

In vitro Anti-Inflammatory Activities and Phytochemical Fingerprints of Fractions of *Detarium microcarpum* Stem Bark

Okugbo, O. T

ookugbo@biu.edu.ng

Benson Idahosa University

Omoriegbe, E. S.

ehimwenma.omoriegbe@uniben.edu

University of Benin

Aghayere, F.

Benson Idahosa University

Ayevbuomwan, M

University of Benin

Adeogun, E

university of Benin

Erhovwosere, O.

University of Delta

Shemishere, U. B

Federal University Birnin Kebbi, Birnin Kebbi,

Research Article

Keywords: Detarium microcarpum, inflammation, membrane stabilization, protein denaturation

Posted Date: May 30th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-6777358/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: The authors declare no competing interests.

Abstract

Chronic inflammation represents a significant global health burden implicated in the pathogenesis of various diseases, including cardiovascular disorders, cancer, type 2 diabetes, and autoimmune conditions. The limitations of conventional non-steroidal anti-inflammatory drugs (NSAIDs), such as adverse effects and diminishing efficacy, necessitate the exploration of alternative therapies, particularly those derived from medicinal plants. This study investigated the anti-inflammatory activity and chemical composition of petroleum ether (PE), dichloromethane (DCM), and hydro-methanol (HMF) fractions of *Detarium microcarpum* stem bark using *in vitro* models and HPLC fingerprinting. Anti-inflammatory activity was assessed via erythrocyte membrane stabilization, inhibition of protein denaturation, and heat-induced albumin denaturation assays, with Aspirin serving as the standard control. HPLC was employed to identify phytoconstituents in each fraction. All fractions exhibited significant ($p < 0.05$) dose-dependent anti-inflammatory activity, with the DCM fraction demonstrating the most potent membrane stabilization effect with IC_{50} value of 0.9524 mg/mL and the HMF fraction showing the greatest heat-induced protein denaturation inhibition ($IC_{50} = 0.4920$ mg/mL). HPLC profiling revealed key anti-inflammatory phytoconstituents such as quercetin, kaempferol, lupeol, betulinic acid, methyl gallate, stigmasterol, and β -sitosterol variably distributed across the fractions. The findings demonstrate that fractions of *Detarium microcarpum* possess significant anti-inflammatory properties, likely attributable to their rich content of flavonoids, triterpenoids, sterols, and phenolic compounds. The results support the plant's traditional medicinal use and underscore its potential as a source of novel anti-inflammatory agents.

Introduction

Inflammation is a pathophysiological response or a biological defence mechanism that enables living cells to protect themselves against diseases that could lead to the bioaccumulation of plasmic fluid and blood cells caused by bacteria, fungi, viruses, physical agents, and defective immune. It may be acute (initial inflammation) or chronic (out of proportion of protection damage). Common symptoms include redness, swelling, pain, loss of function of cells, and heat. (Gonfa et al., 2023). Human living cells naturally develop protective mechanisms in response to body inflammation caused by microbial infections, mechanical injuries, and burns (Anyasor et al., 2019). Acute and chronic inflammatory reactions have been shown to play a significant role in the natural defence mechanisms of the human body's immune system to maintain human health, mainly by stimulating living cells to eliminate harmful agents and removing damaged tissues to heal the affected parts. The inflammatory response is effective because of the secretion of different mediators responsible for the initiation, progression, persistence, regulation, and resolution of inflammation effects (Oguntibeju, 2018).

The inflammatory reactions are usually complex, and the mediators involved can induce, maintain or aggravate several diseases such as arthritis, diabetes, obesity, cancer, neurodegenerative diseases, autoimmune disorders, dementia, scleroderma, allergy, asthma, bronchitis, inflammatory bowel disease, and cardiovascular diseases, which have been increased dramatically over the last few decades (Megid

et al., 2022). Notably, several studies have been carried out on inflammatory diseases. However, the intolerable side effects of the currently available anti-inflammatory drugs are the major problems during their clinical uses. The most commonly used drugs for the management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects, especially gastric irritation leading to the formation of gastric ulcers (Nunes et al., 2020; Dhiman et al., 2021). Hence, various strategies to combat the disease condition through the development of newer and more substantial anti-inflammatory drugs with lesser side effects are developed, and one of them is the intensified efforts towards the discovery of novel drugs from plant sources (Karrat *et al.*, 2021).

Phytochemicals of medicinal plants are the origin of medicines for healing various health disorders of mankind and potential alternative sources of new drug research and development (Bachheti et al., 2020; Gonfa et al., 2023). They remain the most accessible and easily affordable medicines for primary health care in developing countries (Gumisiriza et al., 2019). Some medicinal plants with anti-inflammatory activities include Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Papaya (*Carica papaya*), Blueberry (*Vaccinium corymbosum*), Aloe (*Aloe vera*), Broccoli (*Brassica oleracea*), Olive (*Olea europaea*), Rosemary (*Rosmarinus officinalis*), etc (Das and Mandal, 2018).

Detarium microcarpum is a tree of the Fabaceae family 8 to 12 m high, a species of the wooded savannahs and open forests of the Sudano-Sahelian zone of the African continent. This plant has long been used in traditional medicine by different ethnic groups in Nigeria and several parts of West Africa for the treatment of various diseases such as stomach aches, menstrual pain, dysenteric diarrhoea, dermatitis, meningitis, gonorrhoea, rheumatism, tuberculosis, smallpox, bilharzia, itching, gastric ulcers and diabetes (Habibou et al., 2022; Sanusi et al., 2022). Several studies have provided information on the bioactive compounds in the different parts of the plant, but not so much is known about the plant's *in vitro* anti-inflammatory potential. Hence, the aim of this study. Therefore, this study evaluated the *in vitro* anti-inflammatory potential of dichloromethane, hydro-methanol, and petroleum ether fractions of *D. microcarpum* stem bark.

