

Antisickling and sickling reversal activities of the polyphenol-rich butanol fraction of *Detarium microcarpum* leaf extract and its putative bioactive molecules

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Research Article

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Abstract

Detarium microcarpum (Fabaceae), commonly called 'sweet detar', is an underutilized plant indigenous to Tropical Africa. It is used ethnomedicinally for management of diarrhoea, dysentery, tuberculosis, meningitis, itching and sickle cell disease (SCD). This study aims to investigate the different fractions of methanol extract of the plant's leaf for antisickling and sickling reversal activities. The methanol extract and fractions obtained were subjected to acute toxicity studies in albino mice to determine safety profile, followed by an *in vitro* antisickling assay to assess their potential to prevent or reverse SCD. The phytochemicals present in the butanol fraction of the extract were quantified and putatively identified. Acute toxicity studies indicated that all the four fractions possess an LD₅₀ greater than 5000 mg/kg. The butanol fraction demonstrated a significant ability to reverse the sickling of 70% of the red blood cells. In contrast, the ability of the hexane, chloroform and ethyl acetate fractions to reverse sickled red blood cells were not significantly better than those of control ($p < 0.05$). Colorimetric assays showed the presence of high amount of phenolics (154.34 ± 0.03 mg/g) and flavonoids (119.98 ± 0.19 mg/g). Mass spectrometry analysis of the butanol fraction revealed the presence of phenolic glycosides, flavonoids and triterpenes. The butanol fraction of *sweet detar* methanol leaf extract possesses significant sickling reversal activity, likely attributed to its high polyphenolic and flavonoid content. These results warrant further studies to characterize specific bioactive compounds for potential therapeutic applications in the management of SCD.

Background

Sickle cell disease (SCD) is a genetic blood disorder characterized by the presence of abnormal hemoglobin S, which polymerizes under low oxygen tension, leading to the deformation of red blood cells into a sickle shape (Rees, Williams et al. 2010). These sickled cells are prone to hemolysis and vaso-occlusion, resulting in chronic anemia, pain crises, and organ damage. SCD was first described over one hundred years ago and was the first disease to have its molecular basis elucidated (Vekilov 2007). However, treatment of SCD remains a significant unmet public health challenge, particularly in sub-Saharan Africa (SSA), where up to 40% of the population may carry the sickle cell trait, and up to 89% of global SCD cases occur (WHO 2008, Elendu, Amaechi et al. 2023). One-fourth of the SCD patients in the world are in Nigeria (Ameh, Obodozie et al. 2009, Nwabuko, Onwuchekwa et al. 2022), making Nigeria the country with highest population of SCD disease patients in the world.

The available treatment options for SCD focus on inhibiting haemoglobin polymerisation, rehydrating erythrocytes, and preventing sickle cells from interacting with the endothelium. Curative treatment requires a hematopoietic stem cell transplant, which is very expensive and marred by other challenges (Bhatia and Sheth 2015). The most common management approach in SSA, with over 1.3 billion population, involves prophylaxis of malaria, anaemia, and infection using appropriate drugs (Piel, Rees et al. 2023). The use of hydroxyurea and L-glutamine has demonstrated a significant reduction in SCD crises (Niihara, Miller et al. 2018, Inusa, Hsu et al. 2019). The use of hydroxyurea treatment option was delayed in many parts of SSA (Park, Soni et al. 2019, Opoka, Hume et al. 2020). It became available in

Uganda only after its introduction through a clinical trial in 2014 (Anyanwu, Williams et al. 2016, Park, Soni et al. 2019, Opoka, Hume et al. 2020) and was subsequently adopted across most sickle cell clinics by 2020 (URN 2020). Hydroxyurea and L-glutamine are not affordable to most of the affected population in SSA, coupled with associated adverse effects, including neutropenia, anorexia, bone marrow suppression, hepatic enzyme changes, and dose-dependent leucopenia, nausea, and vomiting (McGann, Williams et al. 2018, Ofakunrin, Okpe et al. 2021, Dexter and McGann 2023, Al-Shami, Maad et al. 2025). The required routine monitoring and individualized dose adjustments further increase the cost, creating an additional barrier to hydroxyurea treatment option in SSA. Urban and rural settings have thus embraced various herbal formulations to manage sickle cell anaemia. The rising popularity of alternative medicines is attributed to affordability and perceived lower toxicity (Busari and Mufutau 2017). Some common herbal recipes use in managing SCD include Niprisan (Marketed as Niclovix and Xickle), Ajaworon HF, and Ciklaviv (Singh, Kaur et al. 2013). Research has identified recipes used by indigenous people to manage SCD (Awor, Bongomin et al. 2023, Awor, Opee et al. 2025). These recipes include several medicinal plants, among them, *Detarium microcarpum* (*D. microcarpum*).

D. microcarpum, commonly known as *sweet detar*, *sweet dattock*, or *tallow tree*, is an under-utilized tree legume that grows naturally in the drier regions of West and Central Africa. It is a small tree up to 10 m tall, with a horizontal root system. It is typically a specie of dry savannah (Leung, Busson et al. 1968, Belem, Nabaloum et al. 2017). The fresh bark or leaves of *D. microcarpum* are applied to wounds to prevent and cure infections (Kouyaté and Van Damme 2006). The leaves, stems, roots, barks, and fruits are widely used in the treatment of various ailments, including tuberculosis, meningitis, itching, and diarrhoea (Obun, Yahaya et al. 2010). Pharmacological studies have shown that *D. microcarpum* leaf extracts possess antimicrobial, antidiabetic, anti-snake venom, antioxidant, and anti-inflammatory properties (Iful 2008, Akinsanmi, Johnson et al. 2017, Semde, Koudou et al. 2018, David, Kolawole et al. 2020, Ihenetu, Ikpa et al. 2020, Kurmi, Modu et al. 2021). The phytochemical composition of the leaf includes sesquiterpenes, diterpenes, steroids, phenolics and fatty acids and their derivatives (Ihenetu et al., 2020; Semde et al., 2018; Meda et al., 2017; Hassan et al., 2010). The plant is ethnomedicinally used in South-western Nigeria as part of recipes for the treatment of SCD (Egunyomi, Moody et al. 2009, Gbadamosi, Adeyemi et al. 2012). The investigated antioxidant, and anti-inflammatory properties of the plant extracts are potential for stabilizing erythrocyte membranes, reducing oxidative stress, modulating ion transport or increasing hemoglobin solubility in SCD conditions (Henneberg, Otuki et al. 2013). Despite its widespread traditional use and relevant pharmacological effects, the fraction-specific antisickling activity of *D. microcarpum* remains underexplored. This study aims to investigate the phytochemical profile of the plant leaf and the potential antisickling activity of its butanol extract. By identifying bioactive compounds and evaluating their in vitro efficacy, this research seeks to evaluate the plant's ethnomedicinal use and to contribute to the early discovery of alternative, affordable, plant-based therapies for SCD.

Materials and Methods

The study was conducted in accordance with guidelines for studies involving natural products, traditional medicine and systems pharmacology (Tveden-Nyborg, Yang et al. 2024).

