamine dihydrochloride) was added at equal volume to the extract mixtures for colour development before the absorbance measured at 546 nm (Multiskan^{TM} Go, USA). Final concentration of the samples and the positive control (catechin) was 0.025 mg/mL.

Determination of the Phytochemicals Content

Total polyphenols content. Total polyphenol assay was conducted as described by Hakiman and Maziah (2009). 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times) was added to 100 µL of 1 mg/mL extract and was left for 5 minutes. Then, 2.5 mL of 7 % (w/v) of sodium carbonate (Na_2CO_3) was added. The mixture was incubated in room temperature (25 ± 2°C) for one hour before the absorbance was measured at 725 nm (Multiskan^{TM} Go, USA). The total polyphenols content of the extract was expressed as mg gallic acid equivalent per gram of sample.

Total phenolic acids content. Total phenolic acids assay was determined as described by Hakiman and Maziah (2009) with minor modification. About 900 µL of distilled water and 100 µL of Folin-Ciocalteu reagent were added to 100 µL of 1 mg/mL extract. The mixture was mixed thoroughly. After 5 minutes, 1 mL of 7 % (w/v) Na_2CO_3 was added. Then, the mixture was diluted to 2.5 mL by adding 400 µL distilled water. The mixture was incubated at room temperature (25 ± 2°C) for 90 minutes and the absorbance was measured at 750 nm (Multiskan^{TM} Go, USA). The total phenolic acids content was expressed as mg gallic acid equivalent per gram of sample.

Total flavonoids content. Total flavonoids assay was determined as described by Hakiman and Maziah (2009) with minor modification. About 400 µL of distilled water was added to 100 µL of 1 mg/mL extract. After that, 30 µL of 5 % (w/v) sodium nitrite was added. After 5 minutes, 30 µL of 10% (w/v) aluminum chloride was added. After 6 minutes, the mixture was then added with 200 µL of 1 M sodium hydroxide and subsequently added with distilled water to a final volume of 1 mL. The absorbance was measured at 510 nm (Multiskan^{TM} Go, USA). The total flavonoids content was expressed as mg catechin equivalent per gram sample.

Total proanthocyanidins content. Total proanthocyanidins assay was conducted as described by Porter (1989) with minor modification. 1 mL of a freshly prepared vanillin solution (1 g/100 mL of 70 % (v/v) sulfuric acid) was added to 500 µL of 1 mg/mL extract and further incubated at room temperature (25 ± 2°C) for 15 minutes. The absorbance was measured at 500 nm (Multiskan^{TM} Go, USA) and the total proanthocyanidins content was expressed as mg catechin equivalent per gram sample.

Antikinase and Antiphosphatase Assay

Both MAPK Kinase (MKK1) and MAP Kinase Phosphatase (MSG5) yeast strains (Table 1) were incubated in broth culture at 28˚C with 220 rpm for 2 days for fermentation purposes and later were incubated at 28˚C for 5 days for screening assay (Watanabe *et al.,* 1995). The glycogen synthase kinase – 3 beta (GSK-3β) screening test employed a transformant of gsk-3 null mutant (Table 1) and conducted according to method described by Cheenpracha *et al*. (2009). The strain was incubated at both 37°C and 25°C for 5 days. All tested extracts were dissolved in respective solvents to a stock concentration of 100 mg/ml and 20 µl of sample aliquots were impregnated on sterile paper dish for the yeast screening assay. Zones of inhibition are measured for data analysis.

Table 1: Genotype of yeast strains used in various type of screening assay.

Screening assay	Strains	Genotype	References
MAP kinase (MKK1)	$MKK1^{P386}$	Transformant from wild type 1788 with mutant type nNV7- $MKK1^{P386}$(*GAL1p- $MKK1^{P386}$*). *MATa/MATa ura3/ura3 leu2/leu2 trp1/trp1 his4/his4 can1/can1* [pNV7-$MKK1^{P386}$].	Watanabe (1995)
MAPK phosphatase (MSG5)	$MKK1^{P386}$_MSG5	*Mat**a** GAL1p-MKK1P386::TRP1 ura3 leu2 trp1 his4 can1*[Pspg14-*MSG5*]	Watanabe (1995); Ho (2001)
GSK-3β	pKT10-GSK-3β	*MATα his3 leu2 ura3 trp1 ade2 mck1::TRP1 mds1::HIS3 mrk1yol128C::LEU2* [pKT10-GSK3b]	Andoh *et al*., (2000); Cheenpracha *et al*. (2009)

Antimicrobial Assay

Microorganisms such as fungi (*Candida albicans* and *Candida krusei*), gram negative bacteria (*Enterobacter aerogenes*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Salmonella typhi*) and gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pneumonia*) were tested in this study. Bacterial cultures were suspended in nutrient broth and incubated for 24 hours at 37°C, while for fungal were in potato dextrose broth and incubated for 24 hours at 28°C (Barbour *et al*., 2004). Inoculum was prepared by diluting the microbial suspension with the broth to 0.5 McFarland standards. Then, 200 µL of inoculum was added into 20 mL of media agar. The agar was poured gently into petri dishes and left to solidify at room temperature. All tested extracts were dissolved in respective solvents to a stock concentration of 100 mg/ml and 20 µl of sample aliquots were impregnated on sterile paper dish for the antimicrobial screening assay. Zones of inhibition are measured for data analysis.

Statistical Analysis

Statistical analysis was carried out using one-way analysis of variance (ANOVA) using SPSS 14.0 version. The significant differences between the mean values were compared using Duncan Multiple Range Test (DMRT) at $p<0.05$.

RESULTS AND DISCUSSION

Previous studies have recognized that antioxidant compounds may play an important role in explaining the benefit of vegetable consumption and thus some of vegetables in Sabah were used in this study to assess their antioxidant activity. This will allow identification of locally available vegetables that has antioxidant capability and developed into value added food. DPPH• scavenging assay is widely used in measuring antioxidant capacity due to its simplicity, rapid and sensitivity (Prior *et al*., 2005; Rafat *et al*., 2010). The DDPH assay shows that although the some vegetable samples shared the same genus but the performance in DPPH• scavenging is different (Table 1). Given an example where only two out of five

species of *Ipomoea* (*Ipomoea batatas* and *Ipomoea batatas* var. star shaped leaf) have high DPPH• scavenging activity. This finding is in line with report by Rasineni *et al*. (2008) on *C. forskhoii*. This may due to the variation of phytochemicals content in response to differences in genetics and growing conditions (Kalt, 2005). Different part of the plant also exhibit different performance on DPPH• scavenging activity. Leaf extract has the highest DPPH• scavenging activity compared to the other parts of vegetable. The biosynthesis of polyphenols which play an important role as antioxidant is a water soluble glycosylated flavonoids and mainly stored in epidermal cell vacuoles whereas the hydroxycinnamic acids are bound to cell walls in leaves (Meyer *et al*., 2006). Of all tested vegetable samples, *Cosmos caudatus* (83.09 ± 3.22), *Eryngium foetidum* (82.63 ± 3.18), *Ipomoea batatas* (89.43 ± 0.37) and *Manihot esculenta* Crantz (84.50 ± 2.75) having a high scavenging performance and comparable to catechin (90.96 ± 1.09%). This finding is in line with reports by Andarwulan *et al*. (2010) and Nur Faezah *et al*. (2013). Interestingly, those species promote better performance as compared to *Centella asiatica* (66.07 ± 4.66) that reported containing asiaticoside and poses high antioxidant activity (Tatmiya *et al*., 2014), and therefore were subjected to different solvents extraction for further analysis.

Scavenging Activities of Selected Vegetables Extracted with Different Solvents

Solvents extraction such as acetone, methanol, ethanol and their combination with water are frequently used for extracting antioxidant compounds from plants (Sultana *et al*., 2009; Dai and Mumper, 2010). In general, methanol extract lower molecular weight (such as polyphenols) while acetone is efficient for higher molecular weight (such as flavanols) (Dai and Mumper, 2010). Meanwhile, ethanol is able to extract polyphenols and tannins, whereas waters extract polypeptides, anthocyanins and tannins (Cowan, 1999). Hence in this study, the air-dried samples were extracted with different solvents to yield extractable polyphenols (combine methanol and acetone), ethanol and boiling water extracts. These extracts were further tested for DPPH•, O_2• and NO• scavenging assays at concentration of 0.5 mg/mL, 0.053 mg/mL and 0.025 mg/mL, respectively. The result revealed that different extracting solvents have different antioxidants performance.

The DPPH• scavenging activitiy of selected vegetables species were ranging from 20.53% to 90.56% (Table 3). Ethanol extract of *I. batatas* exhibit the highest DPPH• scavenging activity (90.56 ± 0.24%) followed by extractable polyphenols of *I. batatas* (89.43 ± 0.37 %), ethanol extracts of *C. caudatus* (84.68 ± 0.29 %) and *E. foetidum* (75.87 ± 8.84 %). Water extract of *Ipomoea batatas* exhibit the lowest DPPH• scavenging activity (6.59 ± 0.66 %). This observation was similar to Moure *et al*. (2000) and Kaur and Kapoor (2002). Ethanol is an organic solvent that effective to extract bioactive compounds from plants and did not toxic to human (Prior *et al*., 2005; Sultana *et al*., 2009; Sulaiman *et al*., 2011). However, the present of total polyphenols, phenolic acids and flavonoids content in this extract are relatively lower (0.002-0.07 mg/g) as compared to extractable polyphenols (0.004-0.52 mg/g) that detailed in Table 4. This suggest that those compounds might not be the main contributor for DPPH• scavenging activity in *C. caudatus* and *I. batatas* in ethanol

extract or *C. caudatus* and *M. esculenta* Crantz in boiled water extract. Previous study has suggested that non-phenolic compounds such as vitamins and carotenoids are capable to act as radical scavenger and thus might be attribute to this variation (Rafat *et al*., 2010; Kaneria and Chanda, 2012).

