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Anti-inflammatory and antioxidant activities and the thin layer chromatographic fingerprinting of the fruit pulp and epicarp extracts of *D. microcarpum*

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Abstract
Extracts from *Detarium microcarpum* have been reportedly used to manage inflammatory and oxidative stress-related disorders such as rheumatism, kidney pains and skin infections in folkloric medicine. This study focused on the *in vitro* antioxidant and anti-inflammatory properties of 70% ethanol extracts of the fruit pulp (FPE) and epicarp (FEE) of *D. microcarpum*. The *in vitro* antioxidant activity of the extracts was assessed using a DPPH radical scavenging assay, while the anti-inflammatory activity of the extracts was assessed by a protein denaturation inhibition assay. The thin layer chromatography (TLC) mobility profiles of the chloroform fractions of each extract were assessed for fingerprinting. The antioxidant activity of FPE and FEE was concentration dependent, with IC_{50} values of 252.40 ± 52.50 µg/ml and 115.80 ± 15.70 µg/ml, respectively. Ascorbic acid used as a control in the antioxidant assay exhibited antioxidant activity with an IC_{50} value of 2.04 ± 0.13 µg/ml. FEE, FPE and Na-Diclofenac also presented significant anti-inflammatory activity, with IC_{50} values of 1.48 ± 0.45 mg/ml, 1.16 ± 0.22 mg/ml and 0.47 ± 0.15 mg/ml, respectively. Qualitative phytochemical screening revealed the presence of saponins, reducing sugars, phenolic compounds, tannins, flavonoids, and phytosterols in both plant extracts. The FFPE and FEE specimens were partitioned into chloroform. The chloroform fraction of each extract displayed seven (7) bands on the TLC plate with R_f values ranging from 0.77 to 0.051 (for FEE) and 0.92 to 0.14 (for FPE). These findings suggest that the fruit pulp and epicarp of *D. microcarpum* hold promise as potential sources for the development of novel antioxidant and anti-inflammatory drugs.

Keywords: *Detarium microcarpum*, anti-inflammation, protein denaturation, Chromatographic fingerprinting, phytochemistry

Introduction
Inflammation, a key factor in diseases such as infections, arthritis, type 2 diabetes, obesity, and cancer, is a complex immune response to harmful triggers. It involves immune cell recruitment, the release of mediators such as cytokines, and heightened blood flow. Chronic inflammation is linked to autoimmune disorders, heart issues, and cancer.

Oxidative stress arises from an imbalance between reactive oxygen species (ROS) production and antioxidant defenses. ROS, such as superoxide radicals and hydrogen peroxide, harm cellular components. They are generated via normal metabolism and can increase due to inflammation, toxins, or diseases. Antioxidants such as superoxide dismutase and vitamins C and E counter ROS, preventing oxidative damage.

Inflammation and oxidative stress are closely linked in physiological and pathological conditions. Inflammation can trigger reactive oxygen species (ROS) production, and ROS can activate signaling pathways that promote inflammation. This interplay forms a vicious cycle, fuelling each other and leading to tissue damage and disease progression.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of pain and inflammatory conditions. NSAIDs are, however, associated with side effects such as gastrointestinal hemorrhage and a stifled function of the immune system [1], thus shifting the attention of the scientific community to alternative pharmacotherapies [2, 3].

Medicinal plants are valuable sources of therapeutic compounds. The scientific literature underscores the biological efficacy of these plant-derived compounds, sparking growing interest in their identification and characterization [4]. The identification of acetyl salicylic acid, a potent anti-inflammatory compound found in aspirin, for instance, was triggered by the traditional use of medicinal plants for treating inflammation [5].

Numerous studies have documented the antioxidant, anti-inflammatory, antimalarial, antimicrobial, and anticancer properties of compounds derived from plants [6]. Secondary metabolites such as phenolic compounds (such as curcumins, flavonoids, and tannins), saponins, terpenoids, and alkaloids have been associated with the bioactive properties of medicinal plants in different investigations [6, 7].

Reports indicate that many phenolic compounds, such as flavonoids, tannins and curcumins, exert their biological effects either via free radical scavenging or by acting as inhibitors of proinflammatory enzymes such as cyclo-oxygenases (COXs) and lipoxygenases (LOXs) in the inflammatory cycle [8, 9].

The composition of phytoconstituents in medicinal plant extracts can differ depending on factors such as geographic location, harvest time, plant part utilized, and the solvent employed for extraction. Chemical fingerprinting techniques offer a thorough chemical overview of medicinal plant extracts [10]. Chromatographic approaches are frequently employed to create distinctive profiles that mirror the intricate chemical makeup of these extracts, thereby enabling their unique identification [11].

