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Phytocomponents from *Anacardium occidentale*, *Psidium guajava*, and *Terminalia catappa* altered membrane osmotic stability of sickle erythrocytes

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Abstract

Background: The present study identified, quantified, and characterized the combinations of phytocomponents from fractionated leaf extracts of *Anacardium occidentale*, *Psidium guajava*, and *Terminalia catappa* that stabilized sickle erythrocyte membrane against osmotic stress, or otherwise, using standard chromatographic-spectrophotometric techniques, namely GC-MS, FT-IR, and UV-visible systems.

Results: The percentage hemolysis of the control sample, in 0.9 g/100 mL NaCl, was 35.08 ± 11.64%, whereas those of the samples containing 40 mg/100 mL, 60 mg/100 mL, and 80 mg/100 mL of ethylacetate extracts of *T. catappa* ranged between 31.82 ± 8.32 and 39.18 ± 6.94%. Ethylacetate extract of *T. catappa* contained comparative high quantities of hexadecanoic acid methyl ester, 9,11-octadecadienoic acid, methyl ester, (E, E)-, trans-13-octadecenoic acid methyl ester, and methyl stearate. FT-IR and UV-visible spectra showed that ethylacetate extract of *T. catappa* contained aromatic compounds as well as nitro-compounds, phenolics, and esters.

Conclusion: To mention but a few, the combinations of major phytocomponents that stabilized sickle erythrocyte membrane against osmotic stress were hexadecanoic acid, methyl ester, 11-octadecenoic acid, methyl ester, dibutyl phthalate, pentacosane, trans-13-octadecenoic acid, methyl ester, whereas the minor phytocomponents include methyl tetradecanoate, methoxyacetic acid, 3-pentadecyl ester, methyl stearate, hexadecanoic acid, isoxazole, 4,5-dimethyl-.

Keywords: Hemolysis, Mean corpuscular fragility, Osmotic stability, Phytocomponents, Sickle erythrocyte

1 Background

The cashew tree (*Anacardium occidentale* Linn.), native to tropical America, is often found growing wild in the central plains of Brazil and later cultivated in many parts of the Amazon rainforest. Stem bark, leaves, and shell oil around the nuts are used for astringent preparations and remedies against inflammatory disorders, and therefore exploited for the treatment of ulcers and diarrhea [1–3]. Although acute, sub-acute toxicity and genotoxic effects of hydro-ethanolic extract of *A. occidentale* on biologic systems have been reported, the crude extract did not produce toxic symptoms in rats in doses up to 2000 mg/kg [3]. However, the report showed that the extract induced frame-shift mutation, base-pair substitution, and chromosomal damage but the toxic effects were less deleterious than the clastogenic effect of cyclophosphamide on Swiss mice micronucleus [3]. The major bioactive principles present in leaves of *A. occidentale* are the flavonoids and tannins [3, 4] along with the fruit phenolic lipids that exhibit toxic effects in biologic systems [3, 5]. The notable flavonoids present in leaves of *A. occidentale* are quercetin-3-O-rhamnoside, kaempferol-3-O-methyl-ether, myricetin-3-O-rhamnoside, kaempferol-3-O-rhamnoside, and amentoflavone [4] as well as the proanthocianidins [6]. Reports also showed that *A. occidentale* leaves are rich in phenols, phlobotannins, cyanogenic glycosides, saponins, (3E)-3,7-dimethylocta-1,3,6-triene, and (1S,6S,7S,8S)-1,3-dimethyl-8-propan-2-yltricyclo [4.4.0.$0^{2,7}$] dec-3-ene [7, 8].

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Guava (*Psidium guajava* Linn.), which belongs to the Myrtaceae family, is often found in tropical and semi-tropical regions of the world. The leaves of *P. guajava* contain substantial quantity of lectin, which is why the leaf extract is used as herbal remedies for intestinal infections caused by pathogens like *Escherichia coli* [9, 10]. Previous reports also showed that mature leaf extracts of *P. guajava* exhibited antimicrobial properties due to their substantial content of flavonoids, namely quercetin-3-O-*α*-L-arabinofuranoside, quercetin-3-O-*β*-D-arabinofuranoside, quercetin-3-O-*β*-D-glucoside, quercetin-3-O-*β*-D-galactoside, and quercetin-3-O-*β*-D-arabinofuranoside [11] along with the presence of squalene and azulene, which are antifungals [12]. The diverse medicinal usefulness of decoctions of guava leaves has been extensively reported elsewhere [11, 13–16].

The Indian almond tree (*Terminalia catappa* Linn.) belongs to the Combretaceae family. Indian almond leaves contain flavonoids such as kaempferol, quercetin, and tannins. Flavonoids are protective against degenerative diseases, such as cancer, diabetes, cardiovascular diseases, and cataract [17–21]. The antioxidant phytochemicals in *T. catappa* leaves include punicalagin, punicalin, terfluvina A and B, chebulic acid, benzoic acid, and cumaric acid and its derivatives [22]. The vast medicinal usefulness, based on empirical data and traditional medicinal practices, of leaf extracts of *T. catappa* are summarized elsewhere [22–24].

When erythrocytes are suspended in hypotonic sodium chloride (NaCl) solution of diminishing osmolality, the uptake of water by the erythrocyte is both instantaneous and quantitative. In practical terms, the quantity of hemoglobin released into the hypotonic solutions following hemolysis is a measure of erythrocyte osmotic fragility index [25–27]. Accordingly, osmotic fragility index defines the capacity of the erythrocytes to withstand hemolysis when subjected to osmotic stress [27]. Osmotic fragility test, in which the population of hemolyzed erythrocytes is measured as a function of variability in osmolality of erythrocyte suspension, is primarily diagnostic of hereditary disorders of erythrocyte membrane cytoskeletal structures such as hereditary spherocytosis and other vast groups of the hemolytic anemias [27–32]. The extrinsic and intrinsic factors that influence erythrocyte osmotic fragility index are summarized elsewhere [27, 30].

Sickle cell disease (SCD) is a hereditary autosomal recessive erythrocyte disorder caused by mutant hemoglobin molecules (sickle cell hemoglobinopathy; $\alpha_2\beta_2^s$) that polymerize under low oxygen tension within the microvasculature. Intra-erythrocytic polymerization of deoxygenated sickle hemoglobin, in turn, promotes distortion of the erythrocytes that take up the sickle morphology [33–35]. The functional and structural defective erythrocytes elicit multiple clinical disorders, notably; severe anemia, vasoocclusion, multiple organ damage, and splenomegaly associated secondary infections [36]. The biological activities and identities of chemical constituents from medicinal plants used for the management of SCD have been exhaustively reviewed elsewhere [37, 38].

Sickle erythrocytes are relatively fragile and frequently undergo spontaneous osmotic lysis because of low elasticity and rigidity of their membrane structures [35]. The enhanced membrane fragility index is further exacerbated by high oxidative stressed status of sickle erythrocytes [39–41]. Stabilization of erythrocyte membrane structures by herbal extracts is attributable to antioxidant activities and membrane chaotropic agent antagonists of phytocomponents from diverse plants [21, 26, 42–44]. Because of limited scope of the previous investigations [21, 26, 43], the combinations of phytocomponents from the herbal extracts that stabilized the erythrocyte membrane against osmotic stress were not identified, quantified, and characterized. Spectrometric methods, viz. Fourier transform-infrared spectroscopy (FT-IR) and ultraviolet-visible spectroscopy (UV-visible) complement gas chromatography-mass spectroscopy (GC-MS) protocols in terms of providing comprehensive insights into the molecular characteristics and quantification of phytocomponents from plant materials [21, 45–49].

In view of the established relatively high fragility of sickle erythrocytes and medicinal usefulness of vast array of phytocomponents, the present study investigated the capacities of fractionated leaf extracts of *A. occidentale*, *P. guajava*, and *T. catappa* to stabilize sickle erythrocyte membrane against osmotic stress using *in vitro* models. Furthermore, the present study identified, quantified, and characterized the combinations of phytocomponents from fractionated leaf extracts of *A. occidentale*, *P. guajava*, and *T. catappa* that stabilized sickle erythrocyte membrane against osmotic stress, or otherwise, using standard chromatographic-spectrophotometric techniques, namely GC-MS, FT-IR, and UV-visible systems.

2 Materials

2.1 Collection of plant materials

Botanical gardens located within the environment that lies on Latitude 5° 30.2237′ N; Longitude 7 2.6277′ E, served as sources of fresh leaves of *A. occidentale*, *P. guajava*, and *T. catappa*. The microclimatic conditions of the botanical gardens are summarized thus; sea-level: 73 m, temperature range: 19.4–30 °C, average annual rainfall volume: 2219 mm, and soil type: sandy loam. The leaves were harvested between the 2nd and 7th April 2019. The leaves of the selected plants were identified and authenticated by Professor F.N. Mbagwu of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. The designated voucher numbers were as follows: *A. occidentale*: IMSUH 009; *P. guajava*: IMSUH 010; *T. catappa*: IMSUH 011. Samples of the plant materials were deposited in the herbarium for reference purposes.

3 Methods

3.1 Preparation, extraction, and fractionation of leaf extracts

The preparation and extraction of the plant materials was according to the methods previously described [50]. Three hundred grams of the ground sample was subjected to repeated cycles of Soxhlet extraction protocol in 2000 mL of ethanol/water mixture; 1:1 *v/v* for 18 h. The hydro-ethanolic leaf extracts were fractionated according to the modified methods previously described [51]. The crude hydro-ethanolic leaf extracts were fractionated by progressive partitioning with equal volumes of solvents in the order of increasing polarities; viz. petroleum ether, *n*-hexane, chloroform, and ethylacetate.

The fractionated leaf extracts were concentrated under reduced pressure for 24 h at 50 °C using a rotary evaporator (Büch Rotavapor R-200). The separate residues were finally dried in anhydrous calcium chloride (desiccant) embedded desiccator. The yield of the extract per 100 g dry leaf sample was calculated thus:

$$\text{Percentile yield} = \frac{\text{Weight of dried extract}}{\text{Weight of dried leaf sample}} \times 100 \quad (1)$$

The fractionated leaf extracts were suspended in given volumes of phosphate-buffered saline (PBS; pH = 7.4), osmotically equivalent to 9.0 g/L NaCl {9.0 g NaCl, 1.71 g $Na_2HPO_4.2H_2O$, and 2.43 g $NaH_2PO_4.2H_2O$ per liter} to give standard solutions of the extracts used for osmotic stability studies of sickle erythrocyte membrane.

3.2 Exclusion criteria

Exclusion criteria for participants were according to guidelines previously proposed [52]. Participants on medications for at least 4 weeks prior to blood sampling were excluded from this study. Additionally, the collected blood samples were thoroughly checked for the presence of clot and hemolysis before used for the experiment.

3.3 Collection and preparation of blood samples

One hundred eight participants of HbSS genotype donated 5.0 mL of venous blood samples. All blood donors were consenting individuals attending clinics between the months of 7th of May and 28th of July, 2019 at Rehoboth Christian Medical Center, Nwaoruebi and Easter Summit Specialist Clinics and Maternity, Amakohia. The clinics are located in Owerri, Imo State, Nigeria. Venous peripheral blood was drawn from the forearm of participants using vacuum blood sampling disodium ethylene diamine tetraacetate (EDTA-Na_2) tubes [52]. The confirmatory test of the blood samples was carried out using electrophoretic methods previously described [53]. The preparation of blood samples was according to previous reports [24]. The erythrocytes were finally re-suspended in 5.0 mL of PBS and used for osmotic stability studies of the sickle erythrocyte membrane.

