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Cyclooxygenase (COX) and 15-Lipoxygenase (15-LOX) Inhibitory Activity and HPTLC Profile of *Asplenium nidus, Diplazium esculentum*, and *Drynaria quercifolia* in Bukidnon, Philippines

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ABSTRACT

Diplazium esculentum, Drynaria quercifolia, and *Asplenium nidus* are among the fern species found in Bukidnon, Philippines which are used as traditional herbal medicines. The HPTLC profile and the anti-inflammatory properties against cyclooxygenase (COX) and 15-lipoxygenase (15-LOX) of the frond ethanolic extracts of *D. esculentum, D. quercifolia*, and *A. nidus* were determined. The High-Performance Thin Layer Chromatography (HPTLC) profile was obtained using ethyl acetate: formic acid: water (16:2:2) as mobile phase and Natural Products (NP) as a derivatizing reagent. The HPTLC profile of the *D. esculentum*, *D. quercifolia*, and *A. nidus* extracts showed 10 (R_f = 0.02-0.97), 13 (R_f = 0.03-0.90), and 14 (R_f = 0.02-0.99) bands, respectively. The profiles for each fern species may be used as a marker for quality evaluation and standardization of herbal formulations containing these plants. For the anti-inflammatory properties, *D. esculentum* and *D. quercifolia* extracts which inhibited more than 50% of the COX enzymes showed significantly higher activity than *A. nidus* and were considered active against COX-2 and COX-1. *D. esculentum*, however, gave a selectivity ratio (COX-2/COX-1) of 1.03 making its inhibitory activity selective against COX-2. The percent 15-LOX inhibitory activity of *D. quercifolia* (58.62%) is significantly higher than that of *A. nidus* (38.70%) but statistically comparable to *D. esculentum* (51.19%). Among the extracts, *D. quercifolia* and *D. esculentum* which inhibited more than 50% of the 15-LOX were considered active. *D. esculentum* and *D. quercifolia* can therefore be potential sources of anti-inflammatory lead compounds for future drug development.

Keywords: anti-inflammatory, COX, ferns, HPTLC, 15-LOX

INTRODUCTION

Inflammation is an essential immune response in higher organisms to infection, injury, and other noxious conditions in the body in an attempt to eliminate or limit the spread of harmful stimuli or agents as well as healing of damaged tissue (Medzhitov, 2010). Symptoms of inflammation are redness, heat, swelling (edema), pain, and loss of mobility or replacement of functional cells with scar tissue. Cyclooxygenase (COX) and lipoxygenase (LOX) are the major enzymes in the biosynthesis of important biological mediators such as prostaglandins and leukotrienes which play a crucial role in stimulating inflammatory reactions (Fiore, 2004). Among the many diseases related to inflammation are chronic diseases, including cardiovascular diseases (CVD), cancer, obesity, and diabetes (Lordan *et al*., 2019).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs to treat inflammatory conditions. However, severe adverse side effects such as gastrointestinal toxicity, cardiovascular adverse effects, and nephrotoxicity are associated with their prolonged use (Wongrakpanich *et al.*, 2018) Therefore, the empirical approach to find new drugs with lesser side effects via the systematic screening of medicinal plants extracts remains a crucial strategy to find new lead compounds (Balunas *et al*., 2005; McChesney *et al*., 2007).

