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INVESTIGATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF ZINGIBER OFFICINALE ROSCOE OLEOSIN ON AIRBORNE PATHOGENIC MICROORGANISMS

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ABSTRACT

Zingiber Officinale Roscoe or ginger has been found to have characteristics that are beneficial to human beings for different purposes. In this study, oleoresins from both young and mature gingers were studied to evaluate their antioxidant and antimicrobial activity on common airborne pathogenic microorganisms. The total phenolic and antioxidant contents were tested using the Folin–Ciocalteu method and DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging activity assay respectively. Meanwhile, antimicrobial activity was determined using the disc diffusion and minimum inhibitory concentration (MIC) against two pathogenic bacteria and fungi; namely S. aureus, P. aeruginosa, C. albicans, and A. niger. Young ginger oleoresin exhibits higher total phenolic contents (129.5869 ± 14.27 GAE) and antioxidant activity (99.06 % ± 1.41) than matured ginger oleoresin. In the antimicrobial study, the result showed that both young and matured oleoresins were able to inhibit the growth of common airborne pathogenic bacteria. However, common airborne pathogenic fungi were resistant to both oleoresin of ginger extract. The oleoresin was able to suppress the growth of S. aureus at the low concentration of 1 mg/ml rather than P. aeruginosa at 10 mg/ml. Young ginger possess higher antioxidant and antibacterial activity than matured ginger.

Keywords: *Zingiber Officinale Roscoe, oleoresin, antioxidant activity, antimicrobial activity, Folin–Ciocalteu method, DPPH assay, pathogenic microorganisms.*

1. INTRODUCTION

The discoveries of potential plant based medicines are increasing prominently due to the adverse health and toxic effects of the synthetic drugs (Bode & Müller, 2005). Researchers are manipulating the traditional knowledge of natural products and herbs passed down from generations to generation, that show promising effects of curing certain diseases; and for the production of new drug. For example, aloe vera (Alemdar & Agaoglu, 2009), turmeric (Chattopadhyay, Biswas, Bandyopadhyay, & Banerjee, 2004), poppy plants or Papaver somniferum (Alavijeh, Chishty, Qaiser, & Palmer, 2005), orchids (Kong, Goh, L. S. Chia, & T. F. Chia, 2003), honey (Alzahrani et al., 2012), black cumin or Nigella sativa L. (S. Singh et al., 2014) and garlic or Allium sativum L. (Ekwenye & Elegalam, 2005) were proven to have high antioxidant and antimicrobial activity.

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Zingiber Officinale Roscoe is the botanical name for Ginger. It has been used for thousands of years as part of herb and spices, as well as for medicinal purposes in various cultures throughout the world. Ginger has been cultivated throughout South□eastern Asia, China and parts of Japan, Austria, Latin America, Jamaica and Africa (Sasidharan & Menon, 2010). Oleoresin and essential oils of ginger have been internationally commercialized in food and pharmaceutical processing (Z. Kamaliroosta, L. Kamaliroosta, & Elhamirad, 2013). Ginger rhizome volatile oil that exhibit antimicrobial activity were found by many researchers (Bellik, 2014; Sasidharan & Menon, 2010). The oil has been found to be able to inhibit the growth of both gram-positive and gram-negative bacteria, including Escherichia coli, Proteus vulgaris, Salmonella typhi, Staphylococcus aureus and Streptococcus viridian (Panpatil, Tattari, Kota, Nimgulkar, & Polasa, 2013). Besides, ginger can be used in the treatment of bacterial infections due to its direct anti-microbial activity (Islam, Rowsni, Khan, & Kab, 2014).

In this study, the evaluation of antioxidant and antimicrobial activity of both young and matured oleoresin of *Zingiber Officinale* Roscoe on common airborne pathogenic microorganisms were evaluated. This study will provide information on oleoresin from ginger to become an alternative natural resources used in medical applications.

2. EXPERIMENTAL

2.1 Plant material and sample preparation

Young and matured *Zingiber Officinale* Roscoe were purchased from Tunas Manja Supermarket in Indera Mahkota, Kuantan, Pahang on April 2015. The gingers were thoroughly washed under running tap water to remove dirt. They were then chopped into small pieces and freeze dried. Finally, the samples of ginger were ground into a fine powder with a grinding machine.

