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Research Article

Total Phenolic Content and Antibacterial Properties of Adlay (Coix *lacryma-jobi* L.) Var Gulian and Ginampay

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ABSTRACT

Coix lacryma-jobi L., commonly known as Adlay or Job's tears is a traditional crop known for its nutritional and medicinal value, attributed to its diverse phytochemicals. Among its varieties, Gulian and Ginampay are widely cultivated in the Philippines, yet limited studies have investigated their bioactive properties. This study aimed to compare the total phenolic content and antibacterial activity of seed oils from Gulian and Ginampay varieties. Phytochemical screening of oil extracted via solvent-extraction revealed that Gulian oil contained significantly higher levels of total phenolics (4.8 mg GAE/g) and terpenoids compared to Ginampay, indicating a richer profile of bioactive compounds. Antibacterial activity, assessed using the Kirby-Bauer Disk Diffusion Assay against *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*, showed that Gulian oil exhibited concentration-dependent inhibition, specifically against Gram-positive bacteria. At 70% v/v dilution, it produced zones of inhibition of 9.01 mm and 6.17 mm against *S. aureus* and *B. subtilis*, respectively. In contrast, Ginampay oil demonstrated minimal or no antibacterial effects. Statistical analysis confirmed significant differences among treatments ($p < 0.05$). Overall, the findings showed the superior phytochemical content and moderate antibacterial potential of Gulian seed oil, supporting its possible application as a natural therapeutic and antimicrobial agent, while Ginampay oil may require enhancement for effective antimicrobial use indicating potential for formulation improvement.

Keywords: Coix lacryma-jobi L., Phytochemical Screening, Antibacterial Assay, Bioactive Compounds.

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1. INTRODUCTION

Coix lacryma-jobi L., commonly known as Adlay or Job's tear is a tall, robust and tillering grass species of the Poaceae family [1]. Adlay is an erect, branching plant that can grow up to 3 meters tall with sword-shaped leaves and glossy bracts with delicate bran and starchy grains [2]. As a short-day plant, it thrives well in tropical areas with a warm climate, high temperature, plenty of rain and fertile soil. It originated and cultivated mainly in Africa and in East and Southeast Asia [3]. In the Philippines, Adlay is cultivated and consumed particularly in Batangas, Isabela, Romblon, the Bicol Region [4].

As a traditional crop, Adlay has been gaining attention due to its adaptability and nutrient value [5]. The grains of this plant are nutrient-rich and possess numerous therapeutic and nutraceutical properties. They are a source of several bioactive compounds and phytochemicals including phenolic acids, flavonoids, anthocyanins, coixenolide, phytosterols, and polysaccharides which contribute to their antioxidant, anti-inflammatory, anticancer, and lipid-lowering effects. In traditional Asian medicine, Adlay has been widely used for managing high cholesterol, promoting weight loss, alleviating menstrual disorders, treating arthritis, and supporting digestive health. Its antioxidant components help neutralize free radicals, reducing oxidative stress and inflammation [6- 9]. Adlay grain is a source of saponins, which possess anti-cancer, anti-inflammatory, and cholesterol-lowering properties (10). It also contains alkaloids like coixol and coixenolide, known for their anti-inflammatory and anti-tumor activities (11). Additionally, it is rich in other phytochemicals such as triterpenoids and sterols, which may support immune function and cardiovascular health (12). Its abundance of polysaccharides further contributes to its immune-enhancing effects (13). These bioactive compounds are key to Adlay's use in herbal medicine and its wide range of health benefits.

Gulian and Ginampay are two distinct and widely cultivated types of Adlay, exhibiting specific characteristics that increase their importance in agricultural activities. Ginampay has become well-regarded for its appeal to consumers when combined with rice, enhancing the nutritional quality by increasing protein and healthy fat levels while maintaining energy values similar to those of plain rice [14]. Studies reveal that Ginampay produced the most significant yield among the varieties examined, attaining 3,413 kg per hectare. Further research indicates that it exhibits strong agronomic characteristics, such as a significant number of productive tillers and effective growth in less fertile soils [15]. On the contrary, Gulian, is recognized for its non-gluten grains featuring a seed coat ranging from white to beige. It produces 2.63 tons per hectare and ripens slightly sooner than other varieties, allowing farmers to receive quicker returns on their investment. It also possesses a strong tillering ability, averaging 7 tillers per plant, which enhances its total productivity [2].

Despite its potential, the existing phytochemical information on particular varieties of Adlay concerning the pharmacological characteristics of its various types is limited to this date. Thus, this study sought to fill this gap by analyzing the phytochemical characteristics and evaluating the antibacterial activities of Ginampay and Gulian varieties of Adlay, in order to explore their pharmaceutical potentials. Additionally, research findings could serve as a foundation for effective breeding strategies of Adlay. This study provides novel findings into their therapeutic potential and contributes valuable data for future applications in natural medicine and product development by comparing the two varieties in terms of both phytochemical content and antibacterial properties.

2. METHODOLOGY

2.1. Place and Duration of the Study

The Phytochemical assays and Antibacterial property screening were conducted at the Tuklas Lunas Development Center Annex Building and in the Natural Products Research Development Center at Central Mindanao University. Permits were obtained for the use of both laboratories. This research took place from January to April 2025.

