ANTISICKLING EFFECT OF ALLIUM SATIVUM AND CARICA PAPAYA

Abstract

Present study deals with those antioxidant molecules might effectively block the polymerization of sickle cell haemoglobin while also enhancing the oxidant status of sickle erythrocytes. The differing concentrations of antioxidant molecules, such as polyphenol and flavonoids, found in the sections of *Carica papaya* and *Allium sativum*, could be the cause of the disparity between the antisickling properties. Additionally, the degree of the active antisickling drugs would be determined by the molecules' affinity with the haemoglobin binding site. The heme pocket and the erythrocyte membrane are the likely sites for binding, as is the case with many known anti-sickling substances. The phenolic compound's antioxidant properties are crucial in the neutralisation of free radicals, which can harm cells if they remain in the system. Therefore, increasing dietary antioxidant intake from sources like *Carica papaya* and *Allium sativum* may help to maintain a sufficient level of antioxidant defence and thereby aid in the management of sickle cell disease.

Keywords: Antisickling agents; phenolic compounds; antioxidants

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I. INTRODUCTION

Sickle cell disease (SCD) is a chronic, inherited condition affecting the red blood cells and various organs in the body through the blood. It is a genetic disease caused by abnormal haemoglobin called sickle haemoglobin (HbS) which polymerises under deoxygenated condition and deform the RBC into 'Sickle' shape. The nucleotide sequencing of β- globin mRNA from sickle β-globin genes revealed that the normal codon GAG at position β6 has been replaced by GUG. People with SCD typically feel good in between periods of sickness. Long-term problems could develop. Sickling can be brought on by a cold, an infection, a lack of fluid in the body (dehydration), or low oxygen levels. Early intervention with effective treatment can reduce the risk of problems. Early diagnosis and specialised care are therefore recommended for SCD.

Sickle cell illness is distinct from sickle cell trait. If someone may have sickle cell trait if your sickle cell gene is present but does not often result in disease. White sickle cell trait (HbAS), which has never been deemed a disease, sickle cell thalassemia, sickle cell HbC illness, and forms of sickle cell anaemia all have one faulty gene. Sickle cell illness and sickle cell trait are two distinct conditions. You may have sickle cell trait if the disease is not typically brought on by the sickle cell gene. One defective gene is present in the forms of sickle cell anaemia HbSS, sickle cell HbC illness, sickle cell thalassemia, and white sickle cell trait (HbAS), which has never been deemed a disease.

The most common variations of the gene include the following:

1. ***Sickle Cell Trait*:** A single faulty gene is present in the individual who has the sickle cell trait. They have both normal HbA and the destructive HbS form of haemoglobin, to some extent. This is known as HbSS. Typically, sickle cell trait carriers don't exhibit any disease-related symptoms. Red cells often have tiny sizes and there may be mild anaemia. Under extremely demanding circumstances, tiredness, hypoxia (poor oxygen), and/or severe infection may ensue, causing sickling of the faulty haemoglobin and some problems related to sickle cell disease. The majority of sickle trait carriers lead typical lives.

2. ***Sickle Cell Anemia:*** The sickle haemoglobin (HbS) has replaced most or all of the individual's normal haemoglobin (HbA). This is known as HbSS. Of all the sickle cell variants, it is the most serious. Due to the shape and thickness of sickled cells, these persons experience a number of difficulties. People with HbSS frequently also experience severe and persistent anaemia.

3. ***Sickle Cell-Hemoglobin SC Disease*:** The individual only possesses one copy of HbS and HbC. HbSC is a common name for this. Red blood cells that are aberrant arise as a result of haemoglobin C. A person with only a small amount of haemoglobin C and normal haemoglobin will typically not have any anaemia symptoms, but they could go on to experience anaemia and difficulties with their eyes and hips later in life. However, a person may get mild to moderate anaemia if they have both HbS and HbC. These patients frequently experience milder versions of some of the difficulties linked to HbSS, or sickle cell disease. Similar characteristics of HbSS and HbSC include vascular cries (blood flow

is impeded because sickled cells have lodged in blood vessels), organ damage from recurrent sickling and anaemia, and a high risk of infection.