Detarium microcarpum, often known as 'tallow tree', 'sweet dattock,' or 'sweet detar,' is an underutilized tree legume native to the arid regions of West and Central Africa. In Ghana, *D. microcarpum* grows naturally in the northern part of the region, where it is locally known as '*Kpala*' (Dagbani), *'Kanankola'* (Kasem), *'Kengagera'* (Gurune) and '*Kpara*' (Kusaal).

The fruits of the plant were found to contain the highest phenolic, flavonoid and antioxidant contents among fourteen wild edible fruits from Burkina Faso [12]. In folkloric medicine, the fruit extracts have been using in managing symptoms of inflammation in the northern part of Ghana.

The primary objective of this study was to generate chromatographic fingerprints and evaluate the anti-inflammatory and antioxidant properties of ethanol extracts derived from both the fruit pulp and epicarp of *D. microcarpum*.

Materials and Methods

Collection and preparation of plant material

Dried fruits of *D. microcarpum* were obtained from the Tamale Central Market in the Northern Region of Ghana between July and August 2021. Authentication was performed by Dr. Abdul Wahab Imoro, Head of the Department of Applied Biology at C.K. Tedam University of Technology and Applied Sciences, Navrongo, Ghana. The fruits were washed, air-dried under shade for two weeks at room temperature, and separated into fruit pulp and epicarp. Both samples were further air-dried under shade for two weeks and then pulverized using a mortar and pestle.

Chemicals and reagents

Analytical grade chemicals and reagents were obtained from various suppliers and used for the bioassays without further purification.

Preparation of plant extracts for biological assays

Approximately 200 g of ground fruit pulp and epicarp were separately extracted in 2 liters of ethanol (70%) for 48 hours at room temperature. The resulting solution was filtered using Whatman No. 1 filter paper. The extracts were then concentrated using a water bath (model HH-S_6) and stored in a refrigerator until further use [13, 14].

Phytochemical screening

The presence or absence of saponins, reducing sugars, phenolic compounds, phytosterols, and flavonoid tannins in both sample extracts were qualitatively assessed using methods employed in the literature [15] and modified where necessary.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity assay

The reported method [16] was modified and adopted. Briefly, 300 mg of each extract (epicarp and pulp) was weighed into 12 ml of distilled water and thoroughly dissolved by shaking to form a uniform mixture. The resultant solution, 25 mg/ml, was serially diluted to obtain concentrations in the range of 1 µg/ml - 0.00195 µg/ml. The same procedure was applied for the preparation of the standard antioxidant ascorbic acid.

DPPH solution (approximately 0.02 mg/ml) was prepared by dissolving 2 mg of DPPH in 100 ml of methanol. The 96-well microplates were filled with 50 µL of the different prepared sample concentrations in triplicate. This was followed immediately by the addition of 150 µL of the constituted DPPH solution, which produced a mixture of 200 µL in each well. The microplate was then incubated for 30 minutes in the dark, after which the absorbance was read and recorded using a microplate reader at a wavelength of 517 nm. The percentage of DPPH inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = \left(\frac{\text{Absorbance of Blank} - \text{Aborbance of Sample}}{\text{Absorance of Blank}}\right) \times 100 \quad (1)$$

The amount of the extract required to cause a 50% decrease in the initial DPPH concentration (IC_{50}) was determined with the aid of GraphPad Prism 8 statistical package.

In vitro anti-inflammatory activity via protein denaturation

The procedure used in the report [17] was modified and adopted. Briefly, eight different concentrations (5–0.0039 mg/ml) of the plant extracts were prepared. A similar procedure was applied for the formulation of different concentrations of the standard drug Na–Diclofenac.

Three millilitres of an egg albumin solution were prepared by dissolving 0.2 ml of the albumin in 2.8 ml of the PBS solution. One hundred microliters of the plant extracts (5 mg/ml – 0.0039 mg/ml) and the positive control were then transferred in triplicate into 96-well microplates, followed by the addition of 150 µL of the egg's albumin (in PBS), which produced 250 µL of each mixture. Distilled water and plant extracts without egg albumin solution were used as negative and color controls, respectively.

The microplate and its contents were incubated for 10 minutes at room temperature and then further heated to 70 °C for 20 minutes. The microplates were then cooled, and the absorbance readings were recorded with a microplate reader at a wavelength of 660 nm.

The percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ Inhibition} = \left(\frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}}\right) \times 100 \quad (2)$$

The amount of the extracts required to cause 50% inhibition of protein denaturation was obtained using the GraphPad Prism 9 statistical package.

Thin layer chromatography profiling of plant extracts

The procedure [18] was adopted and modified where necessary. Thin-layer chromatography was carried out on both hydroalcoholic extracts of the pulp and epicarp of *D. microcarpum* with TLC precoated plates (silica gel $60F_{254}$) using a one-way ascending technique. The plates were cut to an appropriate size with the aid of a pair of scissors and marked with a pencil approximately 1 cm from the bottom and above the plate. Each sample (a workable amount) was then gently dissolved in 70% aqueous ethanol and further partitioned using chloroform. The plates were then uniformly spotted in triplicate with the chloroform fraction of each extract and allowed to dry. The dried spotted plates were developed in a chromatographic tank using a mixture of chloroform and ethyl acetate (30:1).

The developed plates were then dried and immersed in 10% sulfuric acid with the aid of forceps and then dried with hot air from an electric blower. The dried plates were then visualized under daylight. The distinct spots were located and appropriately marked, and their retention factors (R_f values) were calculated using the following formula:

$$Rf = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent front}} \quad (3)$$

Statistical analysis

The descriptive data are expressed as the mean ± standard error of the mean of triplicate entries. All data were analyzed statistically using Microsoft Excel 2016 and GraphPad Prism 8 for Mac OS. The data were further analyzed using one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests, assuming that the variances were equal, using GraphPad Prism 8 for Mac OS. Differences were considered to be statistically significant when $p \leq 0.05$.

Results and Discussions

Phytochemical screening

The phytoconstituents detected in the fruit extracts of *D. microcarpum* are presented in Table 1.

Table 1: Phytochemical screening of the fruit extracts of *D. microcarpum*

Phytochemical	70% EtOH Epicarp extract	70% EtOH Pulp extract
Saponins	+	+
Polyuronides	-	-
Reducing sugars	+	+
Phenolic compounds	+	+
Alkaloids	-	-
Cyanogenic glycoside	-	-
Triterpenes	-	-
Phytosterols	+	+
Flavonoids	+	+
Anthracenosides	-	-
Tannins	+	+
Steroids	-	-
Glycosides	-	-

(+) = phytochemical detected, (-) = phytochemical not detected.

Phytochemicals, which are plant compounds lacking in nutritive value, possess diverse levels of disease-preventing properties. They serve as crucial resources for both traditional and alternative treatments [19]. In this study, standard protocols were employed for preliminary qualitative analysis of some bioactive compounds in *D. microcarpum* fruit pulp and epicarp extracts. Among the phytochemicals screened, saponins, reducing sugars, phenolic compounds, phytosterols, flavonoids and tannins were detected in both extracts, as presented in Table 1. These observations corroborate previous findings [20], who reported the presence of saponins, flavonoids, phenolics, terpenoids and cardiac glycosides. Tannins were visibly absent in their work, while cardiac glycosides were found to be present. These discrepancies may be attributed to the varied geographical locations of the species, extraction methods, and harvest times [21]. The observed results also support other findings [22] with regard to the phenolic and flavonoid contents.

Phenolic acids, polyphenols and flavonoids obtained from plants have been implicated as potent scavengers for free radicals and thus inhibit the oxidative pathways that cause degenerative illness [23, 24]. These compounds are effective antioxidants due to the presence of hydroxyl group(s) attached to the aromatic ring structure [25, 26]. According to reports [19], phenols and phenolic compounds modify the prostaglandin pathways, which prevent the clumping of platelets. They also have the ability to block specific enzymes that cause inflammation.

Saponins have been recognized as bioactive compounds with pharmacological properties such as anti-inflammatory and antipyretic activities [27].

Flavonoids have been reported to exhibit significant antioxidant, anti-inflammatory and analgesic activities[27]. Recent reports have shown that flavonoids have the potential to chelate divalent metal ions, hence preventing free radical formation.

Tannins inhibit lipid peroxidation *in vitro* and have the capacity to scavenge free radicals that are important in prooxidant states. This makes them instrumental in the topical treatment of skin inflammation and injuries [28].

Phytosterols have been reported to possess anti-inflammatory, antioxidant, antimicrobial, anticancer, and cholesterol-lowering activities [21]. Their cholesterol lowering effect stems from the inhibition of cholesterol absorption through displacement from mixed micelles [29].