Extractable polyphenols of *E. foetidum* exhibits the highest NO• scavenging activity (50.93 ± 4.48%) followed by extractable polyphenols of *I. batatas* (46.17 ± 4.75 %), ethanol extract of *I. batatas* (45.97 ± 3.89 %), extractable polyphenols of *M. esculenta* Crantz (45.27 ± 5.30 %) and extractable polyphenols of *C. caudatus* (44.31 ± 5.59 %). Water extract of *M. esculenta* Crantz exhibit the lowest NO• scavenging activity (9.45 ± 6.99 %). Dai and Mumper (2010) also reported that extraction using high polarity organic solvents resulting in an extract with considerably more effective radical scavengers than those with less polarity organic solvents. However, extractable polyphenols in *E. foetidum* relatively compose lower total polyphenols content (0.35 ± 0.01 %), total phenolic acids content (0.39 ± 0.02 %), total flavanoids content (0.08 ± 0.02 %) and total proanthocyanidins content (0.02 ± 0 %) as compared to extracteble polyphenols of *C. caudatus*, respectively. This suggesting that those compounds may not significantly responsible for NO• scavenging activity.

Generally, organic solvents exhibit high antioxidant activity but unlike DPPH• and NO• assays, boiled water extract of *M. esculenta* Crantz exhibits the highest O_2• scavenging activity (62.77 ± 1.56 %) followed by boiled water extracts of *E. foetidum* (48.94 ± 7.19 %), *C. caudatus* (42.62 ± 5.51 %), and *I. batatas* (38.55 ± 10.80 %). Ethanol extract of *M. esculenta* Crantz exhibit the lowest NO• scavenging activity (11.20 ± 4.45 %). Taubert *et al*. (2003) reported that pyrogallol group including proanthocyanidins exhibits highest superoxide scavenging activity. However, in this study the content of total proanthocyanidins content in boiled water extract is relatively lower (0.001-0.12 mg/g) suggesting that other compounds might be responsible for the scavenging activity (Santiago *et al*., 2014). Heat treatment applied on the boiled water extract is expected to degrade cell wall (Miglio *et al*., 2008) and to promote higher solubility of the active compounds by increasing the solubility and mass transfer rate. Heat reduced viscosity and surface tension of the solvents to reach sample matrices and improve the extraction rate (Sultana *et al*., 2009; Dai and Mumper, 2010).

It is commonly known that extract composed high antioxidant compounds will also has high antioxidant activity (Wong *et al*., 2006). However, in this finding although *C. caudatus* has the highest content of antioxidant compounds (total polyphenols, total phenolics, total flavanoids and total proanthocyanidins) but it does not exhibit high antioxidant activity for DPPH•, O_2• and NO• scavenging assays, respectively. This observation was in agreement report by Rafat *et al*. (2010). This variation might be due to the degree of polymerization and the interaction between the diverse chemical structures to the colorimetric assay which affecting the antioxidant capacity (Ismail *et al*., 2004; Sulaiman *et al*., 2011).

Table 2: DPPH• scavenging activities of extractable polyphenol extract of vegetables from Sabah

No.	Vegetable species (local name)	Part tested	DPPH• scavenging activity (%)
1	*Amaranthus tricolor* (Bayam merah hijau), *Lactuca sativa* (Sayur minyak), *Brassica rapa* subspecies *chinensis* var. wavy leaf (Sawi putih keriting), *Sechium edule* (Sayur janggut), *Lactuca sativa* L. var. longifolia (Bola-bola), *Amaranthus gangeticus* var. round leaf (Bayam merah), *Amaranthus oleraceus* (Bayam kampung), *Amaranthus tricolor* var. wavy leaf (Bayam keriting), *Petroselinum crispum (*Daun pasri)	Leaf/stem	0 – 10
2	*Talinum triangulare* (Sam choi), *Spinacia oleracea* (Bayam papai), *Amaranthus paniculatus* (Bayam putih), *Brassica chinensis* var. *parhinensis communis* (Sawi manis), *Momordica charantia* (Pucuk peria), *Solanum nigrum* (Tutan), *Limocharis flava* (Tambung ambung), *Raphanus sativus* (Batang lobak putih), *Cucurbita pepo* L. (Pucuk labu merah), *Basella alba* L. (Gandula), *Monochoria vaginalis* (Tayaan), *Psophocarpus tetragonolobus* (Kacang belimbing), *Brassica chinesis* (Bunga jipun), *Sauropus androgynus* (Sayur manis), *Brassica juncea* (Sawi pahit), *Allium tuberosum* (Bunga kucai), *Brassica alboglabra* (Kailan), *Vigna unguiculata* (Daun kacang), *Brassica rapa* subspecies *chinensis* Tokyo bekana (Sawi keriting)	Leaf/stem/fruit	11 – 20
3	*Manihot esculenta* var. curly (Pucuk ubi keriting), *Crassocephalum crepidioides* (Rumpai merah), *Allium cepa* cv. Aggregatum (Daun bawang), *Carica papaya* L. (Betik), *Ipomoea aquatic* (Kangkung), *Sesbania grandiflora* (L.) Poir. (Kembang turi), *Brassica rapa* subspecies *chinensis* (Sawi putih), *Athyrium esculentum* (Pucuk paku), *Sesbania grandiflora* (L.) Poir. (Kembang turi), *Moringa oleifera* (Daun kilur), *Passiflora foetida* L. (Lapak-lapak), *Ipomoea batatas* (L.) Poir (Pucuk ubi manis)	Leaf/stem/ inflorescent	21 – 30
4	*Amaranthus gangeticus* var. oblong leaf (Bayam merah bukit), *Erechtites valerianifolia* (Sayur jipun), *Stenochlaena palustris* Bedd. (Lemiding), *Ipomoea batatas* 'Black heart' (Pucuk ubi manis merah bukit), *Carica papaya* L. (Betik)	Leaf/stem	31 – 40
5	*Oenanthe javanica* WC. (Daun selom), *Piper sarmentosum* Roxb. (Daun sirih), *Lycium chinensis* L. (Kiugi)	Leaf/stem	31 – 40
6	*Crassocephalum crepidioides* (Rumpai merah)	Root	51 – 60
7	*Centella asiatica* (Pegaga), *Ipomoea batatas* var. green star shaped leaf (Pucuk ubi manis)	Leaf/stem	61 – 70
8	*Cosmos caudatus* (Ulam raja), *Eryngium foetidum* (Bawing), *Ipomoea batatas* (Pucuk ubi rambat), *Manihot esculenta* Crantz (Pucuk ubi kayu)	Leaf/stem	81 – 90
9	Catechin (positive control)		90.96 ± 1.09

Table 3: Antioxidative activities of selected vegetables species extracted with different solvents.

Sample	DPPH• scavenging activity (%)			O_2• scavenging activity (%)			NO• scavenging activity (%)		
	Extractable polyphenols	Ethanol	Boiled water	Extractable polyphenols	Ethanol	Boiled water	Extractable polyphenols	Ethanol	Boiled water
Cosmos caudatus	83.09 ± 3.22^{a}	84.68 ± 0.29^{c}	76.83 ± 2.15^{c}	22.98 ± 8.95^{a}	21.01 ± 7.28^{cd}	42.62 ± 5.51^{ab}	44.31 ± 5.59^{a}	44.29 ± 3.23^{b}	41.66 ± 5.08^{d}
Eryngium foetidum	82.63 ± 3.18^{a}	75.87 ± 8.84^{b}	20.53 ± 0.99^{b}	33.67 ± 5.67^{b}	17.34 ± 6.92^{bc}	48.94 ± 7.19^{b}	50.93 ± 4.48^{b}	38.71 ± 8.78^{ab}	26.14 ± 2.45^{c}
Ipomoea batatas	89.43 ± 0.37^{b}	90.56 ± 0.24^{d}	6.59 ± 0.66^{a}	18.44 ± 8.77^{a}	27.21 ± 7.50^{d}	38.55 ± 10.80^{a}	46.17 ± 4.75^{ab}	45.97 ± 3.89^{b}	17.19 ± 2.57^{b}
Manihot esculenta Crantz	84.50 ± 2.75^{a}	26.58 ± 4.07^{a}	75.54 ± 0.59^{c}	20.19 ± 4.28^{a}	11.20 ± 4.45^{ab}	62.77 ± 1.56^{c}	45.27 ± 5.30^{ab}	34.18 ± 13.64^{ab}	9.45 ± 6.99^{a}
Catechin (positive control)	90.96 ± 1.09^{b}	90.96 ± 1.09^{d}	90.96 ± 1.09^{d}	41.00 ± 9.69^{c}	41.00 ± 9.69^{e}	41.00 ± 9.69^{ab}	64.18 ± 1.43^{c}	64.18 ± 1.43^{c}	64.18 ± 1.43^{e}

Values are mean ± standard deviation of three replicates. ***DPPH• -****tested at the concentration of 0.5 mg/mL.* ***O_2• -*** *tested at the concentration of 0.053 mg/mL.* ***NO• -*** *tested at the concentration of 0.025 mg/mL. Mean values between plant samples were compared using Duncan's multiple range test at $p<0.05$.*

Table 4: Phytochemicals content of selected vegetables species extracted with different type of solvents