4. ***Sickle Cell- Haemoglobin E Disease*:** This variant resembles sickle cell disease type C with the exception that a component of the haemoglobin molecule has been changed. Some patients with haemoglobin E illness do not exhibit any symptoms, and Southeast Asians are the community where this variant is most prevalent. However, mild to moderate anaemia may develop in specific circumstances, including tiredness, hypoxia, severe illness, and/or iron shortage.

Two medicinal plants *Allium sativum* and *Carica papaya* were preferred in this study are of great importance to the health of SCD individuals. The present strategy for treating sickle cell disease focuses on the potential contribution of diet, and this has produced encouraging outcomes (Ekeke, 2000). Other treatment modalities have undoubtedly been created, including the use of stem cells and gene replacement therapy (Kumar and Clark, 1999). Numerous substances have been examined for their potential function in preventing gelation or polymerization and stabilising the erythrocyte membrane, including zinc, short and long chain fatty acids, tucaresol, erythropoietin, amino acids, vitamins, and minerals (Lewis and William, 2008).

In vitro development of dense cells is hampered by aged garlic extract (AGE) and its antioxidant-active components, S-allylcysteine and N alpha-(1-deoxy-D-fructos-1-yl)-L-arginine (fructosyl arginine). Vitamins C and E, as well as the spin-trapping substances alpha-(4-pyridyl-1-oxide)-N-t-butylnitrone and 5-diethoxyphophoryl-5-methyl-1-pyrroline-N-oxide, were all successful in preventing the development of dense cells in vitro. The findings suggested that the membrane of the erythrocyte becomes vulnerable to oxidative damage by reactive oxygen species when extremely stretched sickle-shaped cells are generated by the recurrence of deoxy-oxy cycle. The calcium-activated potassium efflux channel and the development of dense cells are inhibited by protecting the erythrocyte membrane from such oxidative damage that prevents the membranes from becoming leaky to the calcium ion.

Carica papaya is a nutritious powerhouse that is available all year long. *Carica papaya* is one the major fruit crop cultivated in tropical and subtropical zones. Papaya was used to treat common colds, hay fever and asthma. Garlic is nicknamed as Russian penicillin for its widespread use as a topical and systemic antimicrobial agent. The three potent antioxidants vitamin C, vitamin A, and vitamin E are abundant in it. Magnesium, potassium, vitamin B pantothenic acid, folate, and fibre are all examples of minerals. Along with all of this, it also has papain, a digestive enzyme that effectively cures allergies, traumatic injuries, and sports injuries. Collectively, papaya's nutrients strengthen the cardiovascular system, guard against heart disease, heart attacks, and strokes, and guard against colon.

II. MATERIALS AND METHODS

1. **Collection of Blood Samples:** The sickle cell institute Chhattisgarh, Raipur, collected the blood sample from a patient in the age range of 5-35 years old who was known to have SCD for the purposes of this study's evaluation of the antisickling activity of the plant

extract. Written informed consent was received from all participangts with the approval of the Institutional Ethical Committee for study benefits of humans in general. Following HPLC for a multivariant haemoglobin analysis, the electrophoresis test was used to validate the SS status of each of these patients. One patient provided a blood sample in the heparin tubes that was around 3.0 ml in size.

2. **Clinical Laboratory Test:** Clinical laboratory tests such as reticulocyte count, direct bilirubin, lactate dehydrogenase (LDH), and foetal haemoglobin (HbF), were performed according to standard procedure at the Pt. Jawahar Lal Nehru Memorial Medical College, Raipur Chhattisgarh.

