Research Article

Brine Shrimp Lethality and Antioxidant Activity of the Leaf, Rind and Seed Ethanolic Extracts of *Durio zibethinus* L.

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ABSTRACT

Introduction: Substances derived from plants have recently receiving renewed interest due to their versatile applications. Parts of *Durio zibethinus* L. have been used traditionally as remedy to many diseases. To date, limited reports on the cytotoxicity and antioxidative property of *D. zibethinus* parts are available. **Objective:** The study aims to determine the cytotoxic and antioxidative property of the leaf, seed and rind ethanolic extracts of *D. zibethinus* L. **Materials and Methods:** Cytotoxicity of the leaf, seed and rind ethanolic extracts of *D. zibethinus* L. were determined through Brine Shrimp Lethality Assay (BSLA) while antioxidative property were measured via 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity and Reducing Power Assay. **Results and Discussions:** Results of the BSLA showed that the LC_{50} value of the ethanolic leaf, rind and seed extracts are 624.00, 139.31 and 46.28 mg/L, respectively. The data indicate potential cytotoxic property of the rind and seed extracts which can be attributed to the anthraquinones, flavonoids, phenolics, saponins and terpenoids detected in the rinds and seed extracts. The effective concentration (EC_{50}) value for the DPPH radical scavenging activity and reducing power for the seed and rind ethanolic extracts showed significantly higher activity compared to the leaf extract. Similarities in the trend of the DPPH inhibition and reducing power of the ethanolic extracts of the leaves, rinds and seed ethanolic extracts imply direct correlation between DPPH scavenging activity and reducing power ($P<0.01$). **Conclusion:** *D. zibethinus* seeds and rinds can be potential sources of natural cytotoxic and antioxidant compounds.

Key words: *Durio zibethinus,* Cytotoxicity, Antioxidant property, DPPH, BSLA.

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INTRODUCTION

Substances derived from plants have recently receiving renewed interest due to their versatile applications. Medicinal plants are abundant bioresource used in traditional and modern medications, nutraceuticals, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs.[1-2] Nowadays, researches are geared toward discovery of new drugs that possesses potency to combat the menace of drug resistant pathogenic microorganisms, antitumor and anticancer agents.[3] Moreover, researches have indicated that the intake of plant-derived antioxidant helps prevent the development of some diseases. These antioxidants, which can be synthetic or natural, are vital to combat oxidative damage by free radicals in many oxidative stress-mediated disease conditions. However, synthetic antioxidants such as Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) have been recently reported to be dangerous to human health.[4]

Studies devoted to exploring plants as potential sources of natural cytotoxic antioxidant phytochemicals have been conducted. Phytochemicals or secondary metabolites, synthesized from plants, are capable of performing physiological actions in the body.[5-6] Some phytochemicals are found responsible to plants' antioxidant and cytotoxic properties.

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Durio zibethinus L. is known as a king of fruits in Southeast Asia with a distinctive thorn-covered husk and a unique odor. Of the known species that comprises the genus Durio, *D. zibethinus* L. is cultivated to a great extent.[7] About only 30% of the *D. zibethinus* fruit constitutes the only edible portion while the seeds and rinds are treated as food waste.[8] Moreover, numerous studies have reported that different parts of *D. zibethinus* L. trees have been traditionally used as an effective therapy against malaria fever, jaundice, skin diseases, swellings, colds, sores and wounds.[9] Different parts of *D. zibethinus* L. were also reported to exhibit antidiabetic, antimicrobial, anti-inflammatory and anthelminthic properties.[10-13] However, limited reports on the cytotoxic and antioxidant activity among *D. zibethinus* L plant parts are available. Thus, this study aimed to investigate the cytotoxic and antioxidative property of the leaf, seed and rind ethanolic extracts of *D. zibethinus* L. to provide scientific evidence that would support its medicinal properties.

