Annals of Tropical Research 47 (1):103-121(2025)
https://doi.org/10.32945/atr4718.2025

Original Article

Phytochemical extraction and in vitro UV/H_2O_2 photolysis induced DNA damage protection activity potential of cogon grass (*Imperata cylindrica* (L.) P. Beauv.) ground and aerial parts extracts

Lee Andrew L. Gonzales[1*]

ABSTRACT

Cogon grass (*Imperata cylindrica* (L.)P.Beauv.), a globally known invasive grass, has been studied for its application in DNA protective activity. However, most studies only utilized aerial cogon parts leaving the ground parts understudied. This study aimed to compare both ground and aerial cogon phytochemical extracts and its potential protective activity against damage to pUC19 plasmid DNA induced by reactive oxygen species. The total phytochemical analysis showed that the fermented leaf powder showed the highest phenolic and flavonoid content while chloroform root macerate had the lowest yield. Fermented root samples and fermentation control flavonoids were enzymatically hydrolyzed resulting in higher phenolic content. The DNA damage protection assay of the extracts was conducted by photolyzing the UV/H_2O_2 system to produce radical oxygen species inflicting DNA fragmentation. The scored bands showed that all chloroform extracts exhibited DNA damage protective activity. Among the fermented extracts, only fermented leaf macerate exhibited positive protective activity while fermented root samples showed excessive DNA damage, and

Received: 6 Febuary 2025
Revised: 1 June 2025
Accepted: 13 June 2025
Published: 30 June 2025

[1]Department of Biotechnology, Visayas State University, Baybay City, Leyte 6521-Philippines

*Corresponding Author. Address: Department of Biotechnology, Visayas State University, Baybay City, Leyte 6521-Philippines; Email: gonzalesleeandrew@gmail.com

fermented leaf powder with slight DNA damage. The results imply the potential of cogon grass extracts to be developed into cancer-preventive products and apoptotic regulators to minimize cancer proliferation.

Keywords: crude and fermented extracts, cogon grass, DNA protection, phytochemical content

INTRODUCTION

Cancer is a notorious disease known globally due to its lethality and severity. According to the World Health Organization (2022), this disease is one of the leading causes of death worldwide which could account for up to 10 million deaths based on the data from 2020. Such diseases are developed most likely due to activation of the oncogenes present in DNA from unhealthy lifestyles, foreign vectors, or exposure to certain mutagens that could alter the organism's genome (WHO, 2022).

There are several molecular mechanisms exhibited by the mutagens, through mutagenesis, DNA damage, spontaneous damage, chemical adducts, oxidative damage, and DNA intercalation. The most common mutagen in the environment is the sunlight which carries UV radiation. Free radical damage, a known oxidative damage, from radical oxygen species (ROS) in controlled environment can be replicated by photolyzing oxygen-rich solutions. More specifically, UV/H_2O_2 systems can produce ROS to effectively induce damage towards DNA samples (Schrader, 2016). On the other hand, antimutagenic compounds generally have a property known as DNA damage protective activity. Specifically, these are mostly naturally occurring secondary metabolites extracted from an organic source. Such secondary metabolites include phenolic compounds such as flavonoids and tannins; alkaloids; terpenes and other phytochemicals. However, different factors may affect the recoverable amount of such bioactive compounds from natural sources (Kaur et al., 2019).

In this study, cogon grass (*I. cylindrica*) in particular, was used as the sample plant material for the extraction of its bioactive compounds. There are several studies conducted indicating that extracts of the aerial parts of cogon grass exhibited anticancer or antimutagenic potential. Such studies identified that the methanolic leaf extracts were able to inhibit cell proliferation and induce apoptosis in SCC-9 cells (Keshava et al., 2020), exhibit cytotoxic effects over the influence of proliferative prostanoids from colorectal cancer cells (Yan Kwok et al., 2016), and showed DNA damage protection from irradiated lymphocytes, also indicating radioprotective properties (Estrella et al., 2020). Unfortunately, a scientific gap can be observed in these studies due to the fact that these studies only used the aerial parts of the plant. In addition, data on the bioactive compounds in the grass responsible for such properties is insufficient. Hence, in this study, the DNA damage protective property of the aerial and ground parts of cogon grass were assessed.

This study aimed to develop optimized methodologies for extracting, identifying, quantifying, and evaluating the bioactive compounds in cogon grass (*I. cylindrica*) ground and aerial parts, while also validating their potential DNA damage protective activities. Specifically, the objectives include extracting

bioactive compounds using optimized methods, identifying and quantifying phytochemicals in terms of total flavonoid content (TFC) and total phenolic content (TPC), and identifying the DNA damage protective activity of the extracts through DNA damage inhibition assay. The scope of this research is limited to the extraction of bioactive compounds from cogon grass samples collected within the Visayas State University (VSU) main campus, and utilizing chloroform as the sole solvent. The study does not consider growth parameters and edaphic factors of the grass. The fermented samples' alcohol content was not analyzed. While previous researchers have explored various phytochemicals, this study was confined to quantifying the phenolic and flavonoid compounds through specific total phytochemical assays. Additionally, the assessment of DNA protective activity is limited to DNA damage inhibition, with genetic sequencing of the samples falling outside the scope of this research.

MATERIALS AND METHODS

Experimental Design

This study aimed to evaluate and compare the DNA damage protection activity of cogon grass (*I. cylindrica*) extracts from ground and aerial parts using different extraction parameters. The experiment followed a multi-factorial randomized complete block design aimed to determine the best treatment combinations. The factors include two treatment groups; ground parts and aerial parts, two extraction methods; chloroform extraction, and extractive fermentation; and two pre-treatment parameters; oven drying at 90°C, and freshly macerated (soaked for 24h). Overall, there were a total of eight treatment combinations and a negative control for fermentation.