3. **Collection of Plant Material:** The plant material was gathered from nearby Raipur, Chhattisgarh, and air dried for two weeks at room temperature (26ºC). It was then ground into a uniform powder using a mechanical grinding machine to improve the efficiency of the solvent's contact with the plant material's sites.

4. **Extraction of Plant Material:** An exhaustive Soxhlet extraction procedure using aqueous-methanol (1:3, 60–80°C) as the solvent was used to extract dried plant components. The extract was used for additional phytochemical analysis after being freeze-dried and kept at 4°C.

5. **Phytochemical Screening**

- **Qualitative Analysis**

➢ **Alkaloid:** The 500 µL of Hager's reagent (saturated picric acid solution) was added to 500 µL of extract and thoroughly mixed. The production of a yellow-coloured precipitate indicated the presence of alkaloid (Gracelin *et al*., 2013).

➢ **Tannin:** A few drops of 5% freshly made ferric chloride solution were added to 1 mL of extract in 1 mL of water. The presence of tannin in the extract is indicated by the presence of green or black precipitate (Gracelin *et al*., 2013).

➢ **Saponin:** After vigorous shaking, 500 µL of extract was dissolved in 2 mL of distilled water. The presence of saponin in the extract is indicated by the foam's brief persistence (Gracelin *et al*., 2013).

➢ **Flavonoids:** After treating 500 litres of extract with 500 µL of 10% leadacetate solution, the presence of flavonoids was detected by the emergence of a yellow tint (Gracelin *et al*., 2013).

➢ **Total Phenol:** The 500 µL of the extract were combined with 1 mL of freshly made, 2% ferric chloride. The presence of phenol in the extract is indicated by the colour, which can be blue-green or black (Gracelin *et al*., 2013).

- **Quantitative Analysis**

➢ **Alkaloid:** By combining BCG solution with a pH 4.7 phosphate buffer, alkaloid estimation was conducted. An equal amount of each reagent was dissolved. 500 µL of

the aforesaid reagent (phosphate buffer and BCG solution) was added to 500 µL of the extract. Around 2-3 mL of chloroform was added to the tubes at this point, and eliminate the top blue layer before reading the absorbance at 470 nm against a blank. In varied concentrations, atropine was utilised as a standard (Harborne, 1998).

- **Tannin:** The Vanilline-Hydrochloride method was used to estimate the total tannin content. Take an equal amount of 4% vanillin and 8% HCl in methanol. Just before using, the solution must be blended. 500 µL of vanillin hydrochloric reagent were used to process 500 µL of extract. At 500nm, the absorbance was measured. Tannic acid was used as a standard at 1g/mL (Md Ahasun *et al*., 2019).

- **Saponnin:** The amount of total saponin was estimated using 500 µL of extract that had been dissolved in 500 µL of 80% methanol, 1 mLof vanillin in ethanol, and 1 mL of 50% sulphuric acid solution. After 10 minutes of incubation on a water bath at 60 degrees Celsius, the absorbance at 544 nm was measured in comparison to a blank. As a standard, diosgenin dissolved in hot ethanol was employed (1 mg/mL) (Obodoni-Ochuko, 2001).

- **Flavonoids:** A modified version of the aluminium chloride colorimetric method was used to estimate the total flavonoid content. A mixture of 500 µL of sample extract, 1.5 mL of methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M potassium acetate, and 2 mL of distilled water was combined and left at room temperature for 30 minutes. At 420nm, the absorbance was measured. The standard utilised was 1 mg/mL of quercetin. The standard curve was prepared to calculate the flavonoid content, which was then represented as quercetin equivalent (mg/mL) (Arumugam, 2006).

- **Total Phenol:** With few modifications, the Folin-Ciocalteu reagent method was used to estimate total phenol. 0.5 mL of plant extract was combined with 1.5 mL of 10% Folin-Ciocalteu reagent and 1 mL of 2% Na2CO3 and shaken. The resulting combination was allowed to sit at room temperature for 15 minutes. The sample's absorbance was determined at 765 nm. 1 mg/mL of gallic acid was utilised as the standard. The findings were from a curve that was reported as gallic acid equivalence (mg/g of isolated substance) (Makkar *et al*., 1993).

