ANTISICKLING ACTIVITIES OF *CASSIA SIEBERIANA* DC (LEGUMINOSAE)

BY

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ABSTRACT

Sickle Cell Disease (SCD) affects primarily black populations. There are few drugs available for the management of SCD with deleterious side effects complicating the condition. Although many plants including *Cassia sieberiana* have been documented in the management of SCD, there is paucity of scientific evidence for their effectiveness and bioactive constituents. This study was designed to investigate the antisickling activities of morphological parts of *C. sieberiana*, isolate and characterise their constituents.

The powdered samples of *C. sieberiana* (FHI- 112359) root, seed, pericarp and whole fruit were extracted into 70% ethanol by Soxhlet extraction and water by reflux. The extracts were used to either inhibit or reverse sodium metabisuphite-induced sickling of HbSS erythrocytes from SCD patients in steady state, *in vitro*. The ethanol extract of the most active plant part (whole fruit) was successively partitioned to give *n*-hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and aqueous fractions. The fractions were screened for inhibition and reversal of sickling and inhibition of haemoglobin polymerisation. The percentage of inhibition, reversal and rate of decrease in HbSS polymerisation were calculated. The DCM and EtOAc fractions were purified using column and vacuum liquid chromatographic (VLC) techniques. Chromatographic fractions were screened for inhibitory and reversal activities and the active fractions were further purified using column and preparative thin layer chromatography to isolate compounds. Structures of isolated compounds were identified using 1D and 2D NMR (^{1}H and ^{13}C). Data were analysed using one-way ANOVA followed by Student t-test at $\alpha_{0.05.}$

The ethanol extract of *C. sieberiana* whole fruit (CSF) at 180 minutes, exhibited the highest inhibitory activity (83.7±1.3)% compared to seed (65.7±2.2)%, pericarp (10.9±1.2)% extracts and was significantly different from vanillic acid-reference standard (50.4 ± 0.4)% and water whole fruit (51.4±1.6)% extract, while the root ethanol extract lysed the erythrocytes. The root ethanol extract exhibited a reversal activity of (88.9±0.8)%, compared to whole fruit (82.3±0.8)%, seed (82.2±1.0)% and pericarp extracts (55.6±1.4)%; para hydroxy benzoic acid reference standard (86.0±0.6)% and water whole fruit extract (84.4±1.9)%. The CSF exhibited both

inhibitory and reversal activities and also decreased the rate of HbSS polymerisation by (73.5±0.06)%. The DCM and EtOAc fractions exhibited comparable inhibitory (77.9±1.1)%; (77.3±2.9)% and reversal (73.5±1.4)%; (76.8±3.8)% activities, respectively. The EtOAc and aqueous partitioned fractions decreased the rate of HbSS polymerisation by (80.6±0.02)% and (84.2±0.03)%, respectively, which were significantly lower than that of ascorbic acid (92.3±0.002)%. The DCM column fractions B_3 and D_3 exhibited inhibitory activities of (87.9±0.1)% and (91.7±1.5)%, respectively, which were significantly higher than the activities of all other column fractions. Fractions B_3 (70.7±1.6)% and D_3 (86.2±1.4)% also exhibited reversal activities. Nine phenolic compounds were isolated from; EtOAc fraction (E2, E3 and E4) and DCM fraction (M2, M3, G2, G3 and G4). One of the compounds - E3 was characterised as 4′-methoxy-epiafzelechin.

The use of *Cassia sieberiana* in the management of sickle cell disease in ethnomedicine has been justified. The isolated compounds, especially 4′-methoxy-epiafzelechin could serve as potential templates for the development of therapeutic agents with anti-sickling properties.

DEDICATION

I dedicate this project to GOD ALMIGHTY, the all Knowing ONE, who is my strength, shield, pillar and fortress.

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Fatokun Omolola Temitope

CERTIFICATION

I certify that this project was carried out under my supervision, by Mrs Omolola Temitope Fatokun of the Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Oyo state, Nigeria.

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LIST OF ABBREVIATIONS

SCA: Sickle Cell Anaemia

SCD: Sickle Cell Disease

HbSS: Haemoglobin S

RBCs: Red Blood Cells

pTLC: preparative Thin Layer Chromatography

VLC: Vacuum Liquid Chromatography

MeOH: Methanol

NMR: Nuclear Magnetic Resonance spectroscopy

^{1}H: Proton NMR

^{13}C: Carbon 13 NMR

DEPT: Distortionless Enhancement by Polarization Transfer

COSY: ^{1}H-^{1}H Correlation Spectroscopy

HSQC: Heteronuclear Single Quantum Coherence Spectra

HMBC: Heteronuclear Multiple Bond Correlation