MATERIALS AND METHOD

Sample Collection and Preparation

Mature leaves and fruits of *Durio zibethinus* L. were collected from Patag, Alanib, Lantapan, Bukidnon with Global Positioning System (GPS) coordinates of 8°01'57.9" N and 124°58'56.3" E. The leaf samples were washed with distilled water to remove dirt. The rinds and seeds of the fruit were separated from the flesh and washed with distilled water. Identification and authentication of the samples were done at the Botany section of the Central Mindanao University (CMU) Museum, Musuan, Bukidnon. The samples were then air-dried separately until the moisture content is reduced to at least 10 percent. The dried samples were pulverized using a grinder, extracted with ethanol for 72 hrs and filtered. The filtrates were then concentrated in vacuo at 40ºC to yield Concentrated Leaf (LE), Rind (RE) and Seed (SE) ethanolic extracts.

Phytochemical Screening

The LE, RE and SE were screened for presence of phytochemicals, i.e. alkaloids, anthraquinones, flavonoids, phenols, saponins, tannins and terpenoids employing Thin-layer Chromatography (TLC) method with slight modification.[14]

Brine Shrimp Lethality Assay

The brine shrimp lethality assay was carried out with slight modification.[15] A 10,000 mg/L LE, RE and SE stock solutions in ethanol were prepared. Appropriate volumes of 500.0, 250.0, 50.0, 25.0, 5.0 and 2.5 µL of the stock solution were placed into separate test tubes marked up to 5.00 mL to give 1000, 500, 100, 50, 10 and 5 mg/L, respectively. The solution was purged with N2 gas to remove the solvent. Minimal amount of Dimethyl Sulfoxide (DMSO) corresponding to 50.00, 25.00, 5.00, 2.50, 0.50 and 0.25 µL for 1000, 500, 100, 50, 10 and 5 mg/L, respectively, were added as liquid surfactant into each test tube. The mixture was then vortexed to ensure homogeneity. Ten brine shrimp nauplii were transferred from the hatching chamber into each test tubes. Sterilized seawater was added up to the 5 mL mark and the test tubes were incubated under illumination for 24 hrs. Negative and positive controls were run using sterilized seawater with DMSO and potassium dichromate, respectively. The number of surviving shrimps were counted and recorded after 24 hrs. The percentage mortality of nauplii was then calculated.

For sample test solutions giving 0% and 100% mortalities, necessary corrections were done.[16] Using the Probit Table of Finney, the corrected percentage mortality values were transformed to probit values.[17] The best-fitting straight curve was then drawn with the logarithm of the concentration values as the abscissa and the probit values as the ordinate. The log10 concentration value that corresponds to the probit point of 5.00 from the curve was recorded and converted to its antilog value to give the LC_{50}. LC_{50} of less than 200 mg/L was considered cytotoxic.[18] Analysis was carried out in five replicates. Result was then reported as mean of the analysis.

DPPH Radical Scavenging Activity Assay

The DPPH radical scavenging activity assay was carried out in a 96-well microplate.[19] Various concentrations of LE, RE and SE test solutions (5 -500 µg/mL) were prepared in ethanol.

In a well containing 150 µL of sample solution, 50 µL of 0.1 mM DPPH in absolute ethanol was added. The mixture was incubated for 30 mins in the dark at room temperature. The absorbance for the reaction mixtures was measured at 517 nm using a microplate reader. The same procedure was done for the various gallic acid solutions (15-210 µg/mL) and absolute ethanol (blank). The absorbance readings were recorded and the DPPH radical scavenging activity were calculated as percentage DPPH inhibition (Equation 1).

$$\%\ \text{DPPH inhibition} = \left(\frac{A_{cont} - A_{sam\ or\ std}}{A_{cont}} \times 100\right) \quad \text{Equation 1}$$

where:

Acontrol = Absorbance of the control

Asample = Absorbance of the sample solution

Astandard = Absorbance of the gallic acid solution

The Least Squares Method was then employed to obtain the equation of the line. The equation was used to calculate the EC_{50} (concentration required to obtain a 50% DPPH radical inhibition) of the extract solutions. Result is the mean value of the triplicate analysis.

