

## Original Research Article

# Anti-sickling effect of *Senna alexandrina*, *Aerva javanica*, and *Ficus palmata* extracts on sickle cell disorder

Zahraa M Al Yousef<sup>1</sup>, Elaf A Alaswad<sup>2</sup>, M-Zaki M ElAssouli<sup>4</sup>, Dunya A Nori<sup>2</sup>, Faten Z Filimban<sup>3</sup>, Hani Choudhry<sup>2</sup>, Mohammad Z Alam<sup>4,5</sup>, Hadeel Al Sadoun<sup>5</sup>, Nawal MW Helmi<sup>1\*</sup>

<sup>1</sup>Department of Biochemistry, College of Science, University of Jeddah, <sup>2</sup>Department of Biochemistry, College of Science, <sup>3</sup>College of Sciences, Biology Department, Division of Botany, <sup>4</sup>Pre-Clinical Research Unit, King Fahd Medical Research Center, <sup>5</sup>Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

\*For correspondence: **Email:** nmhelmi@uj.edu.sa; **Tel:** +966-12-2334000; **Fax:** +966-12-2334001

Sent for review: 7 November 2022

Revised accepted: 27 February 2023

### Abstract

**Purpose:** To investigate the anti-sickling activity of *Senna alexandrina*, *Aerva javanica*, and *Ficus palmata* extracts.

**Methods:** The leaves were extracted with water and methanol. The anti-sickling effect of extracts was determined using blood from 20 sickle cell disease (SCD) patients by mixing it with 500 and 1000 µg/mL of extract. The treated blood samples were then examined under a light microscope. The osmotic fragility of erythrocytes was determined using 4 mL (0 – 0.9 %) buffered saline mixed with 1 mL extract (500 µg/mL) and 0.05 mL sickled red blood cells (RBCs).

**Results:** *Aerva javanica* extract significantly ( $p < 0.0001$ ) reduced the percentage of sickle cells compared to control. The sickle-shaped cells were 29.47 % when treated with 125 µg/mL concentration of methanol extract of *A. javanica* that was further decreased to 0.35 % on increasing the concentration to 500 µg/mL. A similar trend was also observed with extracts of *F. palmata*. Extracts had protective effect on erythrocyte osmotic fragility. Results of comet assay suggested that extracts did not damage DNA.

**Conclusion:** These plant extracts have promising anti-sickling activity and protect erythrocyte osmotic fragility. However, *in vivo* studies are required to validate these results and for further development of the extracts for therapeutic use.

**Keywords:** Sickle cell disease, Comet assay, *Senna alexandrina*, Antioxidant, RBC

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

## INTRODUCTION

Sickle cell disease (SCD) is a genetic disorder that occurs due to a single gene mutation which results from substitution of the sixth amino acid

valine for glutamine in the beta chain of hemoglobin. The amino acid substitution causes polymerization of hemoglobin tetramers upon deoxygenation. As a result, red blood cells (RBCs) lose flexibility and adopt the

characteristic sickle shape in the capillary circulation, causing ischemia, stroke, multi-organ damage, severe pain, chronic hemolytic anemia, and reduced life expectancy [1,2]. Since the main outcome of sickle-shaped RBCs is the polymerization of hemoglobin, preventing the polymerization of sickle-shaped RBCs has been the most obvious target for the treatment of this disease.