2.2. Sample Preparation

The seeds were obtained from nearby farmers and millers in agricultural areas recognized for growing these particular varieties. Sample selection relied on adequate post-harvest handling and milling techniques to guarantee quality and uniformity. At collection, the seeds were labeled and documented by variety and source, to ensure traceability during the experimental procedure. The seeds were then processed with a mechanical grinder to achieve a fine, uniform powder, enhancing the effective extraction of phytochemicals in the subsequent analyses. These powdered seed samples acted as the main raw material for oil extraction, phytochemical analysis, and antibacterial tests. Samples sourced locally were utilized to guarantee that the study's findings stayed pertinent to the indigenous cultivars, minimizing the variables that could influence the comparative bioactivity and chemical characteristics of these local Adlay varieties. Proper handling and storage of samples were maintained to avoid contamination or deterioration of bioactive compounds prior to laboratory analysis.

2.3. Seed Oil Extraction

The seed oil extraction process was conducted using solvent extraction with 100% ethanol, followed by rotary evaporation for efficient oil recovery [16]. Initially, the seeds were milled into a fine powder and 400 grams of seed powder were soaked in ethanol for 24 hours to facilitate optimal extraction. After soaking, the mixture was filtered to remove solid residues, and the resulting filtrate was transferred to a round-bottom flask for rotary evaporation. Rotary evaporation using ethanolic solvent was used because of its efficiency in dissolving a wide range of polar and non-polar phytochemicals, including phenolics and terpenoids. Ethanol is also generally recognized as safe (GRAS), making it a suitable choice for studies involving bioactive compounds intended for therapeutic or nutraceutical applications. Under reduced pressure and controlled temperature, ethanol was evaporated and collected separately, leaving behind the concentrated seed oil. Approximately 4 mL of oil was obtained, corresponding to an extraction yield of 1% (v/w). The extracted oil was then air-dried and stored for further analysis.

2.4. Total Phenolic Content and Qualitative Test for Terpenoids.

The seed extracts of the two Adlay varieties were processed at the Natural Products Research and Development Center to assess the total phenolic content (TPC) and Qualitative Test for Terpenoids. The TPC was determined using the Folin-Ciocalteu Colorimetric method, where samples were mixed with the reagent and was incubated before measuring absorbance at 765 nm using a spectrophotometer [17]. Lastly, the qualitative test for terpenoids was performed using a developing solvent system composed of ethyl acetate, acetic acid, water, and formic acid in the ratio 100:11:11:26. Samples were applied to a chromatographic medium and developed in this solvent system. After development, the plates were sprayed with 0.5% vanillin-sulfuric acid solution as a derivatizing agent and heated to visualize terpenoid spots by color change [18].

2.5. Antibacterial property screening

The antibacterial activity of Gulian and Ginampay seed oils was assessed using the Kirby-Bauer Disk Diffusion method performed in triplicates, to ensure accuracy and reliability of results. Four bacterial strains; *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa* were obtained as pure cultures and maintained on nutrient agar slants at 4°C. Before testing, each strain was cultivated in nutrient agar and incubated at 37 °C for 24 hours, to achieve optimal growth. Bacterial suspension was prepared for each strain by transferring a loopful of colonies into a sterile nutrient broth, and eventually adjusted and compared its turbidity to 0.5 McFarland standard. Bacterial suspensions were prepared by transferring colonies into sterile nutrient broth, adjusting turbidity to the 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) to standardize inoculum density [19]. Seed oils were extracted and diluted with sterile dimethyl sulfoxide (DMSO) mixed with Tween 20 to achieve 50%, 60%, and 70% concentrations. Each standardized suspension with the test organism was aseptically inoculated in a Mueller-Hinton agar by completely swabbing the surface of the plate. Chloramphenicol (30 µg) was used as the positive control, while the solvent system served as the negative control. Plates were incubated at 37°C for 24 hours, after which zones of inhibition were measured in millimeters using a digital caliper following established protocols [20-21].

2.6. Statistical Analysis

All experimental measurements were conducted in triplicate, and the results were expressed as the average of three analyses. The data was presented as the mean ± standard deviation (SD). To assess the significance of differences between means, one-way analysis of variance (ANOVA) was employed, with a significance level set at $p < 0.05$. The data was analyzed using the Statistical Package for the Social Sciences (SPSS) software, ensuring reliable statistical evaluation of the results.

3. RESULTS AND DISCUSSION

Folin–Ciocalteu Colorimetric *Assay*

The Total Phenolic Content of the Gulian and Ginampay Adlay varieties displayed a significant variation between Gulian (T1) and Ginampay (T2). Gulian exhibited a TPC value of 4.8 ± 0.1 mg GAE/g, which was markedly higher than that of Ginampay, which only registered 0.60 ± 0.04 mg GAE/g (Table 1). Statistical analysis using the F-test indicated that the difference between the two cultivars was highly significant ($p < 0.01$). Furthermore, the coefficient of variation (CV) was low at 3.70%, reflecting the consistency and reliability of the experimental measurements. The mean comparison using Tukey's HSD test confirmed that the values for Gulian and Ginampay were significantly different, as indicated by the different letter groupings (a and b, respectively). The elevated TPC in Gulian suggests a greater abundance of phenolic compounds, which are known to possess antioxidant, anti-inflammatory, and other bioactive properties. This indicates that Gulian may offer more substantial health-promoting benefits, especially in terms of oxidative stress mitigation and potential disease prevention.