Medicinal plants are essential to the health of many individuals and communities. The natural therapeutic properties of these medicinal plants are very useful in healing various diseases (Nostro *et al.*, 2000). According to the World Health Organization (WHO), about 80% of the world's inhabitants, mostly in the developing countries like the Philippines, rely mainly on traditional medicine for their primary health care (Gurib-Fakim, 2006). Among the commonly used medicinal plants in the Philippines are the Pteridophyte species. Out of 1,100 species, more than 50 species were reported to have medicinal values throughout the Philippines, and 41 species of which can be found in Mindanao (Amoroso *et al.*, 2014). *Diplazium esculentum*, *Drynaria quercifolia,* and *Asplenium nidus* are among the medicinal species which can be found in Bukidnon. These fern species are traditionally used as herbal medicines for the treatment of various ailments. *D. esculentum* has been used to cure hemoptysis, cough, acne, tumors, asthma, dysentery, and drying out scars (Pradhan *et al*., 2015; Zannah *et al.*, 2017). *D. quercifolia* was used to treat people from suffering from intestinal worms, abdominal pain, gonorrhea, typhoid, hectic fever, dyspepsia, cough, and phthisis (Mannan *et al.*, 2008; Das *et al.*, 2009; Mollik, 2010) *A. nidus* is considered as depurative, sedative, and was used to treat body aches and pain, fever, headaches, labor pain, and chest pain (Mannan *et al.*, 2008; Nandwani *et al.*, 2008; Lai *et al.*, 2009). Despite the emergence of reports on antioxidant, antitumor, cytotoxic, antibacterial, and anticancer activities in certain species (Rahmat *et al.*, 2003; Lai *et al*., 2011; Kaushik *et al.*, 2012; Chai *et al.*, 2015; Jarial *et al.*, 2018; Prasanna *et al.*, 2019) are available, literature still shows limited scientific data on the anti-inflammatory activity of *D. esculentum*, *D. quercifolia,* and *A. nidus*.

Moreover, medicinal plants are a storehouse of therapeutic phytochemicals that may lead to the development of novel drugs (Azwanida, 2015). As a single herbal medicine may contain various natural constituents, the fingerprints produced by the chromatographic analysis may represent a good representation of various chemical components of herbal medicines (Liang *et al.,* 2004). In this study, the High-Performance Thin Layer Chromatography (HPTLC) of the fern extracts was conducted along with the determination of their anti-inflammatory properties.

MATERIALS AND METHODS

Sample collection and preparation

Fronds of *D. esculentum*, *D. quercifolia*, and *A. nidus* were collected from Central Mindanao University (CMU), University Town, Musuan, Bukidnon, Panadtalan, Maramag, Bukidnon, and the Center for Ecological Development and Recreation (CEDAR), Impalutao, Bukidnon, Philippines, respectively. Plant samples were authenticated by the Botany Section of the CMU Herbarium, CMU, Musuan, Bukidnon, Philippines. The collected fronds were washed with tap water to remove dirt, rinsed with distilled water, and hanged in a net to allow water to drip. The leaf samples were then weighed and air-dried until the moisture content was below 10%. The dried samples were pulverized using an osterizer and sieved to obtain homogenous samples. The powdered samples were then soaked exhaustively in absolute ethanol for 72 h, filtered and the collected extracts were concentrated under *a vacuum* at 40°C. The weight of the concentrated crude ethanolic extracts was then recorded and stored at -20°C until further use.

Determination of High-Performance Thin Layer Chromatography (HPTLC) profile

HPTLC system (CAMAG, Muttenz, Switzerland) equipped with an Automatic TLC Sampler ATS 4, Automatic Developing Chamber ADC 2, Scanner 4, TLC Visualizer, Immersion Device 3, Plate Heater, and visionCATS 2.5 software was used for analysis. Exactly 2.00 uL of sample extracts (0.10 g/mL) in methanol were spotted on an HPTLC aluminum-backed plate (silica gel 60 $F_{254;}$ 20 x 10 cm; Merck) under nitrogen stream. After the sample application, the plate was developed in a pre-saturated 20 x 10 cm twin trough glass chamber (relative humidity: 33%) using ethyl acetate: formic acid: water (16:2:2) as a mobile phase based on the results of the optimization. The chromatogram was then visualized under white light and UV light at 254 and 366. The plate was then immersed in Natural Product (NP) reagent

(immersion speed: 5 cm/s; dwell time: 1 s), air-dried for 5 min under the fume hood, and visualized under UV light at 366 nm.