Many plants have been used in the treatment of sickle cell anemia and they are listed as anti-sickling agents in several researches such as the aqueous extract of *Zanthoxylum heitzii* roots, *Garcinia kola* seed, and unripe fruit of *Carica papaya* [3]. *Senna* plant is a small shrub that belongs to the Fabaceae family. There are many species of *Senna* and one of them is *Senna alexandrina* which is found in Saudi Arabia [4]. The plants of genus *Aerva* are perennial herb, native to North Temperate Zone, especially the Mediterranean regions and Asia. *Aerva javanica* is commonly used for its antioxidant, antiviral, and antidiabetic properties [5]. Genus *Ficus* belongs to the family Moraceae. Most of the members of this family are shrubs, very high trees, and rarely herbs often produce milky juice. *Ficus* species have been reported as antimicrobial, anti-tumor, anti-inflammatory, antioxidant, and tonic medicament [6]. *Ficus palmata* is an extremely variable and common wild fig. It is widely distributed in hot, dry slopes in clay-loam soils in many countries including Saudi Arabia [6]. Phytochemical components of *F. palmata* include steroids, tannins, saponins, fixed oils and fats, flavonoids, alkaloids, proteins, and carbohydrates [7]. The aim of this study was to investigate the antioxidant and anti-sickling activity of the extracts of *S. alexandrina*, *A. javanica*, and *F. palmata* against RBCs having sickled-shaped cells.

## EXPERIMENTAL

### Plant material

*Senna alexandrina* leaves were harvested from Farasan archipelago, Farasan Al-Kabir is eleven meters above sea level with coordinates 16 °49'01.0 N and 41 °53'35.0 E. Farasan archipelago belongs to Jizan Governorate in southwestern Saudi Arabia. *Senna alexandrina* leaves were collected during the flowering season between November 2017 and April 2018. *Aerva javanica* leaves were collected from south of Jeddah City, western Saudi Arabia at 21 °29'194"N and 39°15'282"E on August 2017. *Ficus palmata* leaves were collected from south east of Baljurashi City, south-western Saudi Arabia at 19°48'57"N and 41 °36'54"E in October

2017. Identification of these plants were done by Dr. Faten Zubair Filimban (plant taxonomist), Biology Department, Division of Plant Sciences, King Abdulaziz University, Jeddah, Saudi Arabia.

### Extraction procedure

Air-dried leaves of *S. alexandrina*, *A. javanica* and *F. palmata* were ground to fine powder using mortar and pestle. Extraction was carried out by maceration of dried powdered leaves in water and methanol as described earlier with some modifications [8]. The powder was weighed and soaked in solvent in the ratio 1:10 (1 part sample + 9 parts solvent). The obtained mixtures were incubated with continuous shaking for two days, filtered, concentrated by freeze drying, while the methanol extraction was dried by rotary evaporation process. The crude extracts were stored at 4°C in dried form until used for the study.

### Phytochemical screening and total antioxidant capacity

Qualitative phytochemical screening methods were used to identify the presence of bioactive components such as alkaloids, tannins, saponin, flavonoids and phenols as described previously [9]. The antioxidant potentials of extracts were measured through the total Antioxidant Capacity (TAC) Assay Kit. The kit was purchased from Cell Biolabs, Inc (Cat No. STA-360), San Diego, USA. The TAC assay relies on reducing copper (II) to copper (I) form in the presence of antioxidant. The results were presented as Copper Reducing Equivalent (CRE). The CRE sample values are proportional to the extracts' total Antioxidant Capacity or total Antioxidant Power.

### 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The antioxidant activity of extracts against free radicals were assessed by scavenging method using DPPH as described earlier [9]. Briefly, 3.3 mM of DPPH was prepared in methanol solution as stock and absorbance was taken at a final concentration of 0.157 mM for negative control reading. Water and methanolic extracts were tested at concentrations 476, 357, 238, 119 and 238, 190.5, 167, 143 µg/mL respectively. Due to the colored background of extracts, sample blank was prepared for each concentration extracts, and methanol was added instead of DPPH reagents. For assay optimization, alpha-tocopherol (vitamin E) was prepared at concentration 0.04 µg/mL and served as a positive control. The reaction mixtures were vortexed and incubated for 30 min at room

temperature in the dark. Absorbance (A) was measured at 517 nm against methanol blank. For each extract, test was repeated in triplicate to avoid experimental error and the mean was used to calculate percent inhibition.