3.4 Membrane osmotic stability studies

Erythrocyte osmotic fragility index was measured according to the method described [26]. In a total of four sets (T1–T4) of three test tubes each, 5.0 mL of PBS of varying concentrations equivalent to 0.9–0.3 g/100 mL NaCl were introduced into the corresponding test tubes, such that T1 = 0.9 g/100 mL, T2 = 0.6 g/100 mL, and T3 = 0.3 mg/100 mL. A 5.0 mL of distilled water was added in the fourth set of test tubes (T4). For the test analyses, 0.5 mL of three increasing concentrations (40 mg/100 mL, 60 mg/100 mL, and 80 mg/100 mL) of each of the separate fractionated leaf extracts were added in different sets of test tubes in a corresponding order of concentrations of the fractionated leaf extracts. To each test tube, 0.1 mL of HbSS erythrocyte suspension (10% hematocrit) was added and mixed thoroughly by inverting the tubes several times. For the control experiment, the same procedure was repeated but devoid of suspensions of the fractionated leaf extracts, which was replaced by PBS concentration equivalent to 0.9 g/100 mL NaCl. The erythrocyte suspensions were allowed to stand for 30 min at room temperature (24–27 °C) after which the contents were centrifuged at 1200×*g* for 10 min. The relative quantity of hemoglobin released into the supernatant was measured at λmax = 540 nm using a spectrophotometer (Digital Blood Analyzer; SPECTRONIC 20; Labtech, LabX, Bay Street, Midland, ON, Canada). PBS concentration equivalent to 0.9 g/100 mL NaCl served as blank.

The range of values of percentage hemolysis in PBS of varying concentrations equivalent to 0.9–0.3 g/100 mL NaCl (T1–T3) and in distilled water (T4) were obtained by evaluating the quotient of absorbance of the content of each corresponding test tubes (T1–T3) and the fourth test tube (T4) containing distilled water and multiplied by a factor of 100. Thus:

$$\%H_{T1-T4} = \frac{A_{T1-T3}}{A_{T4}} \times 100 \quad (2)$$

Where;

$\%H_{T1-T4}$: range of values of percentage hemolysis (%).

A_{T1-T3}: absorbance of the supernatant of erythrocyte suspension in PBS of varying concentrations equivalent to 0.9–0.3 g/100 mL NaCl.

A_{T4}: absorbance of the supernatant of erythrocyte suspension in distilled water.

Note: the erythrocytes suspension in distilled water (T4) served as a 100% hemolysis point.

The corresponding PBS concentration in g/100 mL of NaCl, which caused 50% hemolysis, was the mean corpuscular fragility (MCF) index. The MCF values were interpolated from the cumulative erythrocyte osmotic fragility curves obtained by plotting the percentage hemolysis against PBS concentrations in g/100 mL of NaCl.

The relative capacities of the fractionated leaf extracts to stabilize erythrocyte membrane were evaluated as percentage of the quotient of the difference between the MCF values of the test and control samples to that of the control sample as previously described [26]. Thus:

$$\%\mathrm{RMS} = \frac{\mathrm{MCF}_{\mathrm{Control}} - \mathrm{MCF}_{\mathrm{Test}}}{\mathrm{MCF}_{\mathrm{Control}}} \times 100 \quad (3)$$

Where;

%RMS: relative membrane stability (%).

Note: negative value of %RMS is indicative of destabilization by the fractionated leaf extract.

3.5 Spectrometry

The identification, quantification, and characterization of phytocomponents from fractionated leaf extracts of *A. occidentale*, *P. guajava*, and *T. catappa* were carried out using standard chromatographic-spectrophotometric protocols, viz. GC-MS systems {Agilent 7890A GC system set up with 5975C VL MSD, Agilent Technologies, Inc., Santa Clara, CA, USA; The MS system was accomplished in Electron Ionization (EI) mode with Selected Ion Monitoring (SIM)}as well as FT-IR and UV-visible instruments (PerkinElmer Spectrophotometer, USA) according to the methods previously described [48, 54, 55]. The capillary column specification of the GC system was DB-5MS (30 m × 0.25 mm, film thickness of 0.25 μm) with a temperature program set as follows: initial temperature 50 °C held for 1.00 min, 5 °C per min to 100 °C, 9 °C per min to 200 °C held for 7.89 min, and the total run time was 30 min. The injection mode and volume were set at 20:1 and 1.0 μL respectively. The flow rate of helium as a carrier gas was set at 0.811851 mL/min. The ion source temperature and quadruple temperature of the MS system were set at 230 °C and 150 °C, respectively.

3.6 Statistical analyses

The data collected were expressed in means (X) ± SD and analyzed in one-way ANOVA and least significance difference (LSD). The comparison was made between groups and significance was established by ANOVA at 95% confidence level. Difference of $p < 0.05$ was considered statistically significant.

4 Results

4.1 Percentage yields of fractionated leaf extracts

The aggregate yields of the fractionated leaf extracts, which include petroleum ether, *n*-hexane, chloroform, ethylacetate, and residual aqueous extracts were as follows: *A. occidentale* (13.017 g per 100 g dry leaf sample), *P. guajava* (9.627 g per 100 g dry leaf sample), and *T. catappa* (10.060 g per 100 g dry leaf sample). However, the residual aqueous extract fractions gave relatively high yields in the following proportions: *A. occidentale* (10.20 g per 100 g dry leaf sample), *P. guajava* (7.20 g per 100 g dry leaf sample), and *T. catappa* (9.00 g per 100 g dry leaf sample).

4.2 Sickle erythrocytes osmotic fragility in the presence of fractionated leaf extracts of *A. occidentale*

Figure 1a–e showed sickle erythrocytes osmotic fragility in PBS of varying concentrations equivalent to 0.9–0.3 g/100 mL NaCl in the presence of fractionated leaf extracts of *A. occidentale*. Figure 1a showed that the control sample exhibited 38.15 ± 6.31% hemolysis in PBS concentration equivalent to 0.9 g/100 mL NaCl, whereas 80 mg/100 mL petroleum ether extract of *A. occidentale* caused 73.53 ± 10.12% hemolysis. The percentage hemolysis in the presence of 40 mg/100 mL petroleum ether extract of *A. occidentale* in PBS concentration equivalent to 0.9 g/100 mL NaCl was not significantly different ($p > 0.05$) from that of the control sample. The lowest level of percentage hemolysis in PBS concentration equivalent to 0.9 g/100 mL NaCl was in the presence of 60 mg/100 mL ethylacetate extract of *A. occidentale*. Additionally, PBS concentration equivalent to 0.3 g/100 mL NaCl caused 92.98 ± 4.45% hemolysis in the sample containing 80 mg/100 mL ethylacetate extract of *A. occidentale*.

Figure 1b showed that in PBS concentrations equivalent to 0.6 g/100 mL and 0.9 g/100 mL NaCl, the percentage hemolysis of the control sample was not significantly different ($p > 0.05$) from those of the samples containing 40 mg/100 mL, 60 mg/100 mL, and 80 mg/100 mL *n*-hexane extracts of *A. occidentale*. Conversely, 60 mg/100 mL *n*-hexane extract of *A. occidentale* caused 75.53 ± 14.01% hemolysis, which was significantly different ($p < 0.05$) from the control sample as well as the samples containing 40 mg/100 mL and 80 mg/100 mL *n*-hexane extracts of *A. occidentale*.

The percentage hemolysis of the samples containing 40 mg/100 mL, 60 mg/100 mL, and 80 mg/100 mL chloroform extracts of *A. occidentale* in PBS concentrations equivalent to 0.3–0.9 g/100 mL NaCl were not significantly different ($p > 0.05$) from the control sample. Specifically, the percentage hemolysis of the control sample and the test samples in PBS concentration equivalent to 0.3 g/100 mL NaCl varied within a relatively narrow range of 12.22 ± 5.82–19.60 ± 3.43% (Fig. 1c).

The percentage hemolysis of the samples containing 40 mg/100 mL, 60 mg/100 mL, and 80 mg/100 mL ethylacetate extracts of *A. occidentale* in PBS concentration equivalent to 0.9 g/100 mL NaCl showed no significant

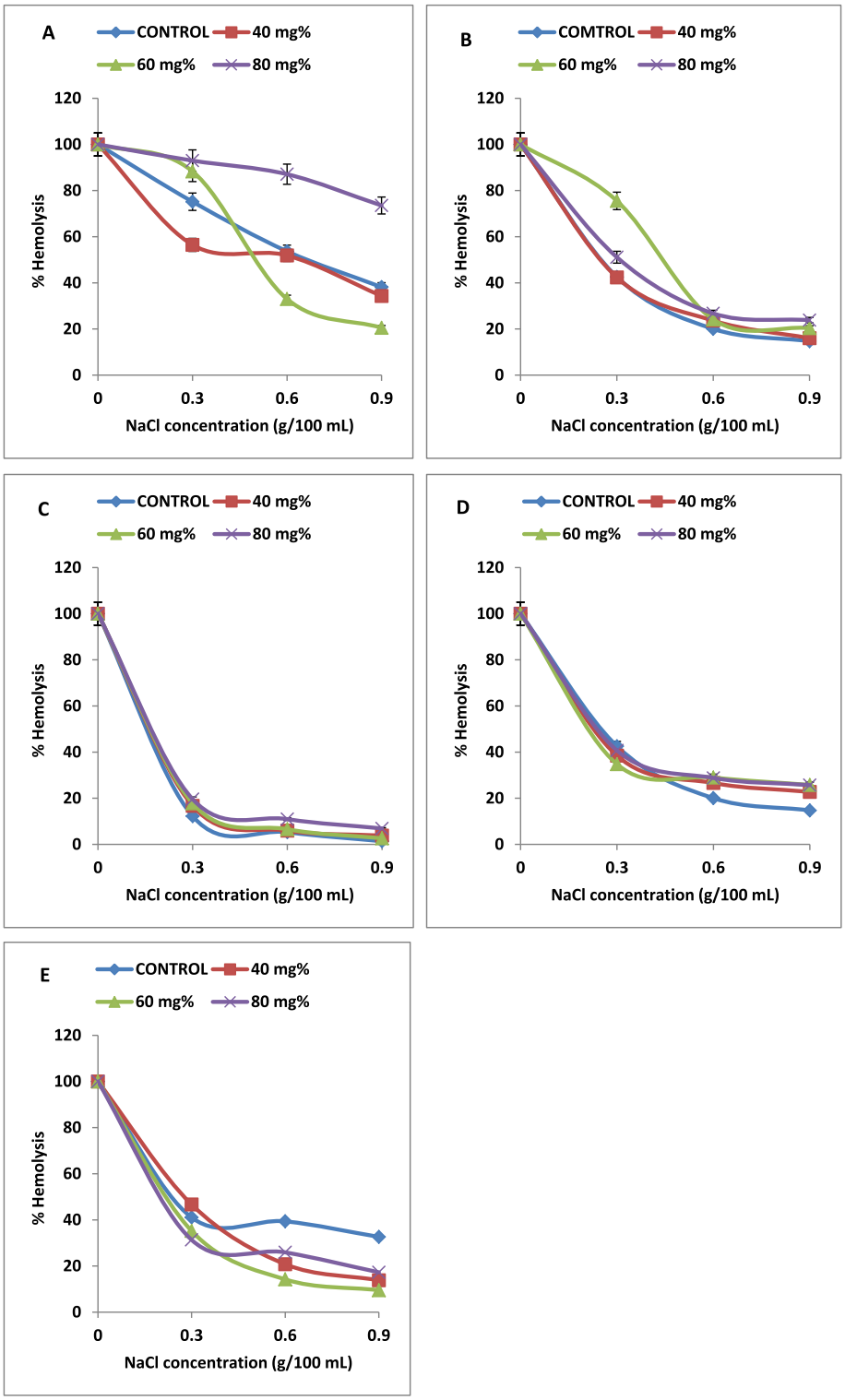

Fig. 1 Erythrocyte osmotic fragility curves: percentage hemolysis of sickle erythrocytes in PBS of varying concentrations equivalent to 0.9–0.3 g/100 mL NaCl in the presence of fractionated leaf extracts of *A. occidentale*. **a** Petroleum ether. **b** *n*-hexane. **c** Chloroform. **d** Ethylacetate. **e** Residual aqueous

difference ($p > 0.05$). Likewise, the percentage hemolysis of the control sample in PBS concentration equivalent to 0.3 g/100 mL NaCl was not significantly different ($p > 0.05$) from the sample containing 80 mg/100 mL ethylacetate extract of *A. occidentale* (Fig. 1d).