Phenolic compounds are known for their antioxidant activity and play critical roles in plant defense, pigmentation, and health-related properties when consumed by humans [22]. The elevated TPC in Gulian suggests that this variety has a higher capacity for antioxidant defense, which could be due to genetic differences affecting phenolic biosynthesis pathways. These biosynthetic differences may be linked to variations in gene expression, enzymatic activity (e.g., phenylalanine ammonia-lyase), or stress responses that stimulate phenolic accumulation [23]. The remarkably low phenolic content in Ginampay could imply lower antioxidant potential, possibly due to a less active phenylpropanoid pathway or differences in storage tissues.

Previous studies have similarly shown wide intraspecific variation in TPC among cereal grains and their wild relatives [24, 25]. For instance, significant differences in phenolic content and antioxidant capacity have been reported between pigmented and non-pigmented varieties of rice and barley.

The significant variation the total phenolic contents of the two Adlay varieties also aligns with results from similar studies on the genetic and environmental influences on phenolic accumulation in plant species. Based on the findings of this study, Gulian exhibited a TPC of 4.8 ± 0.1 mg GAE/g, significantly higher than Ginampay, which is only 0.60 ± 0.04 mg GAE/g. This variation aligns with the results of Wang et al. (2013) who reported similar differences in TPC across different Adlay varieties, with values ranging from 0.5 mg GAE/g to over 5.0 mg GAE/g [26]. This suggests that genetic differences between the two varieties, likely influenced by their inherent biochemical pathways, could explain the higher phenolic content in Gulian. Additionally, Xu et al. (2017) highlighted how the seeds of Adlay tend to have higher phenolic levels compared to other parts of the plant, which could contribute to the higher TPC observed in Gulian if both varieties were analyzed from the same part of the plant [27]. Additionally, studies on other varieties of Adlay such as the wild variety Agrotis in Indonesia have reported varying TPC values. The study conducted by Andriana et al. (2023) found a TPC of 0.35 mg GAE/g in an ethanol extract of Agrotis. These findings suggest that different varieties of Adlay may have distinct phenolic profiles, which could influence their antioxidant activities [28]. Environmental factors, such as soil quality, temperature, and stress conditions, also play a critical role in phenolic synthesis. The study conducted by Martini et al. (2014) also noted that growing conditions, such as drought or high altitudes, could enhance phenolic accumulation in plants, further supporting the idea that environmental conditions may have contributed to the differences in TPC between the two varieties in this study [29]. These findings are consistent with research on other cereal crops, such as barley and wheat, where the phenolic content varied widely between varieties and environmental conditions [32-31].

Moreover, the data suggests that Gulian could be a more suitable candidate for nutritional applications, particularly in the development of functional foods or nutraceuticals. Its higher phenolic content may confer greater health benefits, including anti-inflammatory, anti-cancer, and cardioprotective properties [32]. From a methodological perspective, the use of the Folin–Ciocalteu assay remains a reliable, though non-specific, approach for estimating total phenolics. It is important to note that this method may also react with other reducing substances, including vitamin C and some sugars [33]. However, the controlled nature of this comparative assay ensures that relative differences between Gulian and Ginampay remain valid and meaningful. In summary, the significantly higher TPC observed in Gulian highlights its potential as a superior source of natural antioxidants. The low phenolic content in Ginampay, while still measurable, suggests limited health-promoting compounds in comparison.

Table 1. Total Phenolic Content of Gulian and Ginampay Varieties of *C. lacryma-jobi* .

Treatments	Total Phenolic Content (mg GAE/g)
T1-Gulian	4.8±0.1 a
T2- Ginampay	0.60±0.04 b
F-test	**
C.V. (%)	3.70

Means in a column followed by the same letter are not significantly different at 0.05 level using Turkey's HSD Test **-highly significant

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Qualitative Test for Terpenoids

Terpenoids are known for their wide range of therapeutic effects, including anti-inflammatory, antimicrobial, antioxidant, and anticancer activities [34]. The thin-layer chromatography (TLC) analysis of Adlay oil extracts derived from the Gulian and Ginampay varieties demonstrated the qualitative presence of terpenoids, as revealed by post-chromatographic derivatization with 0.5% vanillin-sulfuric acid reagent. Upon spraying and heating, this reagent reacts with terpenoid compounds to produce violet-colored spots or bands on the TLC plate, a widely recognized method for detecting terpenoids in plant extracts [35-36]. The TLC was conducted in duplicate to ensure the reliability of the results.

In the chromatogram, the Ginampay oil sample (Figure 1 B) displayed more distinct and intensely violet-colored spots compared to the Gulian oil (Figure 1 A), suggesting a richer or more diverse terpenoid content. The presence of multiple spots along the chromatographic path in Ginampay indicates the existence of various terpenoid compounds, which may reflect differences in the biosynthetic pathways of secondary metabolites between the two cultivars [36]. In contrast, the Gulian oil exhibited fewer and fainter violet spots, signifying either a lower concentration or a less diverse profile of terpenoids. These qualitative differences may be attributed to genetic variation, environmental factors, or physiological differences between the cultivars that affect terpenoid biosynthesis.