Materials and Methods

Collection and Authentication of the Plant Part

The stem bark of *Detarium microcarpum* were sourced from a farmer at Osun State, Nigeria. A sample were identified and authenticated at the Department of Plant Biology and Biotechnology (PBB), at the University of Benin, Benin City, Edo State, Nigeria and thereafter deposited as a voucher specimen in the department's herbarium.

Preparation of Stem Bark Fractions of *D. microcarpum*

The stem barks were first subjected to extraction using a modified method described by Azwanida (2015). Freshly obtained *D. microcarpum* stem barks were cleaned and air-dried in the laboratory. The powdered forms of the resulting dried stem barks of weight 2.5 kg were macerated in 10 litres of

methanol solvent over a period of 72 hrs at room temperature after which the mixture was filtered using Whatman No. 1 filter paper into a clean container. The filtrates were concentrated using a rotary evaporator at 40°C. The extracts of weight 210 g were subjected to fractionation using column chromatography with different solvents (petroleum ether, dichloromethane and hydro-methanol). The resultant four fractions were concentrated and stored in clean containers at 4°C for further use.

High-Performance Liquid Chromatography (HPLC) Analysis

The HPLC analysis of petroleum ether, dichloromethane, and hydro-methanol extracts of *Detarium microcarpum* stem bark was conducted using a Shimadzu Prominence-i HPLC system equipped with a quaternary pump, auto-sampler, and UV-Vis detector. Separation was carried out on a reverse-phase C18 column (250 mm × 4.6 mm i.d., 5 µm particle size) maintained at 30°C. Samples were prepared by dissolving each dried extract fraction in HPLC-grade methanol at a concentration of 10 mg/mL. The solutions were sonicated for 10 minutes to enhance dissolution and filtered through a 0.45 µm PTFE membrane syringe filter before injection. The mobile phase consisted of a gradient elution using the proportions of solvent A (0.1% formic acid in water) to solvent B (acetonitrile with 0.1% formic acid) at a constant flow rate of 1.0 mL/min. The gradient profile was programmed as follows: 0 min, 5% B; 10 min, 30% B; 20 min, 50% B; 30 min, 70% B; 35 min, 95% B; and 40 min, back to 5% B. The total run time was 40 minutes. Detection was performed at 280 nm, with an injection volume of 20 µL. Identification of phytochemical constituents was carried out by comparing the retention times of peaks with those of known standards including quercetin, kaempferol, lupeol, betulinic acid, methyl gallate, catechin, epicatechin, β-sitosterol, stigmasterol, and luteolin.

In vitro Anti-Inflammatory Assays

Assay of Membrane Stabilizing Activity Assay

The membrane stabilizing activity assay method was based on the procedure described by Oyedapo et al. (2004) with little modification. The assay mixture consisted of hyposaline (1ml), 0.1M phosphate buffer, pH 7.4 (0.5 ml), varying concentrations of extracts (100–500 µg/ml) and 0.5 ml of 2% (v/v) erythrocyte suspension in a total volume of 3 ml. The control was prepared as above without the drug while the drug control (3 ml) lacked erythrocyte suspension. The standard anti-inflammatory drug for the assay was aspirin. The reaction mixtures were incubated at 56° C for 30 min. The absorbance of the released haemoglobin was read at 560 nm against reagent blank. The percentage membrane stability was estimated using the expression:

$$\text{Percentage membrane stability} = 100 - \frac{(\text{Abs of test drug} - \text{Abs of drug control}) \times 100}{\text{Abs of blood control}}$$

Inhibition of Denaturation of Albumen Activity Assay

Different concentrations of the fractions were assayed for the ability to inhibit denaturation of albumen following the method of Aina and Oyedapo (2013) with slight modifications. The reaction mixtures contained 0.5 ml (1.5 mg/ml albumen) and different concentrations of the fractions, followed by incubation at 37 °C for 20min. The reaction mixtures were heated at 57 °C for 3 min and 2.5 ml of 0.5 M phosphate buffer, pH 6.3 was added. From each of the reaction mixtures, 1 ml was pipetted into clean dried test tubes in triplicates followed by the addition of Copper-Alkaline reagent (1 ml) and 1.0 ml of Folin-Ciocateu's Phenol reagent (1:10). The reaction mixtures were incubated at 55 °C for 10 min. The tubes were cooled and the absorbance as read at 650 nm against reagent blank. The quantity of protein left was calculated using the expression:

$$(\text{Abs of sample} - \text{Abs of blank}) \times 100$$
$$(\text{Abs of standard} - \text{Abs of blank})$$

The percentage inhibition was calculated using the expression:

$$\frac{\text{Quantity of protein left} \times 100}{\text{Total protein}}$$

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 10. Data were analysed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Results were expressed as mean $\pm$ standard error of the mean (SEM). A p-value less than 0.05 ($p < 0.05$) was considered statistically significant.

Results

HPLC RESULTS

Table 1
**IC₅₀ values of Membrane
 Stabilizing Profile of Fractions
 of Stem Bark of *D.
 microcarpum***

Plant	IC ₅₀ value
DCM Fraction	0.9524 ^a
Pet Ether	1.0736 ^b
HMF	1.1601 ^c
Aspirin	0.0004 ^d

Values with the different alphabets as superscripts are significantly different between means at $p < 0.05$, while those with the same alphabet are not significantly different.