Plant Collection and Extraction

D. microcarpum was collected from the wild in its natural habitat in Kano, Northwest Nigeria. The plant was identified and authenticated by a taxonomist (Mr. Namadi Sunusi) at Biological Sciences Department, Ahmadu Bello University, Zaria. A voucher number (901451) was issued, and the herbarium specimen was retained at the department. Freshly collected leaves of *D. microcarpum* were shaded dried, pulverized into powder, and macerated with methanol for 7 days. The resulting extract was subjected to an exhaustive sequential fractionation using a separating funnel with n-hexane, ethyl acetate, chloroform and n-butanol as solvents in order of increasing solvent polarity. The fractions obtained were labelled HF (Hexane fraction), EAF (Ethyl acetate fraction), CF (chloroform fraction), BF (Butanol fraction) and RAF (Residual aqueous fraction). The scientific name *D. microcarpum* (*Detarium microcarpum* Guill. & Perr) was used according to Plants of the World Online (Kew) (Dalziel 1937, R 1938, POWO 2026), ensuring taxonomic consistency and reproducibility. The species is not listed as endangered or protected under the International Union for Conservation of Nature (IUCN) Red List, and no Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)-restricted materials were used (International Union for Conservation of Nature 2026). The voucher documentation allows tracing and reproducibility of the natural product material utilized for this study.

Phytochemical Screening and Acute Toxicity (LD) Test

Preliminary phytochemical screening was carried out according to the method of Evans (Evans 2009). Colorimetric assays were used to estimate the total phenolic content, total flavonoid content, total tannins content and total saponins contents of methanol extract and butanol fraction according to the methods of (Zhishen, Mengcheng et al. 1999, Ribarova, Atanassova et al. 2005, Ainsworth and Gillespie 2007, Makkar, Siddhuraju et al. 2007, Shamsa, Monsef et al. 2008). Acute toxicity of *D. microcarpum* extracts and fractions was assessed by determining the median lethal dose (LD₅₀) using the limit test at 5000 mg/kg, in accordance with OECD Guideline 425 for oral route testing (OECD 2008). Nulliparous, non-pregnant female albino mice (8–9 weeks old; body weight 24.5 ± 2.6 g) were employed for the study. Animals were acclimatized under standard laboratory conditions for five days prior to dosing and subjected to a 5-hour fasting period before administration of the test substances. A total of three animals were used per extract. One animal was dosed at 5000 mg/kg and observed for 48 hours. Two more animals were given the same dose and observed for survival for 48 hours.

Antisickling Activity

Blood Collection and Preparation

The participants were adults (19–25 years), of both sexes, belonging to Hausa-Fulani ethnic group, and confirmed to have sickle cell anaemia (electrophoretic SS (HbSS)). All were in a steady clinical state and were recruited from the Haematology Day Care Unit of Murtala Muhammed Specialist Hospital, Kano, Nigeria. A total of 20 mL of blood was obtained by non-randomization from the first seven (n = 7) consecutive eligible patients into sodium ethylenediaminetetraacetic acid (EDTA) anticoagulant bottles and mixed thoroughly by gentle inversion. The samples were centrifuged to separate plasma, leaving packed erythrocytes, which were subsequently washed with normal saline following the procedure described by (Egunyomi, Moody et al. 2009).

Bioassay of Plant Methanol Extract and Fractions

The study was designed such that the crude methanol extract was administered at three different doses (0.1 mg/ml, 1 mg/ml and 10 mg/ml), n = 3 for each group) using logarithmic scaling after a pre-study validation of extract toxicity level (Boyd 1997, Haas, Manro et al. 2004, Haas, Manro et al. 2012). The control groups included 5 mg/mL p-hydroxybenzoic acid (PHBA) as a positive control (n = 3) and normal saline as a negative control (n = 3). Percentage inhibition of sickling was calculated using the formula described by (Egunyomi, Moody et al. 2009). Antisickling activity of crude extracts and their fractions was evaluated using two approaches: (i) inhibition of erythrocyte sickling and (ii) reversal of sickled erythrocytes. The assays were performed using a modified method published by Sofowora and colleagues (Sofowora, Isaac-Sodeye et al. 1979). For the inhibition assay, 0.5 mL of washed erythrocytes was mixed with an equal volume of each test extract or fraction in uncovered test tubes. Aliquots were taken immediately (0 min), and the remaining mixture was incubated at 37°C (Apex Scientific, South Africa) for 2 h with intermittent shaking. Subsequently, five drops of 2% sodium metabisulfite (to induce sickling) were added, mixed thoroughly, and the tubes sealed with parafilm. Triplicate samples were collected at baseline and at 30-min intervals for two additional readings. Smears were prepared and examined microscopically to count sickled and unsickled cells, following (Egunyomi, Moody et al. 2009). Percentage inhibition of sickling was calculated using the formula described by (Moody, Ojo et al. 2003).

For the reversal assay, 0.5 mL of washed erythrocytes was mixed with 0.5 mL of freshly prepared 2% sodium metabisulfite and incubated at 37°C for 30 min to induce sickling. A drop of this mixture was examined microscopically to confirm sickling. Equal volumes of normal saline or test fraction were then added to separate tubes containing the sickled erythrocyte mixture and incubated at 37°C for an additional 30 min. Samples were collected at baseline and at 30-min intervals for up to 2 h, and smears were prepared and analysed as previously described (Egunyomi, Moody et al. 2009). Reversal activity was evaluated according to the procedure outlined by (Oduola, Adeniyi et al. 2006).

Liquid Chromatography-Mass Spectroscopy of Butanol Fraction

After antisickling assay on the four fractions, the butanol fraction showing most promising activity was analyzed using liquid chromatography (LC) Waters e2695 separation module equipped with a W2998

photodiode array (PDA) detector and coupled to ACQ-QDA tandem mass spectrophotometer (MS) as described by (Piovesana, Rodrigues et al. 2019) with some modifications. The extracted samples were reconstituted in methanol and filtered through polytetrafluoroethylene (PTFE) membrane filter with 0.45 µm size. After filtration, an aliquot (10 µ L) of the filtrate was injected into the LC system and allowed to separate on Sunfire C18 (5.0µm 4.6mm x 150 mm) column. The chromatographic separation was carried out at a flow rate of 1.0 mL/min, with both sample and column temperature at 25°C. The mobile phase consists of 0.1% formic acid in water (solvent A) and 0.1% formic acid in Acetonitrile (solvent B) with a gradient system. The PDA detector was set to scan 210–400 nm with a resolution of 1.2 nm and a sampling rate of 10 points/sec. The mass spectrometric detection was performed in both positive and negative electrospray ionization (ESI) modes at a scan range from m/z 100–1250 with the following instrument parameters: capillary voltage of 0.8 kV (positive) and 0.8 kV (negative); probe temperature 600 °C; flow rate 10 mL/min; nebulizer gas, 45 psi. MS set in automatic mode applying fragmentation voltage of 125 V. Data acquisition and processing were performed using Empower 3 software. The compounds were identified based on fragmentation pattern of each chromatographic peak and corresponding retention time (RT) by comparing with that of National Institute of Standards and Technology (NIST) MS Search Program database (*NIST 23, MS Search v3.0*) for organic compounds. Putative compound identities and confidence levels for LC-MS features were assigned following the Metabolomics Standards Initiative (MSI) four-tier framework, which distinguishes between confirmed identities (Level 1), putatively annotated compounds (Level 2), putatively characterized classes (Level 3), and unknown features (Level 4) (Sumner, Amberg et al. 2007, Salek, Steinbeck et al. 2013, Schymanski and Neumann 2013). Exact masses were interpreted primarily as $[M-H]^-$ ions from negative-mode electrospray and compared with theoretical monoisotopic masses from reference databases and the primary literature.