6. **Osmotic Fragility:** After 24 hours of incubation at 25°C, the extract's ability to stabilise the membrane in response to osmotic stress or hypotonic lysis was measured by the erythrocytes' osmotic fragility (Jaja *et al*., 2000).

7. **Antisickling activity**
   - Evaluation of in vitro induction (Joppa *et al*., 2008).
   - Evaluation of antisickling effect of extract

8. **Statistical Analysis:** The results of each test were carried out in triplicate, and they are shown as mean SEM. The antisickling effect of medicinal herbs was studied using one-way analysis of variance (ANOVA).

III. RESULTS AND DISCUSSION

Bilirubin, a byproduct of liver metabolism, typically ranges between 0.2-1 mg/dL in blood, but the current investigation indicated that its concentration was elevated in 10 samples of patients (2.09±0.3). The discovery is attributed to stress on liver function. Additionally, 10 instances samples (31.2±8.1) beyond the normal limit showed increased SGPT activity. Renal and hepatic dysfunction are also associated with increased SGPT activity. Since bilirubin is a metabolic by-product of haemoglobin, conditions like hepatocellular injury (toxic hepatopathy, tumours), intra- and extra-hepatic biliary tract blockages, intravascular and extravascular hemolysis, etc., result in higher blood total bilirubin levels.

In cholestasis and towards the end of the progression of chronic liver disease, there is an unproportionate rise of direct bilirubin. In the current investigation, enhanced SGPT activity was also identified in 10 samples, suggesting that hepatocytes, myocardial cells, erythrocytes, or skeletal muscle cells may have died. Findings imply that hepatic impairment develops under the strain of sickle cell anaemia. The conclusive conclusion in sickling may result in widespread kidney and liver damage in the community (Pandey et al., 2016).

The concentration of HbS for the sickled patient included in the study is determined to be (75.17±0.88). CBC findings show that people with sickle cell disease are typically anaemic. Hematocrit values for sickled patients are typically lower than normal values (34.53±1.5; 29.68±5.0). As was customary at the time (Allexy *et al*., 2010). As a result of erythrocyte breakdown, higher white blood cell (9.39±0.5), platelet count (331.4±24.72), MCHC (33.53±0.5), and reticulocytes levels are also seen. In the 10 patients, the HbF level was high (16.98±1.13) (Table 1). Few participants reported HbF levels higher than 20%, with the bulk of subjects having levels between 2 and 15% (Tshilolo *et al*., 2012).

In general, less severe illness was linked to greater HbF levels. Children with high HbF levels had much fewer hospitalisations, transfusions, and painful episodes over the course of the previous year, but this link was not statistically significant. Additionally, those who had high HbF levels were less likely to have suffered from dactylitis or a stroke. Those who did get dactylitis were more likely to do so later in life, although this association was not statistically significant. A study discovered a link between LDH and indicators of hemolysis and organ failure. Several indicators of the severity of the hemolysis were substantially linked with LDH.

The levels of haemoglobin and haptoglobin were inversely linked with LDH. Plasma arginase activity, another enzyme abundant in sickle erythrocytes and linked to indicators of hemolytic severity, and plasma haemoglobin levels, reticulocyte counts, haemoglobin S concentration, and LDH all showed significant correlations. Aspartate aminotransferase (AST) and direct or indirect bilirubin were two markers of typically high hemolysis or liver illness that were strongly correlated with LDH (Jun and Yukihiro, 2013). Although this link was seen between LDH and leukocyte count, most likely due to turnover of the enormous numbers of leukocytes produced in patients with SCD, it was also seen with alanine aminotransferase (ALT), a very specific marker of hepatic damage.