Table 2
**IC₅₀ values of Protein
 Denaturation Inhibitory Profile
 of Fractions of Stem Bark of
*D. microcarpum***

Plant	IC ₅₀ value
DCM Fraction	0.5387
Pet Ether	1.1263
Aspirin	0.2897
HMF	0.733

Table 3
**IC₅₀ values of Heat-Induced
 Protein Denaturation Inhibitory
 Profile of Fractions of Stem
 Bark of *D. microcarpum***

Plant	IC ₅₀ value
DCM Fraction	1.0955
Pet Ether	1.1294
Aspirin	0.0773
HMF	0.4920

Discussion

It is well established that inflammation is a part of homeostasis; the fundamental process of life by reacting to changes with opposing measures. More specifically, inflammation is “the vital response to injury”, which may be related to exposure to infection, toxins, damaged cells, waste, and chemical and physical agents. Inflammation helps to repair the damage that results not necessarily from itself, but from over-activation or deviation of the underlying physiological process. The overlap of physiological and pathological elements is the key point of the medical treatment of inflammation (Silvestrini and Silvestrini, 2022).

Inflammation is mediated by many inflammatory mediators such as Cyclooxygenases, Bradykinin, Adenosine triphosphate, Tumor necrosis factor, Prostaglandins, reactive oxygen species and oxidative stress, chemokine's that include tumor necrosis factor (TNF)- α , nuclear factor kappa beta (NFK β), interferon (IFN)- γ , nitric oxide and interleukins, which may originate from different types of cells, plasma or from the damaged tissue itself. Most inflammatory mediators are newly synthesized in the injured tissues or by the migrated immune cells during an inflammatory event (Nadipelly, 2017).

In this study, we evaluated the *in vitro* anti-inflammatory activity of three solvent fractions—dichloromethane (DCM), hydro-methanol (HMF), and petroleum ether (PE)—from the stem bark of *Detarium microcarpum*. This plant has been long used in West African ethnomedicine. Anti-inflammatory activities were assessed through membrane stabilisation and protein denaturation inhibition assays, and bioactive compounds were identified using high-performance liquid chromatography (HPLC) fingerprinting.

Protein denaturation is a biochemical process due to alterations in a protein molecule's secondary, tertiary, or quaternary structures that disrupt covalent bonds. Usually, all known proteins, in their native states, are folded into well-defined and are generally essentially rigid and possess three-dimensional structures. The changes in the protein structure are typically partnered with alterations in chemical, physical and functional properties. In many investigations, few proteins or enzymes lose their activities irreversibly or reversibly when subjected to different natural or man-made conditions. Denaturation can be reversible or irreversible. Denaturation of protein is well documented as a cause of inflammation. Denaturation mostly occurs when the protein is subjected to external elements like inorganic solutes, organic solvents, acids or bases, and by heat or irradiation. The denaturing agents or denaturants widely used in protein folding or unfolding experiments are urea and guanidinium chloride (Lakshmi and Valarmathi, 2020; Acharya and Chaudhuri, 2021).

The membrane stabilizing assay, which reflects the ability of compounds to inhibit hemolysis of red blood cells, revealed that the dichloromethane (DCM) fraction exhibited the highest efficacy among the test samples, with an IC₅₀ value of 0.9524 mg/mL. Although less potent than aspirin (IC₅₀ = 0.0004 mg/mL), the DCM fraction demonstrated meaningful erythrocyte membrane stabilization, suggesting a capacity to prevent lysosomal enzyme release during inflammatory responses.

Similarly, in the protein denaturation and heat-induced albumin denaturation assays—both of which simulate the structural degradation of proteins under inflammatory stress—the hydro-methanol (HMF) fraction demonstrated the greatest activity ($IC_{50} = 0.733$ mg/mL and 0.4920 mg/mL respectively), closely followed by the DCM fraction. These observations align with previous studies indicating that protein denaturation is a key contributor to inflammation and tissue damage, particularly in autoimmune and rheumatic diseases (Lakshmi and Valarmathi, 2020; Singha et al., 2023).

The anti-inflammatory effects observed may be attributed to the abundance of bioactive phytochemicals in the fractions as confirmed by HPLC analysis. The HMF and DCM fractions contained notable amounts of flavonoids (quercetin, kaempferol, catechin, epicatechin, luteolin), triterpenoids (lupeol, betulinic acid), sterols (β -sitosterol, stigmasterol), and phenolics (methyl gallate, p-coumaric acid, melilotoside). These compounds have well-documented anti-inflammatory and antioxidant activities, acting through various mechanisms such as suppression of pro-inflammatory cytokines, inhibition of COX and LOX enzymes, and stabilization of cell membranes (Aghababaei and Hadidi, 2023; Shankar et al., 2021; Gonfa et al., 2023). For instance, quercetin and kaempferol are known to downregulate NF- κ B signaling and reduce levels of TNF- α and IL-6, which are central mediators of inflammation.

The higher performance of the HMF fraction in protein denaturation assays may be linked to its higher concentration of polar flavonoids and polyphenols, which are more soluble in hydro-methanol and contribute strongly to the inhibition of oxidative and inflammatory processes. The synergistic activity of these phytochemicals could enhance the therapeutic potential of the extract beyond what individual compounds may achieve.

Taken together, the *in vitro* models employed in this study demonstrate that *Detarium microcarpum* fractions, particularly those obtained with DCM and HMF, possess promising anti-inflammatory activity that may be developed into phytotherapeutic agents for managing inflammatory diseases.

Conclusion

This study provides scientific validation for the traditional use of *Detarium microcarpum* in the management of inflammatory conditions. The dichloromethane and hydro-methanol fractions exhibited notable *in vitro* anti-inflammatory activity, primarily through membrane stabilization and inhibition of protein denaturation. HPLC analysis revealed the presence of key phytochemicals such as flavonoids, triterpenoids, sterols, and phenolic compounds, which are known contributors to anti-inflammatory effects.