Sample	Total polyphenols content (mg GAE/g sample)			Total phenolics acid content (mg GAE/g sample)			Total flavonoids content (mg CE/g sample)			Total proanthocyanidins content (mg CE/g sample)		
	Extractable polyphenols	Ethanol	Boiled water	Extractable polyphenols	Ethanol	Boiled water	Extractable polyphenols	Ethanol	Boiled water	Extractable polyphenols	Ethanol	Boiled water
Cosmos caudatus	0.40 ± 0.01^{c}	0.05 ± 0^{c}	0.06 ± 0^{c}	0.52 ± 0.01^{c}	0.05 ± 0^{c}	0.07 ± 0^{c}	0.13 ± 0.01^{c}	0.01 ± 0^{a}	0.014 ± 0^{b}	0.12 ± 0^{d}	0.01 ± 0^{c}	0.01 ± 0^{c}
Eryngium foetidum	0.35 ± 0.01^{bc}	0.03 ± 0^{b}	0.02 ± 0^{a}	0.39 ± 0.02^{b}	0.04 ± 0^{b}	0.02 ± 0^{a}	0.08 ± 0.02^{b}	0.02 ± 0^{b}	0.004 ± 0^{a}	0.02 ± 0^{b}	0.01 ± 0^{c}	0.002 ± 0^{b}
Ipomoea batatas	0.31 ± 0.02^{ab}	0.07 ± 0.01^{d}	0.02 ± 0^{a}	0.41 ± 0.01^{b}	0.06 ± 0^{d}	0.02 ± 0^{a}	0.12 ± 0.01^{c}	0.02 ± 0^{b}	0.004 ± 0^{a}	0.004 ± 0^{a}	0.002 ± 0^{a}	0.002 ± 0^{b}
Manihot esculenta Crantz	0.27 ± 0.02^{a}	0.02 ± 0^{a}	0.03 ± 0^{b}	0.29 ± 0.01^{a}	0.02 ± 0^{a}	0.03 ± 0^{b}	0.04 ± 0.01^{a}	0.01 ± 0^{a}	0.002 ± 0^{a}	0.04 ± 0^{c}	0.004 ± 0^{b}	0.001 ± 0^{a}

Values are mean ± standard deviation of three replicates. GAE: garlic acid equivalent. CE: catechin equivalent. Mean values between plant samples were compared using Duncan's multiple range test at $p<0.05$.

Antikinase and Antiphosphatase Activities of Selected Vegetables Species

Free radicals such as reactive oxygen species (ROS) have been associated with a wide array of human diseases including cancer (Waris and Ahsan, 2006). ROS caused an indirect oxidative damage on DNA through lipid peroxidation and affecting cytoplasmic and nuclear signal transduction pathways (Matés and Sánchez-Jiménez, 2000; Waris and Ahsan, 2006). Interference of ROS on signal cascade system such as mitogen activated protein kinases (MAPKs) has led to carcinogenesis (Matés and Sánchez-Jiménez, 2000).

MKK is a cytoplasmic protein kinase that binds specifically to MAPK as activator (Brunet *et al*., 1999). $MKK1^{P386}$ is a hyperactive mutation of MAPK Kinase (MKK1) that has proline instead of serine at the position 386. In galactose media, the presence of galactose will induce the GAL1 promoter which resulting in the overexpression of this mutant gene thus suppressing both the Pkc1 and Bck1 deletion and inhibit the growth of the yeast. As a result, in the present inhibitor will cause yeast growth on galactose media but not on glucose media (Watanabe *et al*., 1995; Pang *et al*., 2009). $MKK1^{P386}$ inhibitor can either target on MKK1 or MPK1 in the PKC1 pathway thus MAP Kinase Phosphatase (MSG5) screening test was carried in order to specify the inhibition (Watanabe *et al*., 1995; Pang *et al*., 2009). MSG5 is a protein tyrosine phosphatase that belongs to a novel subclass of protein phosphatases whose substrates is MAP kinase family members (Doi *et al*., 1994). In the MSG5 screening test, $MKK1^{P386}$-MSG yeast able to grow on both glucose and galactose media thus inhibitory activity for MKK1 is confirmed if no yeast growth on galactose media but growing on glucose media (Doi *et al.,* 1994; Watanabe *et al*., 1995). Meanwhile, glycogen synthase kinase-3 (GSK-3) is a highly conversed protein kinase that involved significantly with diverse physiological process such as cancer (Ougolkov and Billadeau, 2006), diabetes (Ross *et al*., 1999) and neurological disorders (Eldar-Finkelman, 2002). GSK-3 is an unusual protein kinase where it is normally active in cells and it is primarily regulated through the inhibition of its activity (Doble and Woodgett, 2003). In addition to that, compared with the other protein kinases, GSK-3 preference for primed substrates which are previously phosphorylated by another kinase (Doble and Woodgett, 2003). Potential inhibitor will caused the inhibition of the yeast incubated on 37°C due to the *gsk-3* null mutant yeast exhibits temperature sensitivity and no significant activity on 25°C (Andoh *et al*., 2000). However, in this study none of the extracts have inhibition activity towards kinase or phosphatase screening assays.

Antimicrobial Activities of Selected Vegetables Species

Nine microbial species tested in these screening assays which are *Candida albicans, Candida krusei*, *Staphylococcus aureus* and *Streptococcus pneumonia*, *Enterobacter aerogenes*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Those microbes have long history caused a wide variety of human diseases and some are capable of producing highly toxic compounds that adversely affect human health (You, 2006). The results from the tests (Table 5) showed that only *P. aeruginosa* was inhibited by the extracts. This finding is in contrast to Rasdi *et al*. (2010) where the ethanol extract of *C. caudatus* has the ability to inhibit *E. aerogenes* and *C. albican*; and Noumedem *et al*. (2013) on methanol extract of *M. esculenta* Crantz that inhibit growth of *E. aerogenes.* The variation in this result might be due to the differences in the solvent, extraction system, growth condition and plant maturity that might affect the results (Faiza *et al*., 2013).

Pseudomonas aeruginosa is a versatile gram-negative bacterium and one of the top three leading causes of human infection due to its intrinsic resistance to antibiotics and disinfectants (Stover *et al*., 2000). Antibacterial activity of the tested extracts ranging from 8.0 mm to 12.3 mm. Inhibition of bacteria exhibited by both extractable polyphenols and ethanol extracts but not in boiled water extract. Extractable polyphenols of *E. foetidum* exhibits the largest inhibition (12.33 ± 0.47 mm) followed by ethanol extract of *E. foetidum* (11.33 ± 0.47 mm), and extractable polyphenols and ethanol extract of *I. batatas* (9.00 ± 0 mm). The lowest inhibition of *P. aeruginosa* was observed on extractable polyphenols and ethanol extract of *C. caudatus* (8.00 ± 0 mm). This finding is in agreement with report by Ahmad *et al*. (2012) and Faiza *et al*. (2013). The antibacterial properties might be due to the availability of phenolics and flavonoids compounds that able to disrupt bacterial DNA replication (Jayalakshmi *et al*., 2013).

Table 5: Antibacterial activity of selected vegetables species extracted with different type of solvents

Sample	Type of solvents	*Pseudomonas aeruginosa*
Cosmos caudatus	Extractable polyphenols	8.00 ± 0^{b}
	Ethanol extract	8.00 ± 0^{b}
	Boiled water extract	0^{a}
Eryngium foetidum	Extractable polyphenols	12.33 ± 0.47^{f}
	Ethanol extract	11.33 ± 0.47^{e}
	Boiled water extract	0^{a}
Ipomoea batatas	Extractable polyphenols	9.00 ± 0^{d}
	Ethanol extract	9.00 ± 0^{d}
	Boiled water extract	0^{a}
Manihot esculenta Crantz	Extractable polyphenols	8.33 ± 0.47^{bc}
	Ethanol extract	8.67 ± 0.47^{cd}
	Boiled water extract	0^{a}
Penicillin	Positive control	14.67 ± 0.47^{g}

Values are mean ± standard deviation of triplicate analyses. All samples were tested at the concentration of 100 mg/mL. Positive control was tested at the concentration of 1% (w/v), respectively. Extractable polyphenol is the combination of methanol and acetone extracts. Means in each column with different alphabets are significantly different tested using Duncan's multiple range test at $p<0.05$.

CONCLUSION

A total of 55 vegetable samples were screened for their antioxidant activity using the DPPH• scavenging assay. Four selected species with high DPPH• scavenging activity that are *Cosmos caudatus, Eryngium foetidum, Ipomoea batatas* and *Manihot esculenta* Crantz were further extracted with different solvents system. Ethanol extract of *I. batatas*, extractable polyphenols of *E. foetidum* and boiled water extracts of *M. esculenta* Crantz exhibits higher scavenging activity towards DPPH•, O_2• and NO•, respectively. Polyphenols, phenolic acids, flavonoids and proanthocynidins are observed in all extracts, however, the highest total polyphenols content, total phenolics content, total flavonoids content and total proanthocyanidins content were obtained in extractable polyphenols of *C. caudatus*, indicating that these phytochemicals may not significantly contribute to the scavenging

activity in ethanol extract of *I. batatas*, boiled water extract of *M. esculenta* Crantz and extractable polyphenols of *E. foetidum*. No extracts observed as inhibitor to GSK-3β, MKK1 and MSG5, while inhibition of *Pseudomonas aeruginosa* was obtained only on extractable polyphenols and ethanol extracts. This finding has suggested that vegetables found in Sabah have value-added benefits for human health as an alternative for nutraceutical product development.