The antioxidant and anti-inflammatory activities demonstrated by the fruit extracts may be attributed to the presence of the phytochemicals detected.

Thin layer chromatography fingerprinting of extracts

Fingerprint analysis of 70% ethanolic extracts of the pulp and epicarp revealed the presence of seven visible bands (components) in each extract under visible light.

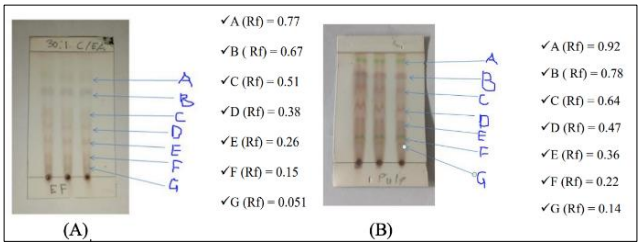

Fig 1: Thin layer chromatograms of the chloroform fractions of the fruit epicarp (A) and fruit pulp (B)

Fingerprints have become an important tool for quality monitoring, particularly in the case of herbal samples, which are increasingly appearing on the consumer market as diet supplements and natural treatments. Fingerprints can be used to identify plant species and assess the quality of a final product, as well as for a variety of other reasons, such as origin, detecting adulteration and controlling the extraction

processes [30]. The genus Detarium comprises three species: *D. macrocarpum*, *D. senegalense*, and *D. microcarpum*. While these species share morphological similarities, they exhibit differences in their ecological distributions [31]. Variances in phytochemical composition due to different ecological conditions may impact their biological significance [32].

While the TLC profiles of the fruit pulp and epicarp extracts of *D. microcarpum* were similar in terms of the number of components or bands, there were variations in their R_f values (Figure 1). The seven components of the epicarp extract had R_f values ranging from 0.77 to 0.051. The pulp extract produced seven spots with R_f values ranging from 0.92 - 0.14. These results agree partially with some sets of R_f values obtained for saponins and flavonoids in previous reports [27].

The similar TLC mobility profiles of the fruit extracts, along with the variations in the retention factors, are indicative of commonalities in the phytochemical composition of both extracts. This suggests that they may share common bioactive compounds with little variation in concentrations or specific compounds. The TLC mobility profiles also serve as reference fingerprints for the identification and authentication of plant extracts.

Anti-inflammatory activity of the fruit pulp and epicarp extracts of *D. microcarpum*

Inhibition of protein denaturation has been identified as one of the major mechanisms of anti-inflammatory activity [33]. The anti-inflammatory activity of *D. microcarpum* fruit extracts were assessed using a heat-induced protein denaturation assay.

The results from the present study revealed that both plant extracts significantly inhibited protein denaturation in a concentration-dependent manner. The maximum percentage inhibition of protein denaturation by the fruit epicarp extract, fruit pulp and standard drug (Na-diclofenac) was 54.53±1.08, 48.63±2.13 and 90.39±0.55%, respectively, at 2 mg/ml. The IC_{50} values of the fruit extracts were comparable to that of the standard drug Na-diclofenac ($p>0.05$) Table 2. This result suggested that fruit extracts of *D. microcarpum* contain bioactive compounds with anti-inflammatory activity similar to that of Na-diclofenac.

A related study [34] reported the biological activity of *D. microcarpum* stem bark extract in carrageenan-induced paw edema. Report [35] indicate that the methanol and ethyl acetate leaf extracts of *D. microcarpum* exhibited greater inhibition of acute and chronic inflammation, respectively, than diclofenac. Our results corroborate these findings and therefore support the traditional use of plant parts in the management of inflammatory-related disorders such as arthritis, pain, and wound healing.

Table 2: Anti-inflammatory activities of *D. microcarpum* fruit extracts via the inhibition of protein denaturation

	Sample % inhibition of protein denaturation		
Conc (mg/ml)	**Epicarp extract**	**Pulp extract**	**Na-Diclofenac**
2	54.53±1.08	48.63±2.13	90.39±0.55
1	51.67±1.83	47.71±0.29	49.79±3.36
0.5	41.12±0.27	45.56±084	42.64±1.16
0.25	27.27±1.03	34.72±0.71	37.42±0.87
0.125	18.11±1.90	28.71±2.85	34.03±1.33
0.0625	13.89±0.95	23.58±0.15	24.14±1.65
0.03125	12.35±1.95	21.95±0.31	17.16±1.64
0.015625	11.04±1.54	14.24±1.28	11.09±0.81
IC_{50}	1.48±0.45[a]	1.16±0.22[a]	0.47±0.15[a]
R^2	0.9316	0.9566	0.8506

The values are presented as the means ± SEMs of triplicate readings and were considered to indicate statistical significance at $p < 0.05$. Values with different superscripted letters across the column are significantly different.