Determination of COX and 15-LOX inhibitory activity

Sample extracts were sent to Bioorganic and Natural Products Laboratory and The Terrestrial Natural Products Laboratory, Institute of Chemistry, University of the Philippines Diliman for the 15-LOX and COX inhibition assays, respectively. Samples that gave ≥ 50% COX-2 inhibition and ≥ 1.00 COX-2/COX-1 ratio are considered active and COX-2 selective. The samples that exhibit ≥ 50% 15-LOX inhibitory effect are considered active against 15-LOX.

COX inhibition assay

The assay was done employing the method described by Bonner and Fry (2012). A 5184 uL of 100 mM pH 8 Tris buffer was added to a clean vial. A 96 uL of 250 U/mL of COX-2 and COX-1 enzymes and 480 uL of 20 uM Hematin were mixed separately and added into the vial with buffer. The mixture constitutes the enzyme-cofactor solution. A 120 uL of the enzyme-cofactor mixture was then placed in each well that had already been dispensed with 50 uL of the same buffer. Then, 10 uL of 200 ug/mL of plant extracts in dimethyl sulfoxide (DMSO) was added to make a final well concentration of 10 ug/mL. An 8 mM indomethacin in 100% DMSO and 5% DMSO (final well concentrations) were used as the positive and the negative control, respectively. After the incubation of the mixture at 25°C for 15 min, a 10 uL of 200 uM Amplex Red (10-acetyl-3,7-dihydroxyphenoxazine) and 10 uL of 2000 uM arachidonic acid were added to each well. The reaction mixture was mixed and purged with N_2. The reaction was then monitored for 2 min using a CLARIOstar® (BMG LABTECH) microplate reader at an excitation wavelength of 535 nm and emission wavelength of 590 nm. The fluorescence intensity was measured at 12 s intervals. The positive control and the % inhibition of the samples were determined based on the average slope of each replicate by using the following formula:

$$\% \text{ Inhibitory Activity} = \frac{\text{Slope}_{\text{uninhibited}} - \text{Slope}_{\text{inhibited}}}{\text{Slope}_{\text{uninhibited}}} \text{ x } 100\%$$

where $\text{Slope}_{\text{uninhibited}}$ is the slope of the line from the fluorescence vs time plot of the negative control group and the $\text{Slope}_{\text{inhibited}}$ is the slope of the line from the fluorescence vs. time plot of the samples/positive control.

15-LOX inhibition assay

The assay used lipoxidase from *Glycine max as* the enzyme source (Axelrod *et al.*, 1981; Auerbach *et al*.,1992). Briefly, a 0.1 M phosphate buffer (pH 7.4) was plated in a 96-well quartz microplate. The samples, enzyme (237 U/mL), and buffer were then shaken and incubated at 25 °C, for 5 min. Linoleic acid was added to start the reaction to make a final well concentration of 33 ug/mL. Absorbance measurements at 234 nm were observed to obtain 22 readings at 30 s intervals between each reading. Lipoxygenase activity was calculated using formula (1). Nordihydroguaiaretic acid (NDGA) was used as the positive control.

Statistical analysis

The determination of the percent inhibitory activity of frond ethanolic extracts of plant samples against COX and 15-LOX enzymes was carried out in four trials. The data obtained were analyzed using One-Way ANOVA in Completely Randomized Design (CRD) and significant differences among the means were determined by Least Significant Difference at 0.05 level of significance.

RESULTS AND DISCUSSION

High-Performance Thin Layer Chromatography (HPTLC) profile

The patterns of phytochemical constituents of the frond ethanolic extracts of *D. esculentum*, *D. quercifolia,* and *A. nidus* were identified based on the color and position of the bands depicted by R_f values after derivatization with the NP reagent (Figure 1; Table I). After derivatization with NP reagent, the HPTLC chromatogram of the frond ethanolic extracts of *A. nidus, D. esculentum,* and *D. quercifolia* displayed 14 (R_f = 0.02-0.99), 10 (R_f = 0.02-0.97), and 13 (R_f = 0.03-0.90) colored bands, respectively.

The profile produced by the plant extracts presents a good integral representation of various chemical components (Liang *et al.*, 2004). The characteristic HPTLC profile of each plant sample with ethyl acetate: formic acid: water (16:2:2) as mobile phase may be utilized as a marker for quality evaluation and standardization of herbal formulations containing these plants.