Among the fractions, the HMF fraction was particularly effective in protein denaturation assays, while the DCM fraction was more active in membrane stabilization, suggesting that the choice of extraction solvent influences the phytochemical profile and bioactivity of the extract. These findings support the further investigation of *D. microcarpum* as a source of potential plant-based anti-inflammatory agents. Future studies should aim at elucidating the precise molecular mechanisms involved, isolating and

characterizing active compounds, and conducting *in vivo* evaluations for pharmacokinetic and safety profiling.

References

1. Acharya VV, Chaudhuri P (2021) Modalities of protein denaturation and nature of denaturants. *Int J Pharm Sci Rev Res* 69(2):19–24
2. Aghababaei F, Hadidi M (2023) Recent Advances in Potential Health Benefits of Quercetin. *Pharmaceuticals* 16(7):1020
3. Aina OI, Oyedapo OO (2013) *In vitro* investigations into the antioxidant and anti-inflammatory potentials of the fractions and ethanolic extract of *Cyclosorus afer* (christ.) Ching, stalks. *Ife Journal of Science*. 15(2): 235–249
4. Anyasor GN, Okanlawon AA, Ogunbiyi B (2019) Evaluation of anti-inflammatory activity of *Justicia secunda* Vahl leaf extract using *in vitro* and *in vivo* inflammation models. *Clinical phytoscience*. 5:1–13
5. Azwanida NN (2015) A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med Aromatic Plants* 4(196):2167–0412
6. Bachheti A, Sharma A, Bachheti RK, Husen A, Pandey DP (2020) Plant allelochemicals and their various applications. In: Mérillon JM, Ramawat K (eds) *Co-Evolution of Secondary Metabolites*. Reference Series in Phytochemistry. Springer, Cham, pp 441–465
7. Das S, Mandal SK (2018) Current developments on anti-inflammatory natural medicines. *Asian J Pharm Clin Res* 11(8):61
8. Dhiman A, Singla C, Vishal, Kumar B (2021) Natural medicinal resources for treating inflammatory conditions. *Int J Med Pharm Sci* 11(9):1–10
9. Gonfa YH, Tessema FB, Bachheti A, Rai N, Tadesse MG, Singab AN, Chaubey KK, Bachheti RK (2023) Anti-inflammatory activity of phytochemicals from medicinal plants and their nanoparticles: A review. *Curr Res Biotechnol* 6:100152
10. Gumisiriza H, Birungi G, Olet EA, Sesaazi CD (2019) Medicinal plant species used by local communities around Queen Elizabeth National Park, Maramagambo Central Forest Reserve and Ihimbo Central Forest Reserve, South western Uganda. *J Ethnopharmacol* 239:111926
11. Habibou HH, Abdoulahi MII, Moctar C, Zakari CO, Rahila HG, Khalid I (2022) Phytochemistry and pharmacology activities of *Detarium microcarpum* (Fabaceae) used in the treatment of parasitic diseases in Niger: A review. *J Pharmacognosy Phytochemistry* 11(2):60–67
12. Karrat L, Abajy MY, Nayal R (2022) Investigating the anti-inflammatory and analgesic properties of leaves ethanolic extracts of *Cedrus libani* and *Pinus brutia*. *Heliyon*. 8(4): 1–8
13. Lakshmi KB, Valarmathi S (2020) *In vitro* anti-inflammatory activity of aqueous extract of *Albizia lebbeck* leaf (L.). *The Journal of Phytopharmacology*. 9(5): 356–360

14. Megid AE, Saydat S, Khamis T, Arisha AH, Rahman AE, Eman O, Fattah AE, Doaa M (2022) Anti-inflammatory, analgesic and ulcerogenic potency of Curcumin in comparison with celecoxib and prednisolone. *Bull Pharm Sci Assiut Univ* 45(1):339–350
15. Nadipelly J (2017) Molecular mechanisms involved in inflammatory cascade: A review. *Texila International J Basic Med Science* 2(1):1–12
16. Nunes CDR, Arantes B, Menezes de Faria Pereira M, Leandro da Cruz S, de Souza Passos L, de Moraes MP, Vieira L, I. J. C. and Barros, de Oliveira D (2020) Plants as sources of anti-inflammatory agents. *Molecules*. 25(16): 3726
17. Oguntibeju OO (2018) Medicinal plants with anti-inflammatory activities from selected countries and regions of Africa. *J Inflamm Res* 11:307–317
18. Oyedapo OO, Akinpelu BA, Orefuwa SO (2004) Anti-inflammatory effect of *Theobroma cacao*, root extract. *J Trop Med Plants* 5(2):161–166
19. Sanusi SB, Lawal SM, Usman A, Musa FM, Ardo B, Way TB (2022) Phytochemical analysis and antibacterial activity of stem bark extracts of *Detarium microcarpum* against bacteria causing gastrointestinal tract infections in humans. *Dutse J Pure Appl Sci* 8(1b):82–89
20. Shankar J, Geetha KM, Wilson B (2021) Potential applications of nanomedicine for treating Parkinson's disease. *J Drug Deliv Sci Technol* 66:102793
21. Silvestrini B, Silvestrini M (2022) Medical implications of the relationships among protein denaturation, necrosis and inflammation: an intriguing story. *Tendons-Trauma, Inflammation, Degeneration, and Treatment*. IntechOpen
22. Singha R, Kanthal LK, Pattanayak S, Maiti M, Bhuniya T, Maity P (2023) *In-vitro* anti-inflammatory activity of *Mimosa pudica* against inhibition of protein denaturation and heat induced haemolysis methods. *International Journal of Pharmaceutical Sciences Review and Research*. 82(1): 48–51

