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Toxicity, anti-inflammatory, and phytochemical properties of *Christella parasitica* (L.) H.Lev. ex Y.H.Chang in Bukidnon, Philippines

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ABSTRACT

Christella parasitica (L.) H.Lev. ex Y.H.Chang is a terrestrial fern traditionally used to treat gout and rheumatism, conditions caused by intense inflammation. Since inflammation is linked to many health problems in humans, investigation on the toxicity and anti-inflammatory potential of *C. parasitica* is of current relevance for drug discovery potential. Crude methanolic extracts of *C. parasitica* fronds and rhizomes were tested for total phenolic content (TPC), total flavonoid content (TFC), DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, cyclooxygenase-2 (COX2) inhibition and toxicity tests against neonatal human epidermal keratinocytes (HEKn) and lung adenocarcinoma (A549). Plant habit, morphological characteristics, and the ribulose-bisphosphate carboxylase (rbcL) region confirmed the plant's identity. Alkaloids and tannins were present only in the fronds, and anthraquinones only in the rhizome while phenolics, saponins, and terpenoids were found in both fronds and rhizomes. Total phenolic content was significantly higher ($P < 0.05$) in the rhizomes compared to fronds. Flavonoids are present in both fronds and rhizomes. Fronds and rhizomes exhibited antioxidant activity based on DPPH radical-scavenging activity relative to ascorbic acid. They also exhibited high anti-inflammatory activity based on the inhibition of COX2. Both frond and rhizome extracts were nontoxic to HEKn and LA A549. These findings indicate that *C. parasitica* is nontoxic and has anti-oxidant and anti-inflammatory activities, which make it a promising natural source of anti-oxidant and anti-inflammatory compounds.

Keywords: anti-oxidants, cyclooxygenase, drug discovery, medicinal ferns, rbcL

INTRODUCTION

Drug development is a highly dynamic process in the history of civilization that has evolved from the knowledge and methods of various indigenous cultures to highly technical processes in many industrial laboratories of pharmaceutical companies. Aside from traditional sources, drug development could also be contingent, as in the case of how Alexander Fleming developed Penicillin.

Many of these development efforts source raw materials from plants. Central Mindanao University (CMU) has been designated as one of Tuklas Lunas Development Center (TLDC) since 2014 and pioneers drug development in the country using pteridophytes. Pteridophytes are a large class of non-flowering plants represented by ferns and lycopods that grow abundantly in many wild areas of Mindanao.

The medicinal importance of pteridophytes has long been established, particularly by the Chinese, who have used these plants in traditional medicine for over 2000 years (Ma et al. 2010). This traditional claim has prompted numerous researchers to investigate their pharmacological values, including their phytochemical composition and bioactivities (Shin and Lee 2010). *Christella parasitica* (L.) H.Lev. ex Y.H.Chang, with synonyms *Cyclosorus parasiticus* (L.) Farw and basionym *Polypodium parasiticum* L. (Evenhuis and Eldredge 2011; Kuo et al. 2019), is one of the 117 reported Philippine fern species listed in the family Thelypteridaceae worldwide (Delos Angeles and Buot 2012). This species is generally a terrestrial fern, found at lower elevations and widespread in tropical areas (Lin et al. 2013). Taxonomically, the genus *Christella* was established by Leveille in 1915 without designating a type species (Li et al. 2013). Holttum (1976) described this genus with *C. parasitica* (L.) Lev. as the type species. Its barcode is also found in the BOLD and NCBI databases using the rbcL gene in the former and trnL-trnF intergenic spacer, matK and psbA-trnH genes in the latter (Ratnasingham and Hebert 2007; NCBI 2020).