Reducing Power Assay

The reducing power was determined employing the method adapted for a 96-well plate assay.[20] Various concentrations of SE, RE and SE test solutions (50, 100, 250, 500 and 750 µg/mL) were prepared in ethanol. In an Eppendorf tube containing 1 mL of the sample test solution, 200 µL of 0.2 M phosphate buffer (pH 6.6) and 200 µL of 1% (w/v) solution of potassium ferricyanide were added. The mixture was then incubated at 50°C for 30 mins. Then 10% (w/v) trichloroacetic acid (200 µL) was added after the solution was cooled to room temperature. The resulting mixture was centrifuged at 11000 rpm for 3 mins. After centrifugation, an aliquot (200 µL) of the upper layer of the solution was transferred to a 96-well plate and 20 µL of 0.1% (w/v) solution of ferric chloride was added. The absorbance of the solution was measured at 620 nm using a microplate spectrophotometer reader. The same procedure was done for the negative control (absolute ethanol) and standard gallic acid test solutions (15, 30, 45, 60, 75 and 90 µg/mL). The percent reducing power of the sample and gallic acid test solutions were then calculated using Equation 2.

$$\text{Reducing Power (\%)} = \left(\frac{A_{\text{sample or std}} - A_{\text{cont}}}{A_{\text{sample}}} \times 100\right) \qquad \text{Equation 2}$$

where:

Acontrol = Absorbance of the control

Asample = Absorbance of the sample test solution

The EC_{50} (mg/L) of the sample solutions was determined by Least Squares Method. The equation of the line was then used to calculate the EC_{50} (concentration required to obtain a 50% DPPH radical inhibition) of the solutions. Result is the mean value of the triplicate analysis.

RESULTS

Phytochemical Screening

The results of the phytochemical screening are shown in Table 1.

Brine Shrimp Lethality

The percent mortality of the A. salina nauplii in LE, RE and SE and the LC_{50} of the sample extracts are presented in Table 2.

Table 1: Phytochemical screening of ethanolic leaf, rinds and seed extracts of *D. zibethinus* L.

PHYTOCHEMICAL	ETHANOLIC EXTRACT			Flesh[21]
	Leaves	Rinds	Seeds	
Alkaloids	+	-	-	+
Anthraquinones	-	+	+	+
Flavonoids	-	+	+	+
Phenolics	+	+	+	+
Saponins	-	+	+	+
Tannins	-	-	-	+
Terpenoids	-	+	+	+

(+) – present; (-) – absent.

Table 2: LC_{50} of the extracts and percent mortality (%) of the brine shrimp nauplii after 24hr exposure to the ethanolic leaf, rind and seed extracts of *D. zibethinus* L.

Sample	Mortality* (%)						LC_{50} (mg/L)	Inference**
	5 mg/L	10 mg/L	50 mg/L	100 mg/L	500 mg/L	1000 mg/L		
Leaves	2.50 ±0.00	2.50 ± 0.00	2.50 ± 0.00	19.50 ± 2.09	43.00 ± 10.06	63.00 ± 2.74	624.00	Non-cytotoxic
Rinds	3.25 ± 0.00	11.88 ± 2.46	30.00 ± 1.77	46.00 ± 9.45	70.63 ± 2.39	85.38 ± 4.89	139.31	Cytotoxic
Seeds	10.32 ± 0.36	25.50 ± 3.26	50.00 ± 10.16	68.50 ± 4.19	83.63 ± 7.20	95.50 ± 2.87	46.28	Cytotoxic
Positive Control ($K_2Cr_2O_7$)	90.00 ± 0.00	97.50 ± 0.00	97.50 ± 0.00	97.50 ± 0.00	97.50 ± 0.00	97.50 ± 0.00	< 5.00	Cytotoxic

*Data are expressed as mean ± SD. Each sample was analyzed five times.