Figures

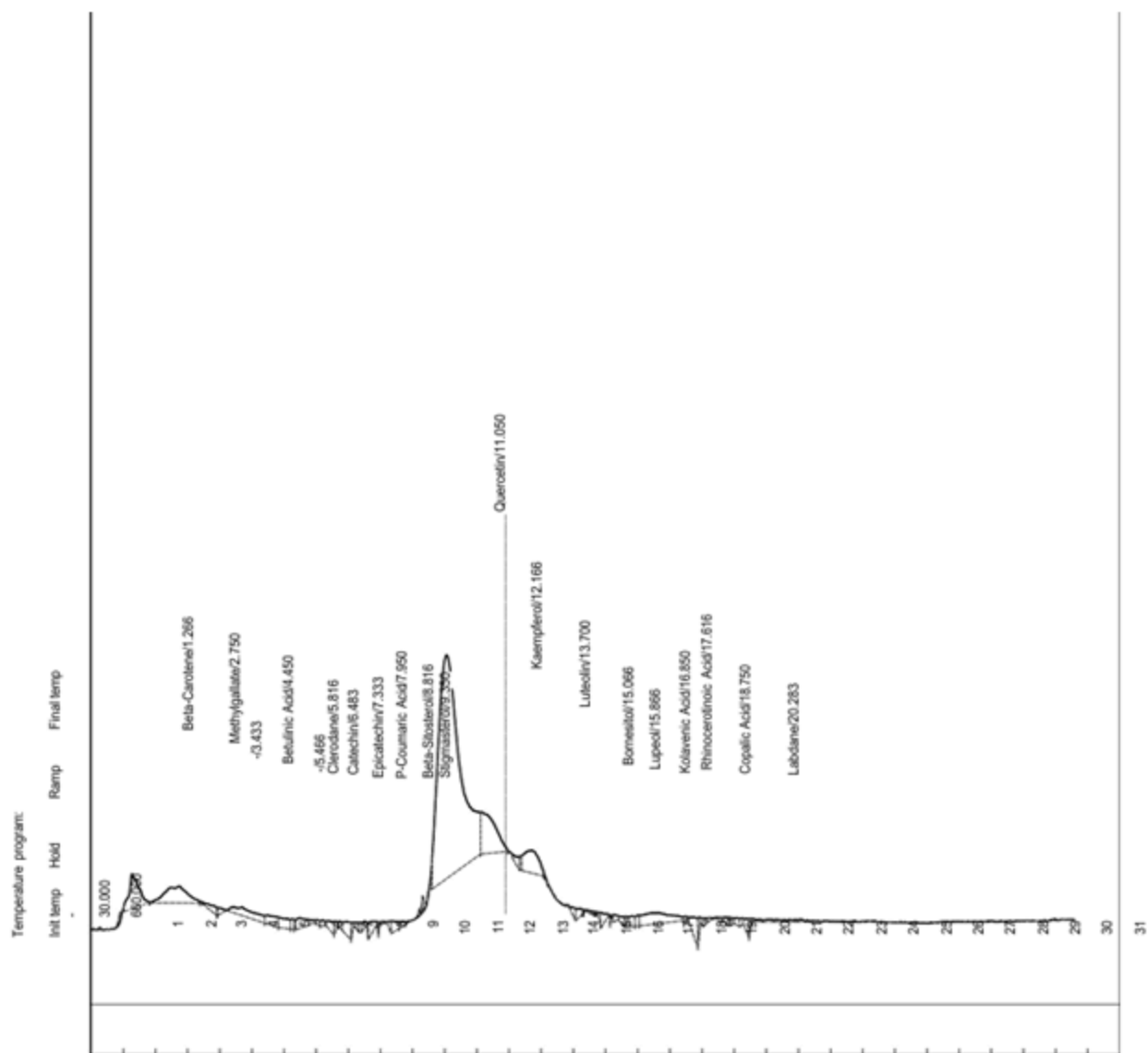


Figure 1

HPLC Analysis of Pet Ether Fraction of Fractions of Stem Bark of *D. microcarpum*