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CHEMICAL AND MICROBIAL EVALUATION OF SOME UNCOMMON INDIGENOUS FRUITS AND NUTS

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ABSTRACT. *Fruits and nuts are essential components of animal and human diets and desert. They represent diverse genetic resources in tropical and subtropical regions of the world. In Nigeria, exotic fruits are more popular as indigenous ones are largely underutilized. This study examined the chemical components of five uncommon fruits: African oil bean [Pentaclathra macrophylla, Fabaceae], Bambara groundnut [Vigna subterranean, Fabaceae], African bush mango [Irvingia gabonensis, Irvingiaceae], African pear [Dacryodes edulis, Burseraceae] and Nigerian walnut [Tetracarpidium conophorum, Euphorbiaceae]. The fresh fruits/nuts were collected from parts of Edo State, Southern Nigeria. Results showed that alkaloid was present in fresh and cooked samples of walnut and pear, and only present in fresh samples of P. macrophylla and V. subterranean. Alkaloid was absent in both fresh and cooked I. gaborensis. The result of mineral composition of the samples suggests highest calcium, potassium and magnesium content was obtained in African pear and P. macrophylla for sodium, zinc and iron. Presence of ascorbic acid, thiamine, riboflavin and lactic acid was confirmed in all the fruits. The highest concentration of ascorbic acid, thiamine, riboflavin and lactic acid was obtained from I. gaborensis, V. subterranean, P. macrophylla and D. edulis respectively. The highest bacterial and fungal count in fresh samples was from Bambara groundnut and P. macrophylla respectively. This study has implicated the relevance of these uncommon fruits and nuts. It is recommended therefore that their awareness should be improved in order to sustain their marketability and food use.*

KEYWORDS: Tropical fruits and nuts, Underutilized fruits, Plant genetic resources, Vegetables, Phytochemicals, Nigeria

INTRODUCTION

Tropical and subtropical regions of the world are endowed with fruits. Fruits and nuts may be classified as vegetables. Botanically they are diverse in characters and circumscription. According to Aigbokhan (2014) a plant may play primary or secondary roles as fruits. Economically, they have the potential to attract foreign exchange. Ethnobotanically fruits are essential components of different cultural, social and spiritual functions especially African and Asia. Fruits are plagued with varying problems, including production, storage, transports and marketability. Many fruits are classified as underutilized or underexploited. More so in the opinion of Acquaah (2007) consumers prefer succulent and juicy fruits.

Most traditional fruits and vegetables have a problem of appealing to consumers who view consider them as unhealthy and unsafe for human consumption. Among these are: African oil bean [*Pentaclathra macrophylla* Benth, Fabaceae], Bambara groundnut [*Vigna subterranean* [Lin.] Verdc, Fabaceae], African bush mango [*Irvingia gabonensis* (Aubry ex O' Rorke) Baill, Irvingiaceae], African pear [*Dacryodes edulis* (G. Don) H.J. Lam., Burseraceae] and Nigerian walnut [*Tetracarpidium conophorum* (Mull. Arg.) Hutch and Dalziel, Euphorbiaceae]. Eze (2012) recognized D. *edulis, V. subterranean and P. macrophylla* as underutilized in spite of their economic oil and suitability to tropical and subtropical climes. V. *subterranean* is a hardy plant able to withstand high temperature and dry conditions (Stone *et al.* 2011). *P. macrophylla* popularly called ugba in southern Nigeria possess edible seed and leaves (Idu *et al.,* 2011). African pear is a fruit tree native to Nigeria and Cameroon, whose fruits may be eaten fresh, boiled or roasted alone or with *Zea mays* (Vivien & Faure, 1996). *D. edulis* is also called butterfruit because of its creamy, delicious, oily pulp rich in amino acid such as leusine and lysine as well as micronutrients and minerals (Stone *et al.,* 2011). The juicy fruit pulp of *I. gabonensis* is rich in Vitamin C and is widely consumed as a dessert fruit or snacks through Western and Central Africa while its pulp can be used in making jam, jelly and juice (Ejiofor, 1994; Leaky *et al.*, 2005 & Okolo *et al.,* 1999). The seeds are sun dried and ground to be used as a thickener in soup in Nigeria. *V. subterranean* is indigenous to tropical African and is highly overlooked by researcher, development agencies and humanitarian programs, even though they are packed with nutrients (Stone *et al*., 2011). Ripe fruits of T. *conophorum* is boiled and eaten as a snack, which is high in protein and polyunsaturated fat (Onimawo, 2010). It is popular for its aftertaste, medicinal properties and source of conophor oil. The study of Ogwu *et al*., (2014) reveal that fruits and nuts represent over 50 % of plant diversity in home gardens in Edo state.

This study aims to conduct microbial assessment and determine the nutritional and chemical composition of cooked and uncooked *P. macrophylla, T. conophorum, I. gaborensis, V. subterranean and D. edulis.* The study will provide supporting data for the food use of these fruits and increase awareness about their health benefits.

MATERIALS AND METHODS

STUDY AREA: The study area has climatic conditions typical of tropical regions and is positioned between Longitude 06^0 04^1 E 06^0 43^1 E and Latitude 05^0 44^1 N and 07^0 34^1 N. Detailed description of the study area have been reported by Osawaru & Ogwu (2014).

COLLECTION OF SAMPLES: All the samples used in this study (Plates 1 – 5) were collected from home gardens, distant farms, markets and as ruderals from Edo state southern Nigeria. A survey was conducted hitherto to implicate them as uncommon consumed fruits in parts of Nigeria. Actual collection was done according to Osawaru & Ogwu (2014).

Plate 1: Bambara groundnut (*Vigna subterranean*)

Plate 2: Africa pear (*Dacryodes edulis*)

Plate 3: African oil bean (*Pentaclathra macrophylla*)

Plate 4: Bush mango (*Irvingia gaborensis*)

Plate 5: Nigerian Walnut (*Tetracarpidium conophorum*)

PREPARATION OF SAMPLES

Tetracarpidium conophorum (Walnut)**:** Walnut samples were categorized into fresh and spoilt. Analytical work on fresh samples were carried out two days after collection while those for spoilt samples were obtained after storing the fresh samples under natural conditions for 10 days. Some samples were left in order to determine the shelf life of the fruits. Samples of walnut were divided into two groups A-B, group A was subjected to mild cooking at boiling water temperature (100 ^{0}C) for 10 minutes. Samples in group B were left in its fresh state and categorized as "uncooked"

Vigna subterranean (Bambara groundnut)**:** The fruits and seeds were carefully cleansed with distilled water and partitioned into two for the purpose analysis (cooked and uncooked). The raw seeds were classified as uncooked seed. The cooked seed was prepared by soaking the raw seeds in water for 14 h at room temperature (24 ± 2 ºC) with a seed to water ratio of 1:5 (w/v). Thereafter, the soaked seeds were washed twice with ordinary water, followed by rinsing with distilled water and then dried in a hot air oven at 50 ºC for 24 h. The soaked seeds were placed in a round-mouthed tall beakers fitted with condensers. The contents of the beaker were cooked. Cooked seeds, along with cooking water, were dried at 50 ºC for 24 h.

Pantaclethra macrophylla (African oil bean seed): The seeds were thoroughly washed to remove extraneous materials and visually inspected in order to discard defective seeds. The selected seeds were dried in an oven at 45 ^{0}C for 8 hours the difference in weight after drying was used to determine the moisture content of the raw seed sample. The dried

samples were ground into powder for subsequent analysis. The cooked sample was obtained by parboiling of the whole seeds for 4 hours, which facilitated the removal of the shell to obtain the cotyledon.

Irvingia gabonensis (African mango): Samples were categorized into fresh and spoilt. Analytical works were carried out within two days after purchase. The spoilt samples were obtained after storing the fresh samples under natural conditions for 10 days. Some samples were left in order to determine the shelf life of the fruits.

Dacryodes edulis (African pear): Samples of African pear were divided into two groups A - B, group A was subjected mild cooking at boiling water temperature (100 ^{0}C) for 10 minutes. Samples in group B were left in its fresh state and categorized as "uncooked". The pulp was separated from the fruit with a sharp knife.

CHEMICAL ANALYSIS OF SAMPLES: The moisture, crude fiber, fat and ash contents were determined according to AOAC method (1996). Crude protein was determined using Kjeldahl method as recommended by AOAC (2000). Carbohydrate content was determined using Anthrone method according to Pearson *et al*. (1976). Mineral analysis was carried out using the methods of Omogbai & Ojeaburu (2010). The method as proposed by Trease and Evans (1983) was adopted for detecting alkaloids. The following micronutrients were determined using standard procedures: ascorbic acid content, thiamine and total titrable acidity

MICROBIOLOGICAL ANALYSIS OF SAMPLES: Nutrient agar was prepared according to Lapage *et al.* (1970); American Public Health Association (2001 & 2004) & Horwitz *et al.* (2007). The method of American Public Health Association (1992; 1993 & 2001) was adopted in the preparation of potato dextrose agar. Serial dilution was done as enumerated by Aneja (2005).

SHELF LIFE STUDY OF AFRICAN MANGO: The shelf life study on African mango fruit was determined by monitoring the microbial load of the fruit stored at room temperature (25.0 ± 2.5^{0}C). Microbial counts were taken at an interval of five days. Physical and sensual characteristics of the samples were also examined.

STATISTICAL ANALYSIS: Data obtained were subjected to descriptive statistical analysis as described by Ogbeibu (2005).

RESULTS

Results are presented in Tables 1 - 10.
Alkaloid was present in fresh and cooked samples of Nigerian walnut and African pear, and only present in fresh samples *P. macrophylla* and *V. subterranean.* Alkaloid was absent in both fresh and cooked *I. gaborensis*.

Table 1: Qualitative examination of alkaloids in fresh and cooked uncommon fruit samples

SAMPLES	FRESH	COOKED
Nigerian walnut seeds	+	+
African pear pulp	+	+
Pantaclethra macrophylla seeds	+	-
African mango pulp(15 days)	-	-
Bambara groundnut seeds	+	-

Key: + = Present
= Absent

The result of mineral composition of the samples are presented in Tables 2. The highest calcium, potassium and magnesium content was obtained from African pear and *P. macrophylla* for sodium, zinc and iron.