Statistical Analysis

Values for the *in vitro* experiments were expressed as mean ± SEM for triplicate independent experiments. Statistical significance was evaluated using one-way ANOVA followed by Bonferroni post-hoc tests using GraphPad Prism (Version 10.5.0). The differences were considered significant at a p-value of less than 0.05.

Results and Discussion

The extraction of 750 g *D. microcarpum* dried leaf sample yielded 140 g of methanol extract. The Qualitative and quantitative phytochemical analysis revealed the presence of saponins, cardiac glycosides, tannins, flavonoids and alkaloids (Fig. 1 and Table S1).

The acute toxicity results of the median lethal doses ($LD_{50} > 5000$ mg/kg)) revealed the absence of observable adverse physical or behavioural alterations in experimental animals. These findings indicate a high marginal safety and support the ethnomedicinal uses of the plant, including in the management of

sickle cell anaemia. Consistent with these results, a study using Lorke method found that *D. microcarpum* leaf extract was safe at doses up to 5000 mg/kg (Estella and Chidiebere 2020).

In the sickling reversal assay (Fig. 2); treatment with 0.1mg/ml of the methanol leaf extract significantly increases sickling reversal activity ($p < 0.05$) at 30, 60, and 90 minutes post-exposure. The reversal effect at 0.1mg/ml was not time dependent. In contrast, results of 1mg/ml of methanol leaf extract demonstrates time dependent increase in the reversal of sickling activity. Similarly, treatment with 10mg/ml of methanol leaf extract significantly increases sickling reversal activity in a time dependent manner (Figs. 2A-3D). The observed time-dependent antisickling effect was not concentration dependent (Fig. 2E and 2F). The activity at 10mg/ml methanol extract was lower than those of 0.1mg/ml and 1mg/ml concentrations of the extract (Fig. 2F). This observation suggests that increasing the concentration beyond 1mg/ml may not confer additional sickling reversal effect. On the contrary, it may produce additional side effects (Alli and Nnodu 2020). An early adaptation of the cells to the high concentration of the extract may also play a role in this phenomenon (Tyson, Park et al. 2020). At 60 minutes following exposure, significant dose-dependent differences in the effect produced by methanol extracts were observed. Thus, the target dose for the extracts to produce antisickling activity can be deduced to be less than or equal to 0.1 mg/ml (Yembeu, Biapa Nya et al. 2022). The percentage of antisickling activity trend over a 90-minute exposure period for five treatments (Fig. 2E) and their corresponding exposure profiles are expressed as Area Under the Antisickling Activity-Time Curve - AUCs (Fig. 2F). All MeOH concentrations and PHBA exhibit a rapid increase in antisickling activity, reaching near complete antisickling activity by 30–60 minutes and maintained throughout the study period (Fig. 2E and 2F).

The sickling prevention assay (Fig. 3) revealed a significant difference ($p < 0.05$) in activity by 30, 60, 90, and 120 minutes (Fig. 3A-3D), and maintained throughout the study period, suggesting that sickling prevention activities of the extract was not concentration or time dependent. Similar time independent antisickling activity was reported in a study on various concentrations of methanol and aqueous extracts from seed, seed pod and leaf of *Garcinia kola* plant (Adejumo, Ayoola et al. 2011). Additionally, a study using methanol leaf extract from *Carica papaya* reported similar time-independent antisickling activity (Imaga 2010). Furthermore, the works of Tshilanda (Tshilanda, Onyamboko et al. 2015) and Pauline (Pauline, Cabral et al. 2013) demonstrated that increasing dose or concentration of anthocyanin rich extracts of *Ocimum canun* and aqueous extract of *Zanthoxylum heitzii* did not significantly increase the antisickling activities. In the trend analysis (Fig. 3E), the methanolic leaf extracts at all tested concentrations and PHBA demonstrated rapid and sustained sickling prevention, achieving near-complete prevention (~ 100%) within first 30-minutes post exposure, and remaining stable throughout 120 minutes, compared to normal saline. The AUC analysis (Fig. 3F) revealed significantly higher overall activity for MeOH and PHBA, with a minor but significant differences between PHBA and some MeOH doses.

The fractionation of 100 g of the extract produced hexane, ethyl acetate, chloroform, butanol and residual aqueous fractions with percentage yields of 1.8%, 5.0%, 1.2%, 38% and 41% respectively (Fig.

S1). Knowledge of the phytochemicals present in a sample extract help to identify the bioactive molecules potentially responsible for its biological activity as well as a plant’s medicinal value (Abbasi and Vishkaei 2022). One of the processes that leads to the sickling of rBCs is low oxygen tension (Nur, Biomond et al. 2011). Polyphenols constitute an important class of exogenous antioxidant molecules. Flavonoids such as rutin and quercetin possess free radical scavenging activity against reactive oxygen species produced during sickle cell crisis (Votano and Rich 1985, Henneberg, Otuki et al. 2013).

The colorimetric assay results show the phenolics and flavonoids contents constituted the majority of the phytochemicals, followed by saponins, alkaloid, and tannins in the butanol fraction (Table 1). Mpiana et al. (Mpiana, Misakabu et al. 2014) have demonstrated that Congolese medicinal plants containing high levels of anthocyanins possessed antisickling properties.

Table 1
Quantitative phytochemical analysis of butanol fraction (Colorimetric Assay)

Phytochemical	Total Alkaloids	Total Phenolics	Total Flavonoids	Total Saponins	Total Tannins
Concentration (mg/g)	78.62 ± 1.23	154.34 ± 0.03	119.98 ± 0.19	81.33 ± 5.37	25.36 ± 0.25
Values are Mean ± SEM, n = 3					

Ethyl acetate and butanol fractions of plant extracts are rich in polyphenols (Oreopoulou, Tsimogiannis et al. 2019). However, ethyl acetate and butanol fractions did not prevent the sickling of sRBC in this study. The sickling prevention activity of the crude fractions of methanol extract of the plant was not statistically significant different from that of negative control. This demonstrates that the crude fractions (hexane, chloroform, ethyl acetate, butanol and residual aqueous) of the methanol extract are not suitable for use in sickle cell prevention. Lack of activity was also observed when the fractions were evaluated for their ability to reverse the sickling of RBCs except for the butanol fraction which demonstrated a significant sickling reversal effect. Phytochemicals exist as complex mixtures in plant and elicit biological responses in a synergistic manner (Chen, Li et al. 2022). Fractionation of the methanol extract may have led to the loss of synergistic antisickling activities observed in the crude methanolic extract. The reversal activity of the butanol fraction was comparable to that of positive control. The marked difference between the activities produced by the crude extracts when compared to methanol fractions may be attributed to the separation process of the antisickling compounds and synergistic influence from fractionation processes (Chen, Li et al. 2022).