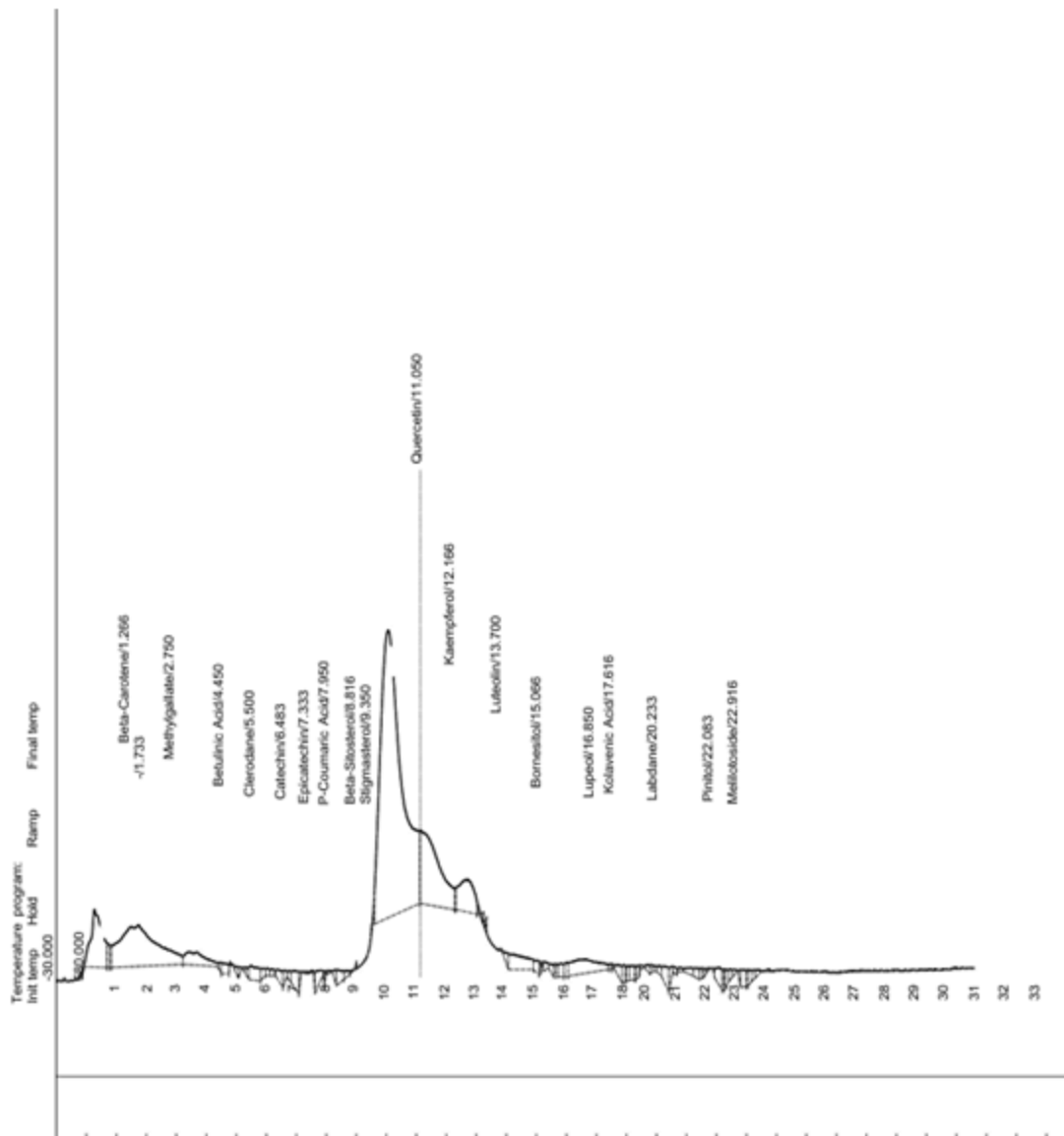


Figure 2

HPLC Analysis of DCM Fraction of Fractions of Stem Bark of *D. microcarpum*

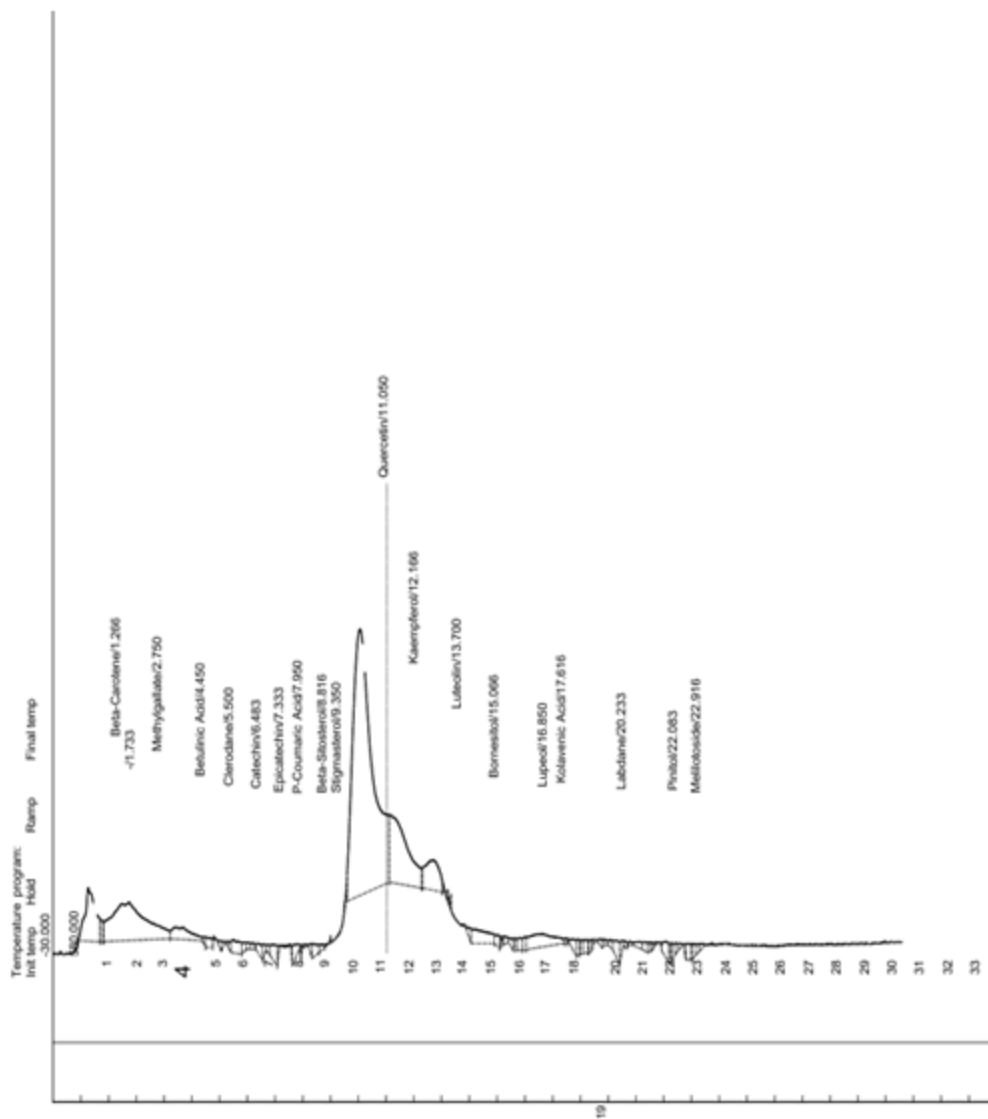


Figure 3

HPLC Analysis of Hydro Methanol Fraction of Fractions of Stem Bark of *D. microcarpum*