2.2 Extraction of oleoresin

Oleoresin compounds were extracted from dry ginger powder using the Soxhlet apparatus method. Firstly, 8.0 g of each young ground samples were weighed and placed inside a paper thimble. The paper thimble was covered with cotton wool and then loaded into the Soxhlet extractor. Two hundred and fifty millilitres of methanol were filled in a solvent vessel. The sample was then extracted at the boiling point of methanol, at 64.7°C for 5 hours. The processes were repeated until all the samples were extracted and allowed to dry completely using a vacuum rotary evaporator. All steps were repeated by using matured ginger. Both samples were stored in the dark at low temperature (4°C) prior to the assessments of their antioxidant and antimicrobial activities.

2.3 Total Phenolic Compound (TPC)

Total phenolic content was quantified using the modified Folin–Ciocalteu's method described by Pawar, Pai, Nimbalkar, and Dixit (2011). The assay mixture was prepared using 0.125 ml of standard gallic acid at different concentrations with 0.250 ml of Folin–Ciocalteu reagent and 1.25 ml of distilled water. It was then incubated for 10 min in the dark. After 10 minutes 1 ml 7% aqueous sodium carbonate and 1 ml of distilled water were added and the reaction mixture was incubated in dark for 90 minutes at room temperature. The absorbance of the

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blue colour was read at 765 nm using methanol instead as blank on a UV / vis spectrophotometer. Finally, the results were compared to the gallic acid standard curve and expressed as mg/gallic or equivalent.

2.4 DPPH (2, 2-diphenyl-1-picrylhydrazyl) Scavenging Activity Assay

The antioxidant activities were determined as the measure of radical scavenging using DPPH assay. Ascorbic acid was used as the standard. Two millilitres of methanolic solution of DPPH (0.004% w/v) was mixed with 1.0 ml of different concentration of young and matured ginger oleoresin. The mixture was then incubated for 30 minutes in the dark. The absorbance at 515 nm was measured using methanol as blank. The inhibition percentage of DPPH (% DPPH) is calculated as follows:

% DPPH Scavenging activity = [(ADPPH – ASample) / ADPPH] x 100%

2.5 Test Microorganism

The microorganisms were provided by the Kulliyyah of Science, IIUM Kuantan Campus. The bacteria used for this study were Gram-positive Staphylococcus aureus and Gram-negative Pseudomonas aeruginosa. The fungi strains were Candida albicans and Aspergillus niger.

2.6 Disc Diffusion Method

The method described by Bellik (2014) was used for disc diffusion test with some modifications. Blank discs were prepared using Whatman filter paper number 3 and were sterilize using an autoclave machine at 121°C for 15 minutes. The diameter of the disc was approximately 6mm in diameter.

The blank discs were then impregnated with 10 µl each of young and matured ginger oleoresin and placed on the surface of the medium previously inoculated with a suspension of microbes. 10µl of 100% methanol was impregnated to the blank disc as a negative control. All the discs were allowed to dry and were consequently placed on the test plate. Tetracyclin and nystatin antibiotic discs were used as positive control for bacteria and fungi respectively. The plates were subsequently incubated overnight at 37°C and 30°C accordingly. Lastly, the zone of inhibition was determined by measuring the diameter in mm of the clear zone around each disc.

2.7 Minimum Inhibitory Concentration (MIC) Method

The incorporation method outlined by Alzahrani et al. (2012) and Bellik (2014) were used in the determination of minimum inhibitory concentration for young and matured ginger oleoresin. Different concentrations of ginger oleoresin were prepared (1, 5, 10, 50, 150, 300 mg/ml). The blank disc (6mm in diameter) was impregnated with 10 µl each of different concentration of both oleoresins. Then all the discs were allowed to dry and were consequently placed on the test plate. Bacteria and fungi plates were subsequently incubated overnight at 37°C and 30°C accordingly. Minimum inhibitory concentration was determined as the lowest concentration of ginger extracts inhibiting the visible growth of each organism on the agar plate.