Table 2: Mineral composition (mg/100g) of fresh and cooked uncommon fruit samples

	Sodium		Calcium		Potassium		Zinc		Iron		Magnesium	
	(mg/100g) ± SD											
Samples	Fresh	Cooked	Fresh	Cooked	Fresh	Cooked	Fresh	Cooked	Fresh	Cooked	Fresh	Cooked
NW	44.570 ± 1.37	37.05± 0.13	48.40 ±3.13	38.07± 2.53	61.35 ±1.61	58.31±1.30	5.44± 0.37	6.52±0.41	2.76± 0.11	1.75±0.05	55.66 ±0.20	47.28± 1.00
AFP	135.065±3.44	108.10 ±8.01	328.7 ±4.02	290.52 ±5.08	472.1 ±21.32	487.65± 8.56	3.89± 0.1	5.28±0.13	2.205 ±0.18	1.71±0.25	247.455±8.13	224.15 ±3.31
PM	320.85±8.81	267.17 ±7.88	201.34±7.96	221.87 ±4.65	270.84±5.39	243.38± 10.85	5.48± 0.32	9.79±0.23	9.61± 0.01	9.73±0.17	190.865±6.51	223.91 ±6.44
BG	9.245 ±0.08	5.53±0.58	219.94±3.39	177.37 ±15.39	250.415±4.36	221.11± 14.74	2.64± 0.13	1.33±0.10	5.46± 0.25	3.37±0.40	112.315±0.73	98.60± 0.49
AM	165.535±1.46	147.615± 0.58	242.58 ± 1.46	218.43 ± 0.51	85.26 ± 1.22	51.475 ± 1.63	2.62 ± 0.13	1.680 ± 0.20	3.325 ± 0.22	1.395 ± 0.29	16.7 ± 1.91	10.475 ± 0.60

Values are means ± standard deviations of duplicates.
Keys: NW (Nigerian walnut), APF (African pear fruit), PM (*Pantaclethra macrophylla* seeds), BG (Bambara groundnut) and AM (African Mango).

The proximate composition of *P. macrophylla* is presented in Table 3. Result suggest high amount of crude fat and protein.

Table 3: Proximate analysis of fresh and cooked *Pantaclethra macrophylla* (African oil bean seed)

Proximate Analysis (%)	Fresh samples	Cooked samples
Moisture	10.135± 0.150	8.535 ± 0.390
Crude fat	45.600 ± 0.180	50.170 ± 0.110
Crude protein	24.700 ± 0.170	22.800 ± 0.030
Carbohydrate	13.230 ± 0.250	11.465 ± 0.150
Ash	3.180 ± 0.060	2.820 ± 0.160
Crude fibre	3.155 ± 0.050	4.210 ± 0.480

Values are means ± standard deviations of duplicates.

The result of proximate composition of Bambara groundnut is presented in Table 4. High amount of carbohydrate and crude protein were recorded.

Table 4: Proximate analysis of fresh and cooked Bambara groundnut (*Vigna subterranean*).

Proximate Analysis (%)	Fresh samples	Cooked samples
Moisture	8.450 ± 0.460	7.435 ± 0.260
Crude fat	6.240 ± 0.090	8.320 ± 0.040
Crude protein	21.355 ± 0.290	19.685 ± 0.090
Carbohydrate	54.505 ± 0.190	57.585 ± 0.250
Ash	3.690 ± 0.370	3.300 ± 0.400
Crude fibre	5.7450 ± 0.110	3.680 ± 0.170

Values are means ± standard deviations of duplicates.

Proximate composition of African bush mango is presented in Table 5. High amount of moisture was obtained which was not reduced by cooking.

Table 5: Proximate analysis of fresh and spoilt *Irvinga gabonesis* fruit pulp (African mango).

Proximate Analysis (%)	Fresh samples	Spoilt samples (15 days)
Moisture	83.610 ± 0.570	85.690 ± 0.810
Crude fat	2.610 ± 0.050	2.240 ± 0.090
Crude protein	3.060 ± 0.270	4.390 ± 0.340
Carbohydrate	3.940 ± 0.250	1.220 ± 0.092
Ash	2.540 ± 0.510	1.520 ± 0.130
Crude fibre	4.250 ± 0.134	4.950 ± 0.510

Values are means ± standard deviations of duplicates.

The proximate composition of Nigeria walnut seeds is presented in Table 6. High moisture content, crude protein and carbohydrate and low crude fat, crude fibre and ash were suggested by the result.

Table 6: Proximate analysis of fresh and cooked *T.* conophorum seeds samples.

Proximate Analysis (%)	Fresh samples	Cooked samples
Moisture	43.760 ± 0.085	42.775 ± 0.250
Crude fat	5.495 ± 0.021	10.300 ±0.200
Crude protein	24.250 ± 0.340	23.385 ± 0.330
Carbohydrate	19.205 ± 0.710	17.340 ± 0.200
Ash	2.940 ± 0.230	2.6150 ± 0.120
Crude fibre	4.350 ± 0.210	3.560 ± 0.420

Values are means ± standard deviations of duplicates determination.

The result of proximate composition of African pear is presented in Table 7. Results suggest high percentage moisture, crude fat and carbohydrate and low crude fibre, ash and crude protein.

Table 7: Proximate analysis of fresh and cooked *Dacryodes edulis* fruit pulp

Proximate Analysis (%)	Fresh samples	Cooked samples
Moisture	46.400 ± 0.156	45.595 ± 0.120
Crude fat	34.445 ± 0.450	39.125 ± 0.120
Crude protein	3.375 ± 0.134	2.470 ± 0.440
Carbohydrate	11.735 ± 0.064	9.275 ± 0.370
Ash	2.590 ± 0.028	2.250 ± 0.100
Crude fibre	1.455 ± 0.064	1.215 ± 0.021

Values are means ± standard deviations of duplicates determination.

The result of micronutrient analysis is presented in Table 8. Highest concentration of ascorbic acid, thiamine, riboflavin and lactic acid was obtained from *I. gaborensis, V. subterranean, P. macrophylla* and *D. edulis* respectively. In African Mango, the results suggest high amounts of ascorbic acid in fresh and cooked samples as well as low in thiamine, riboflavin and lactic acid.

Table 8: Micronutrient composition (mg/100 g) of some fresh and cooked uncommon fruits samples.

	Ascorbic acid(mg/100 g) ± SD		Thiamine (mg/100 g) ± SD		Riboflavin (mg/100 g) ± SD		Lactic acid (mg/100 g) ± SD	
Samples/treatments	Fresh	Cooked	Fresh	Cooked	Fresh	Cooked	Fresh	Cooked
NW	51.900 ± 0.260	47.360 ± 0.530	0.020 ± 0.000	0.001 ± 0.000	0.004 ± 0.000	0.001 ± 0.000	0.070 ± 0.000	0.160 ± 0.004
APF	34.83 ± 0.040	26.590 ± 0.260	0.160 ± 0.030	0.100 ± 0.007	0.650 ± 0.060	0.054 ± 0.003	0.520 ± 0.057	1.135 ± 0.053
PM	18.80 ± 0.270	15.600 ± 0.420	0.072 ± 0.010	15.600 ± 0.420	0.071 ± 0.010	0.040 ± 0.000	0.003 ± 0.000	0.001 ± 0.000
BG	0.058 ± 0.004	0.250 ± 0.002	0.660 ± 0.050	0.565 ± 0.035	0.430 ± 0.028	0.355 ± 0.035	0.091 ± 0.001	0.128 ± 0.003
AM	62.560 ± 0.570	58.830 ± 0.040	0.050 ± 0.000	0.003 ± 0.000	0.003 ± 0.000	0.0205 ± 0.0007	0.210 ± 0.02	0.085 ± 0.010

Values are means ± standard deviations of duplicates

Key: NW (Nigerian walnut), APF (African pear fruit), PM (*Pantaclethra macrophylla seeds*), BG (Bambara groundnut) and AM (African Mango)

The result of microbial count of fresh and cooked samples is presented in Table 9. The highest bacterial and fungal count in fresh samples was from *V. subterranean* and *P. macrophylla* respectively.

Table 9: Microbial load (cfu/g) of some fresh and cooked uncommon fruit samples

	Bacterial count (cfu/g)		Fungal count (cfu/g)	
Samples/treatments	Fresh	Cooked	Fresh	Cooked
NW	7.4×10^2	5.3×10^1	1.1×10^2	2.2×10^1
APF	4.1×10^3	2.4×10^1	2.7×10^3	1.2×10^1
PM	2.5×10^4	6.4×10^3	8.1×10^3	2.3×10^3
BG	7.8×10^4	4.2×10^2	4.9×10^2	8.4×10^1

Value are means ± standard deviations of duplicates
KEYS: NW (Nigerian walnut), APF (African pear fruit), PM (*Pantaclethra macrophylla* seeds) and BG (*V. subterranean).*

Microbial load of the fruit was monitored and expressed in (cfu/g).

Table 10: Shelf life monitoring of *I. gabonensis* stored at 28 ± 2°C.

Time (days)	Microbial load (cfu/g)
One	2.0×10^3
Five	4.7×10^6
Ten	1.3×10^8
Fifteen	5.8×10^{11}

DISCUSSION

The study has investigated the phytochemical composition and microbial status of five uncommon fruits in Edo state southern Nigeria. The fruit samples were collected and analyzed for their proximate composition, mineral composition, micronutrient composition and the shelf life was determined by monitoring the microbial load based on a time interval of five days. Most tropical fruits are underutilize and under exploited regardless of enormous attention from researchers. Rural dwellers depend on wild fruits to meet their daily food needs as well as income generation. Fruits are examples of vegetables and may provide daily energy, protein and vitamins. They are components of balanced diets.

The qualitative examination for alkaloids in fresh and cooked uncommon fruit samples suggest the presence of alkaloids in the samples. *P. macrophylla* seeds, African pear and Bambara groundnut gave a positive result, while negative result was obtained from Fresh African mango pulp and African mango pulp. It was shown that, prolonged cooking had some effect on the qualitative presence of alkaloids in *P. macrophylla* seeds and Bambara groundnut (Table 1).

Fruits are suppliers of minerals and vitamins, which may influence the wellbeing and health status of individuals. The macro and microelemental composition of well-known tropical fruits such as banana, sweet lime, African pear, orange, passion fruit and others have been reported (Eromosele *et al*., 1991; Aremu & Udoessien, 1990 & Burguera *et al*., 1992). The results of the present study are in agreement with similar aspects of Bratte *et al.* (2010) which revealed high crude protein, ether extracts, crude fibre, ash and nitrogen-free extracts, trace amounts of the essential and non-essential amino acids and vitamins, which indicate they can be classified as an energy feed.

