PROCEEDINGS

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"TOWARDS FUNDAMENTAL AND APPLIED RESEARCH FOR SOCIO-ENVIRONMENTALLY SAFE INDUSTRIAL DEVELOPMENT"

Organized By:

Faculty of Industrial Technology, University of Pembangunan Nasional "Veteran" Jawa Timur,
Bali State Polytechnic , and
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PREFACE

Faculty of Industrial Technology, University of Pembangunan Nasional "Veteran" Jawa Timur in collaboration with Bali State Polytechnic and National Cheng-Kung University are organizing an international seminar entitled: Bali International Seminar on Science and Technology (BISSTECH) III 2015, with topic "Towards Fundamental and Applied Research for Socio-Environmentally Safe Industrial Development".

We wish to thank reviewers, keynote speakers, moderators, presenters, and participants for their cooperation and valuable suggestions. We would like to extend our appreciation to members of organizing committees of all events during this seminar.

Finally, we hope that the seminar would give a contribution in creating a better future for mankind.

Thank you.

Denpasar-Indonesia, October 15th, 2015
Editorial Board

BISSTECH III 2015

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EFFECT OF SOLVENT POLARITY ON ANTIOXIDANT ACTIVITY DURING FRACTIONATION OF ETHANOLIC EXTRACT OF *CITRUS HYSTRIX* PEEL

Wenny Irawaty[1] and Aning Ayucitra[2]
Chemical Engineering Department, Faculty of Engineering, Widya Mandala Catholic University Surabaya,East Java, Indonesia
e-mail: wenny_i_s@ukwms.ac.id

ABSTRACT

*Citrus hystrix*has been claimed to exhibit tremendous performance to repair organ damage caused by free radicals developed during metabolism. The present study was undertaken to evaluate the antioxidant power of *Citrus hystrix* peel. Antioxidant activity of several fractions (water, ethyl acetate, and hexane) obtained from the fractionation of ethanolic extract of *Citrus hystrix* peel was investigated. The effect of the solvents on phytochemical content was also evaluated. The research methods involve the *Citrus hystrix* peel preparation (sizing, drying), extraction of the peel by using ethanol 41% for 8 hours, fractionation of the ethanolic extract by different solvents, and then followed by antioxidant activity measurement of the fractions obtained. The results revealed that all fractions of the ethanolic extract of *Citrus hystrix* peel exhibited variable antioxidant activity. Specially, the ethyl acetate fraction showed the highest values of antioxidant capacity (% DPPH scavenging activity). The different activity of the fractions was correlated with phytochemical content in each fraction.

Keywords: *Citrus hystrix*peel, antioxidant, fractionation

INTRODUCTION

Plants and plant products are being used as a source of medicine since long time. The medicinal properties of plants have been widely investigated due to their potent antioxidant activities, no side effect, and economic feasibility (Hui et al., 2009). The natural antioxidants or phytochemicals are the secondary metabolites of plants that are widely distributed in foods of plants and count as phenolic compounds. As antioxidant, phenolic compounds comprising flavonoids and phenolic acids play an important role in the prevention of human pathologies by acting as radical scavenger against degenerative diseases such as cardiovascular diseases, neurodegenerative diseases, blood disorder diseases, diabetes mellitus (Zhao et al., 2012), and cancers. Therefore, there is growing interest toward natural antioxidant from herbal sources (Ebrahimzadeh et al., 2008, Sarepoua et al., 2015, Gorinstein et al., 2001). Phenolic compounds are frequently found in fruits(Nizam Uddin et al., 2014, Ebrahimzadeh et al., 2008, Gorinstein et al., 2001), vegetables(Hui et al., 2009, Widyawati et al., 2014, Harbaum et al., 2008), and grains (Singh et al., 2012, Chiremba et al., 2012). The chemical composition of fruits, including leaves and peels have been widely investigated and it was found that the peel possesses higher antioxidant activity compared to other parts of the fruit (Gorinstein et al., 2001, Li et al., 2006). For example, Gorinstein et al. (2001) found that the amount of phenolic compounds in the peels of orange, lemon, and grapefruit were higher than the peeled fruits. Similar result was reported by Li et al. (2006) when pomegranate was selected in their study (Li et al., 2006).

Citrus fruits contain high content of flavonoids compared to other type of fruits. Citrus fruits contain a wide range of flavonoid compounds which are sub-classed in flavanones, flavones, flavonol, and dihydrochalcone *C*- and/or *O*-glycosides (Roowi and Crozier, 2011, Gattuso et al., 2007). Therefore, since in the early nineties the presence of flavonoids in citrus fruits began to attract a number of researchers. This study focused on *Citrus hystrix*. The leaves of this citrus have commercial importance and its byproduct, i.e. the fruit itself has not been utilized yet.