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2.8 Statistical Analysis

All the experimental values were measured in triplicates (n=3) and was expressed as mean values with their standard errors. Statistical analysis was performed by Statistical Packages for the Social Science Statistics (SPSS). Difference on statistical analysis of the data was considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Total Phenolic Content (TPC)

Table 1 shows the mean absorbance from three replications and the mean of the total phenolic content of *Zingiber Officinale* Roscoe oleoresin extracts. In the present study, oleoresin of young ginger extracts exhibit higher value of total phenolic content (129.59 ± 14.27 mg/g) compared to matured ginger extract (45.23 ± 7.68 mg/g).

Table 1: Total Phenolic Content of Young and Matured Oleoresin of *Zingiber Officinale* Roscoe

Oleoresin Extracts	Mean Absorbance	Total Phenolic content (mg gallic acid equivalent/g)
Young	0.4494 ± 0.0495	129.5869 ± 14.27
Matured	0.1546 ± 0.0301	45.2335 ± 7.682

The values were expressed as mean ± standard deviation (n=3)

3.2 Antioxidant Activity of Zingiber Officinale Roscoe Extracts using Dpph Radical Scavenging Activity Assay

Table 2 shows the IC_{50} value of both young and matured *Zingiber Officinale* Roscoe oleoresin extracts and the positive control (ascorbic acid). Lower value of IC_{50} indicates higher antioxidant activity. According to Patel-Rajesh and Patel-Natvar (2011), IC_{50} represents the effective concentration of sample to be able to scavenge 50% of DPPH radicals and the lower the value, reflects a better protective mechanism against reactive oxygen species (ROS). There is a significant difference between the IC_{50} of young (0.0941 ± 0.0032) and matured (0.1888 ± 0.0117) *Zingiber Officinale* Roscoe oleoresin.

Table 2: IC_{50} Values of Both Young and Matured *Zingiber Officinale* Roscoe Oleoresin Extracts and the Positive Control (Ascorbic Acid)

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Samples	IC_{50} (mg/ml)
Ascorbic acid	0.1258 ± 0.0054
Young ginger	0.0941 ± 0.0032*
Matured ginger	0.1888 ± 0.0117*

The values were expressed as mean ± standard deviation (n=3). Values within the same column are significantly different at $p < 0.05$

3.3 Antimicrobial Activity

3.3.1 Disc Diffusion Test

The screening of antimicrobial assay using the disc diffusion method showed that both young and matured *Zingiber Officinale* Roscoe oleoresin extracts showed inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa* at 100% concentration (Table 3).

Table 3: The Diameter of Zone of Inhibition for Disc Diffusion Test

Micro organisms	Young Ginger (mm)	Matured Ginger (mm)	+ Control (mm)	- Control (mm)
S. aureus	9.7 ± 0.6 (Weak)	9.3 ± 0.6 (Weak)	22.3 ± 2.1 (Strong)	–
P. aeruginosa	7.3 ± 0.6 (Weak)	6.7 ± 0.3 (Weak)	11.7 ± 1.5 (Weak)	–
C. albicans	–	–	14.2 ± 1.3 (Moderate)	–
A. niger	–	–	14.0 ± 1.0 (Moderate)	–

Note: – = No inhibition zone

However, no inhibition zone was found when both oleoresin extracts were tested against *Candida albicans* and *Aspergillus niger.* Both young and matured *Zingiber Officinale* Roscoe oleoresin were considered to have weak antimicrobial activity. There was also no significant difference between the inhibition zone on matured and young ginger oleoresin on bacteria, *S. aureus* and *P. aeruginosa.* The clearing zone on *S. aureus* was bigger compared to *P. aeruginosa.*

3.3.2 Minimum Inhibitory Concentration (MIC) Test

The minimum inhibitory concentration (MIC) was determined by making dilutions of different extracts of garlic and ginger ranging from 1 mg/ml to 300 mg/ml. The results for MIC test is illustrated in Table 4. There was an inhibition on the tested pathogenic bacteria, *S. aureus* and *P. aeruginosa* while *C. albicans* and *A. niger* show negative results. Both young

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and matured ginger oleoresin extracts were able to inhibit the growth of *S. aureus* as low as 1.0 mg/ml while 10.0 mg/ml for *P. aeruginosa.*

Table 4: Minimum Inhibitory Concentration (MIC) of *Zingiber Officinale* Roscoe Oleoresin Extract on Selected Airborne Microorganisms