The use of TLC for phytochemical screening is a standard method in natural product research, providing rapid and cost-effective identification of bioactive compound classes such as terpenoids [34]. The observed violet spots serve as positive indicators of terpenoid presence and support the hypothesis that both oils contain bioactive secondary metabolites with potential pharmacological properties. Terpenoids are well-documented for their antimicrobial, anti-inflammatory, and antioxidant activities [36] which may correlate with any observed biological activity in further assays. Therefore, the more complex TLC profile of the Ginampay oil suggests it may possess greater bioactivity potential compared to Gulian oil, warranting further investigation into its chemical composition and pharmacological applications.

Antibacterial Property Screening

Kirby-Bauer Disc Diffusion Assay.

The antimicrobial efficacy was tested against four bacterial strains, which are *Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Bacillus subtilis,* as shown in Table 2 and Figure 2. The result showed that the positive control, chloramphenicol (T1), had the highest zones of inhibition across all bacterial strains, while the negative control (T2) showed minimal activity. Gulian oil (T3-T5) exhibited dose-dependent antibacterial effects, with the 70% concentration (T5) showing the highest activity, especially against S. aureus and B. subtilis. In contrast, Ginampay oil (T6-T8) showed weaker antibacterial activity across all concentrations. Overall, Gulian oil demonstrated superior antibacterial potential compared to Ginampay.

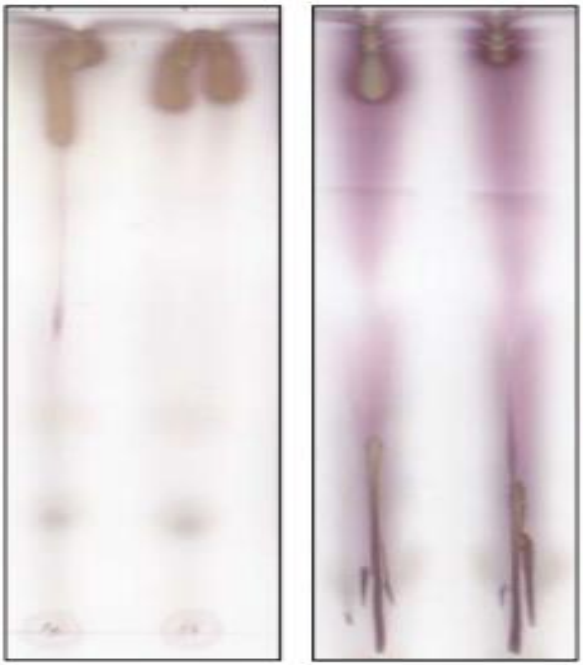

Figure 1. Image of the TLC profile of (A) Gulian Oil and (B) Ginampay Showing Visible Violet Spots/Chromatogram upon Derivatizing with 0.5% Vanillin-sulfuric Acid Indicating Positive Result for Terpenoids.

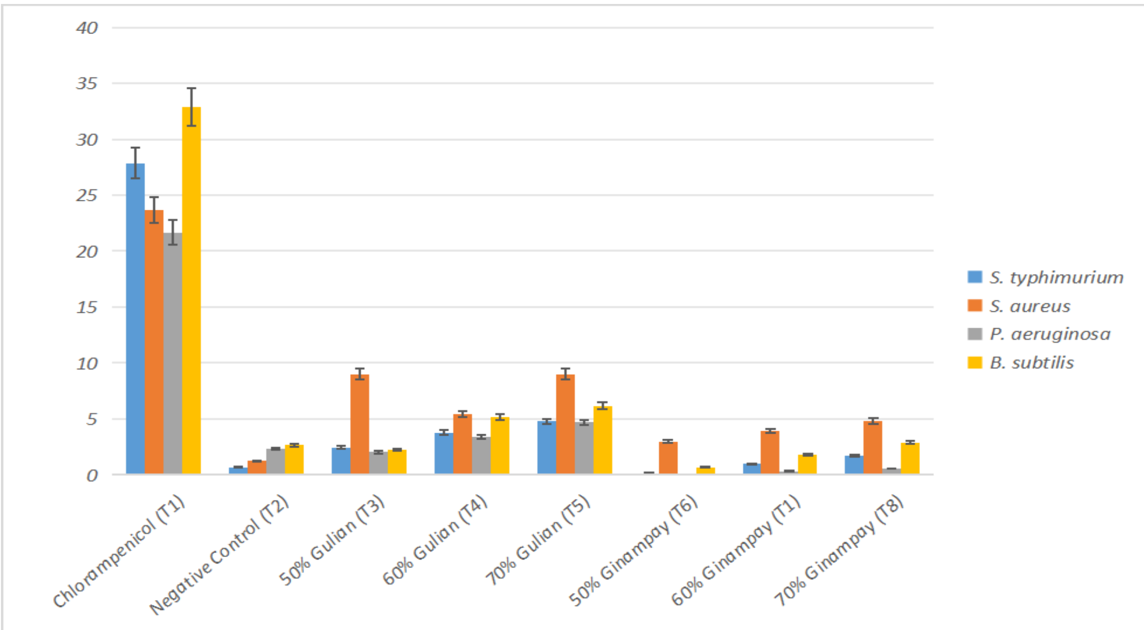

Figure 2. Mean Zones of Inhibition Against Four Bacterial Strains of Gulian and Ginampay Adlay Seed Oil Extracts at Varying Concentrations.