Collection and Preparation of Plant Samples

Cogon grass samples both the ground and aerial parts were collected within the vicinity of VSU, Baybay City, Leyte, (Figure 1). The samples were washed, and wet droplets were dried off followed by the separation of the ground and aerial parts. The individual parts were cut into smaller pieces, packed, weighed and subjected to oven drying at 90°C for 75mins as suggested by Saifullah et al. (2019). The dried samples were pulverized, weighed and stored in a clean dry container for further use. Furthermore, the moisture content of the dried samples was calculated using Equation 1. A second batch of freshly collected cogon roots and leaves were macerated and subjected to immediate solvent extraction or fermentation as the treatments for freshly macerated samples.

Equation 1:

$$\%\text{Moisture Content} = \frac{\text{Initial Weight} - \text{Dried Weight}}{\text{Initial Weight}} x100$$

Figure 1. Location and collection site of the cogon grass samples (*VSU, Department of Biotechnology*, 2024)

Extractive Fermentation

Following a modified method of Li et al. (2018), fresh cogon grass samples were initially mixed with distilled water in a 1:8 mass ratio (samples:dH_2O) and were soaked for 24h before blending. In separate glass vessels, 100g were used as substrate for each of the powdered samples, while 150g were used for the fresh macerated samples. For fermentation, the powdered cogon grass samples were mixed with distilled water in a 1:8 mass ratio. The soaked fresh samples were blended until macerated. Cellulase, pectinase, and sugar were added up to concentrations of 0.01%, 0.005%, and 21%, respectively. Dissolved baker's yeast (100mg mL^{-1}) to give 0.02% concentration was inoculated into the substrate. The cultures were allowed to ferment for a total of 12 days. Three days, in open glass containers at room temperature. Then after the third day, they were sealed and incubated at room temperature for the remaining nine days of fermentation. The fermented samples were filtered and centrifuged, separating the supernatants from the residues. The supernatants were collected and stored in a refrigerator until further analysis. A negative control set-up was prepared indicated as the fermentation control. The fermentation control (F. ctrl) was the same composition of cellulase, pectinase, sugar, and yeast that had been added into the samples, but omitting the cogon grass samples. The method of Li et al. (2019) did not include the specified humidity. Thus, to further supplement the data, the relative humidity for the entire fermentation duration was recorded at an average of 15% and 25°C room temperature.

Solvent Extraction

The powdered and macerated samples (roots and leaves) were soaked in 400mL of concentrated chloroform for 24h with occasional stirring. After soaking, the samples were initially filtered with a cheesecloth. The filtrate was allowed to pass through Whatman No.1 filter paper. The residues from the first filtration were subjected again to another round of extraction until collected filtrates were pooled and concentrated with a rotary evaporator at 80-100rpm at 40-50°C. The crude extracts were weighed, and the percentage yield was computed using Equation 2. A stock solution of 10,000ppm was prepared by dissolving the crude extracts in deionized distilled water and stored at 4oC.

Equation 2:

$$\%yield = \frac{\text{Total mass of crude extract}}{\text{Total mass of dried sample}} x100$$

Phytochemical Analysis

In this study, the total flavonoids and total phenolics in the roots and leaves extracts were quantitatively analyzed using spectrophotometric analysis. The analysis was conducted in the Department of Biotechnology, Visayas State University using a Shimadzu UV-1790 UV-Vis Spectrophotometer. The assays were read in duplicate at their respective wavelengths. The results of the assays were statistically analyzed through one-way Analysis of Variance (ANOVA), and Tukey's Honest Significant Difference (HSD) for post-hoc analysis of the phytochemical difference in the extracts. The equation generated from the standard curve was used to calculate the concentration of the extracts. The one-way Analysis of Variance (ANOVA) showed that at a 5% level of significance, there was a significant difference between the different groups. To determine the significant difference between each group, the results were further analyzed using Tukey's Honest Significant Difference (HSD).

Total Flavonoid Content

Using a modified method of Jaradat et al. (2021), the total flavonoid content of the plant was determined based on the reaction with aluminum chloride. In a tube, 0.25mL of the diluted extracts (1,000ppm for chloroform crude samples and 100% for fermented samples) and standards were mixed with 1.5mL methanol, 0.1mL 10% aluminum chloride, 0.1mL 1M potassium acetate, and 2.5mL distilled water and allowed to stand at room temperature for 30mins. Afterwards, the absorbance was measured at 415nm with the UV-vis spectrophotometer. The analysis was duplicated, and a standard calibration curve was prepared using quercetin at different concentrations, (i.e. 10, 20, 40, 60, 80, 100, 300, and 500µgmL^{-1}). The total flavonoid content of the plant extract was extrapolated from the standard curve and the final value was expressed as microgram of quercetin equivalent per gram of the dry sample (µg QEg^{-1} dry sample).

Total Phenolic Content

Following a modified method of Sari et al. (2023), 0.1mL of the diluted extracts (1,000ppm for chloroform crude samples and 100% for fermented samples) and standards were mixed with 0.1mL of Folin–Ciocalteu reagent and shaken for 1min. The mixture was left to stand for 5mins, and then 1mL of 7% sodium carbonate solution was added. The solution was then diluted to 5mL using dH_2O in a tube. After 120mins in the dark at room temperature, the absorbance was measured at 750nm using the UV-Vis spectrophotometer. The analysis was duplicated and a standard calibration curve was prepared using gallic acid at different concentrations, (i.e. 10, 20, 40, 60, 80, 100, 300, and 500µg mL^{-1}). Phenolic contents were expressed in microgram equivalent of gallic acid per gram of dry sample (µg $GAEg^{-1}$ dry sample).

DNA Damage Protection Activity

Assessment of the extracts' antimutagenic properties was prepared through DNA damage inhibition assay. The ability of the cogon grass extracts, prepared using different combinations of drying and extraction methods, to protect DNA from the damaging ROS or free radicals produced by the photolysis of the UV/H_2O_2 system was analyzed using a modified method of Manalo et al. (2020). Crude chloroform extracts at 100ppm, and 1,000ppm in water were analyzed. On the other hand, the fermented samples were analyzed at 100% and 10% concentrations. The assay was done by photolyzing H_2O_2 with UV radiation in the presence of pUC19 plasmid DNA extracted from transformed E. coli that was purchased from Addgene in the form of an agar stab. The agar stab culture (Plasmid#50005) was produced by the Joachim Messing Lab. The bacterial culture was screened using ampicillin in LB media and was stored at - 80°C in 10% skim milk as a stock culture. The stored culture was revived, and the pUC19 plasmid DNA was extracted using an alkali plasmid extraction protocol (Addgene, 2018).