Among the tested plant extract fractions (Fig. 4), butanol demonstrated the strongest significant antisickling effects (followed by ethylacetate fraction) achieving near-complete reversal comparable to PHBA (p < 0.0001). The activities of the hexane and chloroform fractions were not significant compared to normal saline. The differential antisickling activities observed among plant extract fractions suggest that bioactive compounds responsible for hemoglobin stabilization are concentrated in the butanol fractions.

The lower activity of hexane and chloroform fractions indicates that nonpolar constituents contribute minimally to antisickling effects. These results underscore the importance of polarity in extracting active compounds and provide a basis for further phytochemical characterization and isolation of lead molecules for therapeutic development.

Mass spectrometry-based compound identification and confidence levels

LC-MS analysis of butanol fraction showed presence of diverse classes of phytochemicals which may have combined synergistically to produce the observed antisickling effect. The chromatogram of the butanol fraction revealed six major chromatographic peaks were identified from the fraction and corresponding RT each associated with a molecular ion and a putative structure supported by fragmentation behaviour (Table 2). For the RT peaks 1–4 and 6, measured m/z values and calculated neutral masses matched one dominant candidate within typical LC-MS mass error, and the proposed structures were consistent with known plant metabolites and chromatographic behavior (e.g., polarity and retention time). In accordance with MSI, these features are therefore classified as Level 2: putatively annotated compounds without a confirmation with in-house reference standards. The MS chromatogram peaks suggested the presence of Citrusin C, Corchorifatty acid F, acetyl-11-keto- β -boswellic acid, Quercetin 3-galactoside, a di-C-glycoside and 2-hydroxyethyl octadecanoate (Table 2). Citrusin C and Quercetin 3-galactoside are both phenolic compounds with antioxidant activity.

Table 2
LC-MS Chromatogram of butanol fraction of *D. microcarpum*

S/N	M/Z [M + H] ⁺	Retention Time (Mins)	Proposed Compound	Ontology	Molecular weight(g/mol)
1	325.190	5.980	Citrusin C	Phenolic glycosides	326.137
2	327.403	6.445	Corchorifatty acid F	Lineolic acids and derivatives	328.225
3	511.261	6.755	Acetyl-11-keto- β -boswellic acid	Pentacyclic triterpene	512.350
4	463.251	7.022	Quercetin 3-galactoside	Flavonoid-3-O-glycosides	464.095
5	561.256	7.460	Flavone Di-C-glycoside	C-glycosides	562.524
6	327.629	10.713	2-hydroxyethyl octadecanoate	Fatty acid esters	328.298

Flavonoids are among the most promising antisickling agents from plants (Ameh, Tarfa et al. 2012, Dash, Archana et al. 2013). Chrysin (hydroxyflavone) compound restored haemoglobin function, lowered

osmotic fragility and reduced 2,3-diphosphoglycerate levels of sickled RBCs (Nwankwo, Idowu et al. 2021). Flavonols such as quercetin and kaempferol have also possessed potential antisickling activities (Folashade and Omoregie 2013). Thus, polyphenols present in *D. microcarpum* might be responsible for the antisickling activity of the plant extract. Flavonoids which are a class of polyphenols are also abundant in the butanol fraction. Other phytochemicals such as terpenoids and short chain fatty acids have also demonstrated antisickling potential (Imaga 2010). The proposed structures of the putative bioactive compound based on the LC-MS Chromatogram and drawn in ChemDraw (Fig. 5A-E), represents the most plausible candidate consistent with the observed mass spectral features; however, definitive structural confirmation was not established. The chromatographic peak number at RT of 5.980 min exhibited a precursor ion at m/z of 325.190. Fragmentation suggested phenolic glycoside-type cleavages. The putative structure corresponds to Citrusin C, with the IUPAC name: (2R,3S,4S,5R,6S)-2-(hydroxymethyl)-6-(2-methoxy-4-prop-2-enylphenoxy)oxane-3,4,5-triol (Fig. 5A). The tentative identification of Citrusin C suggests the presence of phenolic glycosides, a phytochemical class widely associated with antioxidant and anti-inflammatory properties (Hao, Chen et al. 2015, Mohammed, Mohammed et al. 2025). Although the available fragmentation pattern supported glycosidic cleavage behavior, the identification is not supported by sufficient data to warrant definitive structural confirmation, underscoring the need for complementary NMR analysis in future studies. The compound appeared at RT of 6.445 min, m/z 327.403 ($[M-H]^-$), produced fragments characteristic of hydroxylated linoleic acid derivatives. The proposed identity is Corchorifatty acid F, with the IUPAC name: (10E,15Z)-9,12,13-trihydroxyoctadeca-10,15-dienoic acid (Fig. 5B). The observed fragmentation was consistent with hydroxylated fatty acid cleavage, but the structural assignment remains tentative. The tentative structural identification of Corchorifatty acid F highlights the contribution of oxidized linoleic acid derivatives, which are often implicated in membrane-associated reduced RBC hemolysis in a SCD pathology and stress responses (Khan, Damanhour et al. 2022, Starlard-Davenport, Palani et al. 2025). The next chromatogram was RT of 6.755 min, with a m/z of 511.261 ($[M-H]^-$). The ion at m/z of 511.261 aligned with neutral losses associated with acetyl and carboxyl functionalities typical of pentacyclic triterpenes. The putative structure is acetyl-11-keto- β -boswellic acid, with an IUPAC name: (3R,4R,6aR,6bS,8aR,11R,12S,14bS)-3-acetyloxy-4,6a,6b,8a,11,12,14b-heptamethyl-14-oxo-1,2,3,4a,5,6,7,8,9,10,11,12,12a,14a-tetradecahydronicene-4-carboxylic acid (Fig. 5C). This putative structural characterization further expands the chemical diversity of the extract. Boswellic acids from different plant extracts are well documented for immunomodulatory anti-inflammatory effects associated with SCD (Sailer, Hoernlein et al. 1996, Rentea 2011, Mannino, Occhipinti et al. 2016), and their presence may contribute to the observed antisickling and sickling reversal bioactivity of the butanol fraction. However, the MS based identification remains putative due to the structural complexity of triterpenes. The next compound appeared at RT = 7.022 min, with a m/z of 463.251 ($[M-H]^-$), corresponding to spectra showing sugar moiety cleavage and retro Diels-Alder transitions typical of flavonoid-3-O-glycosides (Śliwka-Kaszyńska, Anusiewicz et al. 2022). The compound was tentatively assigned as quercetin-3-galactoside, with the IUPAC name: 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one (Fig. 5D). These flavonoid glycosides are known for their antioxidant and cytoprotective effects, relevant to the observed