### Genotoxicity test

The genotoxic effect of *S. alexandrina*, *A. javanica* and *F. palmata* extracts were assessed by alkaline single cell gel electrophoresis assay also known as comet assay. The extracts were incubated with peripheral blood for 2 h at 37 °C to final concentration 1000, 500, 250 and 125 µg/mL as described earlier [10].

### Induction of sickling and determination of anti-sickling activity of extracts *in vitro*

To evaluate the anti-sickling effect of the extracts, whole blood samples were collected in EDTA tubes from twenty adult patients known to have sickle cell anemia disease. All patients were attending Hematology clinics and Day Care unit of the King Abdul-Aziz University Hospital. Written informed consent was obtained from patients according to the guideline no. 9 prepared in 2016 by the Council for International Organizations of Medical Sciences in collaboration with the World Health Organization [11]. The Ethics Committee at King Abdul Aziz University Hospital approved the study (approval no. 611-19).

Blood samples were diluted 1:1 with phosphate buffered saline (PBS) pH 7.4 to decrease RBCs clumping. Induction of sickling *in vitro* was carried out according to the method of Pauline and colleagues [3]. Each sample was incubated with 2 % sodium metabisulphite ( $\text{Na}_2\text{S}_2\text{O}_5$ ) and served as a positive control sample. Different concentrations of extracts were added to the 500 and 1000 µg/mL concentrations. Ten microliters of the mixture were loaded on the slide and covered with coverslip and then sealed with Dibutyl Phthalate Xylene (DPX) and incubated at 37 °C for 2 h. For negative control, the extract was replaced by PBS. A drop of each treated sample was examined at 40x objective lens of light microscope, and five different fields for normal and sickled RBCs were calculated to express the percentage of sickling.

### Evaluation of osmotic fragility

Osmotic fragility of erythrocytes measures the membrane stabilizing effect of the extracts in osmotic stress in hypotonic lysis incubation. The mean corpuscular fragility was determined from the concentration that cause 50 % hemolysis of

RBC as described earlier [3]. Briefly, 4 mL of different concentrations (0 – 0.9 %) of buffered saline with a pH of 7.4 was mixed with 1 mL of each extract having 500 µg/mL concentration and 0.05 mL sickled-shaped RBC blood in a 10 mL reaction vessel. The reaction was carried out separately for each extract and concentration. The mixture was then incubated at room temperature for 24 h and then centrifuged at 3000 rpm for 15 min. The absorbance of the supernatant was read at 540 nm against blank made of 0.9 % buffered saline.

### Statistical analysis

All data are expressed as mean  $\pm$  standard deviation (SD). Statistical analysis including one-way analysis of variance (ANOVA) while pairwise *t*-test was performed using MegaStat Excel (version 10.3, Butler University) and GraphPad Prism (version 8.0.1).  $P < 0.05$  was considered statistically significant.

## RESULTS

### Phytochemicals and total antioxidant capacity of extracts

#### Phytochemical properties

The phytochemical analysis of the plant extracts revealed the presence of flavonoids, tannins, alkaloids, and phenols as summarized in Table 1. The methanolic and aqueous extracts of *S. alexandrina* contained flavonoid compounds only. Methanolic extract of *A. javanica* was positive for tannins and flavonoids, whereas its aqueous extract was positive for alkaloids and phenols. Methanolic extract of *F. palmata* contained phenols, saponins, and tannins, whereas its aqueous extract was positive for phenols and flavonoids.

The total antioxidant activity for aqueous and methanol extract were 14228.5 and 18606 CRE/mg, respectively, for *S. alexandrina*. However, the highest TAC was observed with methanol extract of *F. palmata* (28237.88 CRE/mg) followed by its aqueous extract (Table 2). TACs of *A. javanica* extracts were better than TACs of *S. alexandrina* extract.

#### DPPH radical scavenging activity

The dose-response curve of DPPH radical scavenging activity of *S. alexandrina* extracts at different concentrations compared with vitamin E (control).