Prior to every assay, fresh plasmid DNA was extracted from the transformed E. coli. After acquiring the plasmid DNA (pUC19), in a 96-well plate, 2µL of pUC19 plasmid DNA was added to 10µL of plant extract. A negative control containing 10µL of ultra-pure water was also added into another well. Then 4µL of 3% H_2O_2 was added to all wells containing the samples, including the negative control. To initiate the photolysis reaction, the microtiter plate was placed directly onto the surface of a UV-transilllluminator (302nm) for 10mins at room temperature. Another batch of plasmid DNA was added into the empty wells after the irradiation to serve as positive control. Lastly, 2µL loading dye was mixed into every single well, this included crude samples, fermented samples, negative control, and positive control. All reaction mixtures were analyzed using agarose gel electrophoresis (AGE) with 1% agarose gel with gel red in 1X TAE buffer. The gel was run at 120V for 30mins. The resulting gel was viewed under the UV-transilluminator (302nm), and was band scored based on the positive and negative controls (Manalo et al., 2020). A sample for each treatment combination was ran once and was repeated if non-conformities were identified from the resulting gel.

RESULTS

Crude Extraction from Cogon Grass using Different Methods

Around 289g roots and 504g leaves of cogon grass were initially weighed and oven-dried at the National Abaca Research Center (NARC), VSU Main Campus, Baybay City, Leyte. The dried samples yielded 182g of pulverized roots, and around 250g of pulverized leaves. Using Equation 1, the moisture content lost upon oven drying the cogon grass samples was approximately 37.02% and 50.4% for the roots and leaves, respectively.

The total volume produced from the fermented samples was 800mL from the fermented powdered samples, and 1,200mL from the fermented macerated samples.

Table 1. Percentage yield of the crude extracts

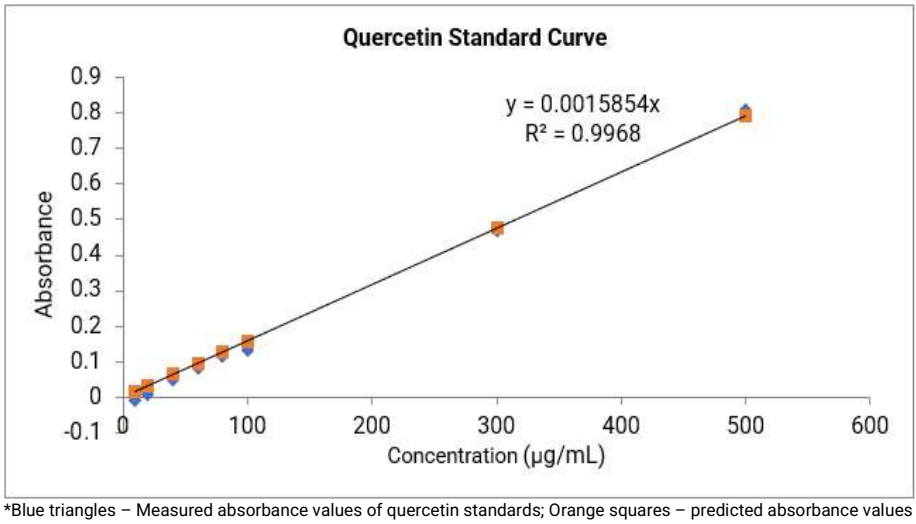
*Samples	Sample (g)	Crude Extract (mg)	Yield (%)
CRP	75	626.7	0.84%
CLP	100	544.0	0.54%
CLM	150	759.2	0.51%
CRM	150	118.0	0.08%

*CRP – Crude Root Powder; CLP – Crude Leaf Powder; CLM – Crude Leaf Macerate; CRM – Crude Root Macerate

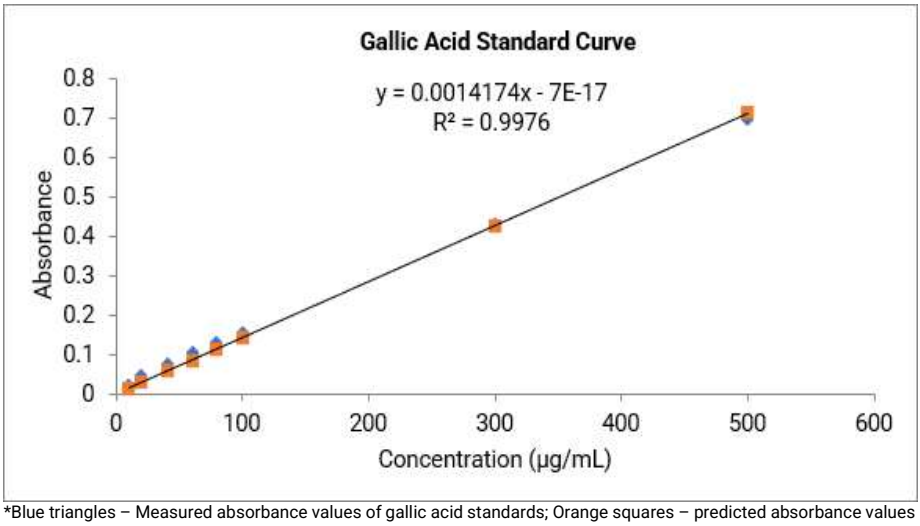
Based on the calculation (Table 1), the sample that had the highest yield of extract was the crude root powder (CRP). Both the crude leaf powder (CLP) and crude leaf macerate (CLM) had an average yield of around 0.52%. However, the crude root macerate (CRM) had the lowest percentage yield of the extract yielding only around 0.08% from the extracted crude sample.

Phytochemical Analysis

Using simple linear regression, the absorbance of the phytochemical standards was analyzed, and a standard curve was generated yielding approximately greater than 0.99r^2 value, which is within the range of accepted value. Since the fermented samples were not concentrated using a rotary evaporator, to assess the effectiveness of the semi solid state fermentation (SSF), the equivalence values were converted to grams of dried sample matter instead of the grams of dried weight of the extract.