There was no correlation between LDH and other organ injury biomarkers such creatinine, alkaline phosphatase, and creatine kinase (Table 1). Plasma concentrations of soluble VCAM-1, a measure of endothelial cell activity, substantially associated with LDH. Other adhesion molecules, such as ICAM-1, P-selectin, and soluble E-selectin, showed weak but significant connection. Multiple hemolysis-related indicators and LDH levels are linked. LDH and HbF levels did not correlate.

Table 1: Blood Parameters Studied in SCD Patients (HbSS) Over Control (HbAA)

Blood parameter	Control	Patients
WBC (10^3/µL)	7.46±0.8	9.39±0.5
Lymph (#10^3/µL)	1.91±0.5	3.52±0.2
Mid (#10^3/µL)	0.52±0.05	0.79±0.6
Gran (#10^3/µL)	5.03±0.7	5.08±o.2
Lymph (%)	28.58±5.05	36.88±1.2
Mid (%)	7.97±0.8	8.68±0.6
Gran (%)	63.45±5.1	54.17±3.2
HGB (g/dL)	11.3±0.5	10.21±0.1
RBC ((10^6/µL)	4.36±0.2	3.67±1.6
HCT (%)	34.85±1.5	29.68±5.0
MCV (fL)	81.91±3.7	83.85±2.1
MCH (pg)	26.61±1.3	28.19±1.7
MCHC (g/dL)	32.25±0.2	33.53±0.5
RDW-CV (%)	14.69±0.3	17.5±0.7
RDW-SD (fL)	47.45±2.1	55.46±2.4
PLT (10^3/µL)	226.2±26.9	331.4±24.72
MPV (fL)	9.78±0.2	8.35±0.2
PDW (%)	14.85±0.2	16.6±0.3
PCT (%)	0.22±0.09	0.27±0.6
LDH (%)	470.20±95.5	764.53±334.7
Bilirubin T (mg/dL)	0.16±0.02	2.09±0.3
Bilirubin D (mg/dL)	0.05±0.01	0.27±0.6
SGOT (U/L)	16.3±3.8	37.5±8.7
SGPT (U/L)	13.8±3.5	31.2±8.1
HbA2 (%)	-	3.72±0.37
HbF (%)	-	16.98±1.13
HbS (%)	-	75.17±0.88

The goal of the standardised extraction process for crude pharmaceuticals (medical plant parts) is to obtain the therapeutically needed portions and to remove undesirable material through treatment with the menstruum, a selective solvent. The thusly acquired extract can then be processed to be added to any dosage form, such as pills and capsules, or utilised as a medicinal agent as is in the form of tinctures or fluid extracts. These products contain a complex blend of many plant metabolites that have therapeutic properties, including alkaloids, flavonoids, tannins, saponins, and phenol.

Alkaloids, flavonoids, tannins, saponins, and phenol were found in a variety of plant extracts from *Allium sativum* and *Carica papaya* when phytochemical screening was done on the extracts in methanol, aqueous-methanol, and water. The investigation's findings, which are summarised in Table 2, indicated the presence of both physiological activity and medicinal action. The methanolic extract of *Carica papaya* was discovered to contain 150 mg of alkaloid content (Michael *et al*., 2021). The highest flavonoids in *Carica papaya* were 210 mg in aqueous-methanol extracts, 120 mg in methanolic extract, and 100 mg in water extract, respectively.

Allium sativum has high flavonoid content in water, at 100 mg, followed by 40 mg in methanolic extract and 30 mg in aqueous extract. More than 2000 distinct flavonoids have been extracted from fruits and vegetables up to this point (Tiaz and Ziegler 2006), and they are taken in the form of fruits and vegetables. They are non-toxic and may have potential health benefits for humans.