This work evaluates the effect of solvent polarity used during fractionation of *Citrus hystrix*peel extract on the antioxidant capacity. The aim of this work was to propose a suitable solvent for the separation of phenolic compounds in crude *Citrus hystrix* peel extract.

LITERATURE REVIEW

Citrus hystrix, commonly known as Kaffir lime or wild lime, is originated from South East Asia and cultivated throughout the tropical regions. *Citrus hystrix* is greenish yellow, acidic flavor, bumpy, and pear-shaped. This citrus is are reported rich in phenolic compounds including flavonoids, limonoids, glycerolglycolipids, furanocourmarins, benzenoid derivative and quinolinone alkaloids with potential health-promoting properties.

Several methods such as maceration (Cha et al., 2010), heat treatment (Xu et al., 2007), microwave (Chiremba et al., 2012), ultrasonic (Ma et al., 2008), far-infrared radiation (Lee et al., 2006), subcritical water (Plaza et al., 2010), high pressure – pulsed electric field (Sánchez-Moreno et al., 2005), fermentation (Harbaum et al., 2008), and cellulases treatment (Kim et al., 2005) have been studied to extract phenolic compounds from plant materials. Among the methods mentioned earlier, maceration is the most economical feasible and this led to commercial application in the future.

Solvent plays an important role in the extraction of plant natural antioxidant compounds. The amount of compounds extracted is influenced by the polarity of both compounds and solvent used. Hegazy and Ibrahium (2012) investigated the effect of solvent (methanol, ethanol,

dichloromethane, acetone, hexane, and ethyl acetate) polarity on the antioxidant capacities of orange peel extracts (Hegazy and Ibrahium, 2012). It was found that the antioxidant capacities vary with solvent polarity and the high polar solvent of ethanol exhibited the highest antioxidant activities. Patel et al. (2011) reported the antioxidant activities of *Hybanthusenneaspermus* (Linn.) F. Muell. (Violaceace) was greatly influenced by solvent polarity that influence phenolic compounds being extracted by certain solvent which in turn its activity (Patel et al., 2011). Similar finding was also reported by other work (Widyawati et al., 2014).

METHODOLOGY

Material: *Citrus hystrix* obtained around East Java was collected in January-March. Chemicals used were ethanol, methanol, hexane, ethyl acetate, ascorbic acid, DPPH, in this work were purchased from Sigma Chemical Co.

Methods: Citrus fruits were manually peeled and the edible portions were carefully separated. The peels were further cut into 0.5 x 0.5 cm and air dried for 2 days. The dry peel was then soaked with ethanol solution 41% for 8 h at room temperature. The solid part was then separated by a What man filter paper. The filtrate was concentrated under a vacuum by evaporating the ethanol. The water residue was then fractionated under solvents of varying polarity. The phytochemical analysis of the various fractions was carried out independently by using methods described in (Harbone, 1973, Adewole et al., 2014). Antioxidant capacity was quantified by the DPPH radical method. For this assay, a solution of DPPH in methanol (0.2 mM) was prepared freshly. 1.25 mL aliquot of this solution was added to 1 mL sample at different concentrations in the range of 0.03-4 mg/mL. The sample solution was shaken and left to react in the dark for 30 min. Then the solution was transferred into a cuvette, and the absorbance was determined at 520 nm using a spectrophotometer (Shimadzu, UVmini-1240). A decrease in absorbance was recorded and the antioxidant capacity was expressed as IC_{50}. The IC_{50} is the concentration where 50% inhibition occurs. The control contained all reagents except the fraction was prepared under the same treatment. The antioxidant capacity was compared to standard compound of ascorbic acid which is already known for its good antioxidant activity. The percentage inhibition of the radicals was calculated using the formula:

$$\% \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100 \quad \ldots\ldots\ldots (1)$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the fraction/standard.

RESULTS

Free radical scavenging activity expressed as IC_{50} ranged from 0.03 to 2.37 mg crude extract/mL as shown in Table 1.

Table-1. DPPH free radical scavenging activity of different fractions of ethanolic*Citrushystrix*peel extract

Fraction	IC_{50} (mg/mL)
Hexane	2.37 ± 0.16
Ethyl acetate	0.03 ± 0.00
Water residue	1.09 ± 0.05
Ascorbic acid (standard)	0.04 ± 0.00

As seen, the three fractions exhibit DPPH radical scavenging capacity and the ethyl acetate shown the best activity among the others with the IC_{50} reached a value of 0.03±0 mg/mL. The water residue exhibited the second highest value in the DPPH free radical test after the ethyl acetate fraction where the IC_{50} was recorded at 1.09±0.05 mg/mL. With the IC_{50} value of 2.37±0.16 mg/mL, hexane fraction occupied the third highest rank in the same test method. Ascorbic acid as the control compound has also exhibited the scavenging activity toward DPPH free radical compound with the IC_{50} value of 0.04 mg/mL.

