EFFECT OF FERMENTATION TOWARDS PRODUCTION OF PHENOLIC COMPOUNDS FROM *Syzygium campanulatum*

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ABSTRACT

Phenolic compounds are plant secondary metabolites that beneficial to the plant and to us human as well such as their anti-oxidant property. Searching and enhancing profile of phenolic compounds were always in action. Wastes such as garden leaf waste can be alternative source of phenolic compounds. *Syzygium campanulatum* is one of the plant in garden whereby pruning activities were done and the leaf waste was discarded. The aim of this study is to quantify the total phenolic content (TPC), total flavonoid content (TFC), anti-oxidant activity and total protein concentration of non-fermented, natural microbes and *Aspergillus brasiliensis* fermented *Syzygium campanulatum* leaf waste. Solid state fermentation at 25°C and 37°C were done on the leaf waste. Centrifugation and shaking incubation were done to extract the contents in the leaf waste. The percent yield of natural microbes and *A. brasiliensis* in production of the phytochemical contents that can be extracted from *S. campanulatum* leaf waste were calculated and compared. The highest mean absorbance of TPC was detected in extract of non-fermented leaf waste. The highest TFC and total protein were detected in natural fermented leaf waste using fermentation temperature of 25°C. The highest anti-oxidant activity was detected in natural fermented leaf waste using fermentation temperature of 37°C. This study showed that fermentations at both temperatures generally decreased the TPC and increased the TFC of *S. campanulatun* leaf waste. The percentage yield of TPC was −40.35% and −23.00% for natural fermentation and *A. brasiliensis* fermentation respectively at 25°C and −49.03% and −26.71% respectively at 37°C. The percentage yield of TFC was +36.81% and +7.40% for Natural fermentation and *A. brasiliensis* fermentation respectively at 25°C and +13.09% and +11.20% respectively at 37°C. Natural fermentation at 25°C increased more TFC in *S. campanulatum* leaf waste.

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LIST OF ABBREVIATIONS

%	Percentage
× *g*	times gravity
°C	degree Celcius
10×	ten times
100×	hundred times
A_0	Absorbance of mixture without the extract at 0 minute in Equation 3.1
A_0	Mean absorbance of the respective phytochemical content in ENOF in Equations 3.2 and 3.3
A_1	Mean absorbance of the respective phytochemical content in ENAF
A_2	Mean absorbance of the respective phytochemical content in EABF.
A_{30}	Absorbance of mixture containing the fermented or non-fermented extract after 30 minutes;
A_{415}	Absorbance at 415 nanometre
A_{517}	Absorbance at 517 nanometre
A_{540}	Absorbance at 540 nanometre
A_{725}	Absorbance at 725 nanometre
AA	Anti-oxidant activity
ABF	*Aspergillus brasiliensis* fermentation
$AlCl_3.6H_2O$	Aluminium chloride hexahydrate
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
CYA	Czapek yeast autolysate
dH_2O	Deionised water
doi	digital object identifier

DPPH	2,2-diphenyl-1-picryhydrazyl
EABF	Extract of *Aspergillus brasiliensis* fermented leaf waste
Ed. / Eds.	Editor / Editors
ENAF	Extract of natural fermented leaf waste
ENOF	Extract of non-fermented leaf waste
et al.	et alia
FRIM	Forest Research Institute Malaysia
g	gram
GRAS	Generally recognized as safe
$KC_2H_3O_2$	Potassium acetate
L	litre
MEA	Malt extract agar
MF0	Mass of conical flasks
MF1	MF0 + 4 g of leaf waste
MF2	MF1 + 5 mL PDA broth
MF3	MF2 + 2 mL of sterile desionised water for NAFR and 2 mL of fungal spores (1×10^6 spores/mL) for ABFR
MF4	Mass of MF3 after four days of fermentation
mg	milligram
mL	millilitre
NAF	Natural fermentation
nm	nanometre
PDA	Potato dextrose agar
PDB	Potato dextrose broth
pH	Potential of hydrogen
pp.	pages

R	Replicate
rpm	revolution per minute
SmF	Submerged fermentation
sp.	species
spp.	several species
SPSS	Statistical Package for the Social Sciences
SSF	Solid state fermentation
TFC	Total flavonoid content
TPC	Total phenolic content
TProt	Total Protein Concentration
uL	microlitre
USFDA	United States Food and Drug Administration
UV	Ultraviolet
v/v	volume/volume
VEGF	Vascular endothelial growth factor
w/v	weight/volume
α	alpha
β	beta

CHAPTER 1:

INTRODUCTION

Wastes, such as food wastes, agro-industrial wastes, garden wastes, and so forth, are generated in large amount every day. As early as 1970s, efforts were done to recover valuable products from wastes mentioned above and utilise wastes as value added-products, for example multifunctional food ingredients, flavours and bioadsorbents, which contributed to nutraceutical, food, and waste water treatment industries (Laufenberg, Kunz & Nystroem, 2003).