Tannin concentration in *Carica papaya* was measured at 440 mg in aqueous-methanolic extracts, with 360 mg and 310 mg of water extract coming in second and third, respectively. The amount of tannin in water extract from *Allium sativum* was reported to be substantial, at 300 mg, 140 mg, and 110 mg in methanolic and aqueous-methanolic extract, respectively. According to Buzzini *et al*. (2008), tannin is typically recommended for the treatment of inflammation, leucorrhoea, gonorrhoea, burns, piles, diarrhoea, and as an antidote for the treatment of alkaloid poisoning.

Allium sativum was found to have the greatest total saponin content, with 80 mg in the methanol and aqueous-methanolic extracts and 70 mg in the water extract. Similarly, *Carica papaya* saponin concentration was found to be greater in methanolic extract, where it was 60 mg, and in aqueous-methanolic extract of *Allium sativum*, where it was 50 mg, with a value of 520 mg. The yields from the water and methanolic extracts were 460 and 260 mg, respectively. *Carica papaya* yielded 40 mg in methanolic and water extracts and 50 mg in an aqueous-methanolic extract. The antioxidant capability of specific plant species can be significantly increased by phenolic compounds (Cai *et al*. 2004) Table 2.

Table 2: Phytochemical Screening of Secondary Metabolites in Microgram (mg)

	Allium sativum			***Carica papaya***		
Solvent → Metabolites ↓	**Methanol**	**Aqueous Methanol**	**Water**	**Methanol**	**Aqueous Methanol**	**Water**
Alkaloids	-	-	-	150	-	-
Flavonoids	40	30	100	120	210	100
Tannins	140	110	300	360	440	310
Saponins	80	80	70	60	50	50
Phenol	460	520	260	40	50	40

Symbol'-'indicated absence; Data given are the mean of three replicates

Regarding erythrocyte osmotic fragility, this study demonstrated a considerable increase in the concentration of NaCl at 250 mg/mL of extracts while decreasing the percentage of hemolysis. The percentage of hemolysis was reduced in all of the studied

extracts. The aqueous-methanolic and water extracts of *Allium sativum* and *Carica papaya* showed the most significant reduction in hemolysis compared to the other extracts of the two plants used, and could therefore have better protective effects on the erythrocyte membrane. Three extracts of *Allium sativum* and *Carica papaya* (Methanol, Aqueous-methanol, Water) were tested.

The extract was able to lessen membrane fragility by preserving the integrity of the erythrocyte membrane and boosting its resistance to osmotic stress/lysis. According to these erythrocyte investigations, *Allium sativum* and *Carica papaya* extracts inhibit hemolysis and have some protective effects on the erythrocyte membrane (Imaga *et al*., 2009) (Table 3).

Table 3: Osmotic Fragility Measured in Percentage

Conc. of NaCl in %	**Blank**	***Allium sativum***			***Carica papaya***		
		Methanol	**Aqueous methanol**	**Water**	**Methanol**	**Aqueous methanol**	**Water**
0.35	100±0.00	72.92±1.8	64.2±5.7	62.16±4.9	75.08±2.4	70.89±5.2	63.56±4.6
0.40	100±0.00	73.51±4.9	69.38±4.5	65.43±6.0	75.59±4.5	73.51±4.9	72.07±6.9
0.45	100±0.00	68.12±6.1	67.86±5.8	61.89±5.0	73.23±2.7	71.43±4.9	69.11±5.8
0.50	100±0.00	70.43±5.0	66.39±3.5	57.85±4.9	68.42±5.3	56.94±5.2	56.21±4.3

Mentioned values are mean±SEM from 10 different SCD patients.