When the concentration of the fractions was increased, it was observed that the antioxidant capacity was improved as shown in Figure 1 for the fraction of ethyl acetate (r^2 = 0.9581). Other fractions showed the similar trend (data not shown).

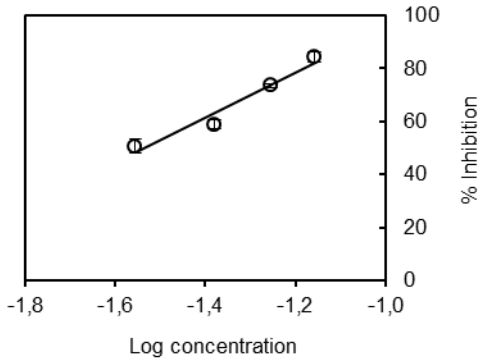

Figure-1. DPPH scavenging activity for different amount of ethyl acetate fraction

In order to get insight into the scavenging activity of each fractions observed in this study, phytochemical analysis of the three fractions were performed and the results are tabulated in Table 2. As seen, each fractions exhibited different compounds that may contributed to the activity of each fractions. Alkaloids, phenolics, and flavonoids were detected in all fractions. While compounds of saponins, tannnins, sugars, and carbohydrates were not identified in the fraction of hexane, however, all the compounds were revealed in the fractions of ethyl acetate and water residue.

DISCUSSIONS

As shown in Table 1, the IC_{50} values varied in the following order: ethyl acetate fraction < water residue < hexane fraction. Similar result was found by other work (Anagnostopoulou et al., 2006) that the fraction of ethyl acetate exhibited the highest antioxidant capacity compared to other fractions (ether, dichloromethane, and water fraction) when sweet orange peel was selected in

their study. The IC_{50} values were then compared to the standard of ascorbic acid. The standard suggests that its activity was 25% lower than the ethyl acetate fraction, while compared to the other two fractions, the standard was 27 and 59% higher for the fractions of water residue and hexane, respectively. The results show that ethyl acetate is regarded as the most effective solvent to extract compounds from the ethanolic extract of *Citrus hystrix* peel. This can be explained by the possible complex formation of phenolic compounds with other components which are more extractable in ethyl acetate than those of other fractions (hexane and water) (Zhao and Hall, 2008, Zhu et al., 2011).

The antioxidant capacity of plants is contributed by the presence of phenolic compounds or phytochemicals. The fractions of *Citrus hystrix* peel extract contain some potent phytochemical constituents such as phenolics, flavonoids, saponins, alkaloids, carbohydrates, and tannins which may be responsible for this activity (Table 2). Phenolic compounds such as phenolic acid, flavonoids, and tannins have been well known contribute the antioxidant activity/capacity (Gorinstein et al., 2001). Flavonoids found in citrus juice were reported flavanone aglycones, flavone aglycones, polymethoxy flavones, flavanone-*O*-glycosides, flavone-*C*-glycosides, and flavone-*O*-glycosides. Earlier investigations on saponins found that the compound reduced the risk factor of atherosclerosis (Rodrigues et al., 2005). Tannins was also reported to possess antioxidant activity (Beninger and Hosfield, 2003). Since the fraction of hexane contains least phytochemical compounds (Table 2), thus it is not surprising if the fraction exhibited the lowest activity to quench DPPH free radical compound. The higher antioxidant capacity exhibited by ethyl acetate fraction cannot only be explained by phytochemical analysis performed. However, the mechanism behind this could be due to the presence of high content of phenolic compounds.

CONCLUSIONS

The fraction of ethyl acetate has shown impressive antioxidant capacity toward DPPH free radical scavenging activity. The comparable antioxidant capacity of ethyl acetate fraction compared to the standard of ascorbic acid indicates that the fraction may have beneficial implication for human health. The mechanism behind this could be due to the presence of high content of phenolic compounds. Accordingly, further investigation on analysis of compounds in the ethyl acetate fraction is required to confirm this.

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Table-2. Phytochemical analysis of various fractions of *Citrus hystrix*peel extract

Fraction	Component						
	Alkaloids	Saponins	Tannins	Sugars	Carbohydrates	Phenolics	Flavonoids
Hexane	+	–	–	–	–	+	+
Ethyl acetate	+	+	+	+	+	+	+
Water residue	+	+	+	+	+	+	+

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