**LC50 < 200 mg/L is cytotoxic.[18]

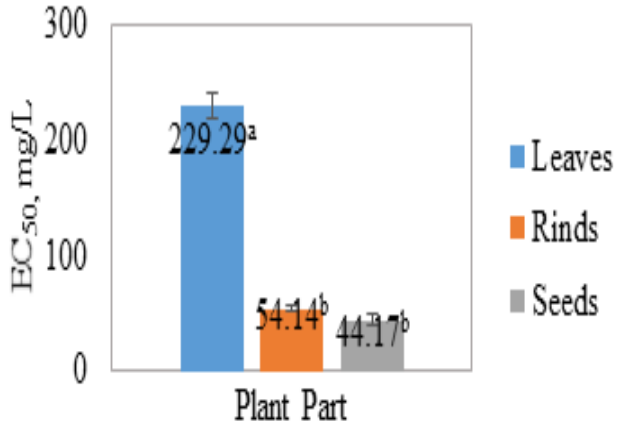

Figure 1: EC_{50} value (mg/L) for the DPPH radical scavenging activity of the ethanolic extracts of *D. zibethinus* L. leaves, rinds and seeds. Error bars are standard error (n=3).

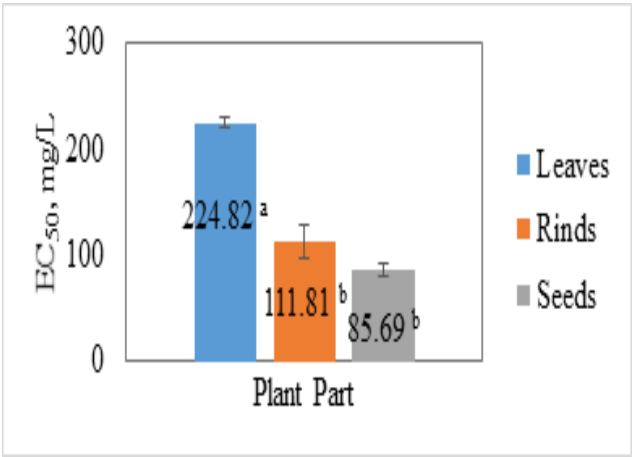

Figure 2: EC_{50} value (mg/L) for the reducing power of the ethanolic extracts of *D. zibethinus* L. leaves, rinds and seeds. Error bars are standard error (n=3).

DPPH Radical Scavenging Activity

The EC_{50} for the DPPH radical scavenging activity of the *D. zibethinus* ethanolic extracts are shown in Figure 1. The mean values of the same letter superscript are not significantly different by Tukey's Test at 0.05 level of significance.

Reducing Power

The antioxidant property of *D. zibethinus* in terms of reducing power is presented in Figure 2. The mean values of the same letter superscript are not significantly different by Tukey's Test at 0.05 level of significance.

DISCUSSION

Phytochemical Screening

Alkaloids and phenolics were detected in the ethanolic leaf extract of *D. zibethinus* L., while anthraquinones, flavonoids, phenolics, saponins and terpenoids were detected in the ethanolic rind and seed extracts (Table 1). The positive results of the screening are consistent with the results of the previous studies using the flesh of *D. zibethinus* L.[12,21]

Several studies have reported that alkaloids, anthraquinones, flavonoids, phenolics, saponins and terpenoids possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflamation, antiatherosclerosis and cardiovascular protection[3,22,23] as well as analgesic, antispasmodic, antibacterial, antimutagenic, antioxidant and antiviral effects.[13,24-25] These findings may support claims on the reported medicinal properties of the leaves, rinds and seeds of *D. zibethinus* L. such as lipid lowering effect, immunomodulatory and antibacterial effect, efficiency in interaction with medicinal entities, bactericidal and antibacterial effects, antimicrobial and antiviral activities, synergism effect with Penicillin G and antioxidative activities.[26-29]

Brine Shrimp Lethality

The Brine Shrimp Lethality Assay (BSLA) was carried out to evaluate the cytotoxicity of the ethanolic leaf, rind and seed extracts of *D. zibethinus* L. against the brine shrimp nauplii. The result of BSLA is summarized in Table 2.