The phytochemical, vitamins and proximate composition of *D. edulis* at different stages of maturation were investigated by Majesty *et al.* (2012), the results obtained reveal the presence of flavonoids, alkaloids, saponins, tannins, cyanogenic glycosides, oxalate, thiamine, riboflavin, niacin, ascorbic acid and tocopherol. The Chemical composition and the effect of heat treatment on seeds of *Dacryodes edulis* were studied by Ujowundu *et al.* (2010) to suggest that among the proximate analysis, the moisture and carbohydrate values were the highest. Potassium, calcium, and phosphorus were also predominant while Sodium, magnesium, selenium, zinc and iron were present in appreciable amounts but manganese was not detected. These results correspond with those obtained in this study. More so, the result agrees with the report of Onyeike *et al.* (1995) that the crude fat is present in *I. gabonensis* seeds. A significant result obtained in this study is the absent of alkaloid in both samples in *I. gabonensis* seeds. This could be attributed to the method used in the analysis. This is similar to the observations of Ogunmefun *et al.* (2013). More so, it could be as a result of the source of the samples.

The seeds of *P. macrophylla* are rich in protein, oil and energy as well as in sodium, potassium, magnesium, calcium and phosphorus while iron, zinc, copper and lead may be of lower concentrations (Oyeleke *et al.,* 2014). This suggests that African oil bean seed has a potential for dietary improvement in food industries. Meanwhile, in the same study, arsenic and cadmium were not detected as tannin, saponin and flavonoid were present while cardiac glycoside and alkaloid are absent. The present study suggests the presence of alkaloids in fresh samples of African oil bean.

Though *T. conophorum* nuts are generally eaten in Nigeria, very little work has been done on the proximate composition and heavy metal content of this nut. The aftertaste of *T. conophorum* could be attributed to the presence of alkaloids. *T. conophorum* is used as a male-fertility agent (Ajaiyeoba & Fadare, 2006). Edem *et al*. (2009) reported the proximate composition, ascorbic acid and heavy metal contents of the nut. Ayodele (2003) reported the presence of oxalates, phylates and tannin in the raw *T. conophorum* nuts. Oyenuga (1997) reported on the amino acid and fatty acid components of the nut and on the use of its leaf juice for the treatment of prolonged and constant hiccups. Nwokolo (1987) also reported on the impact of traditional processing on the nutrient and sensory qualities of the nut. Okpero (2001) reported on the methods of processing the *T. conophorum* nuts while Okafor (1988) reported on the use of *T. conophorum* seeds and processing waste in livestock feed formulation. Walnuts are rich in linoleic and linolenic acids and in other health-related compounds such as high-biological-value proteins (e.g. arginine) fibre, vitamins, tannins, folates and polyphenols which may provide additional antiatherogenic properties (Nus *et al*., 2004). Walnuts contain polyunsaturated fatty acids, which may protect against cardiovascular disease (CVD) and may enhance tocopherol absorption (Jeanes *et al*., 2004).

The results obtained for the mineral composition of fresh and cooked uncommon fruit samples suggest the relative presence of these essential minerals. The mineral composition of fresh fruits is reduced by cooking. Although, it is important to note that the concentration of zinc (mg/100 g) was shown to increase in the cooked samples as against the fresh samples in Bambara groundnut. More so, the concentration of Iron (mg/100g) was shown to increase in the cooked samples as against the fresh samples only in Bambara groundnut. The mineral composition (mg/100 g) of fresh and spoilt African mango sample was shown to decrease significantly with time. The result of proximate analysis of fresh and cooked *Pantaclethra macrophylla* (African oil bean seed) gave credence to the name of the seed (African oil bean seed). It was shown that the oil content of the cooked seed constitute close to 50 % of the seed composition. The crude fibre of the seed was shown to increase on cooking.

In fresh and cooked Bambara groundnut (*Vigna subterranean*), it was shown that carbohydrate constitutes over 50% of the fresh seed proximate composition, although this value was shown to reduce on cooking. The crude protein composition of the fresh seed was shown to reduce on cooking. Other parameters affected by the treatment include; crude fibre, ash, and moisture. The crude fat content was shown to increase significantly on cooking.

The crude fibre content of fresh and spoilt *Irvinga gabonesis* fruit pulp (African mango) was shown to slightly increase in the spoilt sample; the carbohydrate content was also shown to reduce. There was an increase in the moisture content of the spoilt sample, suggesting an increase in the metabolic activity of microorganisms in the spoilt sample. Microorganisms are known to break down carbohydrate with the release of water and carbon dioxide. The levels of moisture, crude protein, ash, carbohydrate and crude fibre in *Tetracarpidium conophorum* (Nigerian walnut) seeds samples were shown to be negatively affected by the treatment. Although, the level of crude fat was shown to be affected positively, suggesting that heat treatment liberated some of the fruit fatty acids from triacylglycerol. For *Dracryodes edulis* fruit pulp, result obtained showed that the fruit is highly rich in oil; the oil content of the fruit was also shown to increase significantly on cooking. In the study by Yusuf *et al.* (2012) the oil extracted from *I. gabonensis* showed high level of saturation and the analysis revealed that it is rich in Myristic and lauric acid, which makes it very suitable for cosmetics and pharmaceuticals.

The micronutrient composition (mg/100 g) of some fresh and cooked uncommon fruit samples suggests the presence of some essential micronutrient. The ascorbic acid content, thiamine levels, riboflavin concentration and lactic acid were present. There was a marked change in the ascorbic acid content of all fresh and cooked uncommon fruit samples analyzed. This suggests that heat treatment has an effect on the Vitamin C content of fruits. Thiamine and riboflavin concentration was observed to be higher in the fresh samples when compared to the cooked samples. The Lactic acid concentration was observed to increase on cooking. The micronutrient composition (mg/100 g) of fresh and spoilt *Irvinga gabonesis* fruit pulp (African mango) showed great difference between the micronutrient composition of fresh African mango pulp and the spoilt fruit. The micronutrient content was shown to decrease as a result of deterioration of the fruit pulp by microorganisms. These micronutrients play essential roles in the human diet.

The bacterial load of the fresh samples was shown to decrease on cooking. Nigerian walnut had the lowest bacterial load, while Bambara groundnut had the highest bacterial load. The fungal load of the fruit samples was comparatively lower to the bacterial load. The shelf life was determined by storing the fruit sample at a temperature of 28 ± 2 °C for a total period of fifteen days when the physical appearance of the fruit does not look appealing for consumption. The microbial load of the fruit samples was used to determine the shelf-life of the sample. The shelf life of the fruit was shown to be at ten and fifteen days when the microbial load was determined to be within the limit of Specific Spoilage Organisms counts of 10^5 to 10^8 cfu/g. The maximum permissible level of total aerobic colony of ready-to-eat foods as given by Fylde Borough Council extracted from manual of PHLSG (2008) was 10^4 to less than 10^6 cfu/g of ready-to-eat food products. Also, according to (Rho & Schaffner, 2007), the limit of microbial growth that determines shelf-life differs according to the food type and storage conditions. Specific Spoilage Organisms counts from 10^5 to 10^8 cfu/g are commonly considered as convenient quality limits. Thus, based on the data collected on the fifth day and tenth day, and following the guidelines of PHLSG (2008) and (Rho & Schaffner, 2007) on ready-to-eat food

Acquaah (2007) recommended that breeders develop new varieties of vegetables and fruits with superior yield, nutritional qualities, adaptation, and general appeal as well as the extension of shelf life through the use of genetic engineering techniques to reduce the expression of compounds associated with fruit deterioration.

In conclusion, the study has shown that these uncommon fruits are rich in chemical composition although often neglected. There is a need to increase awareness of these important aspects of these fruits as well as mobilization of farmers that cultivate them. These farmers should be supported with amenities including credit and storage facilities. At present, these fruits are regarded underutilized and the status deserves a change.

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Growth and Yield Analysis of Sungkai (*Peronema canescens* Jack.) in Kalimantan, Indonesia

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ABSTRACT. *Sungkai (Peronema canescens) is a local commercial tree (native species) that has the potential to be developed as a plantation forest and agroforestry estate. This study aims to determine the percentage of survivality, productivity, optimum cutting cycle, and financial benefits of the Sungkai tree. The study was conducted in the people plantation, Kapuas district, Central Kalimantan Province. The research took the growth parameter of Sungkai which was grown since the year 2001 to 2013 in areas with the ultisol soil type. Data analysis has been using the average value of diameter, height and volume, annual increment (annual), the regression equation of NPV, BCR and IRR. The results showed that in 12 years the percentage of Sungkai survivality reached 89.7%, with an annual increase of 14.10 m^3 $ha^{-1}year^{-1}$ and a density of 997 trees ha^{-1}. The Equation modelling of Sungkai plantation is y = 2.073 + 1.6623x - 0.0165x2 (R^2 = 84.05%). At the level of loan interest of 9% per year, Sungkai have an economic harvest cycle of 15 years with NPV Rp. 58.49 million ha^{-1}, BCR: 7.64 and IRR: 11.75%. Whereas, when the loan interest rate of 6% and 12% per annum, then the cutting cycle of 15 years, the NPV are to Rp. 92.65 million ha^{-1} and Rp. 36.6 million ha^{-1} respectively. In this study, Sungkai tree are very suitable to be developed in agroforestry and to increase the productivity of land such as shifting cultivation area, scrubland and low potential forest areas which were widespread, especially in Kalimantan, Borneo.*

KEYWORDS. Sungkai, Growth and yield, mean annual increment, *Peronema canescens,* and economic cutting cycle, Kalimantan