*Blue triangles – Measured absorbance values of quercetin standards; Orange squares – predicted absorbance values based on linear regression



*Blue triangles – Measured absorbance values of gallic acid standards; Orange squares – predicted absorbance values based on linear regression

Figure 2. Generated standard curves for Quercetin and Gallic acid.

Separate data was calculated to fit with the results of the previous study since the unit for comparison of phytochemical content in this study was adjusted to compare chloroform extract and fermented extracts shown in Table 2.

Lalthanpuii et al. (2018) extracted cogon grass roots by air drying to acquire their powdered samples. Moreover, Roshanak et al. (2016) and Saifullah et al. (2019) concluded that oven-dried extracts yielded more phytochemical content in comparison to air-dried and freshly macerated extracts. Looking at Table 2, the difference in the flavonoid content of the oven-dried root powder (CRP) and air-dried root powder as detected by Lalthanpuii et al. (2018) is only around 10mg QEg^{-1} dry wt. more, while the overall phenolic contents of the extracts in this study were considerably higher in comparison.

Table 2. Comparison of the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) chloroform extracts with previous literature.

*Samples	TPC (mg GAEg^{-1} dry wt.)	TFC (mg QEg^{-1} dry wt.)
CRP	36.49	20.57
CLP	34.48	27.54
CLM	29.56	6.60
CRM	29.90	7.18
Chloroform Extract (Lalthanpuii et al. 2018)	7.54	30.88

*CRP – Crude Root Powder; CLP – Crude Leaf Powder; CLM – Crude Leaf Macerate; CRM – Crude Root Macerate; Chloroform Extract – Data from previous literature (Lalthanpuii et al. 2018)

Total Flavonoid Content

In Figure 3, it was observed that the fermented leaf powder (FLP) obtained the significantly highest flavonoid content with 2,040.04±15.45µg QEg^{-1} dry sample followed by fermented leaf macerate (FLM). Furthermore, the analysis also indicated no significant difference between crude powdered samples (CRP and CLP), and macerated samples (CLM and CRM). However, it is worth noting that CRM had the least detected flavonoid content among all other samples. Moreover, the flavonoids were not detected among the fermented root samples, both powdered and macerated, or in the fermentation control.

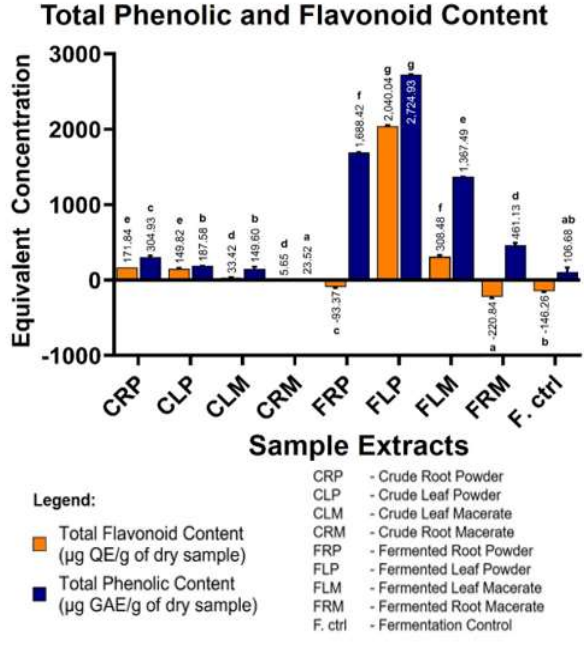


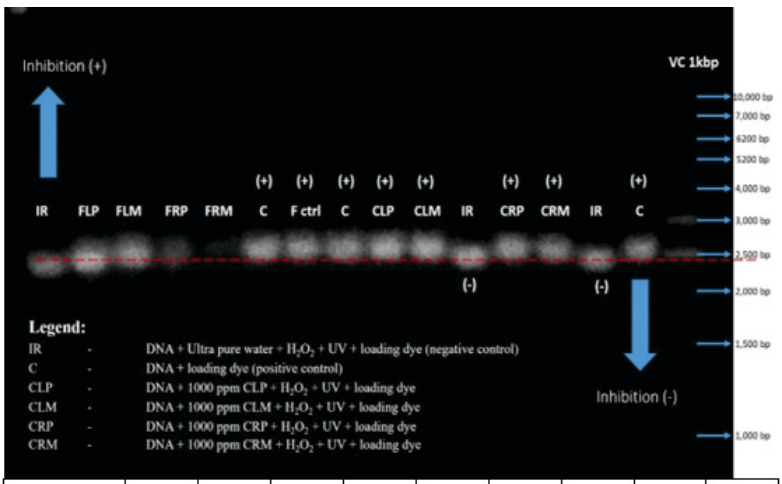
Figure 3. Summary of the total phytochemical (phenolic and flavonoid) content of cogon grass extracts

Total Phenolic Content

The total phenolic contents of the samples were greater in comparison to the flavonoid content. This was observed particularly in the fermented extracts, especially for the fermented root samples where samples having no flavonoids detected showed highly significant levels of phenolic content. As seen in Figure 3, the highest significantly different sample was still FLP at 2724.93±1.84µg GAEg^{-1} dry sample now followed by FRP, FLM, and FRM. Meanwhile, CRM still had the lowest yield in comparison to other samples. The fermented control and CRM, together with CLP and CLM had no significant difference in their concentration.

DNA Damage Protection Activity

The initial AGE reading from the DNA damage inhibition assay is shown in Figure 4. The bands were scored based on the control (C) DNA and irradiated (IR) DNA, which was further separated on the red-line. Bands above the red-line labeled as (+) meaning, an inhibition or DNA was retained. On the other hand, bands below the red line were labeled as (-) indicate DNA damage.