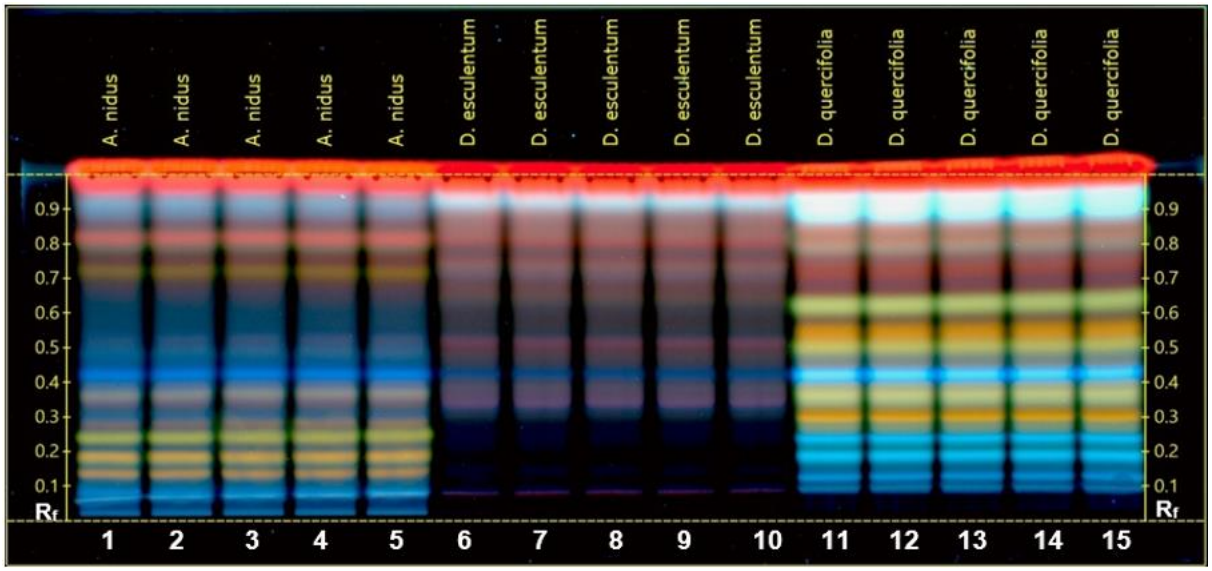


Figure 1. HPTLC Chromatogram of fern extracts under UV light at 366 nm. Tracks 1-5, *A. nidus*; Tracks 6-10, *D. esculentum*; Tracks 11-15, *D. quercifolia* after eluting with ethyl acetate: formic acid: water (16:2:2) and derivatization with NP reagent.

Table I. HPTLC profile of the frond ethanolic extracts of three fern species using Ethyl Acetate: Formic Acid: Water (16:2:2) under 366 nm as mobile phase and NP as a derivatizing agent.

Range of R_f Values	*A. nidus*		*D. esculentum*		*D. quercifolia*	
	R_f	Color	R_f	Color	R_f	Color
0.00 – 0.20	0.02	Blue	0.02	Blue	0.03	Blue
	0.07	Blue	0.05	Blue	0.05	Blue
	0.13	Orange	0.09	Purple	0.09	Blue
	0.18	Orange			0.13	Blue
					0.19	Blue
0.21 – 0.40	0.24	Yellow	0.34	Purple	0.24	Blue
	0.29	Blue			0.29	Orange
	0.36	Purple			0.36	Yellow
0.41 – 0.60	0.42	Blue	0.42	Blue	0.42	Blue
	0.49	Blue	0.51	Purple	0.50	Yellow
	0.53	Blue				
0.61 – 0.80	0.72	Yellow	0.74	Blue	0.63	Yellow
					0.79	Blue
0.8 - 11.00	0.82	Purple	0.82	Purple	0.90	Blue
	0.92	Blue	0.92	Blue		
	0.99	Red	0.97	Red		