Table 2. Summary of the Mean Zones of Inhibition Against Four Bacterial Strains of Gulian and Ginampay Adlay Seed Oil Extracts at Varying Concentrations.

TREATMENTS	*S. typhimurium*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
T1 - Positive control	27.88_{a}	21.67_{a}	23.67_{a}	32.22_{a}
T2 - Negative control	0.68_{d}	2.33_{bcd}	1.24_{d}	2.67_{b}
T3 - 50% Gulian	2.45_{bcd}	2.03_{bcd}	2.40_{cd}	2.23_{b}
T4 - 60% Gulian	3.78_{bc}	3.43_{bc}	5.44_{bc}	5.18_{b}
T5 - 70% Gulian	4.77_{b}	4.68_{b}	9.01_{b}	6.17_{b}
T6 - 50% Ginampay	0.16_{d}	0.00_{d}	2.99_{cd}	0.69_{b}
T7 - 60% Ginampay	0.98_{cd}	0.33_{d}	3.93_{cd}	1.76_{b}
T8 - 70% Ginampay	1.73_{bcd}	0.57_{cd}	4.81_{cd}	2.88_{b}
F-test	**	**	**	**
C.V. (%)	20.34	24.95	20.11	35.98

Values within the same column followed by the same letter are not significantly different from each other at p<0.05, as determined by Turkey's Honest Significant Difference (HSD) test. F-test: ** indicates that the treatment effect is statistically significant at p<0.01. C.V (%): Coefficient of variation, representing the relative variability of the data.

Coix *lacryma-jobi* Oil Against Salmonella Typhimurium

The result of the Kirby-Bauer test shown in table 2 and figure 3 suggests varying levels of antibacterial activity of Gulian and Ginampay extracts against Salmonella typhimurium. Gulian, at concentrations of 50%, 60%, and 70%, exhibited low to moderate antibacterial effects, with inhibition zones of 4.77 mm and 2.45 mm, respectively. This aligns with other studies that have demonstrated the antibacterial potential of plant extracts, including Coix *lacryma-jobi*, against pathogenic bacteria [37]. Higher concentrations of plant extracts are often more effective in inhibiting bacterial growth due to the increased availability of active compounds [38]

Similar results have been reported in other studies where plant extracts showed variable antibacterial potency depending on their composition and preparation methods [38]. Further, the positive control demonstrated a significant zone of inhibition (27.88), consistent with previous findings that conventional antibiotics typically perform better than plant-based treatments [39]. These findings suggest that while Gulian might hold promise as a moderate antibacterial agent, Ginampay may require further investigation or enhancement to demonstrate significant antimicrobial activity.

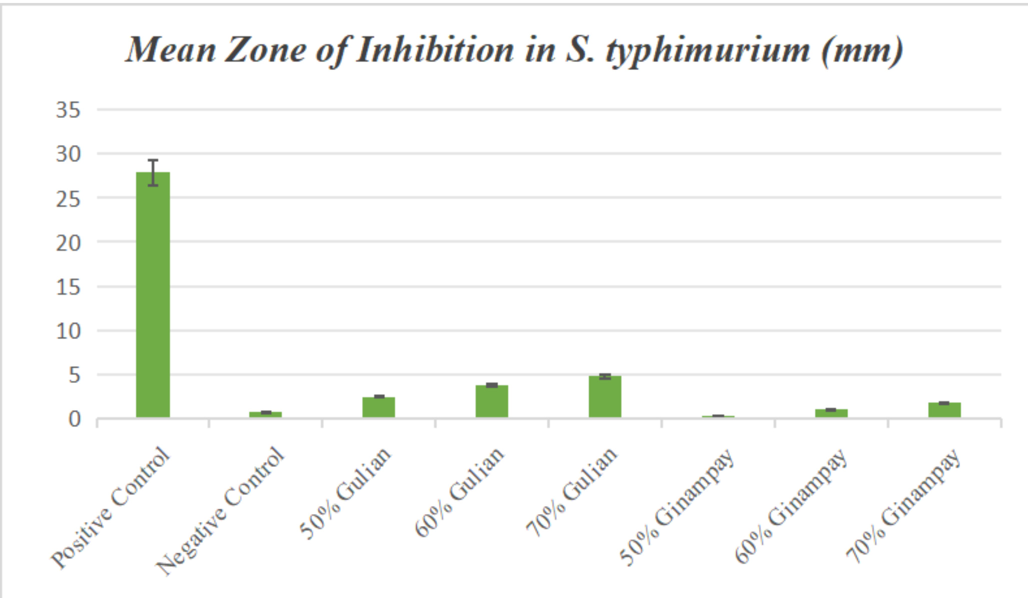

Figure 3. Mean Zones of Inhibition against *Salmonella typhimurium*. of Gulian and Ginampay Adlay Seed Oil Extracts at Varying Concentrations.

Coix lacryma-jobi Oil against _Staphylococcus aureus_

The results for *Staphylococcus aureus* showed that both Gulian and Ginampay extracts exhibit varying degrees of antibacterial activity, with Gulian demonstrating stronger effects than Ginampay as shown in table 2 and figure 4. The positive control, chloramphenicol, exhibited a zone of inhibition of 23.67 mm, confirming its strong antibacterial efficacy against *S. aureus* [37]. In comparison, the negative control showed minimal inhibition (1.24 mm), suggesting no significant antibacterial activity from the substance used in the control.