The percentage hemolysis of the control sample in PBS concentrations equivalent to 0.6 g/100 mL and 0.9 g/100 mL NaCl were significantly higher ($p < 0.05$) than those of samples containing 40 mg/100 mL, 60 mg/100 mL, and 80 mg/100 mL residual aqueous extracts of *A. occidentale*. Conversely, the percentage hemolysis of the control sample in PBS concentration equivalent to 0.3 g/100 mL NaCl was significantly lower ($p < 0.05$) than that of the sample containing 40 mg/100 mL residual aqueous extract of *A. occidentale* (Fig. 1e).

4.3 Sickle erythrocytes osmotic fragility in the presence of fractionated leaf extracts of *P. guajava*

Figure 2a–e showed sickle erythrocytes osmotic fragility in PBS concentrations equivalent to 0.3–0.9 g/100 mL NaCl in the presence of fractionated leaf extracts of *P. guajava*. The percentage hemolysis of the control sample in PBS concentrations equivalent to 0.6 g/100 mL and 0.9 g/100 mL NaCl showed no significant difference ($p > 0.05$) from the samples containing 40 mg/100 mL and 80 mg/100 mL petroleum ether extracts of *P. guajava*. Conversely, the percentage hemolysis in the presence of 60 mg/100 mL petroleum ether extract of *P. guajava* in PBS concentrations equivalent to 0.6 g/100 mL and 0.9 g/100 mL NaCl were significantly higher ($p < 0.05$) than that of the control sample (Fig. 2a).

Figure 2b showed that the percentage hemolysis of sickle erythrocytes in the presence of 40 mg/100 mL *n*-hexane extract of *P. guajava* in PBS concentrations equivalent to 0.6 g/100 mL and 0.9 g/100 mL NaCl were significantly higher ($p < 0.05$) than that of the control sample as well as the samples containing 60 mg/100 mL and 80 mg/100 mL *n*-hexane extracts of *P. guajava*. The percentage hemolysis of the sample containing 80 mg/100 mL *n*-hexane extract of *P. guajava* in PBS concentration equivalent to 0.3 g/100 mL NaCl was significantly lower ($p < 0.05$) than that of the control sample as well as the test samples containing 40 mg/100 mL and 60 mg/100 mL *n*-hexane extracts of *P. guajava* (Fig. 2b).

The percentage hemolysis, in PBS concentration equivalent to 0.3 g/100 mL NaCl, in the presence of 40 mg/100 mL and 80 mg/100 mL chloroform extracts of *P. guajava* as well as the control sample showed no significant difference ($p > 0.05$) but was significantly higher ($p < 0.05$) than that of the sample containing 60 mg/100 mL chloroform extract of *P. guajava*. Additionally, the percentage hemolysis of the control sample was significantly lower ($p < 0.05$) than the test samples (Fig. 2c).

Figure 2d showed that the percentage hemolysis of the control sample in PBS concentrations equivalent to 0.3–0.9 g/100 mL NaCl were significantly lower ($p < 0.05$) than those of the samples containing 40 mg/100 mL, 60 mg/100 mL, and 80 mg/100 mL ethylacetate extracts of *P. guajava*. Conversely, percentage hemolysis in PBS concentrations equivalent to 0.3–0.9 g/100 mL NaCl in the presence of 40 mg/100 mL and 80 mg/100 mL ethylacetate extracts of *P. guajava* showed no significant difference ($p > 0.05$) (Fig. 2d).

The percentage hemolysis in PBS concentration equivalent to 0.9 g/100 mL NaCl of the control and test samples showed no significant difference ($p > 0.05$) (Fig. 2e). On the contrary, the percentage hemolysis of the control and test samples showed significant difference ($p < 0.05$) in PBS concentration equivalent to 0.3 g/100 mL NaCl.

4.4 Sickle erythrocytes osmotic fragility in the presence of fractionated leaf extracts of *T. catappa*

Figure 3a–e showed sickle erythrocytes osmotic fragility in varying NaCl concentrations in the presence of fractionated leaf extracts of *T. catappa*. The percentage hemolysis of the sample containing 60 mg/100 mL petroleum ether extract of *T. catappa* in PBS concentration equivalent to 0.9 g/100 mL NaCl was not significantly different ($p > 0.05$) from that of the control sample (Fig. 3a). Furthermore, the percentage hemolysis of the control sample and the sample containing 60 mg/100 mL petroleum ether extract of *T. catappa* was significantly lower ($p < 0.05$) than those of the samples, in PBS concentration equivalent to 0.9 g/100 mL NaCl, containing 40 mg/100 mL and 80 mg/100 mL petroleum ether extracts of *T. catappa* (Fig. 3a).

Figure 3b showed that the percentage hemolysis of the control sample and the sample containing 80 mg/100 mL *n*-hexane extract of *T. catappa* showed no significant difference ($p > 0.05$). However, the percentage hemolysis of the samples containing 40 mg/100 mL and 60 mg/100 mL *n*-hexane extracts of *T. catappa* were significantly higher ($p < 0.05$) than those of the control sample and the sample containing 80 mg/100 mL *n*-hexane extract of *T. catappa*.

Figure 3c showed that increasing order of percentage hemolysis, in PBS concentrations equivalent to 0.3–0.9 g/100 mL NaCl, in the presence of 40 mg/100 mL, 60 mg/100 mL, and 80 mg/100 mL chloroform extracts of *T. catappa* showed no significant difference ($p > 0.05$). Notably, the percentage hemolysis of the control sample in PBS concentrations equivalent to 0.3–0.9 g/100 mL NaCl were significantly higher ($p < 0.05$) than those of the test samples.

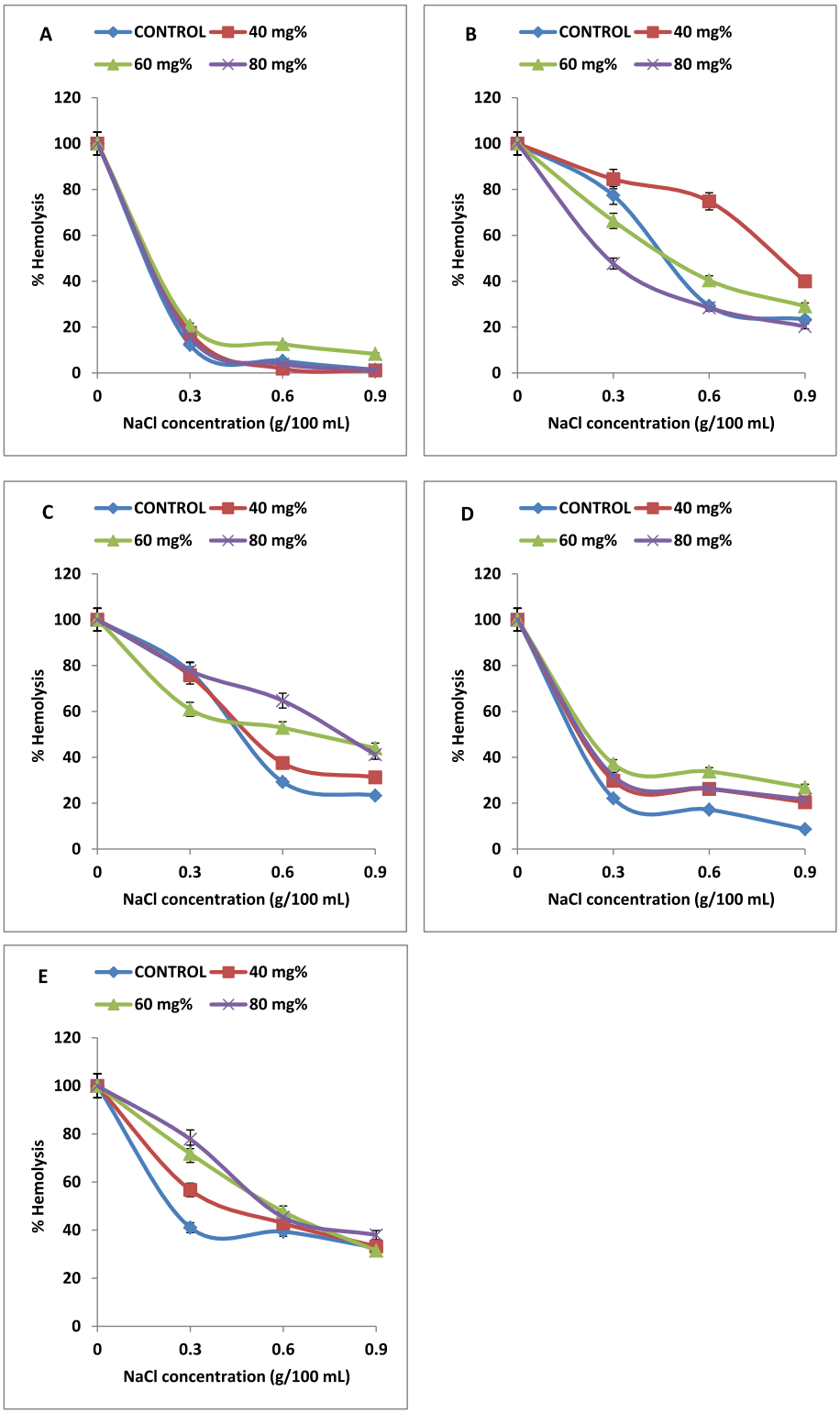

Fig. 2 Erythrocyte osmotic fragility curves: percentage hemolysis of sickle erythrocytes in PBS of varying concentrations equivalent to 0.9–0.3 g/100 mL NaCl in the presence of fractionated leaf extracts of *P. guajava*. **a** Petroleum ether. **b** *n*-hexane. **c** Chloroform. **d** Ethylacetate. **e** Residual aqueous