ABSTRAK. Sungkai (Peronema canescens) adalah sejenis pokok komersial tempatan (native species) yang berpotensi untuk dikembangkan sebagai tanaman dalam hutan perladangan dan kebun tanaman. Penelitian ini bertujuan untuk mengetahui peratus kemandirian, produktiviti, kitaran tebang optimum, dan keuntungan kewangan dari hasil tanaman sungkai. Penelitian dilakukan di hutan tanaman rakyat, Kabupaten Kapuas Provinsi Kalimantan Tengah. Parameter penelitian adalah dari pertumbuhan tanaman sungkai yang ditanam sejak tahun 2001 hingga 2013 pada kawasan yang mempunyai tanah jenis ultisol. Analisa data menggunakan nilai purata diameter, tinggi dan isipadu, pertambahan tahunan (annual), persamaan regresi, NPV, BCR dan IRR. Hasil penelitian menunjukkan bahwa pada umur 12 tahun peratus tanaman yang masih hidup mencapai 89.7%, dengan pertambahan tahunan 10.14 m^3 ha^{-1} $year^{-1}$ dan kepadatan 997 pokok/ha. Model pertumbuhan tanaman sungkai adalah $y = 2.073 + 1.6623x - 0.0165x^2$ (R^2= 84.05%). Pada peringkat faedah pinjaman sebanyak 9% per tahun, tanaman sungkai mempunyai kitaran tuaian ekonomi selama 15 tahun dengan nilai NPV Rp. 58.49 juta/ha, BCR: 7.64 dan IRR: 11.75%. Pada tingkat suku faedah pinjaman 6% dan 12% per tahun, maka pada kitaran tebang selama 15 tahun, nilai NPVnya masing-masing menjadi Rp. 92.65 juta/ha dan Rp. 36.6 juta/ha. Tanaman sungkai sangat sesuai dikembangkan dalam agroperhutanan dan untuk meningkatkan produktiviti tanah bekas perladangan pindah, belukar dan hutan miskin yang tersebar luas, khususnya di Kalimantan.

Kata kunci: Sungkai, Pertumbuhan dan hasil, min pertambahan tahunan, *Peronema canescens*, kitaran ekonomi tebangan, Kalimantan

PENDAHULUAN

A. Latar Belakang Kajian

Luas kawasan hutan di Indonesia sentiasa mengalami pengurangan. Pada tahun 70-an, luas kawasan hutan Indonesia adalah 164 juta ha (Suratmo *et al*. 2003), kemudian menurun menjadi 144 juta ha pada tahun 1981 (Hani'in, 1999) dan menurun kembali menjadi 136.56 juta ha (Ditjen BUK, 2010). Dalam kawasan hutan tersebut, luas kawasan yang berhutan hanya seluas 64% dan kawasan bukan hutan adalah sebanyak 29% (Balitbanghut 2008).

Kecepatan kerosakan hutan sebesar 1.8 juta ha per tahun (1985-1997) dan meningkat menjadi 2.84 juta ha per tahun pada tahun 1997-2000 (Balitbanghut 2008). Selaras dengan penurunan luas kawasan hutan pada kadar yang cepat, kemampuan penghasilan kayu bulat nasional juga mengalami penurunan. Pada tahun 1992 penghasilan kayu bulat nasional sebesar 26.05 juta m^3 $tahun^{-1}$, namun kemudian menurun drastik pada tahun 2001 menjadi hanya 1.81 juta m^3 $tahun^{-1}$. Sejak saat itu penghasilan kayu bulat nasional di Indonesia tidak pernah mencapai angka di atas 10 juta m^3 $tahun^{-1}$.

Untuk meningkatkan kembali penghasilan kayu bulat nasional mampu dicapai melalui pembangunan hutan tanaman ladang maupun kebun tanaman, baik yang dilakukan oleh perusahaan dalam bentuk izin Usaha Pemanfaatan Hasil Hutan Kayu-Hutan Tanaman (IUPHHK-HT) maupun dilakukan oleh masyarakat dalam bentuk Hutan Tanaman Rakyat (HTR), Hutan Rakyat. Pemilihan spesies yang ditanam merupakan komponen penting untuk memastikan keberhasilan program hutan tanaman. Jenis spesies tanaman yang dapat bertoleransi tumbuh di daerah terbuka merupakan pilihan yang tepat untuk dikembangkan dalam kawasan hutan yang telah rosak.

Satu daripada spesies komersial tempatan *(native species)* yang dapat dikembangkan dalam hutan tanaman adalah sungkai (*Peronema canescens* Jack.). Menurut Dephut (1989), kayu sungkai mempunyai berat jenis purata 0.63; kelas awet III dan kelas kuat II-III. Kayu ini sangat sesuai digunakan untuk bahan bangunan, perabot, beruti, papan, lantai, dinding, patung dan ukiran, kerajinan tangan dan venir. Kayu sungkai dikenal mempunyai warna yang cerah dan serat yang indah. Daun sungkai juga dapat digunakan sebagai obat sakit gigi dan menurunkan demam panas (Ditjenhut, 1980).

Dalam rangka mendukung pengembangan hutan tanaman kelas perusahaan kayu sungkai, khususnya di Kalimantan, banyak maklumat tambahan tentang pertumbuhan dan hasil serta analisis kewangan terhadap tanaman ini. Oleh karena itu penelitian tentang tanaman sungkai ini sangat diperlukan.

B. Objektif /Tujuan penelitian

Kajian ini bertujuan untuk mengetahui peratus kemandirian, produktiviti, kitar tebangan ekonomi dan keuntungan kewangan dari tanaman sungkai. Informasi ini diharapkan dapat membantu pihak berkepentingan (stakeholder) dalam membangun hutan tanaman menggunakan spesies sungkai.

KAEDAH KAJIAN

A. Tapak kajian

Penelitian dilakukan di hutan tanaman rakyat yang terletak di Kecamatan Mandau Talawang, Kabupaten Kapuas, Provinsi Kalimantan Tengah. Pengambilan data dilakukan setiap 3 tahun sejak penanaman tahun 2001 sampai tahun 2013.

B. Prosedur Kajian

1. Plot kajian adalah dalam hutan tanaman rakyat jenis sungkai (*Peronema canescens* Jack.) yang ditanam tahun 2001 dengan jarak tanam 3 m x 3 m. Plot kajian merupakan kawasan semak belukar dan hutan potensi rendah serta bekas perladangan berpindah. Jenis tanah Ultisol berwarna kuning kemerahan dengan tekstur lempung liat berpasir (*sandy clay loam*). Tebal lapisan humus dianggarkan sekitar 0.5 cm hingga 21 cm.
2. Plot kajian ditentukan seluas 1 ha yang diambil secara rawak.
3. Pengambilan data dilakukan terhadap diameter (dbh) dan *clear bole height* pada tahun 2004, 2007, 2010, 2013.

C. Analisis Data

1. Umumnya data ditentukan menggunakan rumus:
   $\mu = 1/n \, . \, \Sigma Xi$, Dimana :
   ΣXi = jumlah data dari X_1 sampai X_n = $\sum_{n=1}^{n} \mu.fi$
   μ = nilai tengah atau rata-rata
   n = banyak data

2. Peratusan kemandirian tanaman ditentukan menggunakan pendekatan:
   Peratus hidup = (Σ tanaman hidup / Σ tanaman yang ditanam) X 100%

3. Isipadu pokok dihitung melalui:
   $V = 0.25.\pi.D^2.h.0.7$ dimana Π=3.14; D= diameter dbh dan h= tinggi pohon.

4. Pola pertumbuhan tanaman sungkai dibentuk melalui persamaan polinomial (Brown, 1997; Burkhart, 2003):
   $y = c_1 + c_2x + c_3x^2$
   dimana: y : diameter akhir purata
   x : waktu dalam tahun
   c_1, c_2, c_3 : konstant

5. Analisa kewangan (Financial)

Perhitungan *Net Present Value* (NPV), *Benefit Cost Ratio* (BCR dan Internal Rate of Return (IRR) ditentukan melalui persamaan (Nair, 1993):

$$NPV = \sum_{t=0}^{r} \frac{B_i}{(1+i)^t} - \sum_{t=0}^{r} \frac{C_i}{(1+i)^t}$$

$$BCR = \sum_{t=0}^{r} \frac{B_i}{(1+i)^t} : \sum_{t=0}^{r} \frac{C_i}{(1+i)^t}$$

Internal Rate of Return (IRR) adalah nilai suku bunga (i) pada masa NPV = 0

$$NPV = \sum_{t=0}^{n} \frac{Bt - Ct}{(1+i)^t} = 0$$

dimana:

Bt : penerimaan *(benefit)* tahun ke-t
Ct : pengeluaran *(cost)* tahun ke-t
r, t dan i : siklus tebang, waktu (tahun) dan suku bunga
Proyek dinilai layak bila NPV>0, BCR≥1 dan IRR> suku bunga

Analisis sensitiviti dilakukan untuk mengetahui *output* projek jika terdapat suatu penyimpangan atau perubahan dalam dasar-dasar perhitungan biaya *(cost)* maupun manfaat *(benefit)* (Gray *et al*. 1999). Dalam penelitian ini analisis sensitiviti dilakukan terhadap beberapa tingkat suku bunga, iaitu 6% dan 12%.

KEPUTUSAN DAN PERBINCANGAN

Tanaman sungkai (*Peronema canescens*) pada umur 12 tahun mempunyai pertambahan diameter tahunan purata (MAI diameter) sebesar 1.72 cm/tahun dan pertambahan purata tinggi bebas cabang (*clear bole height)* tahunan purata (MAI tinggi bc) sebesar 0.62 m/tahun. Hasil pengukuran diamater dan tinggi bebas cabang tanaman sungkai dapat dilhat pada jadual 1. Menurut Ditjenhut (1980) tanaman sungkai yang ditanam di Gadungan dengan jarak tanam 3 m x 1 m mempunyai MAI diameter sebesar 1.02 cm/tahun. Dengan demikian tanaman sungkai di lokasi kajian ini mempunyai pertambahan tahunan purata yang lebih besar dibanding di daerah Gadungan.