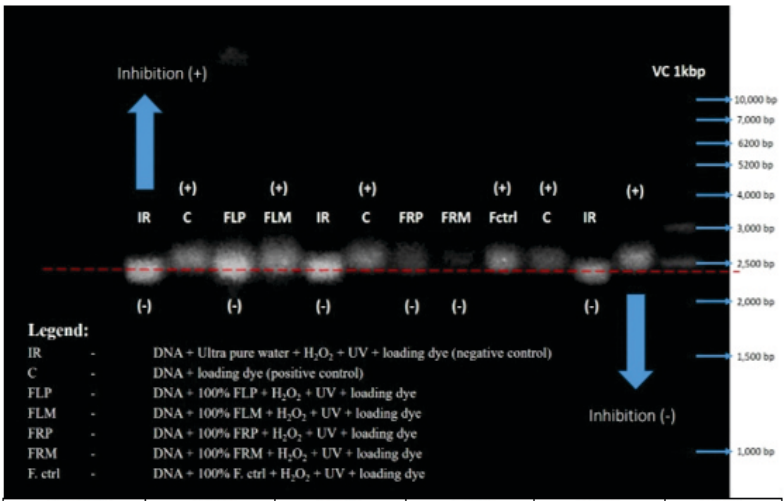


Sample	FLP	FLM	FRP	FRM	F.ctrl	CLP	CLM	CRP	CRM
Inhibition	(-)	(+)	(-)	(-)	(+)	(+)	(+)	(+)	(+)

Figure 4. Initial DNA damage inhibition activity of the extracts.

The irradiated DNA (IR) treated with 302nm UV light and 3% H_2O_2 for 10mins as seen on the left side of the gel, does not align with the rest of the bands indicating DNA degradation through fragmentation resulting in smaller bp size and faster migration of the bands. The plasmid DNA bands treated with the chloroform crude extract solutions align with the control DNA bands which indicate that these extracts possess protective activities.

The fermented root samples (FRP and FRM) showed very faint bands which indicate the possibility of an error, or the result of complete DNA degradation. Thus, a repeat test was performed to confirm these results. However, the fermented control did show inhibition towards DNA damage. To further confirm these results, the fermented samples at 100% concentration were rerun in agarose gel electrophoresis as shown in Figure 5.



Sample	FLP	FLM	FRP	FRM	F.ctrl
Inhibition	(-)	(+)	(-)	(-)	(+)

Figure 5. DNA damage inhibition assay of fermented samples (second run)

Fortunately, the results of the initial run was consistent with the results of the second run as shown in Figure 5. This meant that the fermented root samples (FRP and FRM) had DNA-damaging properties since there was almost complete DNA degradation shown by the faint bands. This indicated that the photolysis of the fermented root solutions produced excessive ROS causing the plasmid DNA to be completely degraded.

To further assess the protective and damaging properties of the extracts, the concentration of the samples was lowered to 10% of the initial concentration; 100 ppm for crude extracts, and 10% for fermented extracts. This was conducted to confirm if the properties of the extracts would vary depending on their concentration. As expected, shown in Figure 6, when the concentrations were lowered, none the samples exhibited any protective activity against ROS produced from the photolysis of UV/ H_2O_2. In addition to that, the fermented root samples which had exhibited excessive DNA damage, now exhibited damaging activity similar to the negative control (IR).

Sample	FLP	FLM	FRP	FRM	F.ctrl	CLP	CLM	CRP	CRM
Inhibition	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)

Figure 6. DNA damage inhibition activity at lower sample concentrations

DISCUSSION

Several factors interplay which affect the yield of the crude extract such as the polarity of the solvent, chemical structure of the extracted poly/phenolic compounds, extraction method, plant matrix, and presence of interfering substances. Most of the time, more polar solvents would produce more extraction yield due to the availability of readily soluble plant materials in higher polarity solvents. The solvent used in this study was chloroform, which is known to be a less polar substance in comparison to water, methanol, and ethanol. However, there is no direct correlation between the yield and phytochemical content of the sample based on the study of Abu et al. (2017) which showed that despite the solvent extraction using water yielding more extracts, it had the lowest total phenolic content compared to the other two polar solvents used (methanol and ethanol). On the other hand, the availability of readily soluble plant matrix can be attributed to the drying method that was employed in the study. Plant samples with harder cellular matrix such as parts of the root system are difficult to extract. Hence, drying the plant samples which can generally weaken its cellular matrix would increase the extraction yield (Abu et al., 2017). Thus, the low extraction yield produced in this study is attributed to the non-polarity of the solvent and the non-totally disrupted cellular matrix of the samples.

There are limited comparison studies between individual plant parts. Moreover, this is one of the first studies that actively compares the leaves and root extracts of cogon grass. In some studies, powdered root extracts generally have higher phytochemical concentrations in comparison to powdered leaves. One of the most recent studies was between leaf and root extracts of *Ligularia fischeri* Turcz by Kim et al. (2022). In addition, older studies also support the implication that root extracts have higher phytochemical yield compared to leaf extracts as reported by Senguttuvan et al. (2014) and Metsopkeng et al. (2020). This trend can also be seen in this study in which CRP has greater phenolic and flavonoid content

compared to other chloroform extracts (CLP, CLM, and CRM). However, the aforementioned comparison studies used ethanol for solvent extraction, which is a polar substance, while in this study, chloroform was used, a non-polar substance. This may imply that phenolics and flavonoids of lesser polarity were extracted from the cogon grass samples through chloroform extraction. Haminiuk et al. (2014) explained that polyphenolic compounds, such as flavonoids, are more soluble in organic solvents that are less polar than water (e.g. chloroform). However, the fermentation set-up in this study is considered to be an aqueous solution, a polar system. One of the factors that can increase the solubility of phenolic compounds in a polar solution is through ionization where ionizable functional groups are ionized when the pH of the system exceeds the pKa value of the compound (Cui et al., 2023). However, fermented products generally have lower pH due to the organic acids that microorganisms produce, especially *S. cerevisiae*, which is an acidophile. Thus, there could be two other reasons why fermentation was able to increase the phytochemical content i.e., by enzymatic hydrolysis and biotransformation (Adebo & Gabriela Medina-Meza, 2020).