**Table 1:** Phytochemical compounds present in the plant extracts

Compound	<i>S. alexandrina</i>		<i>A. javanica</i>		<i>F. palmata</i>	
	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous
Alkaloids	-	-	-	+	-	-
Phenols	-	-	-	+	+	+
Saponin	-	-	-	-	+	-
Tannins	-	-	+	-	+	-
Flavonoids	+	+	+	-	-	+

**Table 2:** DPPH radical scavenging and antioxidant activity of the plant's extracts

Plant	EC <sub>50</sub> (µg/mL) of DPPH		TAC (CRE)	
	Methanol	Aqueous	Methanol	Aqueous
<i>S. alexandrina</i>	178	364	18604.47 ± 0.75	14228.73 ± 0.86
<i>A. javanica</i>	107	408	25828.86 ± 0.80	14665.67 ± 0.56
<i>F. palmata</i>	72	194	28237.88 ± 0.84	26925.26 ± 0.64

**Note:** CRE = Copper reducing equivalent; (n = 3)

**Table 3:** Anti-sickling activity of extracts *in vitro*. Values are presented as mean percent sickle cells ± SD in relation to control (100 %)

Plant	Extract type	Extract concentration (µg/mL)		
		125	250	500
<i>S. alexandrina</i>	Methanol	71.16±4.88	69.86±5.88*	64.69±5.89*
	Aqueous	72.45±4.93	71.20±6.16	67.22±5.96*
<i>A. javanica</i>	Methanol	29.47±5.93**	9.308±0.89***	0.35±0.01***
	Aqueous	36.48±4.16**	22.7±3.19***	1.23±0.1***
<i>F. palmata</i>	Methanol	36.43±3.88**	23.08±1.33***	2.726±1***
	Aqueous	40.59±5.3**	34.44±2.99**	22.41±2.01***

\*0.01 ≤ *p*-value < 0.05; \*\*0.001 ≤ *p*-value < 0.01; \*\*\**p*-value < 0.0001

It was observed that the methanolic extract had higher activity at low concentrations compared to water extract which gave the same activity but at higher concentrations. At the concentration of 238 µg/mL, the DPPH inhibition percentage of methanolic and aqueous extracts of *S. alexandrina* were 61 and 22 %, respectively. In terms of effective concentration, the methanolic extract had EC<sub>50</sub> of 178 µg/mL compared to aqueous extract which had EC<sub>50</sub> of 364 µg/mL (Table 2). The methanolic extract of *F. palmata* had the best EC<sub>50</sub> value (72 µg/mL) among all the plant extracts. The methanolic extracts had better EC<sub>50</sub> values than the aqueous extracts.

#### Anti-sickling activity of extracts *in vitro*

The anti-sickling activity of *S. alexandrina*, *A. javanica*, and *F. palmata* extracts are presented in Table 3. The *A. javanica* extracts significantly (*p* < 0.0001) reduced the percentage of sickle cells compared to the control (no treatment). The treatment of sickled cells with methanol and aqueous extracts of *A. javanica* resulted in a significant decrease of sickled-shaped cells in a concentration-dependent manner. The sickle-shaped cells were 29.47 % when treated with 125 µg/mL concentration of methanol extract of

*A. javanica* which further decreased to 0.35 % on increasing the concentration to 500 µg/mL. A similar trend was also observed with the extracts of *F. palmata*. The extracts of *S. alexandrina* were less effective in anti-sickling activities compared with the extracts of *A. javanica* and *F. palmata*. It was observed that methanol extracts were better in producing anti-sickling effects in comparison to the aqueous extracts.