Sickling increased from 47.01 to 88.08 as a result of the sodium metabisulphite (2%) sickling induction. Significant reduction in the percentage of sickling cells was seen after treating RBCs with various extracts at doses of 250, 500, and 1000 mg/mL. This percentage of sickling changed for the methanolic extracts of *Allium sativum* at 250 mg/mL (53.7 ±5.3), 500 mg/mL (38.78 ±4.1), and 1000 mg/mL (41.03 ±5.9). When compared to previous methods, a 250 mg/mL aqueous-methanol extract of *Carica papaya* demonstrated a significant decrease in the percentage of sickled cells (59.3± 3.4). Both medicinal plants' fruit extracts shown severe deterioration (Table 4). A current trend in the management of tropical diseases and genetic disorders like sickle cell anaemia, with a view to finding less expensive, alternative medicines that the general populace can immediately access, is the primary essential information regarding the research into phytotherapy of disease.

Recent research has demonstrated the antisickling properties of the unripe papaya fruit extract and the whole bulb of *Allium sativum*. The most effective doses of the *Carica papaya* aqueous methanolic extract were 250 and 500 mg/ml. It was discovered that the extract had a sizable amount of powerful antisickling action and significantly influenced the time course for sickling in a dose-dependent way. As part of their antisickling activity, antisickling drugs have been observed to lengthen the Hb polymerization delay time.

This may also suggest that, unlike previous reported substances (Abdulmalik *et al*., 2005) whose antisickling activities are predicated on the interaction with HbS molecules, the effect of the extract is likely at the cell membrane level rather than direct interaction with HbS molecules.

Table 4: Antisickling Effect of Different Solvent Extracts of Plant Measured in Percentage

Dose (µg/mL)	Methanol	Aq. Methanol	Water	p-value
Allium sativum				
250	53.7±5.3	52.59±4.5	37.16±3.2	0.025
500	38.78±4.1	29.13±3.7	27.31±3.0	0.030
1000	41.03±5.9	31.92±4.0	34.62±3.0	0.439
Carica papaya				
250	54.9±4.2	59.3±3.4	29.85±3.4	0.001
500	41.09±3.6	39.49±3.2	34.66±2.7	0.432
1000	40.91±3.3	32.04±3.5	30.72±2.2	0.010

Mentioned values are mean±SEM from 10 different SCD patients

The osmotic fragility test was used to investigate how different concentrations of *Carica papaya* and *Allium sativum* extract affected the erythrocyte membrane. The results showed that the herb had considerable membrane-protective effects and that it had an inhibitory effect on the hemolysis of red blood cells.
Patients who take this herb will experience complete suppression of the sickling effect at modest doses. These findings support the ethnomedical application of the plant and suggest the viability of *Carica papaya* and *Allium sativum* extract as an appealing potential choice for SCD therapy (Imaga *et al*., 2013).

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REFERENCES

[1] Abdulmalik O, Safo MK, Chen Q, Yang J, Brugnara C, Abraham DJ O-FK, and Asakura T. 2005. 5-hydroxymethyl-2-furfural modifies intracellular sickle haemoglobin and inhibits sickling red blood cells. *British Journal of Haematology*. 128(4): 552-561.
[2] Allexy T, Sanqkatumyong S, Connes P, Pais E, Tripette J, Barthelemy JC, Fisher TC, Meiselman HJ, Khoo MC, and Coates TD. 2010. Sickle cell disease: Selected aspects of pathophysiology. *Clinical Hemorheology and Microcirculation*. 44 (3): 155-166.
[3] Arumugam P, Ramamurthy P, Santhiya ST, and Ramesh A. 2006. Antioxidant activity measured in different solvent fractions obtained from *Mentha spicatalinn*. *Asia Pacific Journal of Clinical Nutrition*, 15(1): 199-224.
[4] Bazzini P, Arapitsas P, Goretti M, Brand E, Turchetti B, Pinelli P, Leri F, and Romani A, 2008. Antimicrobial and antiviral activity of hydrolysable tannins. *Mini Reviews in Medicinal Chemistry*. 8 (12): 1179-1187.
[5] Cai Y, Luo Q, Sun M, Corke H. 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinise medicinal plants associated with anticancer. *Life Science*. 74: 2157-2184.
[6] Ekeke GI, Uwakwe AA, and Nwaoguikpe RN. 2000. Edible legumes as beneficial antisickling agents. *Nigerian Journal of Biochemistry and Molecular Biology*, 16: 200-203.
[7] Gracelin DHS, De Britto AJ, and Kumar PBJR. 2013. Qualitative and quantitative analysis of phytochemicals in five pteris species. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5: 105-107.