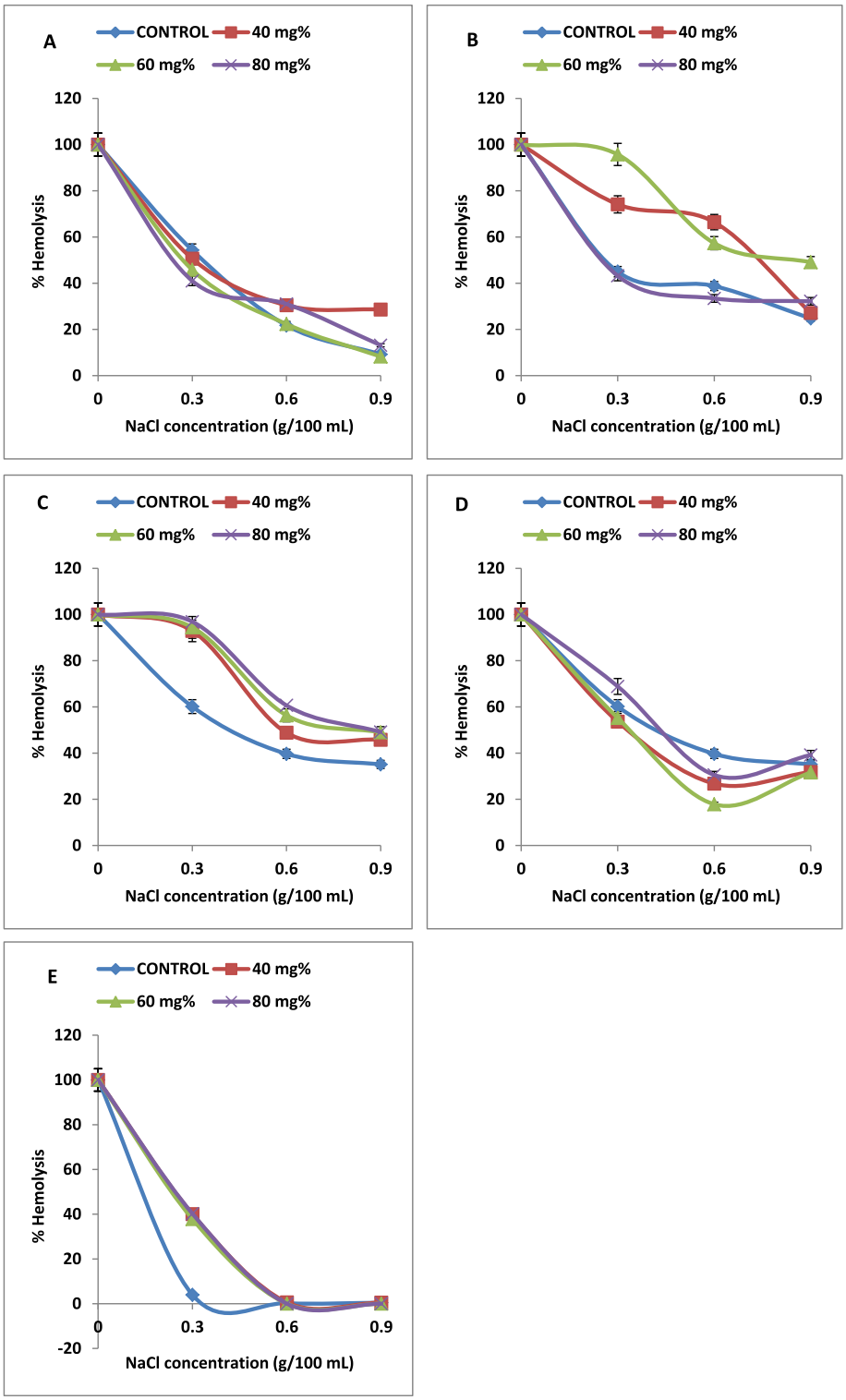

Fig. 3 Erythrocyte osmotic fragility curves: percentage hemolysis of sickle erythrocytes in PBS of varying concentrations equivalent to 0.9–0.3 g/100 mL NaCl in the presence of fractionated leaf extracts of *T. catappa*. **a** Petroleum ether. **b** *n*-hexane. **c** Chloroform. **d** Ethylacetate. **e** Residual aqueous

The percentage hemolysis of the control sample, in PBS concentration equivalent to 0.9 g/100 mL NaCl, was 35.08 ± 11.64%, whereas those of the test samples ranged between 31.82 ± 8.32% and 39.18 ± 6.94% (Fig. 3d). The lowest percentage hemolysis occurred in the sample containing 60 mg/100 mL ethylacetate extract of *T. catappa* in PBS concentration equivalent to 0.6 g/100 mL NaCl.

Figure 3e showed that the sample containing 60 mg/100 mL and 80 mg/100 mL residual aqueous extracts of *T. catappa* in PBS concentration equivalent to 0.9 g/100 mL NaCl exhibited no hemolysis. The percentage hemolysis of the control sample and the sample containing 40 mg/100 mL residual aqueous extract of *T. catappa* were 0.44 ± 0.18% and 0.49 ± 0.09% respectively. The percentage hemolysis of the control sample, in PBS concentration equivalent to 0.3 g/100 mL NaCl, was significantly lower ($p < 0.05\%$) than those of the test samples (Fig. 3e).

4.5 MCF and percentage stability of sickle erythrocytes

The MCF and percentage stability of sickle erythrocytes incubated in fractionated leaf extracts of *A. occidentale*, *P. guajava*, and *T. catappa* is presented in Table 1.

The results showed that 60 mg/100 mL and 80 mg/100 mL petroleum ether extracts of *A. occidentale* stabilized sickle erythrocyte membrane, whereas the sample containing 40 mg/100 mL petroleum ether extract caused the destabilization of sickle erythrocyte membrane. The 80 mg/100 mL petroleum ether extract of *A. occidentale* caused higher erythrocyte membrane stability than that of the sample containing 60 mg/100 mL petroleum ether extract of *A. occidentale*.

Table 1 Mean corpuscular fragility and percentage stability of sickle erythrocytes in the presence of fractionated leaf extracts of *A. occidentale*, *P. guajava*, and *T. catappa*

[Extract fraction]	*A. occidentale*		*P. guajava*		*T. catappa*	
	MCF (g%)	%RMS	MCF (g%)	%RMS	MCF (g%)	%RMS
Petroleum ether						
Control	0.57 ± 0.24	–	0.17 ± 0.01	–	0.34 ± 0.01	–
40 mg%	0.61 ± 0.06	7.02[a]	0.18 ± 0.01	5.88[a]	0.31 ± 0.03	8.82
60 mg%	0.51 ± 0.06	10.53	0.19 ± 0.01	11.76[a]	0.28 ± 0.02	17.65
80 mg%	0.48 ± 0.05	15.79	0.18 ± 0.01	5.88[a]	0.25 ± 0.02	9.00
n-hexane						
Control	0.27 ± 0.05	–	0.47 ± 0.04	–	0.28 ± 0.03	–
40 mg%	0.26 ± 0.02	3.70	0.81 ± 0.02	72.34[a]	0.73 ± 0.04	160.71[a]
60 mg%	0.43 ± 0.06	59.26[a]	0.52 ± 0.09	10.64[a]	0.86 ± 0.01	207.14[a]
80 mg%	0.32 ± 0.06	18.52[a]	0.30 ± 0.06	36.17	0.34 ± 0.15	21.43[a]
Chloroform						
Control	0.17 ± 0.01	–	0.47 ± 0.04	–	0.47 ± 0.06	–
40 mg%	0.18 ± 0.01	5.88[a]	0.51 ± 0.02	8.51 [a]	0.57 ± 0.02	21.28[a]
60 mg%	0.18 ± 0.01	5.88[a]	0.66 ± 0.08	40.43[a]	0.60 ± 0.02	27.66[a]
80 mg%	0.19 ± 0.01	11.76[a]	0.78 ± 0.02	65.96[a]	0.62 ± 0.01	31.91[a]
Ethylacetate						
Control	0.27 ± 0.05	–	0.19 ± 0.02	–	0.47 ± 0.05	–
40 mg%	0.25 ± 0.03	7.41	0.21 ± 0.01	10.53[a]	0.34 ± 0.02	27.66
60 mg%	0.23 ± 0.01	14.81	0.24 ± 0.01	26.32[a]	0.34 ± 0.01	27.66
80 mg%	0.21 ± 0.01	22.22	0.22 ± 0.04	15.79[a]	0.43 ± 0.05	8.51
Residual aqueous						
Control	0.26 ± 0.02	–	0.26 ± 0.02	–	0.16 ± 0.00	–
40 mg%	0.29 ± 0.02	11.54[a]	0.45 ± 0.07	73.08[a]	0.25 ± 0.02	56.25[a]
60 mg%	0.23 ± 0.03	11.54	0.58 ± 0.09	123.08[a]	0.25 ± 0.05	56.25[a]
80 mg%	0.22 ± 0.01	15.38	0.52 ± 0.03	100.00[a]	0.26 ± 0.06	62.50[a]

MCF values are means of 3 determinations ± S.D
%*RMS* relative membrane stability (%), *g*% g/100 mL, *mg*% mg/100 mL
[a]Percentage of membrane destabilization

The sample containing 40 mg/100 mL *n*-hexane extract of *A. occidentale* stabilized sickle erythrocyte membrane by 3.70% (Table 1). Further increase in the concentration of *n*-hexane extract of *A. occidentale* caused the destabilization of sickle erythrocyte membrane. The 60 mg/100 mL *n*-hexane extract of *A. occidentale* exhibited greater capacity to destabilize sickle erythrocyte membrane (Table 1).

Table 1 showed that 40 mg/100 mL and 60 mg/100 mL chloroform extracts of *A. occidentale* exhibited an equal capacity to destabilize sickle erythrocyte membrane. Further increase in the extract concentration exacerbated the destabilizing effect of chloroform extract of *A. occidentale* on the sickle erythrocyte membrane.

The samples containing 40 mg/100 mL, 60 mg/100 mL, and 80 mg/100 mL ethylacetate extracts of *A. occidentale* stabilized sickle erythrocyte membrane in a concentration-dependent manner (Table 1). The capacity of ethylacetate extract to stabilize the sickle erythrocyte membrane was within the range of (7.41–22.22)% (Table 1).

The 40 mg/100 mL residual aqueous extract of *A. occidentale* caused destabilization of the sickle erythrocyte membrane by 11.54%, whereas 60 mg/100 mL residual aqueous extract of *A. occidentale* stabilized sickle erythrocyte membrane by the same magnitude (Table 1). The capacity of the sickle erythrocyte membrane to withstand osmotic stress was further enhanced in the presence of 80 mg/100 mL residual aqueous extract of *A. occidentale* (Table 1).

Table 1 showed that 40 mg/100 mL, 60 mg/100 mL, and 80 mg/100 mL petroleum ether extracts of *P. guajava* caused destabilization of the sickle erythrocyte membrane. A cursory look at Table 1 showed that 40 mg/100 mL and 80 mg/100 mL petroleum ether extracts of *P. guajava* exhibited equal capacity to destabilize sickle erythrocyte membrane.

Table 1 showed that 40 mg/100 mL *n*-hexane extract of *P. guajava* exhibited greater capacity to destabilize sickle erythrocyte membrane than 60 mg/100 mL *n*-hexane extract of *P. guajava*. Further increase in the concentration of *n*-hexane extract of *P. guajava* destabilized sickle erythrocyte membrane by 36.17% (Table 1).

The destabilizing effect of 40 mg/100 mL, 60 mg/100 mL, and 80 mg/100 mL chloroform extracts of *P. guajava* on sickle erythrocyte membrane was in a concentration-dependent manner (Table 1). The capacity of chloroform extract of *P. guajava* to destabilize sickle erythrocyte membrane was within the range of (8.51–65.96)% (Table 1). Likewise, destabilizing effect of ethylacetate extract of *P. guajava* on sickle erythrocyte membrane was within the range of (15.79–26.32)% (Table 1). Furthermore, 40 mg/100 mL, 60 mg/100 mL, and 80 mg/100 mL residual aqueous extracts of *P. guajava* destabilized sickle erythrocyte membrane within the range of (73.08–123.08)%.

Table 1 showed that 40 mg/100 mL, 60 mg/100 mL, and 80 mg/100 mL petroleum ether extracts of *T. catappa* stabilized sickle erythrocyte membrane in a concentration-dependent manner. The maximum stabilizing capacity of sickle erythrocyte membrane by petroleum ether extract of *T. catappa* was 26.47% (Table 1).

The 60 mg/100 mL *n*-hexane extract of *T. catappa* caused the greatest destabilizing effect on sickle erythrocyte membrane compared with the fractionated leaf extracts of *A. occidentale*, *P. guajava*, and *T. catappa* (Table 1). The destabilizing effect of *n*-hexane extract of *T. catappa* on sickle erythrocyte membrane was within the range of (21.43–207.14)% (Table 1). In the same manner, 40 mg/100 mL, 60 mg/100 mL, and 80 mg/100 mL chloroform extracts of *T. catappa* caused destabilization of sickle erythrocyte membrane (Table 1). Table 1 showed that 40 mg/100 mL and 60 mg/100 mL ethylacetate extracts of *T. catappa* exhibited equal capacity to stabilize sickle erythrocyte membrane. However, further increase in the concentration of ethylacetate extract of *T. catappa* reduced the capacity of the extract to stabilize sickle erythrocyte membrane (Table 1).