Flavonoids are intracellular phytochemical compounds produced by plant cells. Most of these compounds are bound to different cellular components known as bound flavonoids which are difficult to extract in an aqueous solution without the use of components that can release these bound residues. The use of cellulase and pectinase in this study as recommended by Li et al. (2019) assists in the decomposition of cellulose and pectin, the main components of the plant cell walls. In fermentation, since *S. cerevisiae* lacks these enzymes, it increased the phytochemical yield in their study.

In this study, the same trend was observed in the fermentation of cogon grass leaves following the recommended fermentation composition and duration for 12 days. However, the fermented cogon root samples exhibited an absence of flavonoids. According to previous studies, cogon grass root extracts have innately low flavonoid content especially when using polar solvents such as ethanol, methanol, and water (Lalthanpuii et al., 2018; Indriyanti et al., 2022). However, bound phytochemicals should be released and extracted into the aqueous system through enzymatic activity in fermentation despite their lack of solubility in polar solvents. The reason why flavonoids were not detected in the fermented cogon roots, other than having low innate flavonoid content, was probably due to the enzymes themselves. *S. cerevisiae* according to Hernández et al. (2003) exhibited β-glucosidase activity which can hydrolyze polyphenols like flavonoids into simple phenols (Tsao, 2010). Hence, the results of total flavonoid content on the cogon grass extracts imply that the flavonoids (polyphenols) present in cogon grass roots are more soluble in organic non-polar solvents and were hydrolyzed into simpler phenols which are usually phenolic acids more soluble in polar solvents. On the other hand, the bound flavonoids present on cogon grass leaves were easily released by fermentation and enzymatic activity leading to more bound residues being released and becoming soluble. This also means that most of these flavonoids were non-hydrolyzable polyphenolic substances, which is why the fermented cogon leaves exhibited the highest flavonoid yield (Singla et al., 2019).

One of the limitations of this study was that the physicochemical composition (e.g. alcohol content) of the fermented extracts was neither analyzed nor purified through distillation or rotary evaporation. Hence, since fermentation generally produces numerous alcoholic compounds due to the presence of yeast in the

mixture, this could explain why there were high phenolic levels in the fermented samples even the fermentation control. As previously explained, two main reasons for the increased phytochemical content in the fermented extracts were enzymatic hydrolysis and biotransformation. Adebo & Gabriela Medina-Meza (2020) reviewed that the use of *Lactobacillus* strains heavily attributed to the corresponding increase of catechin, gallic acid, and quercetin in whole grain cereals. The phenolic degradation exhibited by enzymatic hydrolysis enabled the bioactive compounds of fermenting microorganisms to biotransform released bound phenolic compounds. Another study by De Montijo-Prieto et al. (2023), also concluded that biotransformations conducted by bacterial metabolism were able to modify the phenolic compound profile of avocado leaves with the aid of glycosidases or decarboxylases, hereby, releasing bound phenolics. This is also true for *S. cerevisiae* since this microorganism exhibits β-glucosidase activity (Hernández et al., 2003). Thus, using cellulase and pectinase to decompose cellulose and pectin into individual sugar monomers, paired with β-glucosidase, enables the bound phenolics to be released from such covalent bonds. In addition to that, the hydrolyzable polyphenolic compounds from flavonoids were also hydrolyzed into simpler phenols which was indicated by the loss or undetected presence of flavonoids in fermented cogon roots, and an excessive increase of phenolic content in such extracts.

The DNA damage protection assay determined that the damaging property of the fermented root samples can be further traced back to the phytochemical analysis. It was determined that there was a negative presence of flavonoids in these extracts despite FRP having one of the highest phenol levels. Alcohols are one of the main byproducts of fermentation and it can be further confirmed from the smell of the fermented solutions. The main composition of alcohols are the hydroxyl groups, and through oxidative stress, these could break down and form ROS (Wu & Cederbaum, 2003). Furthermore, it was stated that the flavonoid contents present in the fermented root extracts could be hydrolyzed into simpler phenols. A study by Akbal & Onar (2003), has shown that phenols exhibit photocatalytic degradation when exposed to the UV/H_2O_2 system during photolysis. The phenolic degradation depends on the hydrogen peroxide concentration, UV wavelength, and pH level of the solution. Fermented solutions are generally acidic, and it was shown that after the first 15mins of irradiation, the phenolic decomposition of the samples was at approximately 50% with pH levels 3 and 5 (acidic condition). The pH level requirement for phenolic degradation would also explain why the chloroform crude extracts did not exhibit any DNA-damaging properties. The photocatalytic degradation of phenols and alcohols further enhances the production of ROS in the system. Hence, the reason why the fermented root samples DNA-damaging properties could be due to the alcohol content, negative presence of flavonoids, and photocatalytic degradation of phenolic compounds. This could imply a potential application for regulatory activity against cancer cells by inducing apoptosis caused by the excessive DNA-damaging properties of these fermented root samples.

On the other hand, it was also further confirmed that the fermentation control indeed has an inhibitory property towards DNA damage despite having a negative presence of flavonoids and average phenolic content. The fermentation control was only composed of cellulase, pectinase, sugar, and yeast. This meant that the yeast was still able to perform metabolic activity, while the enzymes remained

unused since there was no lignocellulosic material present in its composition. However, when exposed to oxidative stress from ROS, enzymes start to degrade and cleave individual amino acid residues, like tryptophan, cysteine, and methionine. Such amino acid residues are known for radioprotective effects against plasmid DNA-induced damage, and also for double-strand breaks (DBS) (Yogo et al., 2021). Therefore, since the fermentation control had low phenolic content, the amino acids produced from cleaving the enzymes present in the fermentation control system through oxidative damage could have further interacted with the plasmid DNA preventing the ROS from causing DNA fragmentation.

The fermented leaf samples also had an interesting result regarding their DNA damage inhibition property. The phytochemical analysis on Table 2 has shown that the fermented leaf samples had the highest flavonoid and phenolic contents especially fermented leaf powder (FLP). However, the assay in Figure 6 has shown that FLP had DNA-damaging properties since the fragmentation of the DNA can be observed from the bands, almost aligning with the irradiated DNA. The reason could be similar to the fermented root samples due to the presence of alcohols in the system and the photocatalytic degradation of phenols since FLP has the highest phenolic content at 2724.93±1.84µg $GAEg^{-1}$ dry sample. However, since the flavonoid content of FLP was also high, some of the DNA was retained rather than exhibiting the complete degradation that occurred with the fermented root samples. Lastly, only the fermented leaf macerate exhibited a protective property probably due to its average phenolic and flavonoid content. With these results, the fermented extracts offer a new research gap and potential for extraction of phytochemicals and DNA damage protection activity.