Various colors of bands at different R_f values may correspond to the different phytochemicals. According to Bernardi *et al.* (2019), blue fluorescent and purple spots can be attributed to phenolic acids and anthocyanins, respectively while flavonoids appeared as orange or yellow spots. Chlorophylls are fluorescing bright red (Reich *et al*., 2007). In the study conducted by Reich and Schibli (2007), the flavonoids from St. John's wort separated through HPTLC were evident using the mobile phase ethyl acetate: dichloromethane: formic acid: acetic acid: water (100:25:10:10:11) visualized at 366 nm using NP reagent. The chromatographic profile of the standards further showed characteristic colors such as yellow, blue, and purple bands. In another study, the HPTLC

bands of grape pomace using the solvent system ethyl acetate: acetic acid: toluene (90:10:100) exhibited characteristic orange and yellow colors for flavonoids, while the blue fluorescent color is exhibited by phenolic acids (Bernardi *et al*., 2019). Moreover, the HPTLC fingerprint of various Ginkgo preparations using ethyl acetate: acetic acid: formic acid: water (100:11:11:26) as the mobile phase. The standard flavonoid rutin showed a yellow color band after treatment with NP reagent visualized at 366 nm (Reich *et al*., 2007).

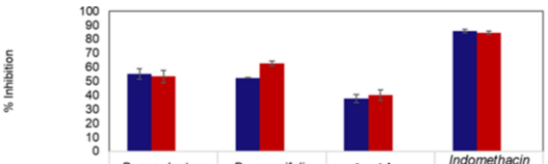
With this, the frond ethanolic extract of *D. esculentum* which showed characteristic colors of blue and purple may contain phenolic acids and anthocyanins, respectively. Tongco *et al*. (2014) reported that frond ethanolic extract of *D. esculentum* is positive for the presence of carbohydrates, anthraquinones, anthranol glycosides, cardiac glycosides, leucoanthocyanins, cyanidin, and phenols. In addition, Chai *et al*. (2015) have also reported a significant amount of phenolic and flavonoids in the aqueous extract of *D. esculentum*. The phytochemical screening *via* the TLC method by Amoroso *et al*. (2017) also reported the detection of phenolics and flavonoids using potassium ferric cyanide-ferric chloride and vanillin/sulfuric acid as a confirmatory test.

The HPTLC chromatogram of the frond ethanolic extract of *D. quercifolia* may contain flavonoids, phenolic acids, and anthocyanins based on the displayed color of bands, i.e. orange or yellow, blue, and purple bands, respectively. Phytochemical analysis of the ethanolic extract of the whole plant showed the presence of flavonoids, phenols, cardioglycosides, betacyanin, and coumarins (Rajesh *et al*., 2014). It was also reported that *D. quercifolia's* dried rhizome contains β-amyrin, Friedelin, naringin, epifriedelinol, β-sitosterol, β-sitosterol, and 3-β-D-glucopyranoside (Ramesh *et al.* 2001). The presence of the flavanone glycoside (naringin) in *D.* quercifolia was established through the HPLC method (Anuja *et al*., 2010). Furthermore, hydroalcoholic extract of *D. quercifolia* fronds was found to contain carbohydrates, phenolic compounds, and flavonoids (Kamboj *et al*., 2013).

On the other hand, the HPTLC chromatogram of frond ethanolic extract of *A. nidus* showed observable characteristic-colored bands such as blue, orange, yellow, and purple which may be accounted for the presence of flavonoids, phenolic acids, and anthocyanins. This is consistent with the results of the study of Jarial *et al*. (2018) who have isolated flavonoids from *A. nidus* through gas chromatography and mass spectrometry (GC/MS) analysis, namely Quercetin 7-O-rutinoside, Globularin, Apigenin 7-O-glucoside, Myricetin 3-O-rhamnoside, Kaempferol 7-O-gentiobioside, Quercetin 7-O-galactoside, Gliricidin 7-O-hexoside, Kaempferol 7-O-rutinoside Kaempferol 3-O-rutinoside, Quercetin Linoleic acid dimer, and Kaempferol 3-O-rhamnoside.