Extract	Microorganisms	Concentration (mg/ml)							
		1	5	10	50	100	150	200	300
Young ginger	*S. aureus*	+	+	+	+	+	+	+	+
	P.aeruginosa	–	–	+	+	+	+	+	+
	C. albicans	–	–	–	–	–	–	–	–
	A. niger	–	–	–	–	–	–	–	–
Matured ginger	*S. aureus*	+	+	+	+	+	+	+	+
	P. aeruginosa	–	–	+	+	+	+	+	+
	C. albicans	–	–	–	–	–	–	–	–
	A. niger	–	–	–	–	–	–	–	–

Notes: + = positive result, there is inhibition zone
– = Negative result, no inhibition zone

In the present study, oleoresin of young ginger extract exhibits higher value of total phenolic content (TPC), which was almost triple the TPC amount of matured ginger extract. However, the levels of TPC value for methanolic extract of matured *Zingiber Officinale* Roscoe reported by Zachariah (2008) and young *Zingiber Officinale* Roscoe reported by Ghasemzadeh, Jaafar, and Rahmat (2010) were different from the present study. According to them, matured *Zingiber Officinale* Roscoe possess higher TPC value than young *Zingiber Officinale* Roscoe. This might be because of the differences in variety of gingers used in this study. According to Fahmi (2014), most of the ginger variety sold in Malaysian markets are of Chinese variety. Young ginger probably has more active compounds, such as phenolic compounds, because of its freshness and age. Meanwhile, to reach its maturity of eight to nine months, there might be the degradation of phenolic compounds occurring in matured gingers, which result in low of phenolic contents.

The antioxidant activity of *Zingiber Officinale* Roscoe young and ginger oleoresin extracts were tested using DPPH assay. In this study, the positive control used was ascorbic acid. The higher the percentage of percent inhibition (I%), the higher the ability to scavenge free radicals. Thus the higher the antioxidant activity that the extract occupies. Based on the result, percent inhibition (I%) value of young *Zingiber Officinale* Roscoe extract was higher than matured oleoresin *Zingiber Officinale* Roscoe extract and positive control, ascorbic acid in all tested concentrations. This result states that oleoresin of young *Zingiber Officinale* Roscoe

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was more active compared to the positive control, ascorbic acid and matured oleoresin *Zingiber Officinale* Roscoe extract. Even at the lowest experimental concentration (0.2 mg/ml), oleoresin extract of young ginger is more than 90%. This result signifies that solvent plays the greatest role in extracting the bioactive antioxidant compounds in ginger extract. It was stated that methanol extracts were the most effective DPPH radical scavengers compared to acetone and ethyl acetate (Miliauskas, Venskutonis, & van Beek, 2004). Thus, this finding supports the previous study by Miliauskas et al. (2004) where both of the oleoresin extracts were extracted using methanol as their extracting solvent gave great amount of antioxidant compounds, from both young and matured oleoresin.

The IC_{50} of young oleoresin was better than synthetic antioxidants, ascorbic acid and matured *Zingiber Officinale* Roscoe oleoresin extract. This shows that oleoresin of young *Zingiber Officinale* Roscoe is more effective as an antioxidants against ROS than a well-known antioxidant, ascorbic acid. The higher amount of bioactive compounds present in the young *Zingiber Officinale* Roscoe oleoresin affects excellent ability to scavenge free radicals. This study also corresponds to the previous study by Ghasemzadeh et al. (2010) where the ginger extracts show promising IC_{50} value even at young age.

In this study, young and matured *Zingiber Officinale* Roscoe rhizome were extracted using methanol. According to G. Singh et al. (2008), methanol is the most suitable solvent that is able to extract a high amount of oleoresin compounds and methanol is able to extract active compound such as tannins, alkaloids, glycosides, lignans and terpinoids. Nevertheless, the compounds that exist in both extracts have not been elucidated in this study. In the future, it is best to screen and indentify compounds involved in affecting the antioxidant activity of the ginger oleoresin.