antisickling and sickling reversal activities of the butanol extract fraction (Thangaswamy, Branch et al. 2021, Vona, Sposi et al. 2021). The compound at RT of 7.460 min ($M \approx 562.26$, ESI-[M-H]⁻) with a m/z of 561.256 ([M-H]⁻) corresponding to accurate-mass and chromatographic characteristics compatible with a flavone di-C-glycoside, but the measured deprotonated mass did not match the canonical formula for a known compound. Therefore, RT at 7.460 min is reported as putative apigenin di-C-glycoside or 4-[3-(4-formylphenyl)-phenyl]-benzaldehyde based on fragmentation pattern, in line with MSI guidance that Level 3 (Sumner, Amberg et al. 2007, Salek, Steinbeck et al. 2013). No structure is proposed for other minor co-eluting ions at this retention time, which remain unassigned Level 4 unknowns. Those apigenin di-C-glycoside and polyphenyl aldehydes contribute to the extract's redox chemistry and inhibition of HbS polymerization (Eaton and Bunn 2017, Yembeau, Biapa Nya et al. 2022), resulting in the observed effects on SCD. An appearing compound was observed at 10.713 min RT, with a m/z of 327.629 ([M-H]⁻) and fragmentation behavior, suggesting fatty acid ester cleavage, with putative identity of ethylene glycol monostearate, corresponding to an IUPAC based structural fragment: 2 hydroxyethyl octadecenoate (Fig. 5E). The tentative identification suggests the presence of long chain fatty acid esters. These compounds may originate from natural lipid metabolism or from processing related surfactant residues (Luo, Xu et al. 2007, Telen, Malik et al. 2019, Khan, Damanhoury et al. 2022, Mohammed, Mohammed et al. 2025); this would require further confirmation and evaluation studies.

Implications and Future Directions

The results from both reversal and prevention assays demonstrate that methanolic extract and butanoic fraction exhibit rapid and sustained antisickling effects, achieving near-complete reversal and prevention activity within 30 minutes and maintained the activity throughout the study period. These findings are consistent with previous reports that hydroxylated and phenolic compounds stabilize hemoglobin and inhibit HbS polymerization under hypoxic conditions (Noguchi and Schechter 1985, Syed, Doshi et al. 2018, Santana, Pitanga et al. 2020). The putative bioactive compounds discovery results from this study have documented antioxidant, anti-inflammatory, and membrane-stabilizing properties (Imaga 2010, Chen, Li et al. 2022). Their presence in a single fraction raises the possibility of synergistic interactions that enhance antisickling efficacy. This supports the concept of multi-target phytotherapy, which may offer advantages over single-compound drugs in complex diseases like SCD (Ekor 2014). The chemical diversity of the putative moieties provides a strong biochemical rationale for the observed activities.

While the study demonstrates significant antisickling activity, the underlying mechanisms remain to be fully elucidated. Flavonoids and polyphenols may act by stabilizing erythrocyte membranes, reducing oxidative stress, or modulating ion transport (Henneberg, Otuki et al. 2013, Nwankwo, Idowu et al. 2021). Furthermore, these bioactive molecules likely act by increasing hemoglobin solubility and favoring the relaxed state through non-covalent interactions such as hydrogen bonding and π - π stacking, thereby disrupting fibre formation (Zagrean-Tuza, Igescu et al. 2024). The minimal differences among dose levels suggest a saturation effect, indicating that structural compatibility, not concentration alone plays significant roles in the observed antisickling efficacy. The significantly higher AUC values underscore

their therapeutic potential as adjuncts or alternatives to hydroxyurea, which primarily induces fetal hemoglobin rather than directly reversing or preventing sickling.

Findings from this study have important clinical implications, particularly for populations in low-resource settings where access to conventional therapies for SCD remains an unmet clinical need. Current pharmacological treatments such as hydroxyurea, voxelotor, and L-glutamine, while effective, are often inaccessible due to cost, availability, and infrastructure constraints (Ware, de Montalembert et al. 2017, Niihara, Miller et al. 2018). Thus, the translational implications in resource-limited settings like SSA countries where the prevalence of SCD is highly consequential. Moreover, the promising safety profile observed in acute toxicity studies ($LD_{50} > 5000$ mg/kg) supports its suitability for further preclinical and clinical evaluation. If validated *in vivo*, standardized formulations of *D. microcarpum* extracts could be developed into nutraceuticals or adjunct therapies and integrated into community-based SCD management programs, offering a culturally acceptable and economically feasible alternative to hydroxyurea or stem cell transplantation (Ekor 2014, Estella and Chidiebere 2020).

This rapid onset from reversal and prevention assays demonstrate that it is highly relevant for managing acute vaso-occlusive crises in SCD, where timely reversal of sickling can prevent severe complications. Unlike hydroxyurea, which primarily acts by inducing fetal hemoglobin and requires prolonged administration, the putative identified compounds offer the potential for rapid onset therapeutic effects during SCD crises. The saturation effect across fraction doses suggests that structural compatibility rather than concentration drives efficacy, highlighting the importance of molecular interactions, which was implicated in the earlier discussion.

Furthermore, the overall results have cultural relevance and ethnopharmacological alignment with the traditional use of *D. microcarpum* in the management of SCD, a practice deeply rooted in West African ethnomedicine. Ethnobotanical surveys have consistently reported the use of *D. microcarpum* in polyherbal formulations for anemia and related blood disorders (Kouyaté and Van Damme 2006, Busari and Mufutau 2017). By demonstrating fraction-specific antisickling activity and identifying bioactive compounds, this study bridges the indigenous herbal medicine knowledge with modern pharmacological evidence, supporting integration of traditional medicine into formal healthcare systems. However, caution is warranted in promoting the use of *D. microcarpum* in traditional medicine for SCD as the current findings are based solely on *in vitro* assays, and the pharmacological effects, safety, and efficacy in humans remain unverified. Without standardized dosing, compound characterization and clinical validation, indiscriminate use may pose risks or lead to ineffective treatment outcomes.

While our study provides valuable insights, study has limitations to be considered when interpreting its findings. First, the antisickling assays were conducted exclusively *in vitro*, which may not fully capture the complexity of SCD pathophysiology *in vivo*. Second, the study did not isolate individual compounds from the butanol fraction, making it difficult to attribute the observed activity to specific phytochemicals. Third, the lack of mechanistic assays limits understanding of how the active compounds interact with hemoglobin or erythrocyte membranes. Moreover, the osmotic fragility assay results suggest potential

membrane-disruptive effects that could be associated with saponins content, which warrants further investigation. Finally, inter-individual variability among blood donors was not explored.

Future work will include MS/MS spectra for all features and compare them with authentic standards under identical LC-MS conditions to upgrade annotations to Level 1 where possible, as recommended by MSI and subsequent best-practice documents for high-confidence metabolite identification (Sumner, Amberg et al. 2007, Salek, Steinbeck et al. 2013). Future studies should focus on *in vivo* studies using animal models of SCD are essential to validate efficacy and safety under physiological conditions. Mechanistic studies, including molecular docking, membrane interaction assays, and oxidative stress profiling, could elucidate the pathways through which these compounds exert their effects. Additionally, formulation development and pharmacokinetic studies will be necessary to assess bioavailability and optimize dosing. Collaborative clinical research could facilitate early-phase translation of ethnomedicinal knowledge into evidence-based therapeutic options.

Conclusion

The findings of this study support the folkloric use of *D. microcarpum* as component of a polyherbal formulation and highlight its potential for development into safe, affordable, plant-based therapies, particularly in resource-limited settings. Importantly, this research lays critical groundwork in the field of ethnopharmacology by scientifically validating a traditionally used plant and identifying promising leads for drug development. In regions with high SCD prevalence and limited access to conventional therapies, such studies are essential for expanding the therapeutic landscape. This work contributes to the long-term goal of developing accessible, acceptable, and evidence-based interventions for the unmet health and clinical need.