Cyclooxygenase inhibition activity

Samples that gave ≥ 50% COX-2 inhibition and ≥ 1.00 COX-2/COX-1 ratio are considered active and COX-2 selective. *D. quercifolia* extract gave the highest % percent COX-1 inhibition while *A. nidus* gave the lowest value while for % COX-2 inhibition the inhibitory effect increases in the order of *A. nidus* < *D. quercifolia* < *D. esculentum* (Figure 2). However, among the extracts, only *D. esculentum* and *D. quercifolia* were considered active against COX-2 and COX-1. ANOVA revealed significant differences in the percent COX-1 and COX-2 inhibition (P_r < 0.05). The percent COX inhibition of *D. esculentum* and *D. quercifolia* frond ethanolic extracts are statistically comparable but significantly higher than that of *A. nidus.* Furthermore, *D. esculentum* showed a 1.03 selectivity ratio making its inhibitory activity against COX-2 selective.



	D. esculentum	D. quercifolia	A. nidus	Indomethacin (+)
COX-2	55.26[a]	52.34[a]	37.38[b]	85.80
COX-1	53.44[a]	62.66[a]	40.06[b]	84.48
COX-2/COX-1	1.03	0.84	0.93	1.01

Figure 2. Inhibitory activity of the frond ethanolic extracts of *D. esculentum*, *D. quercifolia*, and *A. nidus* against COX-1 and COX-2. The means of the same letter superscript within the row are not significantly different by Tukey's post hoc test at 0.05 level of significance; error bars are SEM; n=4.

The observed anti-inflammatory property of *D. esculentum* and *D. quercifolia* can be attributed to the presence of phytochemicals that prohibit the occurrence of inflammation via enzyme inhibition. Flavonoids, anthraquinones, phenols, alkaloids, cyanidins, saponins, and proteins have been reported present in both the ethanolic and aqueous leaf extracts (Tongco *et al.* (2014). On the other hand, diterpenes, leucoanthocyanins, triterpenes, and phytosterols were detected in the ethanolic

extract of *D.* esculentum. Junejo *et al*. (2015) also reported the presence of flavonoids, carbohydrates, glycosides, alkaloids, saponins, tannins, and phenolic compounds in the leaves of *D. esculentum*. Preliminary phytochemical investigation of *D. quercifolia* has shown the presence of alkaloids, glycosides, saponins, phytosterols, and tannins (Korwar *et al*., 2010). The said phytochemicals: i.e. stilbenes, phenolics, alkaloids, flavonoids, terpenoids, chalcones, and catechin derivatives have been evaluated as naturally derived COX inhibitors (Jachak, 2006). Moreover, the HPTLC profile of *D. esculentum* and *D. quercifolia* extracts further supports the presence of phytochemicals with known anti-inflammatory properties. The obtained results may also provide additional scientific evidence for the reported ethnomedicinal uses of *D. esculentum* and *D. quercifolia*. *D. esculentum* has been used to cure hemoptysis, cough, dysentery, fever, dermatitis, and measles (Roosita *et al.* 2008; Pradhan *et al*., 2015). *D. quercifolia* was used by locals to treat diarrhea, abdominal pain, hectic and intermittent fever, cough, baldness, bleeding gums, cardiac tonic, peptic ulcer, and use as a poultice on swellings and anti-inflammatory setting of fractured bones (Padhy *et al*., 2015).

In the study conducted by Kaushik *et al.* (2011), *D. esculentum's* chloroform and acetone frond extracts gave positive results when tested for the anti-inflammatory property using carrageenan-induced paw edema assay and a significant anti-inflammatory effect appears to be similar to that of ibuprofen, which could be related to its prostaglandin inhibitory activity. The findings of the study further support the reported inhibitory activity of *D. esculentum* methanolic extract against the COX-2 enzyme (Nair *et al*., 2015). In another study, Anuja *et al*. (2014) reported that the ethanolic extract of fertile fronds of *D. quercifolia* exhibited anti-oedematous properties. The fertile fronds, in a dose-dependent manner, significantly inhibited the carrageenan-induced paw edema in rats. This can be attributed to the inhibition of cyclooxygenase leading to inhibition of prostaglandin synthesis. Thus, *D. esculentum* and *D. quercifolia* ethanolic frond extracts, which shows promising anti-inflammatory property can be potential sources of anti-inflammatory compounds.