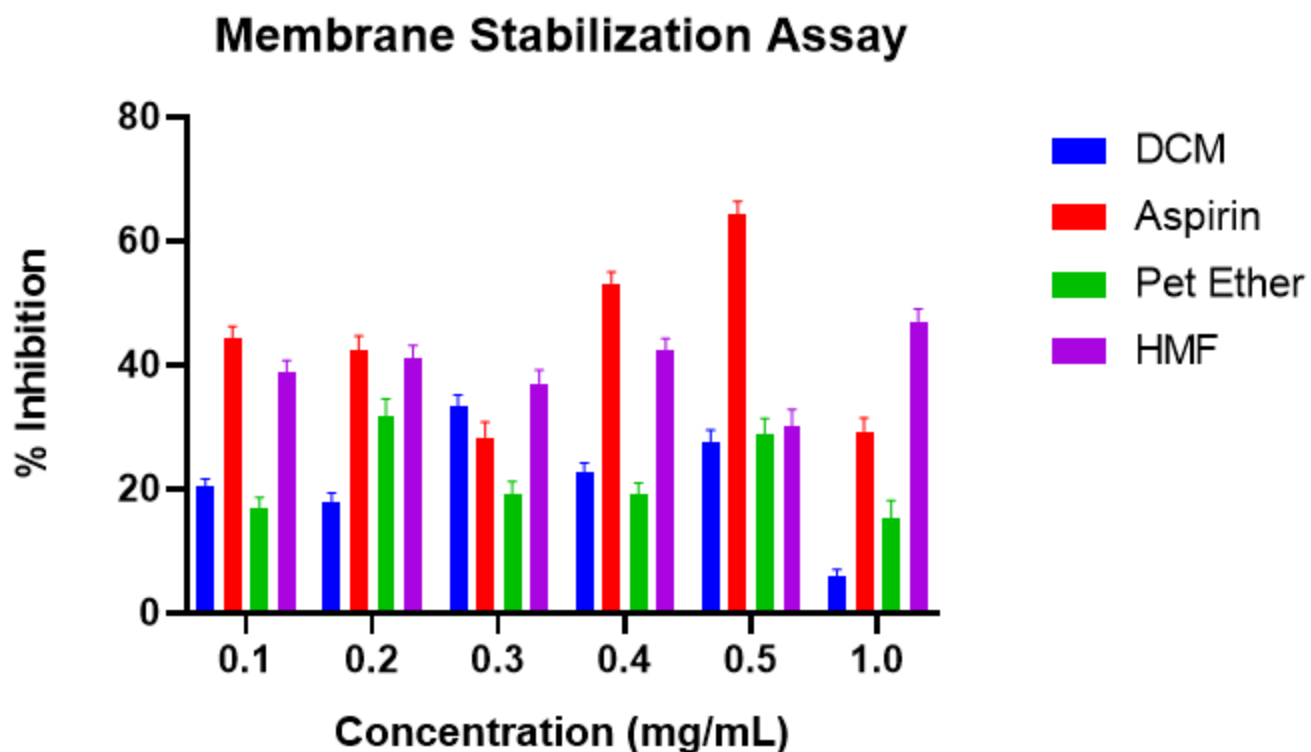


Figure 4

Membrane Stabilizing Profile of Fractions of Stem Bark of *D. microcarpum*

Values are expressed as mean $\pm$ SEM, n = 3/ group. DCM = Dichloromethane Fraction, Aspirin = Standard drug, HMF = Hydro-methanol fraction, Pet. Ether = Petroleum Ether Fraction