Gulian demonstrated a concentration-dependent antibacterial effect against *S. aureus*, with inhibition zones of 9.01 mm, 5.44 mm, and 2.40 mm at 50%, 60%, and 70% concentrations, respectively. These results suggest that Gulian has moderate antibacterial properties, with the highest concentration producing the largest zone of inhibition. Similar to the result of other bacteria, these findings have been reported in the study conducted by Smith et al. (2020) [37] where plant extracts exhibited stronger antibacterial activity at higher concentrations due to the higher concentration of active compounds. However, even at the highest concentration, Gulian's activity was still weaker than that of the positive control.

Ginampay also demonstrated antibacterial activity, though weaker compared to Gulian. The inhibition zones at 50%, 60%, and 70% concentrations were 4.81 mm, 3.93 mm, and 2.99 mm, respectively. While these values are smaller than those observed for Gulian, they still indicate a degree of activity against *S. aureus*. This aligns with the study of Brown & Lee (2019) that have shown varying degrees of antibacterial potential in plant extracts, with some showing moderate to low activity against Gram-positive bacteria like *S. aureus* [39]. Therefore, both Gulian and Ginampay exhibit antibacterial activity against *S. aureus*, with Gulian being more effective, especially at higher concentrations.

Coix lacryma-jobi Oil against _Pseudomonas aeruginosa_

For gram-negative *Pseudomonas aeruginosa*, both Gulian and Ginampay extracts exhibit minimal antibacterial activity, with Gulian also showing slightly better results compared to Ginampay (Table 2 & Figure 5). The positive control demonstrated a zone of inhibition of 21.67 mm, indicating strong antibacterial activity against *P. aeruginosa*, consistent again with the results of previous studies showing the high effectiveness of conventional antibiotics [37]. Same with the other bacteria, the negative control showed a minimal zone of inhibition (2.33 mm), suggesting that there is negligible or no antibacterial activity in the control substance.

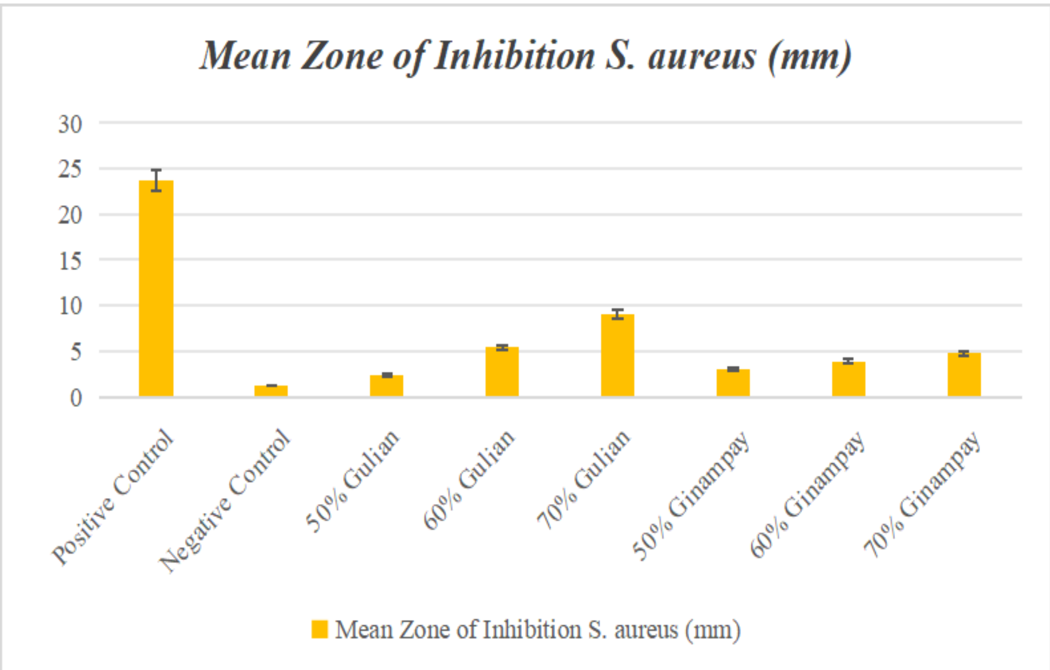

Figure 4. Mean Zones of Inhibition against *Staphylococcus auereus* of Gulian and Ginampay Adlay Seed Oil Extracts at Varying Concentrations.

For Gulian, inhibition zones of 4.68 mm, 3.43 mm, and 2.03 mm were observed at concentrations of 50%, 60%, and 70%, respectively. This suggests that Gulian has a moderate antibacterial effect on *P. aeruginosa*, with a decrease in activity as the concentration decreases. However, the inhibition zones for Gulian remain relatively small compared to the positive control, indicating that its antibacterial effect is weaker. On the other hand, Ginampay demonstrated significantly weaker antibacterial activity, with inhibition zones of only 0.57 mm, 0.33 mm, and no inhibition at all at 50% concentration. These results suggest that Ginampay is less effective against *P. aeruginosa*, even at higher concentrations, which is again consistent with reports of varying antibacterial effects for plant extracts depending on their chemical composition and the target pathogen [40].