In traditional medicine, *C. parasitica* is recognized for treating gout and rheumatism (Benjamin and Manickam 2007; Singh and Upadhyay 2014), conditions caused by intense inflammation (Dalbeth and Haskard 2005). Reports on its phytochemicals (Paul et al. 2011; Mithraja et al. 2012) support its traditional medicinal use. Pursuing the anti-inflammatory potential of *C. parasitica* is of current relevance because inflammation is linked to many health problems in humans. Inflammation is classically viewed as an acute response to tissue injury, but contemporary revelations show it can be chronic and a major factor in developing diseases such as arthritis, atherosclerosis, cancer, heart valve dysfunction, obesity, diabetes, congestive heart failure, digestive system diseases, and Alzheimer's disease (Karin et al. 2006). A total of 410,244 studies on inflammation are indexed on PUBMED of the National Coalition for Biotechnology Information (NCBI). Most of these studies are very recent (Cervellati et al. 2020; Woolbright 2020; Nunes et al. 2020), suggesting that anti-inflammatory therapeutics have primary importance in the drug discovery process. The high volume and recency of these studies underscore the urgent need for new and effective anti-inflammatory agents.

The ongoing search for new therapeutic agents is crucial due to the rising prevalence of various health conditions and the need for effective treatments with fewer side effects. In inflammation, the goal is to achieve anti-inflammatory efficacy with few side effects. Corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) are used as anti-inflammatory drugs; however, several harmful side effects are recorded (Buchman 2001; Jones and Tait 1995). The former reduces inflammation by suppressing the immune system, while the latter inhibits cyclooxygenase, thereby preventing the production of prostaglandins, which is a key molecule in the inflammatory response. NSAIDs selective to COX2 inhibitors are preferred since they produce fewer digestive problems as side effects (Green 2001).

Recognizing the potential of *C. parasitica* for drug development, this study undertook a comprehensive examination of the species' frond and rhizome parts. The first objective was to establish its biological identity through traditional taxonomy and modern barcoding methods, ensuring accurate validation of field samples for subsequent laboratory analysis. The second objective was to verify the presence of phytochemicals in the frond and rhizome, particularly for Philippine species, where such data are currently insufficient. Additionally, the study aimed to gather empirical evidence on the efficacy and safety of *C. parasitica* using standardized methods and a tiered approach in testing (McKim and James 2010; Bacskay et al. 2018). These steps are critical in the drug discovery process. The resulting highlights are discussed in this report.

METHODS

Collection, Identification, and Preparation of Plant Material

Whole plant samples of *C. parasitica* (L.) H. Lev. ex Y.H. Chang (Figure 1) were collected from Mt. Musuan, Maramag, Bukidnon. These were identified through morphology by keying out using the Fern Flora of the Philippines (Copeland 1958), confirmed by the taxonomist in the research team, and deposited at the University Herbarium (CMUH). Deoxyribonucleic acid (DNA) barcoding using the rbcL gene was employed to confirm the species identity of the plant sample at the genetic level. The DNA extraction and amplification were conducted at the CMU Tuklas Lunas Development Center. Young fronds of *C. parasitica* were silica-dried and processed for total genomic DNA extraction using a modified cetyltrimethylammonium bromide (CTAB) method of Rogers and Bendich (1994). The amplification of the rbcL region was done using the primer pair rbcLaF and rbcLaR. This was amplified through a polymerase chain reaction (PCR) machine, Veriti® thermal cycler

(P/N 4375786, Life Technologies). The amplicons from thermal cycling were resolved using agarose gel electrophoresis, stained with Gel Red®, and visualized in GelDoc™ EZ documentation (Bio-Rad Technologies, Inc.). The PCR amplicons were sent to Macrogen, South Korea for bidirectional capillary electrophoresis sequencing (Sanger). BioEdit™ was used to edit the sequences. Edited sequences were submitted for homology to the Basic Local Alignment System Tool (BLAST) and Barcode of Life Database (BOLD) Identification System then deposited to Genbank.

The mature frond and rhizome parts were washed thoroughly with distilled water before air drying for 3-6 days at room temperature. Fresh and dry weights of the collected samples were recorded and the percent moisture loss was also calculated using the following formula adapted from Jin et al. (2017):

$$Mn = ((Ww - Wd)/Ww)\ x\ 100$$

Wherein, M_n = moisture content (%) of material, W_W = wet weight of the sample, and W_d = weight of the sample after drying.