Declarations

Ethical statements

All research procedures were conducted under ethical approval granted by the Ethical Committee of Kano State Ministry of Health (MOH/Off/797/TI/759) and Ahmadu Bello University, Zaria (ABUCAUC/2021/115). Venous blood was withdrawn from each participant following informed consent and in accordance with approved institutional study protocols.

Conflict of Interest statement

The authors have no conflicts of interest to declare.

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Author contributions

Research conceptualization and study design were conceived by AIM, HI, AAA, TY, and YA. AIM and HI conceived and designed the experiments along with AAA and TY. AIM acquired the data. AIM and YA conducted the statistical analysis and drafted the manuscript. All authors contributed intellectual content during the analysis, manuscript drafting, or revision, and approved the final version of the manuscript.

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Data Availability

Data will be available upon reasonable request.

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Figures

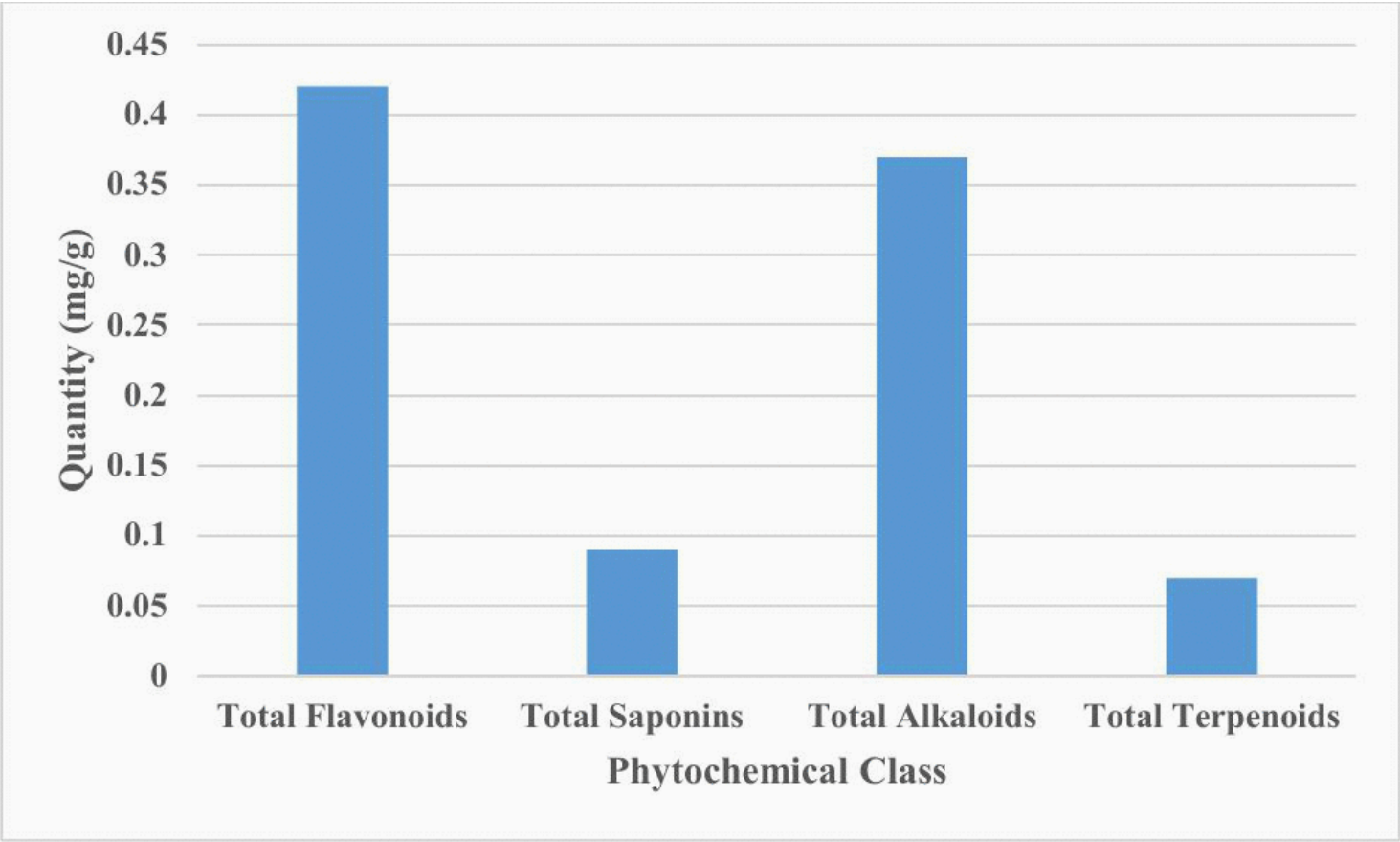


Figure 1

Quantitative Phytochemical Analysis of Crude Extracts (Gravimetric Assay)