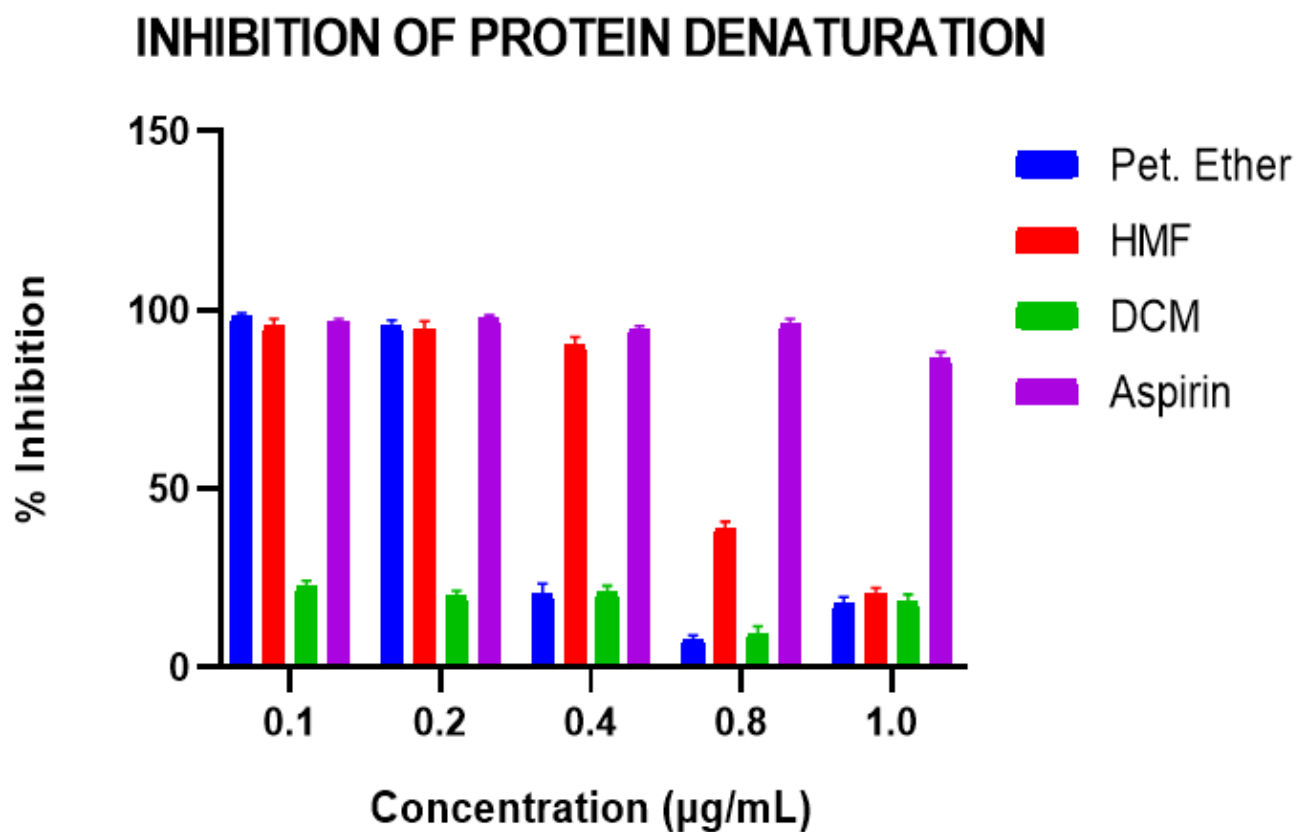


Figure 5

Protein Denaturation Inhibitory Profile of Fractions of Stem Bark of *D. microcarpum*

Values are expressed as mean $\pm$ SEM, n = 3/ group. DCM = Dichloromethane Fraction, Aspirin = Standard drug, HMF = Hydro-methanol fraction, Pet. Ether = Petroleum Ether Fraction

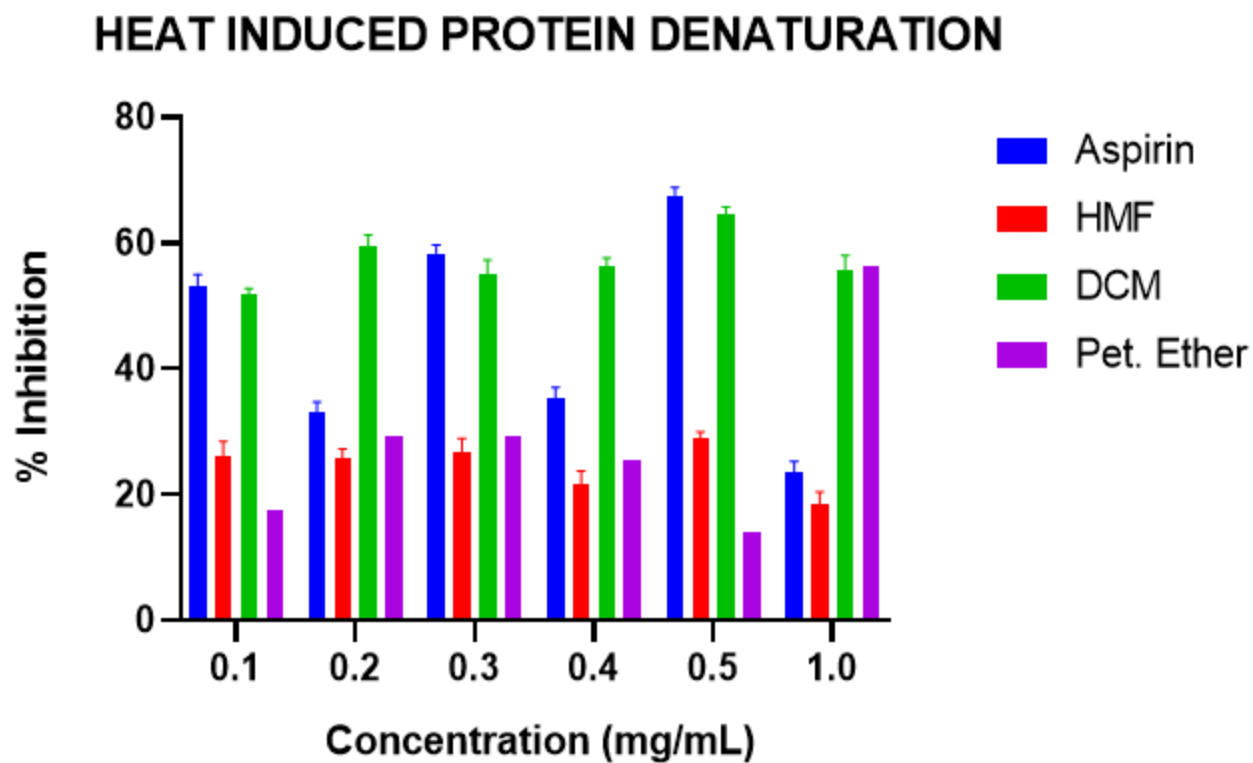


Figure 6

Heat Induced Protein Denaturation Inhibitory Profiles of Fractions of Stem Bark of *D. microcarpum*

Values are expressed as mean $\pm$ SEM, n = 3/ group. DCM = Dichloromethane Fraction, Aspirin = Standard drug, HMF = Hydro-methanol fraction, Pet. Ether = Petroleum Ether Fraction