The dried frond and rhizome parts of *C. parasitica* (L.) H.Lev. ex Y.H.Chang, below 10% moisture content, were ground separately using a heavy-duty miller and then sieved to produce a more homogeneous product.
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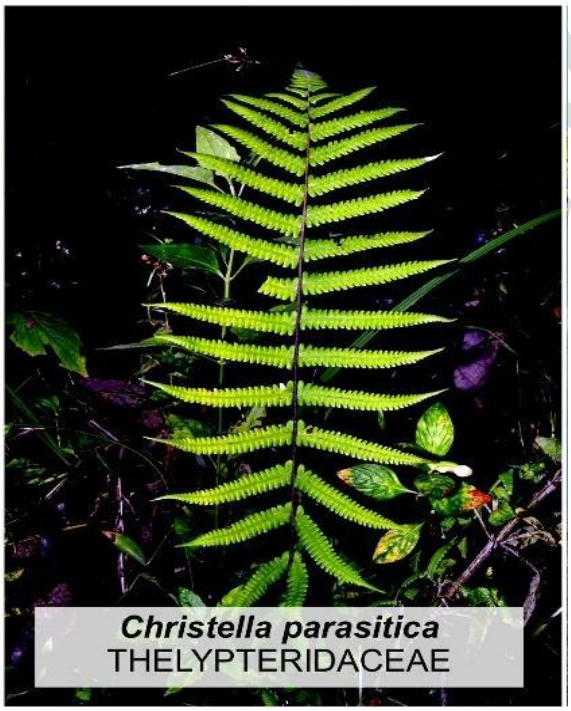

Figure 1. Frond of *Christella parasitica* (L.) H.Lev. ex Y.H.Chang (Thelypteridaceae) located at Mt. Musuan, Bukidnon.

Extraction and Phytochemical Studies

Methanolic extraction. The frond and rhizome samples were dried and powdered. Then, they were soaked in 99.99% HPLC grade methanol at room temperature for 72 hours (100 g sample / 500 mL solvent). The methanolic mixtures were then filtered through Whatman No. 1 filter paper and the filtrates were dried in a vacuum at 40°C using a rotary evaporator (Porquis et al. 2018). The extracted concentrates from the frond and rhizome samples were stored separately in an air-tight container at 4°C until further use.

Qualitative phytochemical analysis. To perform phytochemical screening of the crude methanolic extracts, a bioautographic assay via thin-layer chromatography (TLC) was used. This method was adapted from Brinda et al. (1981) and Gracelin et al. (2013) to detect the presence of important phytochemical constituents such as alkaloids, anthraquinones, phenolics, saponins, tannins, and terpenoids. Ten (10) µL of crude extracts were applied to the TLC plates using capillary tubes and air-dried before placing the TLC plates in a chamber. The plates were developed using chloroform: methanol (5:1) as the mobile phase and observed under UV light (254 nm).

Quantitative Phytochemical Analysis

Determination of total phenolic content (TPC). The total phenolic content was analyzed using the Folin–Ciocalteu colorimetric method in a 96-well microtiter configuration (Ainsworth and Gillespie 2007). The concentrated extracts of the frond and rhizome parts were dissolved in a DMSO (dimethyl sulfoxide): Methanol: Water (15:5:2) solution at 2 mg/mL (Amoroso et al. 2014). Then, 20 µL aliquot of the solution was mixed with Folin-Ciocalteau (1:10) reagent (Sigma) and incubated for 30 minutes. To modify the procedure, a five percent (5%) sodium carbonate solution was added to the solution as described in Bayili et al. (2011). After 2 hours of incubation at room temperature, the absorbance value was measured at 750 nm using a MultiSkan Go (ThermoScientific) UV/VIS spectrophotometer. A standard calibration curve was obtained using Gallic acid (R^2 = 0.9998).