#### Genotoxicity

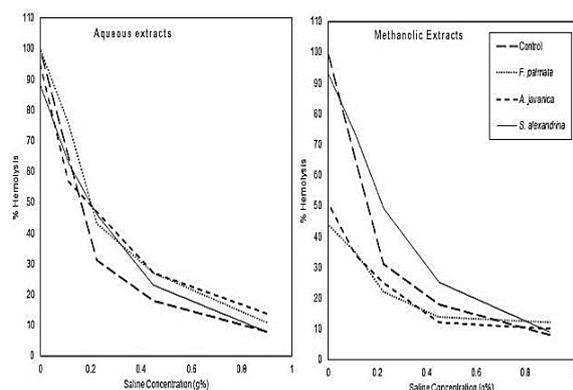
Around 300 leukocytes were analyzed for comet formation following treatment with the extracts at various concentrations. The comet result on the human peripheral blood leukocytes with methanolic and water extracts of *S. alexandrina*, *A. javanica*, and *F. palmata* at concentrations of 125, 250, and 500 µg/mL failed to produce any visible genotoxic effect on blood leukocytes. The tail moments as presented by EDRI images were very low at each indicated concentration. The number of comet cells at each treated concentration was less than 1 in control and treated samples. These comet results suggest that both methanolic and aqueous extracts are safe and do not damage DNA (Table 4).

**Table 4:** Comet assay parameters in peripheral blood leukocytes treated with leaf extracts

Extract type	Concentration (µg/mL)	Comet parameter	Plant		
			<i>S. alexandrina</i>	<i>A. javanica</i>	<i>F. palmata</i>
Methanol	125	% comet cells	≤ 1	≤ 1	≤ 1
		Tail moment	0.00	0.00	0.00
	250	% comet cells	≤ 1	≤ 3.5	≤ 1
		Tail moment	0.00	0.03±0.01	0.01±0.001
	500	% comet cells	≤ 1	10	≤ 1.1
		Tail moment	0.00	0.09±0.01	0.06±0.001
Aqueous	125	% comet cells	≤ 1	≤ 1	≤ 1
		Tail moment	0.00	0.00	0.00
	250	% comet cells	≤ 1	≤ 2	≤ 2
		Tail moment	0.00	0.01±0.004	0.01±0.002
	500	% comet cells	≤ 1	30	18
		Tail moment	0.00	0.15±0.01	0.35±0.09
Control	No treatment	% comet cells		≤ 1	
		Tail moment		0.0	

### Osmotic fragility

Aqueous and methanolic extracts of *S. alexandrina* have shown protective effect on erythrocyte osmotic fragility when treated with different saline concentrations. Both extracts have better osmotic fragility as compared to control at 500 µg/mL under the pressure of decreasing percent salinity (Figure 1). In other words, percent hemolysis was lesser in blood cells treated with methanolic and aqueous extracts of *S. alexandrina*, *A. javanica*, and *F. palmata* compared with the control.



**Figure 1:** The effect of extracts on stability of erythrocyte cell membrane in saline of (0 – 0.9 %) at 500 µg/mL

### DISCUSSION

The total antioxidant status is reported to be reduced in sickle cell anemia due to increased release of oxygen free radicals generated in the body by metabolic processes. Therefore, sickle cell disease individuals require greater glutathione (antioxidant) to facilitate metabolic processes than healthy individuals [12]. Hence, proper administration of antioxidants may greatly

help improve the well-being of such patients. Oxidative stress is the most important feature of SCD; hence, antioxidants are widely used to treat various disorders including SCD [13]. Several beneficial effects have been reported with the use of antioxidants such as protection of RBC against lipid peroxidation, increased levels of reduced glutathione as well as reduction in the levels of reactive oxygen species [13].

DPPH is a free radical compound that is reduced by accepting electrons or hydrogen and converted to a stable form. This reaction is irreversible, which gives an accurate way to evaluate the ability of compounds to act as free radical scavengers or hydrogen donors, and produce antioxidant activity [14]. Several studies have revealed the ability of plant extracts to reduce DPPH to a more stable form and hence it has been classified as free radical scavenger and antioxidant [15]. Although the extracts (methanol and aqueous) of *F. palmata*, *S. alexandrina*, and *A. javanica* exhibited significant anti-oxidant activities, the values were lower than that of vitamin E.