The percent mortality of the brine shrimp nauplii increases as the concentration of the test solutions are increased. Apparently, the percent mortality of the nauplii was in the order of leaves < rinds < seeds extracts for all concentrations of the test solutions. This indicates that the mortality of the nauplii in sample extracts is concentration-dependent The highest percent mortality values in 5 mg/L to 1000 mg/L test solutions were consistently recorded for the ethanolic seed extracts while the lowest percent mortality values were observed for the ethanolic leaf extracts. These findings indicate that the ethanolic extracts of the various plant parts

Table 3: Pearson's correlation test between DPPH radical scavenging activity and reducing power of the ethanolic extracts of *D. zibethinus* L. leaves, rinds and seeds.

		DPPH	Reducing Power
DPPH	Pearson Correlation	1	.950**
	Sig. (2-tailed)		<.001
	N	9	9
Reducing Power	Pearson Correlation	.950**	1
	Sig. (2-tailed)	<.001	
	N	9	9

** Correlation is significant at the 0.01 level (2-tailed).

of *D. zibethinus* L. exhibit cytotoxicity against the brine shrimp nauplii and that its toxicity to the nauplii is plant part-dependent. The relatively high cytotoxicity of the ethanolic rind and seed extracts than the leaf extracts can be due to the different phytochemicals in *D. zibethinus* L. rinds and seeds. Apart from the alkaloids and phenolics which are present among the three plant parts, the seeds and the rind ethanolic extracts contain anthraquinones, flavonoids, saponins and terpenoids as well. Studies have shown that these phytochemicals are known to exhibit analgesic, antispasmodic, antibacterial, antimicrobial, anti-inflammatory, antioxidant, anti-allergenic, diuretic, antiviral, anticancer and antitumor properties.[14,30-33]

For the LC_{50}, the ethanolic leaf extract gave the highest value (624.00 mg/L), followed by the rind (139.31 mg/L) and the seed (46.28 mg/L). Since the LC_{50} value and the plant extract toxicity are inversely proportional, the cytotoxic property of the plant parts increases in order of leaves < rinds < seeds. Plant extracts with LC_{50} less than 200 ppm are considered significantly active and had the potential for further investigation.[18] Thus, rinds and seeds exhibited potential cytotoxicity that would warrant further studies.

DPPH Radical Scavenging Activity

The results of the determination of the DPPH radical scavenging activity, in terms of EC_{50} value, of the ethanolic extracts of D. *zibethinus* L. leaves, rinds and seeds show that among the plant parts, the leaves gave the highest EC_{50} value, followed by the rinds and seeds which recorded of 229.29, 54.14 and 44.17 mg/L respectively (Figure 1). On the other hand, pure gallic acid recorded a value of less than 15.00 mg/L. The result show that *D. zibethinus* L. leaves, rinds and seeds exhibit antioxidative property via DPPH free radical scavenging mechanism.

The EC_{50} is the efficient concentration of the substrate which is required to cause an amount of 50% loss in DPPH radical activity.[34] The lower the EC_{50} value, the higher the scavenging activity.[35]

The results of the Analysis of Variance (ANOVA) at 0.05 level of significance and the subsequent Post Hoc Tukey's Test indicate that the ethanolic extracts of *D. zibethinus* L. rinds and seeds have statistically similar EC_{50} value but is significantly lower than that of *D. zibethinus* L. leaves. This indicate that the rind and seed extracts have higher DPPH radical scavenging activity than the leaf extract. Thus, the DPPH radical scavenging activity of *D. zibethinus* L. is plant-part dependent.