On the other hand, non-steroidal anti-inflammatory drugs (NSAIDs) differ in their ability to inhibit COX-1 and COX-2. NSAIDs are, therefore, generally characterized based on their *in vitro* COX selectivity (Wong, 2019). This study determined the COX-2 selectivity of the frond ethanolic extracts of plant samples, which has been described as the ratio of percent COX-2 inhibition to percent COX-1 inhibition. A selectivity ratio of ≥ 1.00 would imply the relative selectivity of sample extract against the COX-2 enzyme. The frond ethanolic extract of *D. esculentum* which gave a selectivity ratio of 1.03 may be considered both COX-2 active and selective. Moreover, *D. esculentum* can be a potential source of COX-2 selective anti-inflammatory compound that can be developed into a drug in the future.

According to Vane (1996), drugs that have higher potency against COX-2 and a better COX-2/COX-1 activity ratio will have considerable anti-inflammatory activity with fewer side effects in the stomach and kidney. COX-2 selective drugs are used for the relief of acute pain associated with dental surgery and primary dysmenorrhea and for the treatment of rheumatoid arthritis and osteoarthritis (Patrignani *et al*., 2005). Furthermore, Koki and Masferrer (2002) reported that COX-2 inhibitors also possess an inhibitory effect against tumor growth and metastasis in various relevant animal models in cancer. Consequently, selective COX-2 inhibitors constitute promising agents for both cancer therapeutics and chemoprevention (Howe *et al*., 2002). On the other hand, the frond ethanolic extract of *D. quercifolia* which shows potent inhibitory activity against the COX-2 enzyme but is non-selective for COX-2 can be a valuable source of potential compounds for the development of NSAIDs.

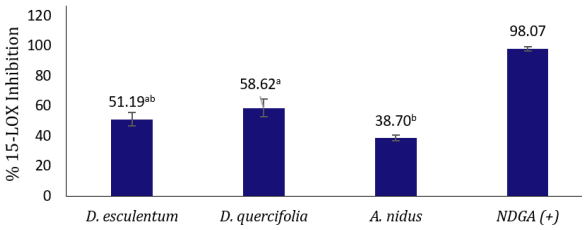


Figure 3. Inhibitory activity of the frond ethanolic extracts of *D. esculentum*, *D. quercifolia*, and *A. nidus* against 15-LOX. The means of the same letter superscript are not significantly different by Tukey's post hoc test at 0.05 level of significance; error bars are SEM; n=4.

15-Lipoxygenase inhibition activity

Among the extracts, *D. quercifolia* and *D. esculentum* inhibited more than 50% of the 15-LOX (Figure 3). Thus, both extracts can be considered active. ANOVA revealed significant differences in the % 15-LOX inhibition values of the frond

ethanolic extracts ($P_r < 0.05$). The % 15-LOX inhibition exhibited by *D. quercifolia* is significantly higher than that of *A. nidus* but statistically comparable to that of *D. esculentum*.

The results may suggest anti-inflammatory activity of the sample extracts *via* inhibition of 15-LOX activity. The 15-LOX enzyme has been identified as an attractive target for therapeutic intervention because of its expression in atherosclerotic lesions (Kühn *et al*., 1994). The 15-LOX is also overexpressed in adenocarcinoma tissues, as well as in all human prostate cancer cell lines (Kelavkar *et al*., 2000). The 15-LOX pathway generates pro-inflammatory eoxins in mast cells, eosinophils, and nasal polyps from allergic subjects. Consequently, this suggests that 15-LOX is a potential target for the treatment of inflammatory respiratory disorders such as rhinitis, asthma, and chronic obstructive pulmonary disease (COPD) in humans (Feltenmark *et al*., 2008). Inhibitors of 15-LOX can be classified according to their mode of inhibitory action. Some are grouped as redox inhibitors which reduce the active site iron or trap the radical intermediates. While others are called iron-chelating inhibitors and non-redox inhibitors which compete with fatty acid substrates for binding to 15-LOX active sites (Sadeghian *et al*., 2015). Thus, frond ethanolic extracts of *D. quercifolia and D. esculentum* can be a source of compounds that can be developed into an anti-inflammatory drug that potentially inhibits 15-LOX.