[8] Harborn JB. 1998. Phytochemical methods. A guide to modern techniques of plant analysis. Chapman and Hall publication. 4th edition.
[9] Imaga NA. 2013. Phytomedicine and nutraceuticals: Alternative therapeutics for sickle cell anemia. *Scientific World Journal*. 2013:269659.
[10] Imaga NOA, Gbenle GO, Okochi VI, Akanbi SO, Edeoghon SO, Oigbochie V, Kohinde MO, and Bamiro SB. 2009. Antisickling property of carica papaya leaf extract. *African journal of Biochemistry Research*. 3(4): 102-106.
[11] Jaja SI, Kehind MO, Gbenebites S, Mojiminyi FBO, and Ogungbemi AI. 2000. Effect of vitamin C on arterial blood pressure, irreversible sickled cells and osmotic fragility in sickle cell anemia subjects. *Nigerian Journal of Physiological Sciences*. 16(1): 14-18.
[12] Joppa KM, Vovor A, Eklu-Gadegbeku K, Agbonon A, Akikokou K, and Gbeassor M. 2008. Effect of Morinda lucida Benth. (Rubiaceae) and Newbouldialeavis P. Beauv. (Bignoniaceae) on sickling of red blood cells. *Journal of Tropical Medicine*. 68(3): 251-256.
[13] Jun M and Yukihiro S. 2013. Hepatic Manifestations in Hematological Disorders. *International Journal of Hepatology*. 2013:484903.
[14] Kumar P, and Clark M. 1999. Hematological Diseases. Clinical Medicine (4thedn). W.B. Saunders: Edinburg; 353-362.
[15] Lewis H, William M. 2008. Diet and Nutrition in Sickle Cell Disease. SEARCH drSpock; 1-3.
[16] Makkar H, Bluemmel M, Borowy N, and Becker K. 1993. Gravimetric determination of tannin and their correlation with chemical and protein precipitation methods. *Journal of the Science of food and Agriculture*. 61: 161-165.
[17] Md Ahasun H., Arrafy R., Fahmida K., Md Abdul H., Syed M., Al S., Md Tajul I., Harun R., and Md Sharifull I. 2019. Comparative Analysis on Antioxidant Activities of Barks from *Sonneratiacaseolaris* Grown in Three Saline Zones of Sundarbans. *Biological Medicinal Chemistry*, 1: 1-6.
[18] Michael H, Jeffrey M, and Vafa A. 2021. Alkaloids Used as Medicines: Structural Phytochemistry Meets Biodiversity—An Update and Forward Look. *Molecules*. 26: 1836-1854.
[19] Obodoni BO, and Ochuko PO. 2001. Phytochemical studies and comparative efficiency of the crudeextracts of some Homostatic plants in Edo and Delta states of Nigeria. Global journal of Pure Applied Science, 8: 203-208.
[20] Pandey, MM., Khatoon, S., Rastogi, S., and Rawat, AKS. 2016. Determination of flavonoids, polyphenols and antioxidant activity of T*ephrosia purpurea*: a seasonal study. *Journal Integrative Medicine*. 14 (6): 447-455
[21] Tiaz L, and Ziegler E, 2006. Plant Physiology. Sinauer Associates, Publisher, Massamchusetts, 4th ed 13: 315-344.
[22] Tshilolo L, Summa V, Gregorj C, Kinsiama C, Bazeboso JA, Avvisati G and Labie D. 2012. Foetal haemoglobin, erythrocytes containing foetal haemoglobin, and hematological features in congolese patients with sickle cell anaemia. *Anemia*. 1-7.