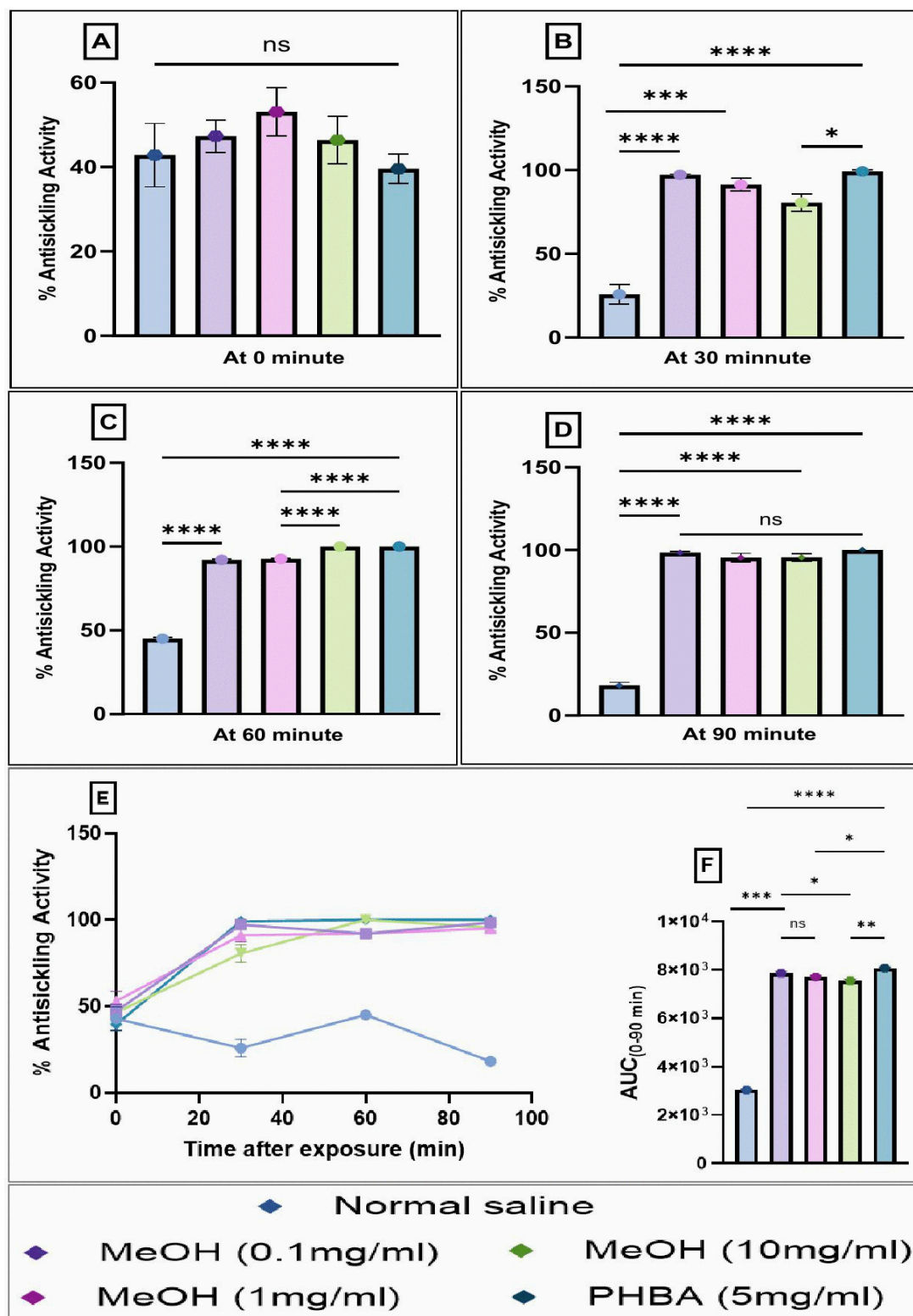


Figure 2

Antisickling activity at different time points (2A-D), overall trend (2E), and corresponding AUCs (2F) for treatment with MeOH (0.1 and 10 mg/ml), PHBA (5 mg/ml), and normal saline. Compared to normal saline treatment, MeOH and PHBA treatments rapidly increased antisickling activity after exposure, reaching near-complete reversal by 60 min and maintaining steady-state through 90 minutes. N=3, $P < 0.05$, $P^{**} < 0.01$, $P^{***} < 0.001$, $P^{****} < 0.0001$.

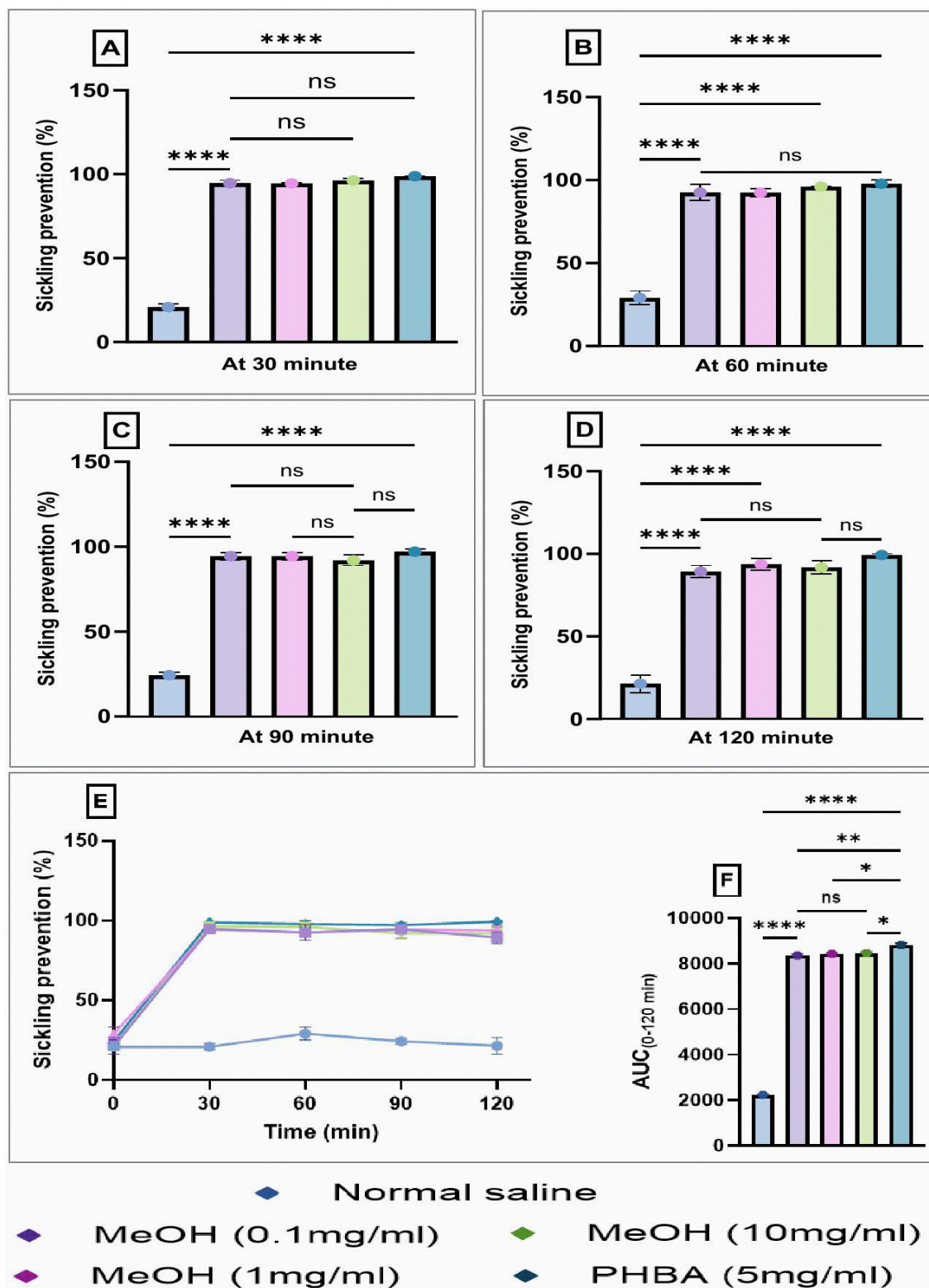


Figure 3

Sickling prevention activity at different time points (3A-D), overall trend (3E), and corresponding AUCs (3F) for different treatments. Compared to normal saline treatment, MeOH and PHBA rapidly achieved near-complete prevention (~100%) by 30 min and remained at steady for 120 min post exposure (****p<0.0001) with slight differences among active treatments.