The samples containing 40 mg/100 mL and 60 mg/100 mL residual aqueous extracts of *T. catappa* caused equal level of destabilization of sickle erythrocyte membrane (Table 1). The destabilizing effect of residual aqueous extract of *T. catappa* on sickle erythrocyte membrane increased with corresponding increase in the concentration of the extract.

4.6 Spectrometry

4.6.1 GC-MS of fractionated leaf extracts of A. occidentale, P. guajava, and T. catappa

The fractionated leaf extracts of *A. occidentale*, *P. guajava*, and *T. catappa* that stabilized sickle erythrocyte membrane against osmotic stress, summarized from Table 1, were as follows: *A. occidentale* (petroleum ether and ethylacetate extracts), *P. guajava* (*n*-hexane extract), and *T. catappa* (petroleum ether and ethylacetate extracts).

Table 2(A–E) summarized the combinations of phytocomponents present in petroleum ether extract of *A. occidentale*, ethylacetate extract of *A. occidentale*, residual aqueous extract of *A. occidentale*, *n*-hexane extract of *P. guajava*, petroleum ether extract of *T. catappa*, and ethylacetate extract of *T. catappa*. Aliphatic compounds in corresponding fractionated leaf extracts of *A. occidentale*, *P. guajava*, and *T. catappa* are also summarized in Table 2(A–E). The corresponding GC-MS chromatograms of the selected extracts are illustrated in Fig. 4a–e.

The five major phytocomponents present in *A. occidentale* were hexadecanoic acid methyl ester, 11-octadecenoic acid methyl ester, pentadecanoic acid 14-methyl-methyl ester, 9,12-octadecadienoic acid (Z, Z)-methyl ester and 9-octadecenoic acid, methyl ester, (E)—(Table 2(A)). Table 2(B) showed that pentacosane, in comparative terms, represented the highest abundant phytocomponent

Table 2 Phytocomponents of (A) petroleum ether extract of *A. occidentale*, (B) ethylacetate extract of *A. occidentale*, (C) *n*-hexane extract of *P. guajava*, (D) petroleum ether extract of *T. catappa*, (E) ethylacetate extract of *T. catappa*

S/No.	R_T (min)	Phytocomponents	MF	MW (g/mol)	%PA
(A)					
1.	5.262	o-Xylene	$C_6H_4(CH_3)_2$	106.16	2.30
2.	6.811	Benzene, 1-ethyl-4-methyl-	C_9H_{12}	120.1916	2.13
3.	7.652	Benzene, 1, 2, 4-trimethyl-	C_9H_{12}	120.1916	2.33
4.	19.205	Methyl tetradecanoate[a]	$C_{15}H_{30}O_2$	242.3975	1.53
5.	23.437	Hexadecanoic acid, methyl ester[a]	$C_{17}H_{34}O_2$	270.4507	49.59
6.	23.921	Pentadecanoic acid, 14-methyl-, methyl ester[a]	$C_{17}H_{34}O_2$	270.50	10.34
7.	27.129	9, 12-Octadecadienoic acid (Z, Z)-, methyl ester[a]	$C_{19}H_{34}O_2$	294.4721	8.49
8.	27.399	11-Octadecenoic acid, methyl ester[a]	$C_{19}H_{36}O_2$	296.4879	14.14
9.	27.661	9-Octadecenoic acid, methyl ester, (E)-[a]	$C_{19}H_{36}O_2$	296.49	6.52
10.	28.042	Methyl stearate[a]	$C_{19}H_{38}O_2$	298.5	2.62
(B)					
1.	5.262	Benzene, 1, 3-dimethyl-	C_8H_{10}	106.1650	3.97
2.	6.826	2, 4-Nonadiyne	C_9H_{12}	120.19	1.33
3.	7.684	Benzene, (1-methylethyl)-	C_9H_{12}	120.1916	1.31
4.	18.562	Hexacosane[a]	$C_{26}H_{54}$	366.718	2.27
5.	18.617	Methoxyacetic acid, 3-pentadecyl ester[a]	$C_{18}H_{36}O_3$	300.5	1.71
6.	19.618	1-Hexacosanol[a]	$C_{26}H_{54}O$	382.7	2.38
7.	20.039	Tetracosane[a]	$C_{24}H_{50}$	338.7	4.66
8.	22.817	2-Hexyl-1-octanol[a]	$C_{14}H_{30}O$	214.39	< 0.01
9.	24.001	Diisooctyl phthalate[a]	$C_{24}H_{38}O_4$	390.6	< 0.01
10.	24.478	Bicyclo [4.1.0] heptane-3-cyclopropyl-7-hydroxymethyl [cis]	$C_{11}H_{18}O$	166.26002	< 0.01
11.	24.636	Tricosane[a]	$C_{23}H_{48}$	324.6	10.55
12.	24.747	Dibutyl phthalate[a]	$C_{16}H_{22}O_4$	278.34	29.34
13.	27.605	Undec-10-ynoic acid, undecyl ester[a]	$C_{22}H_{40}O_2$	336.5518	1.53
14.	27.629	3-Cyclopentylpropanoic acid, but-3-yn-2yl ester[a]	$C_{12}H_{18}O_2$	194.2701	< 0.01
15.	28.360	4-Nonanol 2, 6, 8 trimethyl-[a]	$C_{12}H_{26}O$	186.3342	< 0.01
16.	29.717	Pentacosane[a]	$C_{25}H_{52}$	352.7	40.95
(C)					
1.	25.001	D-Erythro-sphinganine[a]	$C_{18}H_{39}NO_2$	525	< 0.01
(D)					
1.	5.262	o-Xylene	C_8H_{10}	106.16	10.25
2.	5.310	Nonane	C_9H_{20}	128.25	4.15
3.	5.477	Cyclohexane, 1-ethyl-2-methyl-	C_9H_{18}	126.2392	1.29
4.	5.540	Trans-1, 3-diethylcyclopentane	C_9H_{18}	126.24	0.43
5.	5.747	Cyclononene	C_9H_{16}	124.22	0.87
6.	5.913	Benzene, (1-methylethyl)-	C_9H_{12}	120.1916	1.37
7.	5.985	Cyclohexane, propyl-	C_9H_{18}	126.2392	0.61
8.	6.048	Octane, 2, 6-dimethyl-	$C_{10}H_{22}$	142.2817	1.01
9.	6.176	1, 15-Pentadecanediol[a]	$C_{15}H_{32}O_2$	244.4134	0.39
10.	6.596	Benzene, propyl-	C_9H_{12}	120.1916	2.30
11.	6.715	Nonane, 4-methyl-	$C_{10}H_{22}$	142.2817	0.41
12.	6.819	Benzene, 1-ethyl-3-methyl-	C_9H_{12}	120.1916	11.17
13.	6.946	Nonane, 3-methyl-	$C_{10}H_{22}$	142.2817	0.39
14.	7.017	Benzene, 1, 2, 3-trimethyl-	C_9H_{12}	120.1916	4.44

Table 2 Phytocomponents of (A) petroleum ether extract of *A. occidentale*, (B) ethylacetate extract of *A. occidentale*, (C) *n*-hexane extract of *P. guajava*, (D) petroleum ether extract of *T. catappa*, (E) ethylacetate extract of *T. catappa (Continued)*

S/No.	R_T (min)	Phytocomponents	MF	MW (g/mol)	%PA
15.	7.263	Benzene, 1-ethyl-2-methyl-	C_9H_{12}	120.1916	2.59
16.	7.652	Benzene, 1, 2, 3-trimethyl-	C_9H_{12}	120.1916	12.76
17.	8.407	Spiro [bicycle [2.2.1] hept-5-ene-2, 1'-cyclopropane][a]	C_9H_{12}	120.19	7.66
18.	8.732	Indane	C_9H_{10}	118.18	2.77
19.	9.010	Indene	C_9H_8	116.16	0.60
20.	9.089	Benzene, 1, 4-diethyl-	$C_{10}H_{14}$	134.2182	0.97
21.	9.153	Benzene, 1-methyl-3-propyl-	$C_{10}H_{14}$	134.2182	1.16
22.	9.264	p-Mentha-1, 5, 8-triene	$C_{10}H_{14}$	134.2182	1.16
23.	9.375	Benzene, 2-ethyl-1, 3-dimethyl-	$C_{10}H_{14}$	134.2182	1.90
24.	9.518	3a, 4, 5, 6, 7, 7a-Hexahydro-4, 7-methanoindene	$C_{10}H_{14}$	134.2182	1.51
25.	9.876	Benzene, 4-ethyl-1, 2-dimethyl-	$C_{10}H_{14}$	134.2182	1.03
26.	9.939	Benzene, 1-ethyl-2, 4-dimethyl-	$C_{10}H_{14}$	134.2182	1.53
27.	10.011	1-Penten-3-yne, 2-methyl-	C_6H_8	80.1277	0.84
28.	10.114	Benzene, 2-ethyl-1, 4-dimethyl-	$C_{10}H_{14}$	134.2182	3.65
29.	10.122	Benzene 1-methyl-4-(-2-propenyl-)	$C_{10}H_{12}$	132.2023	< 0.01
30.	10.225	4, 7-Methano-1H-indene, octahydro-	$C_{10}H_{16}$	136.2340	2.02
31.	10.415	Undecane	$C_{11}H_{24}$	156.313	0.59
32.	10.471	1-Penten-3-yne, 2-methyl-	C_6H_8	80.1277	0.67
33.	10.693	o-Cymene[a]	$C_{10}H_{14}$	134.2182	0.49
34.	10.995	Benzene, 1, 2, 3, 4-tetramethyl-	$C_{10}H_{14}$	134.2182	0.87
35.	11.122	Benzene, 1, 2, 3, 5-tetramethyl-	$C_{10}H_{14}$	134.2182	1.60
36.	11.591	(E)-1-Phenyl-1-butene	$C_{10}H_{12}$	132.2023	1.35
37.	11.876	2,4-Dimethylstyrene	$C_{10}H_{12}$	132.2023	2.73
38.	12.043	Benzene, 1-butynyl-	$C_{10}H_{10}$	130.1864	0.73
39.	12.170	1-Hexen-3-yne	C_6H_8	80.13	0.67
40.	12.480	Benzene, 1-methyl-4-(1-methylpropyl)-[a]	$C_{11}H_{16}$	148.2447	0.41
41.	12.797	Naphthalene	$C_{10}H_8$	128.17	2.54
42.	13.036	Benzene, (1-methyl-1-butenyl)-	$C_{11}H_{14}$	146.2289	1.62
43.	13.838	Benzene, (1-ethyl-1-propenyl)-	$C_{11}H_{14}$	146.2289	0.56
44.	14.179	Benzene, (3-methyl-2-butenyl)-	$C_{11}H_{14}$	146.2289	0.48
45.	14.449	Benzene, 2-ethenyl-1, 3, 5-trimethyl	$C_{11}H_{14}$	146.2289	0.63
46.	15.148	Benzocycloheptatriene	$C_{11}H_{10}$	142.1971	0.99
47.	15.457	Naphthalene, 2-methyl-	$C_{11}H_{10}$	142.1971	0.54
48.	21.087	Isoxazole, 4, 5-dimethyl-[a]	C_5H_7NO	97.12	0.47
49.	23.993	Hexadecanoic acid[a]	$C_{16}H_{32}O_2$	256.4241	< 0.01
50.	27.606	10-Octadecenoic acid, methyl ester[a]	$C_{19}H_{36}O_2$	296.5	0.82
51.	28.336	Methyl stearate[a]	$C_{19}H_{38}O_2$	298.5	< 0.01
(E)					
1.	24.009	Hexadecanoic acid, methyl ester[a]	$C_{17}H_{34}O_2$	270.4507	20.03
2.	27.494	9, 11-Octadecadienoic acid, methyl ester, (E, E)-[a]	$C_{19}H_{34}O_2$	294.5	18.26
3.	27.701	Trans-13-octadecenoic acid, methyl ester[a]	$C_{19}H_{36}O_2$	296.4879	49.49
4.	28.368	Methyl stearate[a]	$C_{19}H_{38}O_2$	298.5	12.22