Leaves from, for example, fruits, shrubs, and trees contain large amount of bioactive phytochemicals, such as phenolic compounds (Wahle, Rotondo, Brown & Heys, 2009), which are well known for their health benefits (Martins et al., 2011). Assessments of phytochemicals were done on leaves of creosote bush (Aguilar et al., 2008), passion fruit (Silva et al., 2013), white butterfly (Adefegha & Oboh, 2015), mango fruit (Fernandez-Ponce, Casas, Mantell, Rodriguez & Ossa, 2012), and tea plant (Nor Qhairul Izzreen & Mohd Fadzelly, 2013), which all concluded high amount of total phenolic and polyphenolic compounds.

Syzygium campanulatum is a plant commonly cut into hedges and shrubs and usually planted for landscaping (Waysidetrees, 2011). Similar to other species of genus *Syzygium*, they are characterized by having yellow, orange, pink, red and maroon coloured young leaf shoots (Forest Research Institute Malaysia (FRIM), 2014). Although common, with some other species under the same family Mrytaceae, there were limited researches on their phytochemical constituents. With promising data on other species that researched showing the beneficial bioactivity as well as data on phytochemical of *S. campanulatum* (Abdalrahim et al., 2013; Abdul et al., 2014; Abdul et al., 2015; Abdul et al., 2016), *S. campanulatum* could be another rich source of phytochemicals such as phenolic compounds.

Bacterial or fungal fermentation on above mentioned wastes and leaves can enhance the extraction and production of phenolic compounds (Martinez-Avila,

Aguilera, Saucedo, Rojas, Rodriguez & Aguilar, 2014). However, certain processing, such as in the olive processing prior to fermentation decreased the phenolic compounds extracted after fermentation (Hur, Lee, Kim, Choi & Kim, 2014). Kim et al. (2013) reported that certain individual flavonoids such as epigallocatechin gallate decreased which contributed to decrease in total flavonoid content. The individual flavonoids were degraded either to other identified non-flavonoid compounds such as gallic acid, other flavonoid compounds and other unidentified compounds which concluded by the authors that all contributed to increase in anti-oxidant capacity.

Leaves of *S. campanulatum* can serve as a source of natural waste due to pruning activities. Leaves were usually discarded during pruning activities and these leaf wastes can be investigated for compounds such as phenolic compounds with wide benefits, such as anti-oxidant and anti-cancer activity (Martins et al., 2011).

Hence, with interest on the phenolic compounds of leaf wastes of *Syzygium campanulatum* in garden of INTI International University (INTI-IU), the aims of this study are to:

1. quantify phytochemical contents, namely total phenolic content, total flavonoid content, and total protein and anti-oxidant activity in the non-fermented and fermented *S. campanulatum* leaf waste

2. determine the effect of fermentation towards the changes and production of phenolic compounds from *S. campanulatum* leaf waste.

3. compare the percentage yield of phenolic compounds using *Aspergillus brasiliensis* and natural microbes as the organism for fermentation of *S. campanulatum* leaf waste.

CHAPTER 2:

LITERATURE REVIEW

2.1 PHENOLIC COMPOUNDS

Phenolic compounds can be classified either into monophenolic or polyphenolic compounds, which having one or more aromatic ring, respectively, where most of the phenolic compounds, having hydroxyl and oxygen group (Wahle et al., 2009). They also can be grouped based on number of hydroxyl group as di-, tri- or polyhydric. They also can be classified as flavonoid or non-flavonoid, where flavonoid stands as the largest group among over 8000 phenolic compounds discovered (Martins et al., 2011). Subcategories of non-flavonoid includes phenolic acids, stilbenes, coumarins and tannins, while different substitution in ring C in the backbone gives different types of flavonoid flavanols, flavonols, flavones, flavanones, anthocyanidins, and isoflavones.

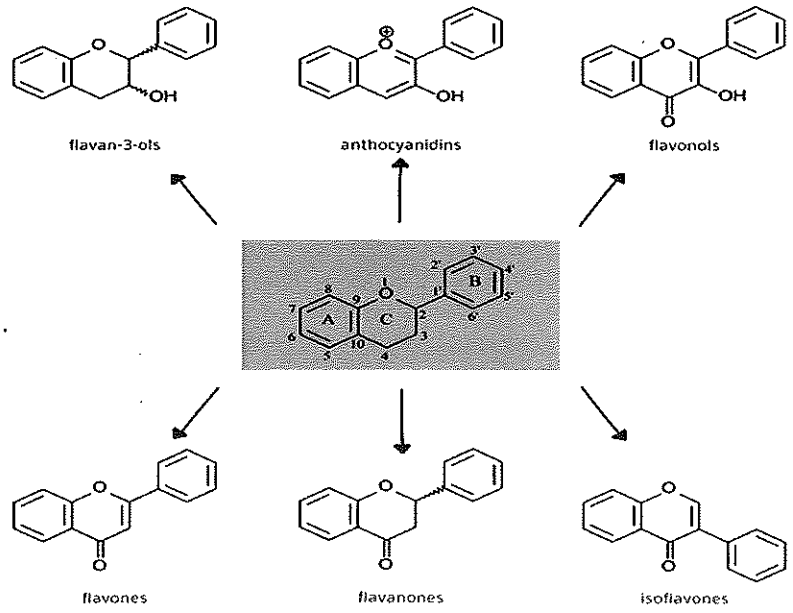

Figure 2.1 Example of backbone of flavonoids compounds formed by variation in ring C.