Overall, all of the chloroform crude extracts at 1000 ppm exhibited DNA protective activity against the free radicals produced from the photolysis of UV/H_2O_2. In addition, the fermented control (F. ctrl) and fermented leaf macerate (FLM) exhibited a protective property towards DNA damage while the fermented leaf powder caused slight DNA damage to the plasmid DNA. Lastly, the fermented root samples (FRP and FRM) were shown to cause excessive degradation of the plasmid DNA.

The results imply that the cogon grass chloroform crude extracts and fermented leaf macerate (FLM) offer potential towards anti-cancer technology. Moreover, even the damaging properties of FRP and FRM have potential to be utilized as an apoptotic regulator. These are recommended for future studies. Specifically, a more thorough analysis of cogon grass extractive fermentation and further assessment of DNA damage protection activity through quantitative assays are recommended.

CONCLUSION

The study aimed to validate optimized methodologies derived from relevant literature, leading to several key conclusions. First, cogon grass extracts that underwent oven drying and fermentation showed the highest phytochemical contents, particularly in the aerial parts. Specifically, the fermented leaf powder (FLP) yielded the highest total phenolic and flavonoid content. Additionally, the increased phenolic content of fermented samples indicates that microbial activity

and enzymatic hydrolysis of polyphenols significantly elevated the phenolic yield. Furthermore, the presence of flavonoids in chloroform crude extracts and FLM, along with amino acids in the fermented control (F. ctrl), contributed to the DNA damage protective activity of these samples. Lastly, the high phenolic contents found in fermented root samples and FLP were linked to DNA damage due to an increased production of reactive oxygen species (ROS) resulting from the photodegradation of phenols under UV/ H_2O_2photolysis.

ACKNOWLEDGMENT

The author is grateful to Mr. Lourd Franz Gabunada for guiding the author during his entire thesis duration and for allowing him to use the laboratory resources and facilities of the Department of Biotechnology at Visayas State University, Baybay City, Leyte. He would also like to extend his thanks to the department staff and faculty, and friends who supported this research.

AUTHOR CONTRIBUTIONS

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

FUNDING SOURCE

This study was financed by the sole author.

AVAILABILITY OF DATA AND MATERIALS

The required data are presented in the paper and supplementary materials. For any questions regarding the data, interested readers should contact the author directly.

ETHICAL CONSIDERATIONS

This article did not include human subjects or animal studies.

COMPETING INTEREST

The author declared no potential competing interest concerning the research, authorship, and/or publication of this article.

REFERENCES

Abu, F., Mat Taib, C. N., Mohd Moklas, M. A., & Mohd Akhir, S. (2017). Antioxidant properties of crude extract, partition extract, and fermented medium of D*endrobium sabin* flower. *Evidence-Based Complementary and Alternative Medicine*, *2017*(1), 2907219. https://doi.org/10.1155/2017/2907219

Adebo, O. A., & Medina-Meza, I.G. (2020). Impact of fermentation on the phenolic compounds and antioxidant activity of whole cereal grains: A mini review. *Molecules*, *25*(4), 927. https://doi.org/10.3390/molecules25040927

Adebo, O. A., & Gabriela Medina-Meza, I. (2020). Impact of fermentation on the phenolic compounds and antioxidant activity of whole cereal grains: A mini review. *Molecules*, *25*(4), 927. https://doi.org/10.3390/molecules25040927

Akbal, F., & Nur Onar, A. (2003). Photocatalytic Degradation of Phenol. *Environmental Monitoring and Assessment*, *83*(3), 295–302.https://doi.org/10.1023/A:1022666322436

Cui, X., Liao, J., Liu, H., Tang, W., Tie, C., Tian, S., & Li, Y. (2023). Adsorption of phenols from aqueous solution with a pH-sensitive surfactant-modified bentonite. *Separations*, *10*(10), 523. https://doi.org/10.3390/separations10100523

De Montijo-Prieto, S., Razola-Díaz, M.C., Barbieri, F., Tabanelli, G., Gardini, F., Jiménez-Valera, M., Ruiz-Bravo, A., Verardo, V., & Gómez-Caravaca, A. M. (2023). Impact of lactic acid bacteria fermentation on phenolic compounds and antioxidant activity of avocado leaf extracts. *Antioxidants*, *12*(2), 298. https://doi.org/10.3390/antiox12020298

Estrella, M., Lu, A. L., & Lasin, S. (2020). *Antimutagenic Potential of Cogon (Imperata cylindrica) Methanolic Leaf Extract in Irradiated Human Male Peripheral Blood Lymphocytes* [Research, Philippine Science HighSchool]. https://www.researchgate.net/publication/351108056_Antimutagenic_Potential_of_Cogon_Imperata_cylindrica_Methanolic_Leaf_Extract_in_Irradiated_Human_Male_Peripheral_Blood_Lymphocytes

Haminiuk, C. W. I., Plata-Oviedo, M. S. V., de Mattos, G., Carpes, S. T., & Branco, I. G. (2014). Extraction and quantification of phenolic acids and flavonols from *Eugenia pyriformis* using different solvents. *Journal of Food Science and Technology*, *51*(10), 2862–2866. https://doi.org/10.1007/s13197-012-0759-z

Hernandez, L., Espinosa, J.C., Fernandez-Gonzalez, M. & Briones, A. (2003). β-Glucosidase activity in a *Saccharomyces cerevisiae* wine strain. *International Journal of Food Microbiology*, *80*(2), 171–176. https://doi.org/10.1016/S0168-1605(02)00149-6

Indriyanti, R. A., Ariyanto, E. F., Usman, H. A., Effendy, R. R., & Dhianawaty, D. (2022). Quantification of total polyphenols and flavonoids, antioxidant activity, and Sinensetin and Imperatorin contents of Imperata cylindrica root ethanol extract. *Pharmacognosy Journal*, *14*(4), 327–337. https://doi.org/10.5530/pj.2022.14.103