Coix lacryma-jobi Oil against _Bacillus subtilis_

Consistent to other bacterial samples, Gulian demonstrated a concentration-dependent antibacterial effect on *B. subtilis*, with inhibition zones of 6.17 mm, 5.18 mm, and 2.23 mm at concentrations of 50%, 60%, and 70%, respectively as shown in Table 2 and Figure 6. These results indicate that Gulian has moderate antibacterial properties, with stronger activity at higher concentrations.

Ginampay, while still showing antibacterial activity, exhibited much weaker effects on *B. subtilis* compared to Gulian. The inhibition zones at 50%, 60%, and 70% concentrations were 2.88 mm, 1.76 mm, and 0.69 mm, respectively. These results suggest that Ginampay has relatively limited antibacterial activity.

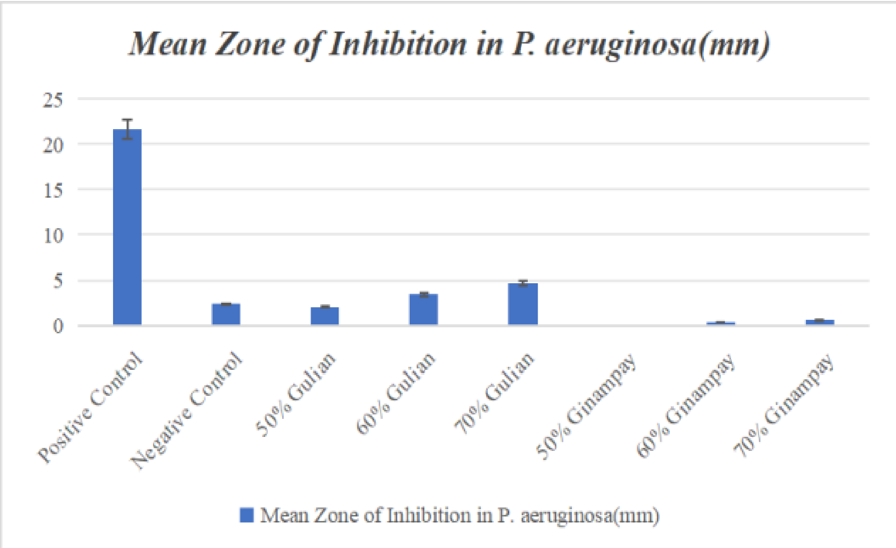

Figure 5. Mean Zones of Inhibition against *Pseudomonas aeruginosa* of Gulian and Ginampay Adlay Seed Oil Extracts at Varying Concentrations

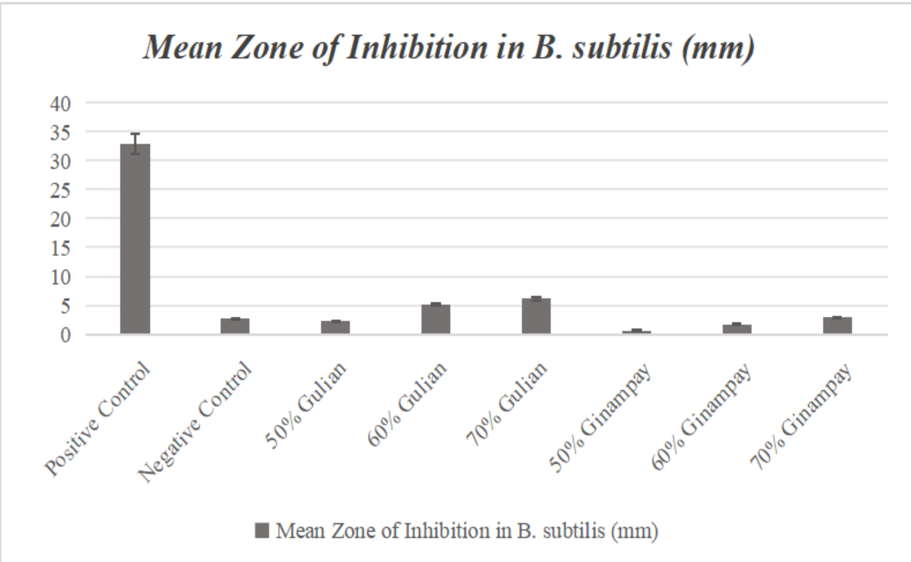

Figure 6. Mean Zones of Inhibition against *Bacillus subtilis* of Gulian and Ginampay Adlay Seed Oil Extracts at Varying Concentrations.

The results of the Kirby-Bauer Disk Diffusion assay, summarized in the table 2, show significant variation in the antibacterial activity of Gulian and Ginampay Adlay seed oils against four bacterial isolates, *Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Bacillus subtilis*. Treatments varied in terms of cultivar and concentration (50%, 60%, and 70%), and results were analyzed using ANOVA followed by Tukey's HSD post hoc test at a 0.05 significance level.

Across all bacterial strains, the positive control (T1) exhibited the highest zones of inhibition, confirming the effectiveness of the standard antibiotic used. In contrast, the negative control (T2) had minimal to no inhibitory effect, as expected.