Determination of total flavonoid content (TFC). The total flavonoid content of the samples was determined using a 96-well microtiter configuration (Sahu and Saxena 2013). Thirty (30) µL of fern extract at a concentration of 2 mg/mL in DMSO: methanol: water (15:5:2) was mixed with 30 µL of 10% aluminum chloride and 30 µL of 1M sodium acetate. Then, 110 µL of ultrapure water was added (Porquis et al., 2018). After 30 minutes of incubation at room temperature, the absorbance value of the samples was

read at 415 nm in the MultiSkan Go (ThermoScientific) UV/VIS spectrophotometer. The total TFC was expressed as µg quercetin equivalents per gram samples (µg QE/g).

Determination of antioxidant activity. The antioxidant activity of the extracts was initially determined through the DPPH radical scavenging assay. In a 96-well microtiter plate, 50 µL of concentrated extracts dissolved in a solvent consisting of 15 DMSO: 5 methanol: 2 water with a final concentration of 0.33 mg/ml were added with 150 µL DPPH (2,2,1-diphenyl-1-picrylhydrazyl). A 0.4 mg/mL ascorbic acid (AA) and the solvent (15 DMSO: 5 methanol: 2 water) were used as positive and negative controls, respectively. The plate was incubated at room temperature for 30 min, then absorbance was read at 517 nm. Percent DPPH radical scavenging activities (%DPPH) of the sample extracts and percent DPPH radical scavenging activities relative to ascorbic acid (%DPPH relative to AA) were computed using Equations 1 and 2, respectively (Amoroso et al. 2014):

$$\%\ DPPH\ \ Radical\ Scavenging\ Activity = \ [(A0 - A1)/A0]\ x\ 100 \quad (1)$$

$$\%\ DPPH\ relative\ to\ AA\ = \ (\%DPPHsample\ /\ \%DPPHAA)\ x\ 100 \quad (2)$$

where: A0 and A1 are the absorbance of the solvent and sample extract/ascorbic acid, respectively.

Anti-inflammatory assay (COX-Inhibition Assay). The inhibition activity of the extract on the cyclooxygenase 2 (COX-2) enzyme was determined using a COX (Ovine/Human) Inhibitor Screening Assay kit (Cayman Chemicals, Inc., USA) following the manufacturer's instructions. The extracts were assayed via enzyme-linked immunosorbent assay (ELISA) at a final concentration of 100 ppm. Two trials, each with 4 replicates were done for every extract. Celecoxib (Celebrex) at a 100-ppm concentration was used as a positive control.

Cell Viability Assay

MTT cell proliferation assay. The proliferative activity on neonatal Human Epidermal Keratinocytes (HEKn) was determined using the MTT assay 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide kit (Vybrant, Invitrogen). Cells at passage 3 were plated in a 96-well plate with a concentration of 5,000 cells/well, then incubated for 24 hours. The cells were cultured in EpiLife® basal media (M-EPI-500-CA) supplemented with Human Keratinocyte Growth Supplement (HKGS). The basal media were subsequently changed before adding the plant extract at final concentrations of 20 ppm and 200 ppm. The plated cells with extracts were incubated again at 37˚C for 48 hrs. Ten (10) µL of 12 mM MTT stock solution was added to label the cells, then incubated at 37ºC. After 4 hours of incubation, the media were removed, leaving only 25 µL in the wells. Then, the cells were added with 50 µL of dimethyl sulfoxide (DMSO) and incubated at 37ºC for 10 minutes. Absorbance values per well were read at 540 nm using a microplate reader. Cell viability was calculated as a percentage with untreated cells (Calderón-Montaño et al. 2021). The assay was carried out in two trials with three replicates per extract.