Jaradat, N., Hawash, M., & Dass, G. (2021). Phytochemical analysis, in-vitro anti-proliferative, anti-oxidant, anti-diabetic, and anti-obesity activities of Rumex rothschildianus Aarons. Extracts. *BMC Complementary Medicine and Therapies*, *21*(1), 107. https://doi.org/10.1186/s12906-021-03282-6

Jung, Y-K., & Shin, D. (2021). *Imperata cylindrica*: A review of phytochemistry, pharmacology, and industrial applications. *Molecules*, *26*(5), 1454. https://doi.org/10.3390/molecules26051454

Kaur, P., Purewal, S. S., Sandhu, K. S., & Kaur, M. (2019). DNA damage protection: An excellent application of bioactive compounds. *Bioresources and Bioprocessing*, *6*(2). https://doi.org/10.1186/s40643-019-0237-9

Keshava, R., Muniyappa, N., & Gope, R. (2020). Bioactivity guided fractionation and elucidation of anti-cancer properties of *Imperata cylindrica* leaf extracts. *Asian Pacific Journal of Cancer Prevention*, *21*(3), 707–714. https://doi.org/10.31557/APJCP.2020.21.3.707

Kim, T-H., Truong, V-L., & Jeong, W-S. (2022). Phytochemical composition and antioxidant and anti-inflammatory activities of *Ligularia fischeri* turcz: A

comparison between leaf and root extracts. *Plants*, *11*(21), 3005. https://doi.org/10.3390/plants11213005

Lalthanpuii, P.B., Zarzokimi, & Lalchhandama, K. (2018). Some phytochemical analyses of different extracts of the cogon grass *Imperata cylindrica* from Mizoram, India. *Science Vision*, *18*(4), 120–124. https://doi.org/10.33493/scivis.18.04.03

Li, Q., Chang, X., Guo, R., Wang, Q., & Guo, X. (2019). Dynamic effects of fermentation on phytochemical composition and antioxidant properties of wampee (*Clausena lansium* (Lour.) Skeel) leaves. *Food Science & Nutrition*, *7*(1), 76–85. https://doi.org/10.1002/fsn3.795

Manalo, C.A.M., Arguelles, J.A.P., Bendaña, E.M., Comia, A.F.M., Ribao, M.R.B., & Dumaoal, O.S.R. (2020). In vitro antioxidant and DNA damage inhibition activity of ethanolic extract of *Araucaria heterophylla* (Salisb.) Franco (Norfolk Island pine). *The Steth*, *14*, 31–45. https://research.lpubatangas.edu.ph/the-steth-vol-14-2020/

Metsopkeng, C.S., Nougang, M.E., Nana, P A., Arfao, A.T., Bahebeck, P.N., Djimeli, C.L., Eheth, J.S., Ewoti, O.V.N., Moungang, L.M., Agbor, G. A., Perrière, F., Sime-Ngando, T., & Nola, M. (2020). Comparative study of *Moringa stenopetala* root and leaf extracts against the bacteria *Staphyloccocus aureus* strain from aquatic environment. *Scientific African*, *10*, e00549. https://doi.org/10.1016/j.sciaf.2020.e00549

Sari, K.R.P., Ikawati, Z., Danarti, R., & Hertiani, T. (2023). Micro-titer plate assay for measurement of total phenolic and total flavonoid contents in medicinal plant extracts. *Arabian Journal of Chemistry*, *16*(9), 105003. https://doi.org/10.1016/j.arabjc.2023.105003

Schrader, T. J. (2016). Mutagens. In *Encyclopedia of Food and Health* (pp. 20–28). Elsevier. https://doi.org/10.1016/B978-0-12-384947-2.00476-1

Senguttuvan, J., Paulsamy, S., & Karthika, K. (2014). Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for in vitro antioxidant activities. *Asian Pacific Journal of Tropical Biomedicine*, *4*(1), S359–S367. https://doi.org/10.12980/APJTB.4.2014C1030

Singla, R.K., Dubey, A.K., Garg, A., Sharma, R.K., Fiorino, M., Ameen, S.M., Haddad, M.A., & Al-Hiary, M. (2019). Natural polyphenols: Chemical classification, definition of classes, subcategories, and structures. *Journal of AOAC International*, *102*(5), 1397–1400. https://doi.org/10.5740/jaoacint.19-0133

Tsao, R. (2010). Chemistry and biochemistry of dietary polyphenols. *Nutrients*, *2*(12), 1231–1246. https://doi.org/10.3390/nu2121231

Verma, K., Shrivastava, D., & Kumar, G. (2015). Antioxidant activity and DNA damage inhibition in vitro by a methanolic extract of *Carissa carandas* (Apocynaceae) leaves. *Journal of Taibah University for Science*, *9*(1), 34–40. https://doi.org/10.1016/j.jtusci.2014.07.001

VSU, Department of Biotechnology. (2024). [Google Maps]. Google; Google. https://www.google.com/maps/place/10%C2%B044'50.9%22N+124%C2%B047'31.8%22E/@10.7474047,124.7921791,53m/data=!3m1!1e3!4m4!3m3!8m2!3d10.74746!4d124.792163?entry=ttu

WHO. (2022). Cancer. https://www.who.int/news-room/fact-sheets/detail/cancer

Yan Kwok, A.H., Wang, Y., & Ho, W.S. (2016). Cytotoxic and pro-oxidative effects of *Imperata cylindrica* aerial part ethyl acetate extract in colorectal cancer in vitro.

Phytomedicine, *23*(5), 558–565. https://doi.org/10.1016/j.phymed.2016.02.015

Yogo, K., Murayama, C., Hirayama, R., Matsumoto, K., Nakanishi, I., Ishiyama, H., & Yasuda, H. (2021). Protective effects of amino acids on plasmid DNA damage induced by therapeutic carbon ions. *Radiation Research*, *196*(2), 197-203. https://doi.org/10.1667/RADE-21-00033.1