In interpreting antibacterial activity, the zone of inhibition (ZOI) serves as a critical measure. According to standard classification, ZOIs greater than 20 mm are considered indicative of strong antibacterial activity, 10 -20 mm as moderate, and below 10 mm as weak or negligible [41]. Based on this scale, all Adlay oil treatments regardless of concentration or variety, fell within the weak category, with the exception of Gulian oil at 70% (T5) against *S. aureus*, which approached moderate activity at 9.01 mm. This benchmark further emphasizes the comparatively limited antibacterial effect of Adlay seed oils, especially when evaluated alongside the significantly larger inhibition zones produced by the positive control. Nevertheless, even small ZOIs may indicate the presence of bioactive compounds with potential synergistic effects or broader-spectrum applications at higher concentrations or in combination with other agents.

On the other hand, Gulian oil showed a concentration-dependent increase in antibacterial activity against *S. typhimurium,* with the highest growth inhition observed at 70% (T5, 4.77mm). This value was significantly lower than the positive control (27.88 mm) but significantly higher than all Ginampay treatments, which displayed minimal activity across all concentrations. This trend confirms that Gulian oil is more effective than Ginampay against this Gram-negative bacterium.

Against *P. aeruginosa*, the most resistant strain overall, the oil treatments again demonstrated dose-dependent efficacy. However, the zones of inhibition were markedly small across all treatments, ranging from 0.00 to 4.68 mm. Gulian oil at 70% (T5) again recorded the highest among the treatments (4.68 mm), suggesting that while Coix oil exhibits some inhibitory potential, its activity against this particular pathogen is limited.

In contrast, activity against *S. aureus* which is a Gram-positive bacterium was substantially higher. Gulian oil at 70% (T5) demonstrated a zone of inhibition of 9.01 mm, which, although lower than the positive control (23.67 mm), was significantly higher than all Ginampay treatments and the negative control. This supports the notion that phenolic-rich plant extracts like Gulian oil are more effective against Gram-positive bacteria due to the lack of an outer membrane, which in Gram-negative bacteria often prevents the penetration of antimicrobial agents [40].

For B. subtilis, the positive control (T1) recorded the highest inhibition zone (32.22 mm), while Gulian oil at 70% (T5) exhibited the highest among the treatments (6.17 mm), significantly outperforming the Ginampay treatments. The trend suggests that Gulian oil's bioactive compounds possibly phenolics and terpenoids contribute to its antimicrobial potency [42].

The F-test results indicated that treatment effects were highly significant (**), confirming that the observed differences among treatments were not due to chance. Meanwhile, the coefficient of variation (C.V.) values ranged from 20.11% to 35.98%, indicating acceptable levels of experimental variability, especially for biological assays.

The data reveal two important conclusions, which are first, the antimicrobial activity of Adlay oil is dose-dependent, and Gulian oil is consistently more effective than Ginampay. These findings correlate with the phytochemical data that show Gulian oil has higher total phenolic content (4.8 ± 0.1mg GAE/g) and antioxidant capacity (37 mg AAE/g), as well as a richer profile of terpenoids. Thus, its enhanced antimicrobial action is likely attributable to these bioactive compounds.

Comparative Analysis of *C. lacryma-jobi* var. Gulian and Ginampay

In phytochemical assessments, the two cultivars showed difference between each other. Gulian exhibited significantly higher total phenolic content (4.8 ± 0.1 mg GAE/g) compared to Ginampay (0.60 ± 0.04 mg GAE/g), implying greater antioxidant and health-promoting potential. The antibacterial activity of Adlay varietes Gulian and Ginampay was tested against *Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aeruginosa,* and Bacillus subtilis using 50%, 60%, and 70% seed oil extracts. Results *Bacillus subtilis* showed that Gulian exhibited stronger antibacterial effects than Ginampay, particularly at 60% and 70% concentrations. The highest inhibition was observed against S. *aureus* showing 9.01 mm of bacterial inhibition at 70% Gulian. Gulian also showed moderate inhibition against *Salmonella sp. and B. subtilis*, while both cultivars had limited effects on *P. aeruginosa*. Ginampay's antibacterial activity remained consistently weak across all concentrations and test organisms. These findings suggest that Gulian possesses superior antibacterial potential, especially against Gram-positive bacteria, and that its efficacy increases with extract concentration.

4. CONCLUSION

This study provides a comparative analysis on the phytochemical and antibacterial properties of two *C. lacryma-jobi* varieties, Gulian and Ginampay. Phytochemical assays revealed that Gulian possessed a total phenolic content (TPC) of 4.8 ± 0.1 mg GAE/g, eight-fold higher than Ginampay's 0.60 ± 0.04 mg GAE/g. TLC profiling detected terpenoids in both oils, with Ginampay displaying more numerous and intense violet spots. Finally, Kirby–Bauer tests against four pathogens demonstrated dose-dependent antibacterial activity: at 70 % seed-oil concentration, Gulian produced inhibition zones of 4.77 mm *(S. typhimurium*), 9.01 mm *(S. aureus),* 4.68 mm (*P. aeruginosa),* and 6.17 mm *(B. subtilis),* whereas Ginampay's corresponding zones were 1.73 mm, 4.81 mm, 0.57 mm, and 2.88 mm. Thus, this study concludes that *Coix lacryma-jobi* var. Gulian outperforms Ginampay in terms of its phytochemical, and antibacterial properties showing the significance and the differences of both phytochemical profiles and antibacterial properties in evaluating the potential of these cultivars for commercial and therapeutic use.

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