Anti-cancer assay. The following procedure for MTT cytotoxicity assay was adapted from Mosmann (1983). Human lung adenocarcinoma (A549) cell lines were seeded into sterile 96-well microtiter plates using a seeding density of 6 x 10 cells/mL then incubated overnight at 37°C and 5% CO_2. Serial dilutions of the samples at 4mg/mL DMSO were performed to which four different concentrations (1000 µg/mL, 500 µg/mL, 250 µg/mL, and 125 µg/mL) in a master dilution plate (MDP) were used. From the MDP, 10 µL of each concentration was dispensed onto the plated cells to obtain the final screening concentrations of 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL. Three replicate wells were used per concentration. Doxorubicin (17nM) and DMSO were used as the positive control and negative control, respectively. The treated cells were then incubated in 5% CO_2 at 37°C for 72 hours. After incubation, the media were removed from the 96-well microtiter plate and 20 µL of 3-(4,5-dimethylethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) at 5 mg/mL in Phosphate Buffer Saline (PBS) was added. The treated cells were incubated again at 37°C and 5% CO_2 for 4 hours. Then, DMSO was added to each well to dissolve the formazan crystals formed by the reduction of the dye by the live cells. Absorbance was read at 570 nm. Cell viability was calculated as a percentage in relation to untreated cells, while IC50 was obtained through linear regression analysis (Calderón-Montaño et al. 2021; Norberg-King 1993). Three trials with 3 replicates for each concentration were done for each sample. The US National Cancer Institute Plant Screening Program sets a standard of IC 50 ≤ 20 ppm for plant extracts having an active cytotoxic effect (Kaewpiboon et al. 2012). Samples with an IC50 value less than 30 µg/mL are considered active (Jokhadze et al. 2007).

RESULTS

Morphological and Molecular Identification

Based on plant habit and morphological characteristics such as sori shape and distribution, frond type, rhizome characteristics, and the presence or absence of scales or hair, the collected plant specimens were identified as *C. parasitica* (L.) H.Lev. ex Y.H.Chang. Furthermore, the rbcL region with an average length of 561 base pairs was successfully sequenced and confirmed the initial identification with 100% identity when compared to GenBank and BOLD databases. The GenBank accession number for the sequence of this species is MZ501574.

Qualitative Phytochemical Analysis of Crude Methanolic Extracts

Thin-layer chromatography showed the preliminary detection of phytochemicals in crude methanolic extracts of *C. parasitica* through positive color reactions of the spots. This revealed the presence of phenolics, saponins, and terpenoids in both the frond and rhizome parts. Alkaloids and tannins were observed only in the frond while anthraquinones were present only in the rhizome (Table 1).

Quantitative Phytochemical Analysis of Crude Methanolic Extracts

Both the frond and rhizome parts of *C. parasitica* contained phenolic and flavonoid compounds as indicated by the total phenolic content (TPC) and TFC of the crude methanolic extracts. The TPC found to be higher in the rhizome compared to the frond (Table 2).

Antioxidant and Anti-inflammatory Assays

The methanolic extracts of *C. parasitica* frond and rhizome exhibited antioxidant activity by scavenging DPPH radicals, although the activity was lower than that of ascorbic acid (Table 3). On the other hand, both frond and rhizome extracts exhibited high anti-inflammatory activity (≥50%) by inhibitingcyclooxygenase-2 (Table 3). Moreover, the rhizome showed higher antioxidant activity compared to the frond.

Table 1. Phytochemicals in crude methanolic extracts of *Christella parasitica* (L.) H.Lev. ex Y.H.Chang using thin-layer chromatography (TLC). Legend: +/- (presence/absence of the phytochemical).

Phytochemicals	Plant Part	
	Frond	Rhizome
Alkaloids	+	-
Anthraquinones	-	+
Phenolics	+	+
Saponins	+	+
Tannins	+	-
Terpenoids	+	+

Cell Viability Assay

The effects of *C. parasitica* frond and rhizome crude methanolic extracts on the viability of human epidermal keratinocytes (HEKn) were determined using the MTT cell proliferation assay. The extracts were tested at two concentrations: 20 and 200 ppm (Table 4). The frond methanolic extract showed high cell viability at both 20 ppm (88.98 ± 4.81) and 200 ppm (85.95 ± 10.53). The rhizome methanolic extract also showed relatively high cell viability at both 20 ppm (129.14 ± 21.54) and 200 ppm (99.17 ± 3.54).