Result in Table 3 was in accordance with the previous study conducted by Bellik (2014), that reported ginger oleoresin showed antimicrobial activities against *S. aureus* and was inactive against *A. niger*. However, in the present study, ginger oleoresin contradicted the study conducted by G. Singh et al. (2008), whereby they observed antimicrobial activities on oleoresin methanolic extract against *A. niger* but there was no inhibition zone against bacteria, *S. aureus* and *P. aeruginosa*. It is believed that origin of *Zingiber Officinale* Roscoe affects biological properties of phytochemical because the study conducted by Bellik (2014) where the *Zingiber Officinale* Roscoe used was Chinese origin while G. Singh et al. (2008) studied Indian varieties. Table 4 also states the strength of antimicrobial activity for all extracts. Both oleoresin extracts can be have weak antimicrobial activity against *S. aureus* and *P. aeruginosa*. This result also contravenes with the study conducted by Joe, Jayachitra, and Vijayapriya (2009) where *Zingiber Officinale* Roscoe shows moderate antimicrobial activity against *S. aureus* and *P. aeruginosa*. Bigger clearing zone on *S. aureus* than *P. aeruginosa* was found where this result was supported by Joe et al. (2009) where the zone of inhibition of methanolic extract of ginger against *S. aureus* was bigger compared to *P. aeruginosa*.

Table 4 shows that bacteria species exhibit different levels of sensitivity towards ginger oleoresin. It is anticipated in MIC result that both oleoresin of *Zingiber Officinale* Roscoe will be able to inhibit the growth of *S. aureus* at lower concentration than *P. aeruginosa* because previous disc diffusion result shows bigger zone of inhibition, when both oleoresin extracts were tested against *S. aureus* and *P. aeruginosa*. The present study also supports the study by

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Bellik (2014) whereby, *Zingiber Officinale* Roscoe oleoresin was able to inhibit the growth of *S. aureus* at a low concentration.

For the overall discussion, it is found that young *Zingiber Officinale* Roscoe exhibits higher total phenolic content than matured gingers. Surprisingly, oleoresin of young *Zingiber Officinale* Roscoe also surpassed the inhibition value of positive control, synthetic antioxidant ascorbic acid. This result supports earlier study by G. Singh et al. (2008) in which oleoresin of *Zingiber Officinale* Roscoe also shows high antioxidant activity compared to synthetic antioxidant, Butylhydroxyanisole (BHA). Hence, it can be said that *Zingiber Officinale* Roscoe extract shows promising results to replace synthetic antioxidants in the future. Young ginger oleoresin also shows higher antimicrobial activity than matured gingers. Therefore, it can be concluded that young *Zingiber Officinale* Roscoe exhibits more antioxidant and antimicrobial activity than matured *Zingiber Officinale* Roscoe.

4. CONCLUSION

In conclusion, young *Zingiber Officinale* Roscoe oleoresin exhibits higher total phenolic content, antioxidant and antibacterial activity than matured *Zingiber Officinale* Roscoe oleoresin. Data from the present study agrees with the existing studies on *Zingiber Officinale* Roscoe, which exhibit higher antioxidant activity and possesses the ability to inhibit the growth of pathogenic bacteria. However, it is also indicated that oleoresin of *Zingiber Officinale* Roscoe was unable to suppress the growth of airborne pathogenic fungi and failed to support previous studies in which ginger held the characteristics of an antifungal agent.

It can also be said that the higher total phenolic content of certain compounds signifies their antioxidant and antimicrobial activity. Hence, in this study, it shows that oleoresin of young ginger bears more active constituents than matured ginger that have the ability to prevent free radical damage and growth of pathogenic microorganisms. This is due to the considerably high antioxidant value of ginger oleoresin extract. Even at a very low concentrations, both oleoresin extracts are capable to surpass the antioxidant activity of synthetic antioxidant compounds such as ascorbic acid. Hence, ginger oleoresin extract can be regarded as a potential antioxidant to replace synthetic antioxidants.

At the same concentration of 1.0 mg/ml and 10.0 mg/ml, both ginger oleoresin extracts are able to prevent the growth of *S. aureus* and *P. aeruginosa.* This is probably due to the high amount of active compounds present in the ginger oleoresin extract. Despite the ability to hinder the growth of airborne pathogenic bacteria, fungi shows resistance against both oleoresin extracts since *C. albicans* and *A. niger* seem to have developed a resistance against many antifungal agents and antibiotics.

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