Various medicinal plants are reported for their anti-sickling activities, and research on these plants has been proved rewarding. Phytomedicines not only ameliorate the severity of SCD but are also reported to reverse sickling conditions *in vitro* [16]. Many herbal drugs are already on the market to treat SCD such as Niprisan, (renamed Nicosan) which contains *Piper guineense*, *Pterocapus osun*, *Eugenia caryophyllum*, and *Sorghum bicolor* as active components. Another herbal drug is Cikalvit in which the active components are *Cajanus cajan* seed extract and aqueous extracts of *Zanthoxylum zanthoxyloides* roots [16].

The plant extract to be used as therapeutic agent should be safe, and does not damage DNA as well as non-mutagenic. Similar to these results, many researchers have also reported with certain other plant extracts are used as therapeutic agents. The ethanol extract of *Portulaca oleracea* aerial parts has been observed not to be DNA damaging in lymphocytes by comet assay but has protective effects on H<sub>2</sub>O<sub>2</sub>-induced DNA damage [17]. The Vitotox test has revealed the absence of genotoxicity for an extract of aerial parts of *Fumaria officinalis*, which was further confirmed in the comet assay performed on C3A human cells [18]. Antioxidants have been shown to have several beneficial effects such as protection of RBC against lipid peroxidation, increases the level of reduced glutathione, and reduces levels of reactive oxygen species. The effect on RBC membrane permeability, however, has been largely unexplored [13]. Decreased percent hemolysis has also been reported in blood cells treated with other plant extracts such as *Zanthoxylum heitzii* [3]. Caffeic acid and *Clitoria ternatea* flower petal extract have been reported to possess remarkably high anti-hemolytic activity against chemically induced hemolysis [19].

## CONCLUSION

Aqueous and methanol extracts of *S. alexandrina*, *A. javanica*, and *F. palmata* demonstrate significant anti-sickling and antioxidant properties, and are not damaging to DNA. Furthermore, the extracts exhibit protective effect on erythrocyte osmotic fragility. Thus, in vivo studies are required to validate these results and for further development of the extracts for therapeutic use.

## DECLARATIONS

### Acknowledgements

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through project number IFPRC-168-141-2020 and King Abdulaziz University, DSR, Jeddah, Saudi Arabia.

### Funding

None provided.

### Ethical approval

None provided.

## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Conflict of Interest

No conflict of interest associated with this work.

## Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Nawal Helmi and Hani Choudhry conceived the idea, M-Zaki ElAssouli and Zahraa Al Yousef collected the plant samples, Faten Filimban identified the samples, Zahraa Al Yousef, Elaf Alaswad, and Dunya Nori conducted the experiments, Nawal Helmi and Hani Choudhry analyzed the results and Mohammad Alam wrote the manuscript. All authors read and approved the manuscript for publication.

## Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

## REFERENCES

1. Ilboudo Y, Bartolucci P, Rivera A, Sedzro JC, Beaudoin M, Trudel M, Alper SL, Brugnara C, Galactéros F, Lettre G. Genome-wide association study of erythrocyte density in sickle cell disease patients. *Blood Cells Mol Dis* 2017; 65: 60–65.
2. Poletti V, Urbinati F, Charrier S, Corre G, Hollis RP, Fernandez BC, Martin S, Rothe M, Schambach A, Kohn DB, et al. Pre-clinical development of a lentiviral vector expressing the anti-sickling  $\beta$ AS3 globin for gene therapy for sickle cell disease. *Mol Ther Methods Clin Dev* 2018; 11: 167–179.
3. Pauline N, Cabral BNP, Anatole PC, Jocelyne AMV, Bruno M, Jeanne NY. The in vitro anti-sickling and antioxidant effects of aqueous extracts *Zanthoxylum heitzii* on sickle cell disorder. *BMC Complement Altern Med* 2013; 13: 162.