Based on the total phenolic content, antioxidant activity, and cell viability, the rhizome methanolic extract performed better than the frond extract. Therefore, the rhizome extract was prioritized for further testing in the MTT cell viability assay against lung adenocarcinoma A549. In comparison to the chemotherapeutic agent doxorubicin, which only allowed 29-32% growth of cells at concentrations of 6.25 - 50 µg/ml, the rhizome extract did not hinder the proliferation of LA A549 cells (Table 5). Moreover, while the mean IC50 of Doxorubicin is 2.19 µg/ml, no linear interpretation can be achieved with the rhizome extract.

Table 2. Phenolic and flavonoid content of crude methanolic extracts of *Christella parasitica* (L) H.Lev. ex Y.H.Chang using the Folin-Ciocalteau and Aluminum Chloride method, respectively. Values are presented as mean ± SD (n=3); *** p-value < 0.001; ns - differences between the means not significant at p-value > 0.05.

Plant Part	Total Phenolic Content*** (mg GAE/ g sample)	Total Flavonoid Content^{ns} (µg QE/ g sample)
Frond	41.88 ± 2.22	14.17 ± 2.83
Rhizome	149.37 ± 0.59	2.01 ± 0.44

Table 3. DPPH radical scavenging activity (%) and percent inhibition on cyclooxygenase-2 in crude methanolic extracts of *C. parasitica* (L) H.Lev. ex Y.H.Chang. Values are mean ± SE (n=3); Value for ascorbic acid is 75.6%.; ***p-value < 0.001; ns – no significant differences between the means at p-value > 0.05.

Plant Part	Antioxidant Activity*** (% DPPH Radical Scavenging Activity Relative to Ascorbic Acid; P=0.000)	Anti-inflammatory Activity^{ns} (Cyclooxygenase or COX-2)
Frond	16.90 ± 0.58	60.15 ± 4.82
Rhizome	29.77 ± 0.89	74.97 ± 2.49

Table 4. Percent cell viability in human epidermal keratinocytes (HEKn) treated with *C. parasitica* methanolic extracts at 200 and 20 ppm extract concentration.

Plant part	Concn. (ppm)	Trial 1	Trial 2	Mean ± SD
Frond	20	92.38	85.58	88.98 ± 4.81
	200	93.39	78.51	85.95 ± 10.53
Rhizome	20	113.91	144.37	129.14 ± 21.54
	200	101.67	96.67	99.17 ± 3.54

Table 5. Mean cell viability (%) of the rhizome methanolic extract subjected to anticancer preliminary assay using MTT *in vitro* cell proliferation assay. *p-value (treatment) ≤ 0.001; p-value (concentration) ≤0.05; NLI - no linear interpretation.

Concentration (µg/mL)	% Cell Viability*	
	Doxorubicin	Rhizome
50	29 ± 9.17	105 ± 13.56
25	32 ± 9.76	104 ± 30.26
12.5	29 ± 9.17	108 ± 25.37
6.25	30 ± 10.85	106 ± 21.07
0	100 ± 0.00	100 ± 0.00
IC50 (ug/ml)	2.19	NLI

DISCUSSION

Morphological and Molecular Identification

Drug discovery from plants and plant identification are inseparable, especially when a potential drug is being studied and analyzed. Traditionally, plant identification relies solely on the plant's morphology. However, relying solely on morphology can be challenging, especially for an untrained eye, due to the wide range of plant forms. This challenge is particularly amplified with ferns and their allies, as they lack flowers and fruits that could facilitate easy identification. In this study, the traditional identification method for *C. parasitica*, using morphological characteristics, is supplemented with DNA barcoding. This involves using a short section of DNA from a standardized region of the genome, specifically the rbcl gene (Kress and Erickson 2007), which codes for the large subunit of ribulose 1,5 bisphosphate carboxylase/oxygenase (RUBISCO). The rbcl gene is considered a benchmark locus in phylogenetic investigations (Kress and Erickson 2007).