Antioxidant activity of the fruit pulp and epicarp extracts of *D. microcarpum*

The antioxidant activities of the *D. microcarpum* fruit extracts are presented in Table 3.

Table 3: Antioxidant activities of *D. microcarpum* fruit extracts via a free radical scavenging activity assay

	Sample % inhibition		
Conc (µg/ml)	**Epicarp extract**	**Pulp extract**	**Ascorbic acid**
250	62.36 ± 0.66	43.38 ± 1.49	69.30 ± 1.68
125	44.79 ± 4.14	26.68 ± 0.85	68.76 ± 1.60
62.5	20.49 ± 1.57	12.91 ± 0.22	68.54± 2.60
31.25	8.67 ± 2.16	4.66 ± 0.67	68.87 ± 2.29
15.625	4.98 ± 2.61	3.04 ± 0.99	67.57 ± 1.93
7.8125	3.19± 0.10	2.97 ± 0.94	62.47 ± 2.11
3.9062	2.60 ± 2.17	2.54 ± 0.05	58.35 ± 0.65
1.9531	0.65 ± 0.03	1.52 ± 0.93	31.77 ± 4.46
0.9766	0.21 ± 0.01	0.54 ± 0.07	9.54 ± 3.79
0.4883	0.68 ± 0.05	0.46 ± 0.09	3.25 ± 1.42
IC_{50}	115.80 ± 15.70[a]	252.40 ± 52.50[a]	2.04 ± 0.13[b]
R^2	0.9920	0.9952	0.9906

The values are presented as the means ± SEMs of triplicate readings and were considered to indicate statistical significance at $p < 0.05$. Values with different superscripted letters across the column are significantly different.

The fruit pulp and epicarp extracts of the plant displayed significant antioxidant activity, with IC_{50} values of 252.40±52.50 and 115.80±15.70 µg/ml, respectively. The antioxidant activities of both extracts were comparable. However, ascorbic acid exhibited superior antioxidant activity (IC_{50} = 2.04±0.13 µg/ml) to that of the fruit extracts.

These results are partly in agreement with those reported in a previous work [36]. The essential oil from the leaves of *D. microcarpum* is reported as a potent source of antioxidants[16]. Similar studies [25] conducted on the leaf extract of *D. microcarpum* suggested that the ethanolic extract exhibited excellent DPPH radical scavenging activity with an IC_{50} value of 4.84 mg/ml in comparison to standard ascorbic acid, which has an IC_{50} of 2.94 mg/ml. These findings suggest that extracts from *D. microcarpum* are a rich source of phytochemicals with significant antioxidant activity.

Conclusion

This research assessed the Thin Layer Chromatography (TLC) mobility profile, the antioxidant and anti-inflammatory properties of 70% ethanolic extracts derived from both the fruit pulp and epicarp of *D. microcarpum*. These findings indicate that both extracts contain bioactive compounds with antioxidant and anti-inflammatory effects. They demonstrated significant inhibition of protein denaturation in a concentration-dependent manner, comparable to the standard drug Na-diclofenac. Moreover, the extracts exhibited antioxidant activity, although they were less potent than ascorbic acid. This study revealed a similar TLC mobility profile for the fruit pulp and epicarp extracts of *D. microcarpum*, indicating a consistent TLC fingerprint. The presence of phytochemicals such as saponins, reducing sugars, phenolic compounds, phytosterols, flavonoids, and tannins suggests the potential therapeutic value of *D. microcarpum* fruit extracts.

Further studies could focus on isolating and characterizing specific bioactive compounds responsible for the observed antioxidant and anti-inflammatory activities. Additionally, exploring the mechanism of action of these compounds and conducting *in vivo* studies to assess their efficacy and safety profiles would be valuable for their potential use as natural anti-inflammatory and antioxidant agents.

Data Availability Statement

The data associated with this study are included within the published article. Additional files are available from corresponding the author upon request.

Conflicts of Interest

The author(s) declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

LAO conceived the research, provided guidelines for selecting protocols, performed statistical analysis, prepared the initial and final drafts of the manuscript. BA assisted in sample collection, carried out laboratory procedures and prepared results for statistical analysis.

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