4. Tounekti T, Mahdhi M, Khemira H. Ethnobotanical study of indigenous medicinal plants of Jazan Region, Saudi Arabia (Internet). *Evid Based Comple Alternat Med* 2019 (cited 2020 Feb 9). Available from: <https://www.hindawi.com/journals/ecam/2019/3190670/>
5. Khan AW, Jan S, Parveen S, Khan RA, Saeed A, Tanveer AJ, Shad AA. Phytochemical analysis and enzyme inhibition assay of *Aerva javanica* for ulcer. *Chem Cent J* 2012; 6: 76.
6. Alqasoumi SI, Basudan OA, Al-Rehaily AJ, Abdel-Kader MS. Phytochemical and pharmacological study of *Ficus palmata* growing in Saudi Arabia. *Saudi Pharm J* 2014; 22: 460–471.
7. Nasar S, Murtaza G, Mehmood A, Bhatti TM. Green approach to synthesis of silver nanoparticles using *Ficus palmata* leaf extract and their antibacterial profile. *Pharm Chem J* 2017; 51: 811–817.
8. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga LL. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *Afr J Tradit Complem Altern Med AJTCAM* 2011; 8: 1–10.
9. Locatelli M, Gindro R, Travaglia F, Coïsson J-D, Rinaldi M, Arlorio M. Study of the DPPH-scavenging activity: Development of a free software for the correct interpretation of data. *Food Chem* 2009; 114: 889–897.
10. ElAssouli SM, AlQahtani MH, Milaat W. Genotoxicity of air borne particulates assessed by comet and the salmonella mutagenicity test in Jeddah, Saudi Arabia. *Int J Environ Res Public Health* 2007; 4: 216–223.
11. International Ethical Guidelines for Health-related Research Involving Humans, Fourth Edition. Geneva. Council for International Organizations of Medical Sciences (CIOMS); 2016.
12. Ifeanyi OE, Stella EI, Favour AA. Antioxidants in the management of sickle cell anemia. *Int J Hematol Blood Disord (Internet)* 2018 (cited 2021 Mar 4); 3. Available from: <https://symbiosisonlinepublishing.com/hematology/hematology25.php>
13. Al Balushi H, Hannemann A, Rees D, Brewin J, Gibson JS. The effect of antioxidants on the properties of red blood cells from patients with sickle cell anemia. *Front Physiol (Internet) Frontiers*; 2019 (cited 2020 Nov 17); 10. Available from: <https://www.frontiersin.org/articles/10.3389/fphys.2019.00976/full>
14. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. *J Food Sci Technol* 2011; 48: 412–422.
15. Batool R, Khan MR, Sajid M, Ali S, Zahra Z. Estimation of phytochemical constituents and in vitro antioxidant potencies of *Brachychiton populneus* (Schott & Endl.) R Br *BMC Chem* 2019; 13: 32.
16. Imaga NA. Phytomedicines and nutraceuticals: alternative therapeutics for sickle cell anemia. *Sci World J (Internet). Hindawi*; 2013 (cited 2020 Nov 17); 2013: e269659. Available from: <https://www.hindawi.com/journals/tswj/2013/269659/>
17. Behravan J, Mosafa F, Soudmand N, Taghiabadi E, Razavi BM, Karimi G. Protective effects of aqueous and ethanolic extracts of *Portulaca oleracea* L. Aerial parts on H<sub>2</sub>O<sub>2</sub>- induced DNA damage in lymphocytes by comet assay. *J Acupunct Meridian Stud* 2011; 4: 193–197.
18. Edziri H, Guerrab M, Anthonissen R, Mastouri M, Verschaeve L. Phytochemical screening, antioxidant, anticoagulant and in vitro toxic and genotoxic properties of aerial parts extracts of *Fumaria officinalis* L. growing in Tunisia. *South Afr J Bot* 2020; 130: 268–273.
19. Phruksanan W, Yibchok-anun S, Adisakwattana S. Protection of *Clitoria ternatea* flower petal extract against free radical-induced hemolysis and oxidative damage in canine erythrocytes. *Res Vet Sci* 2014; 97: 357–363.