Qualitative Phytochemical Analysis of Crude Methanolic Extracts

Numerous secondary metabolites, primarily from the phenolic, flavonoid, terpenoid, and alkaloid classes, have been identified as active components in fern species. Among these, terpenoids are the most abundant chemical group present in ferns (Ho et al. 2010). Terpenoids have a variety of biological uses, with triterpenoids acting as antioxidants (Garcia et al. 2006), diterpenoids as anti-inflammatory agents (Kim et al. 2016), and sesquiterpenoids as cytotoxic compounds (Ge et al. 2008).

The presence of phenolics, saponins, and terpenoids in both fronds and rhizomes, alkaloids and tannins in fronds, and anthraquinones in rhizomes may confirm the medicinal potential of these ferns. These phytochemical compounds are known to support various biological activities in medicinal plants and contribute to their antioxidant properties. Tannins, saponins, and triterpenes have all been reported to have antitumor, mutagenic, anti-inflammatory, and anti-ulcer activities (Chung et al. 1998; Ferguson et al. 2006; Lemeshko et al. 2006; Roy et al. 2007; Ye et al. 2007; Mohammed 2014). Similarly, naturally occurring anthraquinones exhibit a broad spectrum of bioactivities such as cathartic, anticancer, anti-

inflammatory, antimicrobial, diuretic, vasorelaxant, and phytoextrogent activities (Chien et al. 2015).

In this study, the localization of phytochemicals to a specific plant organ is observed. Alkaloids and tannins were found exclusively in the frond, while anthraquinone was localized in the rhizome. Dela Cruz et al. (2017) also documented the localization of these phytochemicals in certain fern species. Alkaloids and tannins were observed only in the frond of *Drynaria quercifolia* and *Pyrrosia adnascens*, while anthraquinones were found only in the rhizome of *Drynaria quercifolia*. However, *Microsorum punctatum* (L.) and *Pyrrosia adnascens* contained anthraquinones in both the frond and rhizome (Dela Cruz et al. 2017). At the cellular level, secondary metabolites were localized in the parenchymal cells such as secretory tissues, vacuoles, and cytosol of the frond and rhizome in *Pteris* species (Sulisetijono et al. 2020). Tannins are particularly abundant in the xylem of leaves in many plants (Badria and Aboelmaaty 2019). The presence of phytochemicals in multiple plant organs can result in varying amounts and rates of bioactivity. For example, leaves exhibited higher total phytochemical content, total flavonoid content, and DPPH radical scavenging activities compared to stems (Raya et al. 2015). In this study, the rhizome exhibited a high amount of total phenolic content, antioxidant activity, and anti-inflammatory activity but these properties were limited to the frond. These findings imply that the nature of the plant part should be considered in pharmacological studies, as it can influence the production of secondary metabolites. The strategic localization of these compounds optimizes chromatographic explorations and facilitates the cost-effective isolation of natural products for drug development (El Babili et al. 2021).