R_T retention time, *MF* molecular formula, *MW* molecular weight, *PA* peak area, %PA < 0.01 trace amount [56]
[a]Plant metabolite

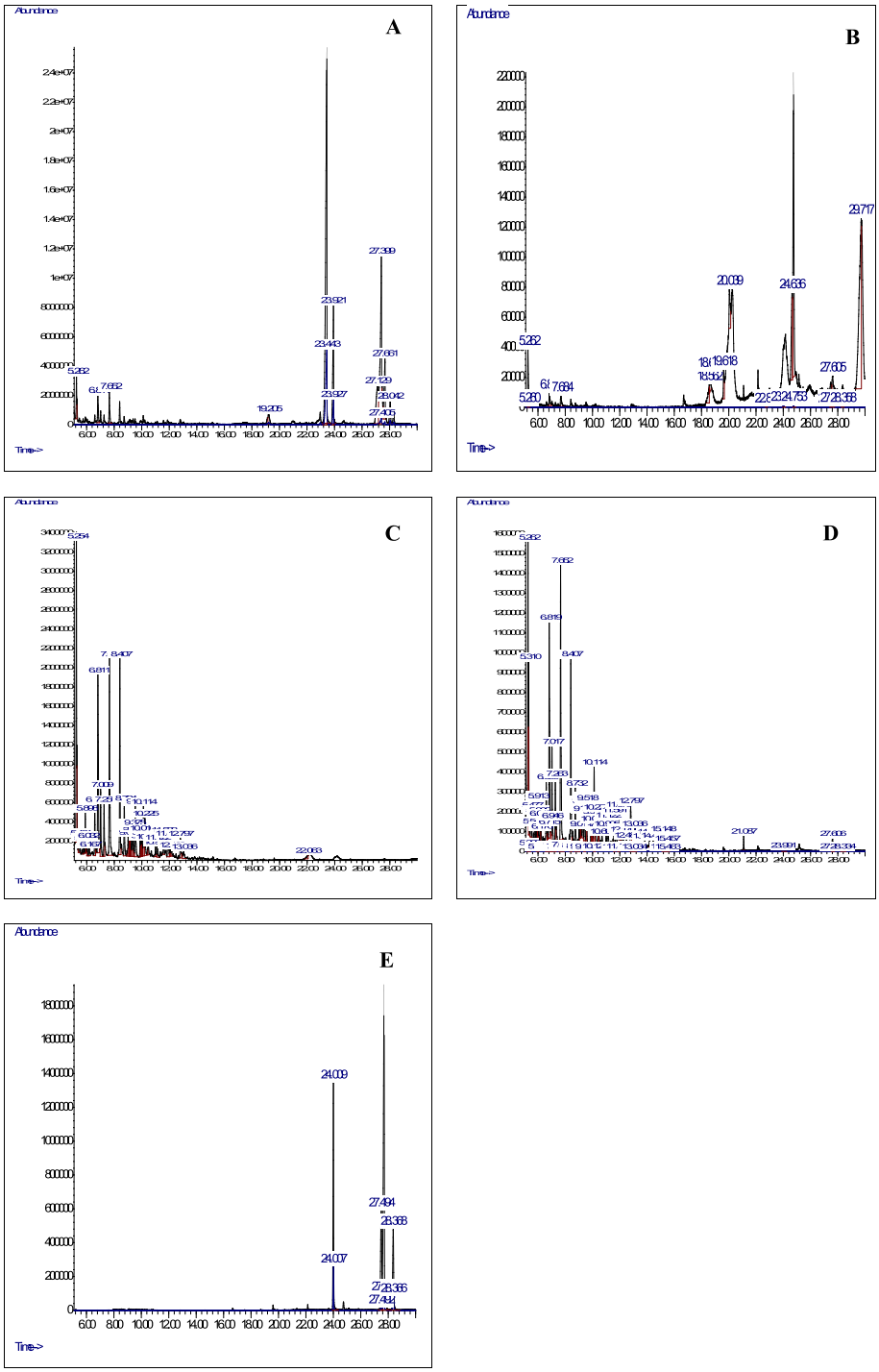

Fig. 4 GC-MS chromatograms: **a** Petroleum ether extract of *A. occidentale*. **b** Ethylacetate extract of *A. occidentale*. **c** *n*-hexane extract of *P. guajava*. **d** Petroleum ether extract of *T. catappa*. **e** Ethylacetate extract of *T. catappa*

present in ethylacetate extract of *A. occidentale*. Other notable phytocomponents present in ethylacetate extract of *A. occidentale* were dibutyl phthalate, hexacosane, 1-hexacosanol, tetracosane, 2-hexyl-1-octanol, diisooctyl phthalate, tricosane, undec-10-ynoic acid undecyl ester, and 3-cyclopentylpropanoic acid but-3-yn-2yl ester.

Table 2(C) showed the presence of D-erythro-sphinganine in *n*-hexane extract of *P. guajava.* Table 2(D) showed the

presence of 51 phytocomponents in petroleum ether extract of *T. catappa*. Notable phytocomponents of petroleum ether extract of *T. catappa* were 1,15-pentadecanediol, 4,7-methano-1H-indene, octahydro-, hexadecanoic acid, 10-octadecenoic acid methyl ester, and methyl stearate. Ethylacetate extract of *T. catappa* contained comparative high quantities of hexadecanoic acid methyl ester, 9, 11-octadecadienoic acid, methyl ester, (E, E)-, trans-13-octadecenoic acid methyl ester, and methyl stearate.

4.6.2 FT-IR of fractionated leaf extracts of A. occidentale, P. guajava, and T. catappa

The FT-IR spectra of the selected leaf extracts, namely ethylacetate and petroleum ether extracts of *A. occidentale*, *n*-hexane extract of *P. guajava*, and petroleum ether and ethylacetate extracts of *T. catappa* are illustrated in Fig. 5a–e and summarized in Table 3(A–E).

Petroleum ether extract of *A. occidentale* exhibited characteristic strong and medium bands within the range of (2955.8–2855.1) cm^{-1} and (905.7–693.3) cm^{-1} (Fig. 5a), which were indicative of the presence of aliphatic compounds and aromatic mono- and di-substituted aromatic compounds (Table 3(A)). Weak band at 1748.1 cm^{-1} was indicative of the presence of ester carbonyl group bearing molecules. Other notable molecular constituents present were aldehyde, phenolics, acyl halides, and alkoxy functional groups containing compounds.

Figure 5b showed that ethylacetate extract of *A. occidentale* contained alcohol functional group (O-H) bearing molecules. Peaks within the range of (2989.1–2907.3) cm^{-1} were indicative of the presence of saturated aliphatic compounds (Table 3(B)). The presence of aromatic compounds in the extract was typified by peaks around 1446.2 cm^{-1}, 786.5 cm^{-1}, and 733.0 cm^{-1} (Table 3(B)). A medium band at 1376.9 cm^{-1} and peaks within the range of (1889.8–1654.9) cm^{-1}suggested the presence of acyl group, phenolics, as well as carboxylic acid, esters, and amide functional groups in ethylacetate extract of *A. occidentale* (Table 3(B)).

The presence of saturated aliphatic and aromatic compounds in *n*-hexane extract of *P. guajava* was typified by multiple weak and strong bands within the range of (2952.8–2858.9) cm^{-1} and (909.5–83.08) cm^{-1} (Fig. 5c). The presence of aromatic compounds as exemplified by weak band around 1457.4 cm^{-1}. Furthermore, weak bands within the range of (1990.4–697.0) cm^{-1} suggested the presence of conjugated alkenes, primary and tertiary amines, di-substituted aromatic compounds, and *cis*-alkenes (Table 3(C)).

The weak band around 3734.80 denotes N-H and O-H stretch, which suggested the presence of amide and alcohol functional groups bearing molecules (Fig. 5d). Peaks within the range of (3112.3–2851.4) cm^{-1} were indicative of the presence of aliphatic compounds in petroleum ether extract of *T. catappa* (Table 3(D)). Petroleum ether extract of *T. catappa* contained carbonyl and alkoxy functional groups as indicated by weak bands at 1733.2 cm^{-1} and 1039.9 cm^{-1} respectively. Additionally, peaks around (887.1–801.4) cm^{-1} were indicative of the presence of aromatic compounds (Table 3(D)).

The broad band at 3410.5 cm^{-1} was characteristic of the presence of alcohol functional group (O-H) in ethylacetate extract of *T. catappa* (Fig. 5e). Additionally, the peaks within the range of (2981.9–2907.3) cm^{-1} and (939.3–849.8) cm^{-1} suggested the presence of aliphatic compounds. Table 3(E) showed the presence of aromatic compounds as well as nitro-compounds, phenolics, and esters.

4.6.3 UV-visible of fractionated leaf extracts of A. occidentale, P. guajava, and T. catappa

The selected fractionated leaf extracts of *A. occidentale*, *P. guajava*, and *T. catappa* gave characteristic UV-visible spectra (Fig. 6a–e). Petroleum ether extract of *A. occidentale* exhibited multiple λmax around region of 272.00 nm, which suggested the presence of nitro-containing chromophores in the extract (Fig. 6a). The λmax of ethylacetate extract of *A. occidentale* was at 205.00 nm (Fig. 6b). Figure 6c showed multiple peak values of UV-visible spectra of *n*-hexane extract of *P. guajava*. Petroleum ether extract of *T. catappa* exhibited multiple distinct peaks within a broad range of 204.00 nm and 671.00 nm (Fig. 6d). Ethylacetate extract of *T. catappa* gave single λmax at 363.00 nm (Fig. 6e).

5 Discussion

The capacity of phytocomponents to alter erythrocyte membrane stability is measured in vitro when a given population of erythrocyte is suspended in hypotonic solutions of varying NaCl concentrations [25, 26, 42]. Previous studies had reported distortions in membrane osmotic stability of human erythrocyte genotypes, in health and disease, and the capacities of plant extracts to reverse those alterations [26, 42, 44, 57–59]. Certain phytocomponents are associated with stabilization of erythrocyte membrane integrity or, on the contrary, cause exacerbated hemolysis in hypotonic solutions [44, 60–62]. For instance, certain classes of the tannins, aminoglycosides, and saponins from verse collections of plant species have been implicated in eliciting erythrocyte membrane destabilization by their chaotropic interaction with biomembrane components [1, 21, 57, 62–64]. The destabilizing effects elicited by phytochemicals are analogous to hemolytic actions of intra-erythrocytic denatured hemoglobin precipitate—Heinz bodies. According to Jarolim et al. [65], Heinz bodies form an attachment with erythrocyte membrane components, which elicits striking membrane damage, evidenced by increased membrane lipid peroxidation and cross-linked membrane proteins. The damaged sickle erythrocyte membrane is characterized by calcium-activated increased permeability of potassium, phospholipids bilayer