Tabel 1: Pertambahan purata diameter dan tinggi bebas cabang (*clear bole height*) tanaman sungkai sehingga berumur 12 tahun

Tahun	Umur	Diameter (cm)	MAI diameter (cm/th)	Tinggi bebas cabang (m)	MAI Tbc (m/th)
2001	0	1.86	0.00	0.3	0.00
2004	3	7.39	2.46	2.2	0.73
2007	6	15.80	2.63	3.8	0.63
2010	9	17.65	1.96	5.2	0.58
2013	12	20.58	1.72	7.4	0.62

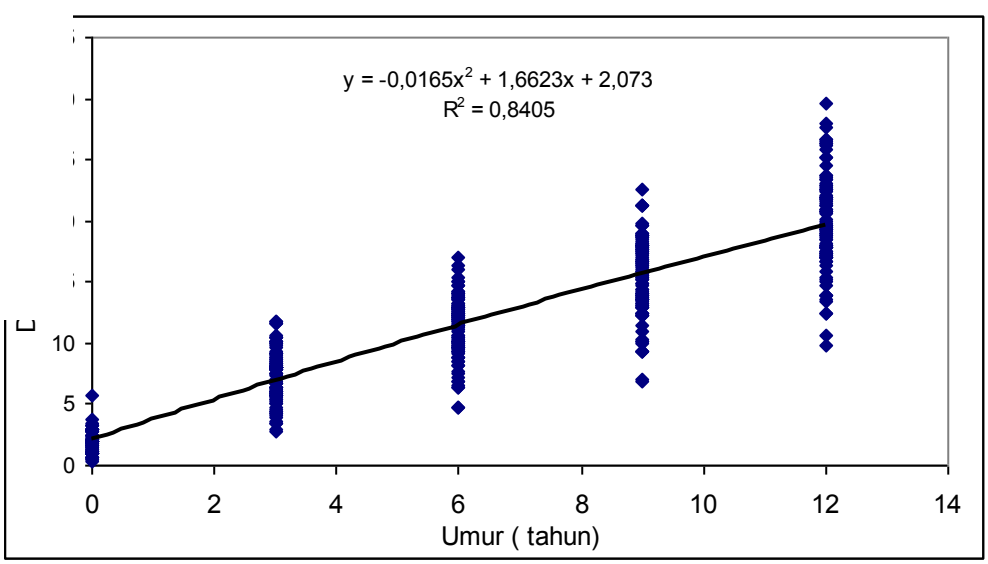
Peratusan kemandirian tanaman sungkai pada umur 12 tahun mencapai 89.71%. Tanaman sungkai merupakan jenis asli Kalimantan yang relatif tahan pada kondisi tanah marginal, sehingga jarang pokok/pohon yang mati. Beberapa tanaman ada yang terserang serangga perosak kumbang moncong (*Alcides* sp) yang menyebabkan daun berlubang-lubang, namun tidak menyebabkan kematian pokok. Secara umum, penurunan peratusan kemandirian tanaman disebabkan adanya persaingan dalam memperoleh unsur-unsur makanan dalam tanah dan ruang tumbuh.

Pola pertumbuhan tanaman sungkai membentuk persamaan polinomial (Brown, 1997; Burkhart, 2003) sebagai berikut:

$y = 2.073 + 1.6623x - 0.0165x^2$ (R^2= 84.05%)

dimana: y : diameter akhir purata

x : waktu dalam tahun



Rajah 1: Model persamaan polinomial pada pertumbuhan tanaman sungkai

Berdasarkan model persamaan di atas, dapat dianggarkan pertambahan diameter dan tinggi bebas cabang ke depan, demikian pula dengan isipadunya. Berdasarkan pendekatan tersebut, MAI jumlah isipadu tanaman sungkai pada kajian ini sebesar 10.14 $m^3ha^{-1}tahun^{-1}$ dengan kepadatan 997 pokok ha^{-1} pada umur 12 tahun. Menurut Ditjenhut (1980) tanaman sungkai yang ditanam di Gadungan dengan jarak tanam 3 m x 1 m mempunyai MAI volume total 11.5 $m^3ha^{-1}tahun^{-1}$. Dengan demikian tanaman sungkai di lokasi kajian ini mempunyai purata pertambahan tahunan yang lebih kecil dibanding di daerah Gadungan.

Rajah 2: Tanaman sungkai yang berumur 12 tahun (kiri) dan papan sungkai (kanan)

MAI diameter pada kajian ini lebih besar dari MAI diameter di Gadungan, namun MAI isipadunya lebih kecil. Hal ini disebabkan peratusan kemandirian tanaman sungkai di lokasi penelitian ini lebih kecil di bandingkan peratusan kemandirian tanaman sungkai di daerah Gadungan. Pada kajian ini peratusan kemandirian tanaman sungkai sebesar 89.71% dengan kepadatan tanaman 997 pokok ha^{-1} pada umur 12 tahun. Jumlah tanaman sungkai pada awalnya penanaman sebesar 1.111 pokok ha^{-1}.

Analisis kewangan (financial) tanaman sungkai dilakukan dengan menjangka pencapaian isipadu tanaman sungkai per ha sehingga berumur 35 tahun. Dengan menggunakan anggaran harga jual kayu sungkai berdiameter 10-20 sebanyak Rp. 850.000, per m^3 dan berdiameter 20 cm ke atas sebanyak Rp. 1.500.000 per m^3, maka dengan kadar faedah sebanyak 9% (berlaku saat ini), kitaran tuaian ekonomi dicapai pada umur 15 tahun dengan nilai NPV sebanyak Rp. 58.49 juta ha^{-1}, BCR sebesar 7.64 dan IRR 11.75% (Jadual 2). Apabila kadar faedah turun menjadi 6%, maka kitaran tuaian ekonomi dicapai pada umur 15 tahun dengan nilai NPV sebanyak Rp. 92.65 juta ha^{-1}, BCR sebesar 10.62 dan IRR 11.79% dan apabila kadar faedah naik menjadi 12%, maka kitaran tuaian ekonomi dicapai pada umur 15 tahun dengan nilai NPV sebanyak Rp. 36.6 juta/ha, BCR sebesar 5.47 dan IRR 11.66% (Jadual 2).

Jadual 2: Analisis kewangan tanaman sungkai pada tingkat suku bunga 9%, 6% dan 12%

Faedah	Umur	NPV	BCR	IRR
9%	5	-6,722,000	0.00	8.3%
	10	10,381,813	2.29	11.87%
	15	58,489,927	7.64	11.75%
	20	46,398,275	5.96	10.86%
	25	38,299,379	4.95	10.34%
	30	25,426,666	3.53	10.06%
	35	19,451,165	2.92	9.89%
6%	5	-6,083,933	0.00	8.31%
	10	15,823,816	2.87	12.21%
	15	92,651,088	10.62	11.79%
	20	86,899,446	9.26	10.95%
	25	85,247,273	8.63	10.46%
	30	82,229,511	7.83	10.04%
	35	78,111,598	7.35	9.87%
12%	5	-6,580,139	0.00	8.31%
	10	6,398,036	1.84	11.24%
	15	36,601,492	5.47	11.66%
	20	23,872,147	3.80	10.67%
	25	15,642,235	2.79	10.04%
	30	4,855,190	1.55	10.06%
	35	1,094,425	1.12	9.92%

Berdasarkan maklumat pada jadual 2, tanaman sungkai telah layak dituai pada umur 10 tahun namun belum memberi keuntungan kewangan yang maksimum. Apabila tanaman dituai pada umur lebih dari 15 tahun, maka tidak diperoleh keuntungan yang maksimum karena kos perawatan tanaman serta kos faedah yang dikenakan pada tiap komponen pengeluaran lebih tinggi dibanding penambahan keuntungan dari pertambahan tanaman.

Menurut Sutisna dan Ruchaemi (1995), tanaman sungkai dapat dituai sebagai kayu perkakas pada umur 20-30 tahun. Berdasarkan hasil kajian ini, tanaman sungkai masih layak dituai pada umur 20-30 tahun juga, namun berdasarkan keadaan semasa, dimana teknologi pengolahan kayu telah berkembang pesat dan kayu sungkai berdiameter 10-20 cm mempunyai nilai ekonomi, maka penuaian pada umur 15 tahun adalah yang paling layak dengan keuntungan kewangan (financial) yang tertinggi. Penundaan penuaian akan menambah kos pembiayaan projek dan kurang cekap.

KESIMPULAN

Sungkai (*Peronema canescens* jack.) adalah spesies komersial asli Kalimantan yang bersifat intoleran dan dapat tumbuh dengan baik pada kawasan bekas perladangan, belukar dan hutan gambut yang tersebar luas dalam kawasan hutan. Pada umur 12 tahun peratusan hidup tanaman sungkai mencapai 89.7%, pertambahan tahunan purata 11.90 m^3 ha^{-1} $year^{-1}$ dengan kepadatan 997 pokok ha^{-1}. Model pertumbuhan tanaman sungkai adalah $y = 2.073 + 1.6623x - 0.0165x^2$ (R^2= 84.05%). Pada kadar faedah pinjaman 9% per tahun, tanaman sungkai mempunyai kitaran tuaian ekonomi selama 15 tahun dengan nilai NPV Rp. 58.49 juta ha^{-1}, BCR: 7.64 dan IRR: 11.75%. Apabila kadar faedah pinjaman turun menjadi 6% atau naik menjadi 12% per tahun, maka kitaran tuaian ekonomi masih tercapai pada umur 15 tahun dengan nilai NPV-nya masing-masing sebanyak Rp. 92.65 juta ha^{-1} dan Rp. 36.6 juta ha^{-1}. Tanaman sungkai dapat dipergunakan untuk kegiatan penanaman semula hutan dan penghijauan dalam rangka meningkatkan produktiviti tanah serta boleh dikembangkan dalam hutan tanaman industri kelas perusahaan kayu pertukangan dalam skala luas karena permintaan kayunya yang semakin meningkat.

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