Phytochemicals in different plant parts is speculated to affect their bioactivity. This was demonstrated in a study wherein total phenolic and flavonoid content and antioxidant activity of Betula utilis D. Don vary among plant parts.[36] Moreover, these findings support results of previous studies which detected presence of polyphenols like phenolics and flavonoids in the pulp of *D. zibethinus* which are contributors to the plant's antioxidant capacity.[37] Presence of polyphenol and Superoxide dismutase (SOD) in the durian seed, sarcocarp and peel extracts.[37-38]

Reducing Power

Reductive capabilities of plant extracts can serve as a significant indicator of their potential antioxidant activities.[39]

As shown in Figure 2, seed ethanolic extract exhibited the highest reducing power followed by rind and leaf extracts. However, pure gallic acid showed higher activity. Results imply that *D. zibethinus* L. leaves, rinds and seeds possess antioxidative property specifically reduction capability.

The results of the Analysis of Variance (ANOVA) at 0.05 level of significance and the subsequent Post Hoc Tukey's Test reveal that *D. zibethinus* L. rind and seed ethanolic extracts demonstrated significantly higher reducing power and consequently higher antioxidant activity. This can be accounted to the differences in the type and amount or concentration of phytochemicals present in the different plant parts.[40] Thus, antioxidant activity, of *D. zibethinus* L., in terms of reducing power, vary with plant part.

However, the similarities in the trend of the DPPH inhibition and reducing power in *D. zibethinus* L. leaves, rinds and seeds is interesting to note. Using Pearson's correlation test, which quantifies the linear association between two quantitative variables, it is possible to estimate the relationship between DPPH inhibition and reducing power. The results, as shown in Table 3, reveal that there is a positive correlation between DPPH and reducing power ($P < 0.01$) in *D. zibethinus* L. leaves, rinds and seeds. This implies that increase in reducing power results in a corresponding increase in the DPPH radical scavenging activity of the ethanolic extracts of *D. zibethinus* L. leaves, rinds and seeds.

The same correlation between DPPH radical scavenging activity and reducing power was observed in Porphyra indica ($P<0.01$) and Porphyra vieatnamensis ($P<0.05$).[41] This observation indicates that the substances responsible to reduce Fe^{3+} to Fe^{2+} are capable of scavenging DPPH radical satisfactorily and suggest that these substances might react with DPPH radical through electron transfer reaction mechanism.[42]

CONCLUSION

The present data obtained showed that ethanolic rind and seed extracts of *D. zibithenus* possess cytotoxic and antioxidant properties. Results of the study may also serve as scientific evidence to support traditional use of *D. zibethinus* L. rinds and seeds. Rrinds and seeds of *D. zibethinus* L., which are considered as food waste, may be utilized as potential source of natural cytotoxic and antioxidant compounds. Thus, studies to further establish the bioactivity of *D. zibethinus* L. and to isolate the compounds responsible for its bioactivity is recommended.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVITAIONS

ANOVA: Analysis of Variance; **BHA:** Butylated; hydroxyanisole; **BHT:** Butylated hydroxytoluene; **BSLA:** Brine Shrimp Lethality Assay; **DPPH:** 2,2-diphenyl- 1-picrylhydrazyl; **DMSO:** Dimethylsulfoxide; **EC_{50}:** Effective Concentration; **LC_{50}:** Lethal Concentration; **SOD:** Superoxide dismutase; **TLC:** Thin Layer Chromatography.

SUMMARY

The study evaluates the cytotoxic and antioxidative property of the leaf, seed, and rind ethanolic extracts of *Durio zibethinus* L. The results obtained indicate potential cytotoxic property of the rind and seed ethanolic extracts. Moreover, a significantly higher DPPH radical scavenging activity and reducing power was exhibited by the seed and rind extracts as compared to the leaf extracts. These can be attributed to the phytochemicals, i.e. anthraquinones, flavonoids, phenolics, saponins, and terpenoids, detected in the said plant extracts. Thus, *D. zibethinus* seeds and rinds can be potential sources of natural cytotoxic and antioxidant compounds.

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