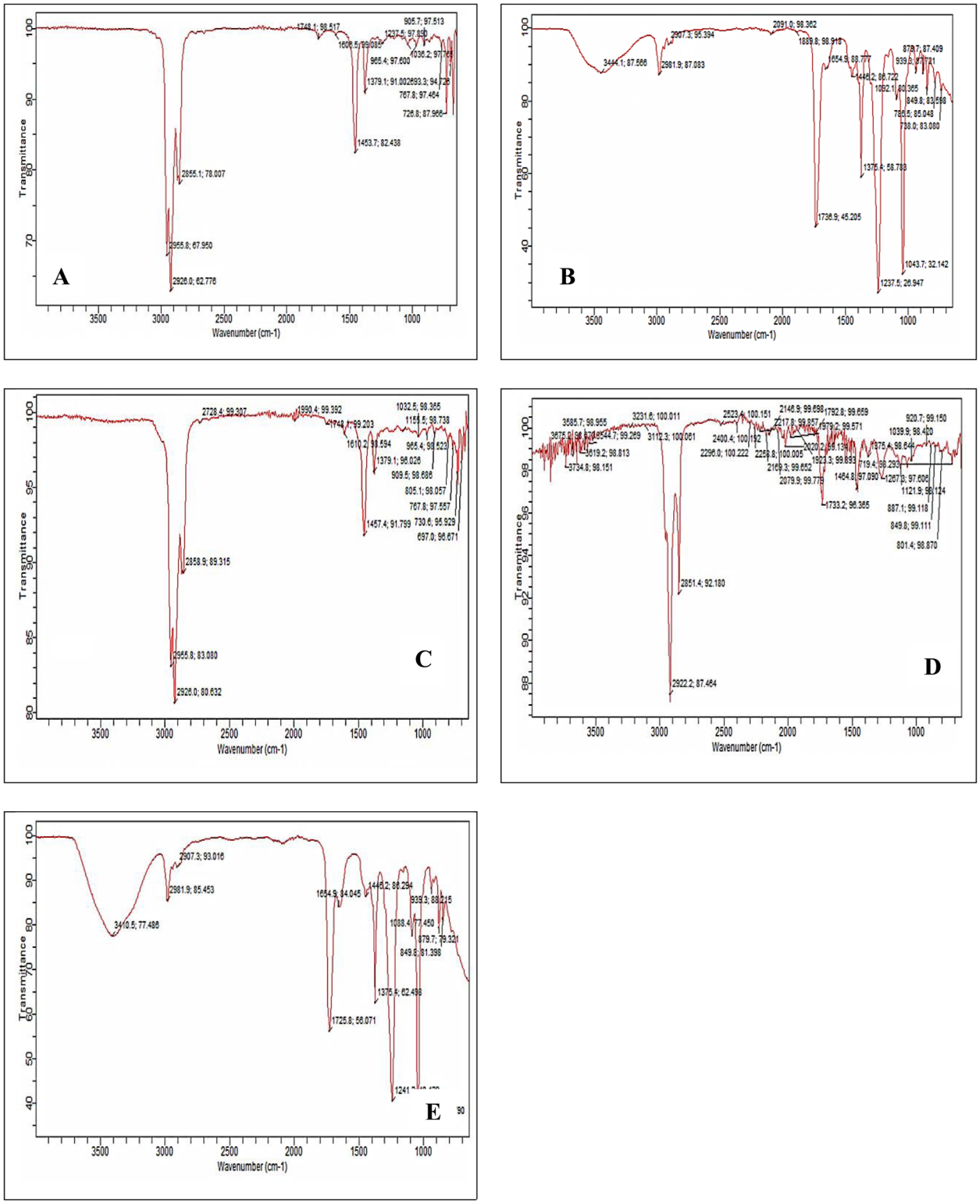

Fig. 5 FT-IR spectra of **a** Petroleum ether extract of *A. occidentale*. **b** Ethylacetate extract of *A. occidentale*. **c** *n*-hexane extract of *P. guajava*. **d** Petroleum ether extract of *T. catappa*. **e** Ethylacetate extract of *T. catappa*

Table 3 FT-IR spectra peak values: (A) petroleum ether extract of *A. occidentale*, (B) ethylacetate extract of *A. occidentale*, (C) *n*-hexane extract of *P. guajava*, (D) petroleum ether extract of *T. catappa*, (E) ethylacetate extract of *T. catappa*

S/No	Peak/band (cm^{-1})	%T	Functional groups/assignment	Origin
(A)				
1.	2955.8	67.950	Alkanes sp3 C-H stretch	C-H
2.	2926.0	62.776	Alkanes sp3 C-H stretch	C-H
3.	2855.1	78.007	Aldehyde C-H stretch	C-H
4.	1748.1	98.517	Ester carbonyl C-O stretch	C=O
5.	1606.5	99.085	Conjugated alkenes C-C	C=C
6.	1453.7	82.438	Aromatic compounds C-C stretch	C=C
7.	1379.4	91.002	Acyl C-O; phenol C-O stretch	C=O; C-O
8.	1237.5	97.513	Aromatic ethers, aryl-O stretch	Ar-O-C
9.	1036.2	97.765	Alkoxy C-O stretch	X-O-C
10.	905.7	97.513	Mono-substituted alkene sp2 C-H bend	=C-H
11.	767.8	97.464	Di-substituted aromatic sp2 C-H bend	=C-H
12.	726.8	87.966	Mono-substituted aromatic sp2 C-H bend	=C-H
13.	693.3	94.726	Di-substituted aromatic sp2 C-H bend	=C-H
(B)				
1.	3444.1	87.566	Dimeric O-H stretch	O-H
2.	2989.1	87.083	Alkanes sp3 C-H stretch	C-H
3.	2907.3	95.394	Alkanes sp3 C-H stretch	C-H
4.	2091.0	98.362	Isothiocyanate (-SCN) stretch	-SCN
5.	1889.8	98.918	Carboxylic acids C-O stretch	C=O
6.	1736.9	45.205	Ester C-O stretch	C=O
7.	1654.9	88.777	Amides C-O stretch	C=O
8.	1446.2	86.722	Aromatic C-C stretch	C=C
9.	1376.9	45.205	Acyl C-O; phenol C-O stretch	C=O; C-O
10.	1237.5	26.947	Skeletal C-C vibration	C-C
11.	1092.1	80.365	Alkoxy C-O stretch	X-O-C
12.	1043.7	32.142	Primary amine C-N stretch	C-N
13.	939.3	87.721	Alkenes sp2 C-H bend	=C-H
14.	878.7	87.409	Alkenes sp2 C-H bend	=C-H
15.	849.8	83.588	Tri-substituted alkenes sp2 C-H bend	C-H
16.	786.5	85.048	Di-substituted aromatic sp C-H bend	C-H
17.	733.0	83.080	Mono-substituted aromatic C-H bend	C-H
(C)				
1.	2952.8	83.080	Alkanes sp3 C-H stretch	C-H
2.	2926.0	60.632	Alkanes sp3 C-H stretch	C-H
3.	2858.9	89.315	Alkanes sp3 C-H stretch	C-H
4.	2728.4	99.307	Aldehyde C-H stretch	C-H
5.	1990.4	99.392	Cyanate C-N stretch	-C≡N
6.	1748.1	99.203	Esters C-O stretch	C=O
7.	1610.2	98.594	Conjugated alkenes C-C stretch	C=C
8.	1457.4	91.799	Aromatic ring stretch	C=C-C
9.	1379.1	96.026	Alkanes sp3 C-H bend	C-H
10.	1155.5	98.738	Tertiary amine C-N stretch	C-N

Table 3 FT-IR spectra peak values: (A) petroleum ether extract of *A. occidentale*, (B) ethylacetate extract of *A. occidentale*, (C) *n*-hexane extract of *P. guajava*, (D) petroleum ether extract of *T. catappa*, (E) ethylacetate extract of *T. catappa (Continued)*

S/No	Peak/band (cm^{-1})	%T	Functional groups/assignment	Origin
11.	1032.5	98.385	Primary amine C-N stretch	C-N
12.	909.5	98.686	Vinyl C-H bend	C-H
13.	805.1	98.057	Di-substituted aromatic C-H bend	C-H
14.	767.8	97.557	Di-substituted aromatic C-H bend	C-H
15.	730.6	95.929	Cis-alkene sp2 C-H bend	C-H
16.	697.0	96.671	Cis-alkene sp2 C-H bend	C-H
(D)				
1.	3734.8	98.151	Amide and alcohol N-H and O-H stretch	N-H; O-H
2.	3112.3	100.06	Alkynes sp C-H stretch	≡C-H
3.	2922.2	87.464	Alkanes sp3 C-H bend	C-H
4.	2851.4	92.180	Alkanes sp3 C-H bend	C-H
5.	2079.9	99.779	Isothiocyanate (-NCS) stretch	N≡C-S
6.	2020.2	99.134	Isothiocyanate (-NCS) stretch	N≡C-S
7.	1733.2	96.365	Aldehyde C-O stretch	C=O
8.	1464.8	97.090	Aromatics compounds sp2 C-C stretch	C=C
9.	1121.9	96.124	Alkyl substituted alkoxy C-O stretch	C-O-C
10.	1039.9	98.420	Alkoxy C-O stretch	X-O-C
11.	920.7	99.150	Alkenes sp2 C-H bend	C-H
12.	887.1	99.118	Di-substituted aromatics sp2 C-H stretch	C-H
13.	849.8	99.111	Di-substituted aromatics sp2 C-H stretch	C-H
14.	801.4	98.870	Di-substituted aromatics sp2 C-H stretch	C-H
(E)				
1.	3410.5	77.486	Alcohols O-H stretch	O-H
2.	2981.9	85.453	Alkanes sp3 C-H stretch	C-H
3.	2907.3	93.016	Alkanes sp3 C-H stretch	C-H
4.	1725.8	56.071	Aldehyde C-O stretch	C=O
5.	1446.2	86.294	Aromatic compounds C-C stretch	C=C
6.	1375.4	62.498	Nitro compounds NO_2 stretch	-N=O
7.	1241.2	40.429	Acyl C-O; phenol C-O stretch	C-O
8.	1088.4	77.450	Alkoxy C-O stretch	X-O-C
9.	1043.7	35.790	Alkoxy C-O stretch	X-O-C
10.	939.3	88.215	Mono-alkene sp2 C-H bend	C-H
11.	879.7	79.321	Alkene sp2 C-H bend	C-H
12.	849.8	81.398	Alkene sp2 C-H bend	C-H

%T percentage transmittance

destabilization, decreased deformability and unusual assemblies, and interactions of membrane proteins as well as unusual oxidation of protein thiols and cytoskeletal dysfunction [66, 67]. Furthermore, the use of mathematical models had revealed the relationship between sickle hemoglobin (HbS) polymerization and membrane morphology, osmotic properties, viscoelasticity, rheology, and related physicochemical characteristics of sickle erythrocytes as empirically described [67–69]. By implication, phytocomponents that deterred HbS aggregation and polymerization improved sickle erythrocyte membrane stability [35, 59, 70, 71] within limits of their permeability across the biomembrane system as previously described [72]. In line with the present findings, the presence of isothiocyanates, a notable anti-HbS polymerization phytochemicals [70, 71], in ethylacetate extract of *A. occidentale* obviously contributed, in parts, in stabilizing the sickle erythrocyte membrane against osmotic stress. Additionally, aromatic compounds