Moreover, the bioactivity of *D. esculentum* and *D. quercifolia* may provide science-based evidence for their reported ethnomedicinal uses. *D. quercifolia* has been used as a treatment of rheumatism, osteodynia, and dentagia (Tran *et al*., 2015). *D. quercifolia* has been also used to treat diabetes by the Marakh sect of Garos, tuberculosis and throat infections in Assam, cardiac problem and a useful poultice on fractured bones after setting up the bones by the Reang tribe of Tripura (Shil *et al*., 2009; Sen *et al*., 2011; Rahmatullah *et al.*, 2012). Anuja *et al*. (2010) have worked on the anti-inflammatory property of *D. quercifolia* using granuloma formation in rats and carrageenan-induced paw edema where significant inhibition of inflammation which was almost comparable to that of positive control indomethacin was observed.

The potential activity of *D. esculentum* and *D. quercifolia* ethanolic frond extracts against 15-LOX may be attributed to the polyphenolic content of the extracts. Anuja *et al*. (2014) have reported the detection of coumarins, flavonoids, glycosides, phenolics, saponins, steroids, tannins, and terpenoids in the fronds of *D. quercifolia*. In the study by Junejo *et al.* (2015), flavonoids, tannins, saponins, carbohydrates, glycosides, alkaloids, proteins, and phenolic compounds are the most prominent components of the sequential leaf extract of *D. esculentum* in the phytochemical screening has been reported. In the literature, flavonoids (Malterud and Rydland, 2000), terpenoids (Amagata *et al*., 2003), allyl benzenes, allyloxy benzenes, heterocylic, and phenolic compounds (Sadeghian *et al*., 2015) are reported as 15-LOX inhibitors. Nevertheless, phytochemical screening of *A. nidus* also revealed the presence of compounds claimed to inhibit 15-LOX such as phenolic, terpene, and flavonoids (Amoroso *et al*., 2017). The presence of phytochemicals with known anti-inflammatory properties in the *D.* esculentum, D*. quercifolia,* and *A. nidus* extracts is further supported by their HPTLC profile. However, in a study conducted by Wangensteen *et al*. (2004), the 15-LOX inhibitory activity is positively correlated to the number of phenolic compounds it contains. The total phenolic content of the crude methanol extract of *D. quercifolia* fronds has been reported to be 2939 ± 469 mg GAE / 100 g (Tan and Lim 2015), while *A. nidus* was 305 ± 13 mg GAE / 100 g (Lai *et al.,* 2009). Thus, this may account for the low inhibitory effect of *A. nidus* ethanolic extract against the 15-LOX activity.

CONCLUSION

The HPTLC profile of the fond ethanolic extracts of *D. esculentum*, *D. quercifolia*, and *A. nidus* may be utilized for quality evaluation and standardization of herbal formulations containing these plants. Moreover, the HPTLC profile of the frond ethanolic extracts suggests the possible presence of phenolic acids and anthocyanins in *D. esculentum* while flavonoids, phenolic acids, and anthocyanins may be present in *A. nidus* and *D. quercifolia*. These phytochemicals may account for the observed anti-inflammatory properties of *D. esculentum, D. quercifolia,* and *A. nidus* frond ethanolic extracts. Although *D. quercifolia* and *D. esculentum* were both active inhibitors of COX and 15-LOX enzymes, only *D. esculentum* was found COX-2 selective. Results suggest further investigation to identify compounds responsible for their anti-inflammatory properties.

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