Phenolic compounds, particularly those derived from plants, have been found to possess anti-inflammatory and anti-cancer properties. They achieve this by inhibiting oxidative stress and conditions associated with inflammation (Tatipamula and Kukavica 2021). Reports have shown that phenolic compounds have significant value in preventing the development and progression of various human diseases (Rahman et al. 2021). In this study, a high TPC of 149.37 ± 0.59 mg GAE/g sample was observed in the *C. parasitica* rhizome methanolic extract. Total phenolic content values greater than 10 mg gallic acid/g are considered high (Zakaria et al. 2010). The flavonoid content varies among different fern species. In this particular study, the frond and rhizome samples had relatively lower observed TFC values of 14.17 and 2.01 µg QE/g, respectively, when compared to other fern species. For example, *Lycopodium cernua* had 11.46 µg QE/g in the fronds and 5.22 µg QE/g in the rhizomes (Porquis et al. 2018). The *M. punctatum* contains 6.69 µg QE/g in the fronds and 17.38 µg QE/g rhizomes, while *D. quercifolia* had 36.74 µg QE/g in the frond samples (Dela Cruz et al. 2017). Flavonoids, which are polyphenols, exhibit several biological effects such as anti-inflammatory and anticancer potential. They achieve this by inhibiting pro-inflammatory cytokines and activating antioxidant transcription factors (Ferraz et al. 2020). Hence, the fern species under investigation in this study may possess anticancer, anti-inflammatory, and antioxidant activities that are correlated to their total flavonoid contents.

Antioxidant and Anti-inflammatory Assays

The DPPH radical scavenging assay was used to evaluate the antioxidative properties of the crude methanolic extracts. The DPPH is commonly used to evaluate the antioxidant potential of herbal extracts. Antioxidant compounds help reduce oxidative damage caused by reactive oxygen species, which are free radicals that can harm nucleic acids and proteins and potentially leading to cell death (Gulcin and Alwasel 2023). In this study, DPPH data aided in prioritizing the extracts for the COX-2 inhibition assay as well as gave a glimpse into the mechanism underlying the bioactivity of the extracts. The anti-inflammatory activities of drugs are mediated by the inhibition of cyclooxygenases which catalyze the bioconversion of arachidonic acid to prostaglandins (Badieyan et al. 2012). The COX-2 isozyme is involved in the anti-inflammatory response, as it is induced by mitogenic and proinflammatory stimuli. Moreover, COX-2 expression is triggered by inflammation and carcinogenesis. The COX-2 is overexpressed in many solid tumors such as colon, breast, prostate, liver, and lung cancers (Badieyan et al. 2012).

Cell Viability Assay

This study showed that the crude methanolic extracts of *C. parasitica* did not exhibit cytotoxic activity, as they failed to inhibit the proliferation of HEKn cell lines (normal cells) and LA A549 (cancer cells). The MTT assay is a cytotoxicity assay used to assess the metabolic activity of cells. Viable cells can produce mitochondrial enzymes that convert tetrazolium into formazan, making the MTT assay an effective method for evaluating the toxicity of materials on cell growth (Tolosa et al. 2015). Also, the MTT in vitro proliferation assay is widely used to preliminarily evaluate the anticancer activity of natural product extracts (McCauley et al. 2013). In this study, the methanolic extracts were assessed for preliminary anticancer activity based on the viability of lung adenocarcinoma A549 cells. Compared to doxorubicin, a chemotherapeutic drug used to treat several cancer types, the rhizome extract did not prevent the proliferation of the cells. Unlike

doxorubicin which prevents or slows down the growth of cancer cells by blocking the enzyme topoisomerase (Kciuk et al. 2023), the rhizome extract may be devoid of metabolites that may damage the DNA of cancer cells.

The presence of phenolic and flavonoids as well as the antioxidant and anti-inflammatory activities of *C. parasitica* extracts may imply potential of this plant for future product development, although confirmatory assays are needed to ensure safety. The present investigation revealed the presence of medicinally important constituents of *C. parasitica* and provided useful information on its cytotoxicity and anti-inflammatory potential. It is nontoxic and has anti-inflammatory activities which make it a promising natural source of anti-inflammatory compounds.

FUNDING

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ETHICAL CONSIDERATIONS

Gratuitous permit for plant collection was approved by the Department of Environment and Natural Resources (DENR) Region 10 (Wildlife Gratuitous Permit No. R10 2019-57) after complying necessary requirements. Prior arrangement and courtesy visits with the municipal and barangay officials were observed.

DECLARATION OF COMPETING INTEST

The authors declare that there are no conflicting interests to any authors.

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