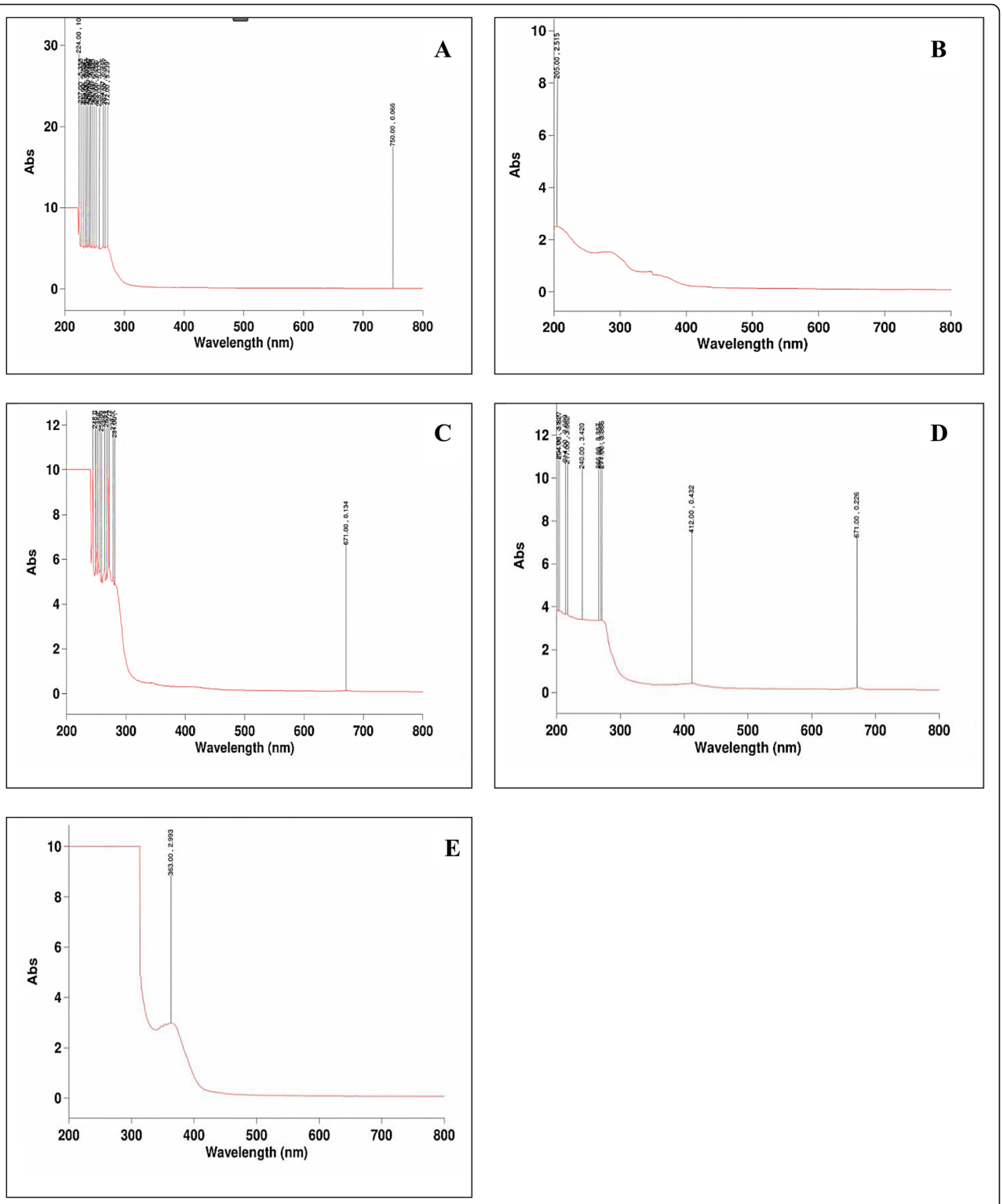

Fig. 6 UV-visible spectra of **a** Petroleum ether extract of *A. occidentale*. **b** Ethylacetate extract of *A. occidentale*. **c** *n*-hexane extract of *P. guajava*. **d** Petroleum ether extract of *T. catappa*. **e** Ethylacetate extract of *T. catappa*

such as L-phenylalanine and its derivative L-phenylalanine benzyl ester are effective agents, even at relatively low concentrations, against HbS polymerization and have been proposed to be effective therapeutic agents for the management of SCD [72–76]. The improved sickle erythrocyte osmotic stability in samples containing petroleum ether

extract of *T. catappa* appeared to suggest that aromatic compounds in petroleum ether extract of *T. catappa* attenuated HbS polymerization-induced hemolysis in hypotonic solutions as previously described [35, 59, 70, 71]. From another perspective, the antioxidant phytocomponents from plant materials viz. the anthocyanins, anthraquinones, flavonoids, and antioxidant vitamins (vitamins C and E) generally stabilized membrane systems against molecular and biological agents that compromised membrane integrity [21, 30, 42, 44, 57, 59, 72].

In that regard, the present study showed membrane osmotic stability of sickle erythrocytes were altered in the presence of fractionated leaf extracts of *A. occidentale*, *P. guajava*, and *T. catappa*, such that the extracts exhibited differential capacities to either stabilize sickle erythrocyte membrane integrity or promote membrane destabilization. Based on the percentile yields of petroleum ether, *n*-hexane, chloroform, and ethylacetate as well as residual aqueous extracts, the results of the present study indicated relatively high content of hydrophilic phytocomponents in leaf extracts of *A. occidentale*, *P. guajava*, and *T. catappa*. Nevertheless, the findings of the present investigations did not implicate hydrophilic phytocomponents of *A. occidentale*, *P. guajava*, and *T. catappa* as agents that promoted sickle erythrocyte stability since, to a large extent, fractions of their residual aqueous extracts promoted membrane destabilization. Rather, hydrophobic fractions of the leaf extracts, namely petroleum ether extract of *A. occidentale*, ethylacetate extract of *A. occidentale*, *n*-hexane extract of *P. guajava*, petroleum ether extract of *T. catappa*, and ethylacetate extract of *T. catappa* exhibited comparative high capacity to promote membrane osmotic stability of sickle erythrocytes. The present findings were in agreement with previous reports, in which the authors noted that there was an established positive correlation between hydrophobicity of short-chain aliphatic alcohols and their capacity to alter membrane integrity [77]. For instance, ethylacetate extract of *A. occidentale* contained short-chain aliphatic alcohols, namely 1-hexacosanol, 2-hexyl-1-octanol and 4-nonanol 2,6,8-trimethyl-, which further explain, in part, the stabilizing effects of the fraction on sickle erythrocyte membranes subjected to osmotic stress. In concord with the present findings, previous report noted that flavonoids from ethylacetate extract of *Lantana camara* stabilized normal erythrocyte membrane against osmotic stress [42].

In addition, short-chain aliphatic ester derivatives from the fractionated leaf extracts of *A. occidentale*, *P. guajava*, and *T. catappa* appeared to have promoted sickle erythrocyte osmotic stability as previously reported [77]. Earlier study had posited that hydrophobicity of short-chain aliphatic esters facilitated their integration into the membrane system, eliciting structural rearrangement that promoted membrane stability [77, 78]. In agreement with the present results, previous findings had reported that short-chain aliphatic acids (octadecanoic acid and 16-octadecenoic acid), short-chain aliphatic esters (pentadecanoic acid, 14-methyl ester, and 9-octadecadienoic acid (Z)-, 2, 3-dihydroxypropyl ester), aldehyde (*α*-campholene aldehyde), nitro compound (cyclohexanespiro-5′,4′-methyl-2′-phenyl-2′-oxazoline), alcohol (borneol), and aliphatic compound (*α*-phellandrene) in silica gel fractionated aqueous and methanol extracts of *Telfairia occidentalis* stabilized sickle erythrocyte membrane against osmotic stress and deterred sickling [79].

The present study noted that diisooctyl phthalate and aromatic compounds from ethylacetate extract of *A. occidentale*, in part, stabilized sickle erythrocyte membrane under osmotic stress. The molecular configurations of diisooctyl phthalate and aromatic compounds appeared to have contributed to their stabilizing effect on sickle erythrocyte membrane system. A related study had previously reported that despite inter-species differences in erythrocyte membrane properties, dicarboxylic acids with cyclic hydrocarbons, namely bis (2-ethylhexyl) phthalate, 1,2-cyclohexanedicarboxylic acid, and phthalic acid with a benzene ring significantly stabilized cattle erythrocytes subjected to osmotic pressure due to their wedge-like effect on membrane phospholipids [80].

Additionally, the polarities of antioxidant vitamins and related low molecular weight antioxidants (LMWAs), which protect biological systems against oxidative damage to cellular components, dictate their distribution patterns in cellular regions. For instance, hydrophobic antioxidants such as the tocopherols are restricted to act within the hydrophobic regions of the cell, e.g., biomembrane systems, whereas the hydrophilic antioxidants effect their free radical quenching activities within the aqueous environments of the cell. Accordingly, the current information of cellular distribution of LMWAs suggest that the tendency of phytocomponents from the fractionated leaf extracts of *A. occidentale*, *P. guajava*, and *T. catappa* to interact and interfere with sickle erythrocyte membrane systems appeared to correlate with hydrophobicity of molecular species of the leaf extracts.

The present study of GC-MS system protocol in conjunction with sickle erythrocyte osmotic stability-guided analysis revealed vast array of hydrophobic phytocomponents, namely short-chain aliphatic esters, methylated aromatic hydrocarbons, cycloalkanes, hexahydro-methanoindene, isoxazole, and short-chain aliphatic carboxylic acids as molecular species from leaf extracts of *A. occidentale*, *P. guajava*, and *T. catappa* that caused relative osmotic stability of sickle erythrocyte membrane. Furthermore, FT-IR and UV-visible protocols further confirmed the alkanes, aldehydes, esters, conjugated alkenes, aromatic compounds, aromatic ethers, isothiocyanates, primary and tertiary amine, cyanate, alkynes, amide, alcohols, and nitro compounds as phytocomponents from fractionated leaf extracts of *A. occidentale*, *P. guajava*,

and *T. catappa* that promoted membrane osmotic stability of sickle erythrocytes.

6 Conclusion

The selected groups of fractionated leaf extracts that stabilized sickle erythrocyte membrane against osmotic stress were ethylacetate and petroleum ether extracts of *A. occidentale*, *n*-hexane extract of *P. guajava*, and petroleum ether and ethylacetate extracts *T. catappa*. GC-MS, FT-IR, and UV-visible spectrometry systems protocols indicated that short-chain aliphatic esters, methylated aromatic hydrocarbons, cycloalkanes, hexahydro-methanoindene, isoxazole, and short-chain aliphatic carboxylic acids and alkanes were phytocomponents from leaf extracts of *A. occidentale*, *P. guajava*, and *T. catappa* that stabilized sickle erythrocyte membrane integrity. Other phytocomponents, namely the aldehydes, esters, conjugated alkenes, aromatic compounds, isothiocyanates, primary and tertiary amine, cyanate, alkynes, amide, alcohols, and nitro compounds, were also considered as stabilizers of sickle erythrocyte membrane subjected to osmotic stress.

Specifically, to mention but a few, the combinations of major phytocomponents that stabilized sickle erythrocyte membrane against osmotic stress were hexadecanoic acid, methyl ester, 11-octadecenoic acid, methyl ester, dibutyl phthalate, pentacosane, trans-13-octadecenoic acid, methyl ester, whereas the minor phytocomponents include methyl tetradecanoate, methoxyacetic acid, 3-pentadecyl ester, methyl stearate, hexadecanoic acid, isoxazole, and 4, 5-dimethyl-.

The present research recommend further study on isolation and purification of the phytocomponents suggested, in the present study, to have stabilized osmotic stressed sickle erythrocytes and apply the isolated and purified phytocomponents in membrane osmotic stability studies in vitro in order to establish their membrane stabilizing activity and, possibly, mode of action.

Abbreviations

EDTA-Na_2: Disodium ethylene diamine tetraacetate; FT-IR: Fourier transform-infrared spectroscopy; GC-MS: Gas chromatography-mass spectroscopy; HbS: Sickle hemoglobin; MCF: Mean corpuscular fragility; $Na_2HPO_4.2H_2O$: Disodium hydrogen phosphate dihydrate; $Na_2S_2O_5$: Sodium metabisulfite; NaCl: Sodium chloride; $NaH_2PO_4.2H_2O$: Sodium dihydrogen phosphate dihydrate; PBS: Phosphate-buffered saline; SCD: Sickle cell disease; UV-visible: Ultra violet-visible spectroscopy

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Authors' contributions

PCC conceived and designed the research and supervised the laboratory work. PCC prepared the manuscript. PCC/RCE/ABC-A analyzed the data. PCC/RCE/ABC-A collected the plant samples and carried out the laboratory work. All authors have approved the manuscript in the present form and gave the permission to submit the manuscript for publication.

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Availability of data and materials

All the data generated and analyzed during the study are included in the main manuscript.

Ethics approval and consent to participate

Collection of the blood samples was in accordance with the ethical principles that have their origins in the October 2008 Declaration of Helsinki. The present study was approved by the Ethical Committee for Research, Department of Biochemistry, Imo State University, Owerri, Nigeria. Ethics Approval Number: ODVC/REN/544/19. All the participants filled and signed Informed Consent Form.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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