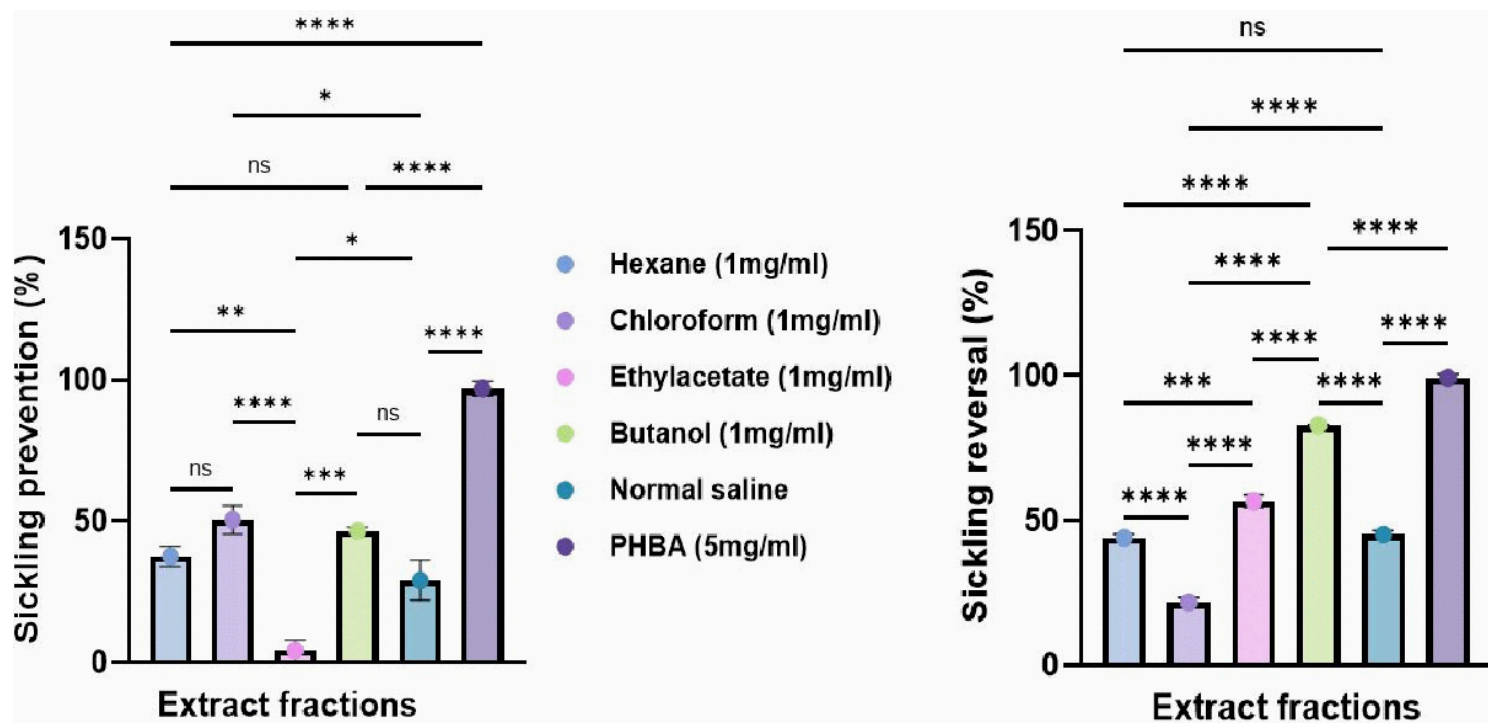


Figure 4

Antisickling activities of different fractions of plant extract fractions showing percentage sickling prevention (left) and reversal (right) effects at 1 mg/ml compared to PHBA (5 mg/ml) and normal saline.

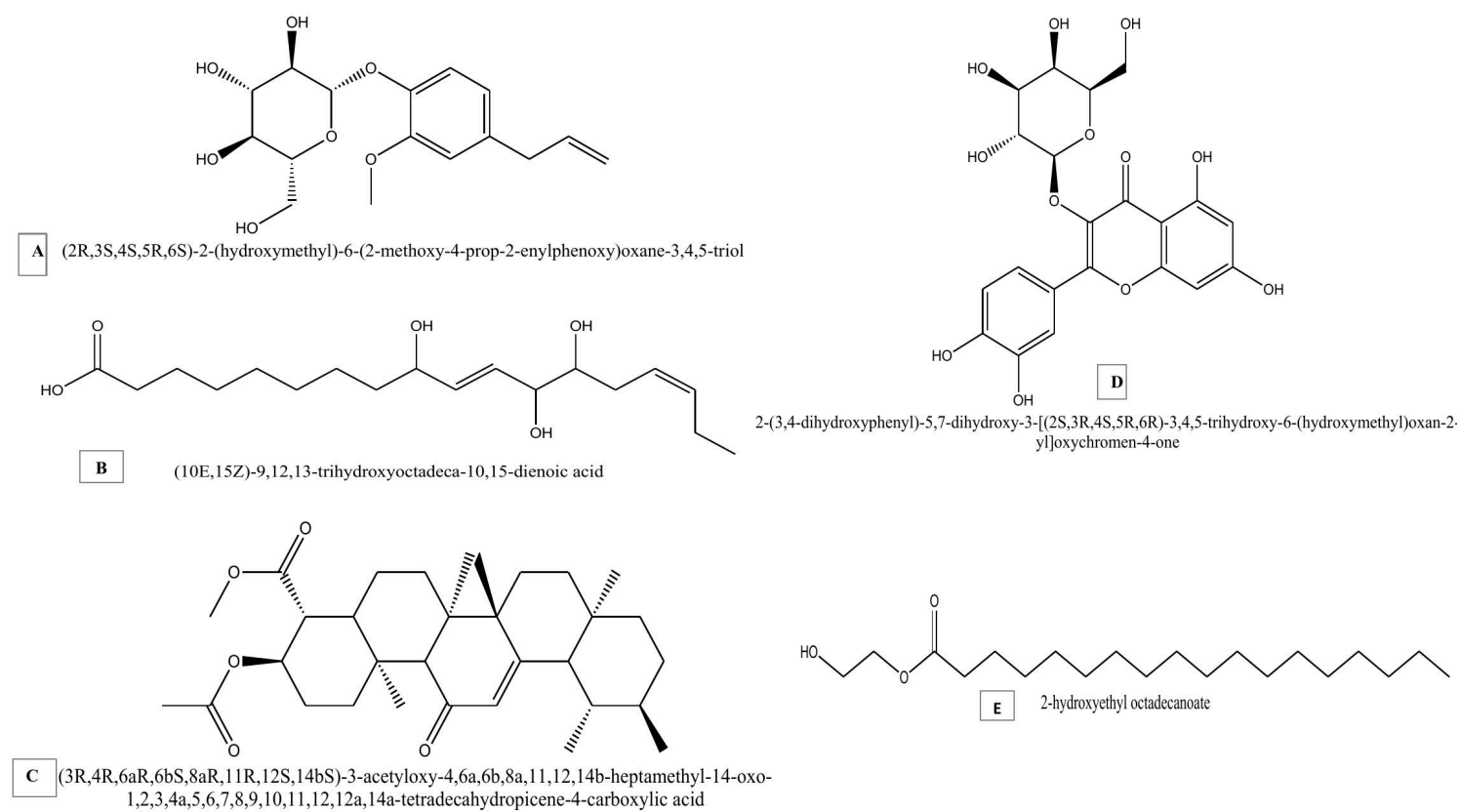


Figure 5

LC–MS chromatogram and proposed structures of compounds detected in the butanol fraction of the D. microcarpum leaf extract. The putative structures were deduced from MS fragmentation behavior and are shown with their corresponding IUPAC names. Structural assignments remain tentative pending NMR confirmation.

Supplementary Files

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