In vitro anti-sickling activities of *Sorghum bicolor* (L.) Moench

ABSTRACT

The use of medicinal plants to cure and prevent human ailments, including Sickle Cell Disease (SCD), is an old traditional method. *Sorghum bicolor* has been used traditionally in the management of SCD. The objective of the current study was phytochemical evaluation and screening of anti-sickling activity of various fractions on sodium metabisulphite induced sickling of HbSS erythrocytes. Chemical screening of extract of *Sorghum bicolor* seeds revealed the presence of flavonoids, alkaloids, saponins, phenol, and tannin. The sickle reversal test results showed that ethyl acetate soluble fraction had highest anti-sickling activity following n-hexane, butanol and water-soluble fractions. In hypoxia, sickle erythrocytes reverse into their usual biconcave form was observed after treating with the extract. These results further indicate *Sorghum bicolor* seed extract as potential phytotherapy for sickle cell anemia.

Keywords: Anti-sickling, Phytochemical, *Sorghum bicolor*.

1. Introduction

Sickle Cell Disease (SCD) is a challenging genetic recessive disorder that affects millions of individuals worldwide, particularly those of African, Mediterranean, Middle Eastern, and Indian descent [1]. In India, central and western states like Madhya Pradesh, Maharashtra, Gujarat, Kerala, Tamil Nadu, Odisha, and Chhattisgarh have startlingly high rates of sickle cell anemia [2]. This genetic mutation affects the β-globin gene, which encodes a subunit of hemoglobin, a protein that transports oxygen in the bloodstream. In individuals with SCD, a point mutation causes the substitution of a single amino acid (valine for glutamic acid) in the β-globin chain, leading to the production of abnormal hemoglobin known as hemoglobin S (HbS) [3]. Under specific conditions, such as inadequate oxygen supply or dehydration, HbS molecules become polymerized, forcing red blood cells to form a sickle shape. These sickled cells are rigid and prone to sticking to blood vessel walls, obstructing blood flow and triggering a cascade of complications [4]. Although there are currently no particular medications available to treat the genetic hereditary disease, However, the first line of clinical care for sickle cell disease involves the use of hydroxyurea, antibiotics, antimalarial prophylaxis, folic acid, amino acid supplementation, blood transfusions, and bone marrow transplants. First-line clinical treatments are expensive and come with risks. [5].

There is therefore a need for continuous search for alternative ways of treating SCD. In recent decades, there has been an increasing interest in investigating natural substances for potential therapeutic effects in a variety of disorders, including SCD. The concept of anti-sickling activity revolves around the ability of certain compounds to inhibit the polymerization of hemoglobin S prevents red blood cells from sickling. By maintaining the normal biconcave shape of erythrocytes, these compounds mitigate the risk of vaso-occlusive crises and improve blood flow, thereby alleviating symptoms associated with SCD. Across various cultures, indigenous healers have long utilized numerous medicinal plants with purported anti-sickling properties as part of their therapeutic arsenal. Some of them are; *Bombax pentadrum, Ficus capensis, Parinari mobola* and *Ziziphus mucronate* [6]. Since *Sorghum bicolor* has a rich phytochemical composition and proven pharmacological characteristics, it has become one of the more appealing options. *Sorghum bicolor* represent one of the major vegetal sources of phenolic compounds such as phenolic acids (ferulic, p-coumaric, and protocatechuic acids), flavonoids (Luteolinidin, apigeninidin, 5-methoxyluteolinidin), carotenoids (lutein, zeaxanthin, and β-caroteneis), Phytosterols (β-Sitosterol, campestero and stigmasterol) and tannins which produce various pharmacological effects [7-10].

In World traditional medicine, *Sorghum bicolor* has been used for the treatment of several diseases such as Cardiovascular Diseases, Diabetes, Cancer, Inflammation, Oxidative Stress, Dyslipidemia, Obesity and antiradical activities of the plant extracts have been validated in recent pharmacological studies [11-12]. Though *Sorghum bicolor* has been widely used in traditional medicine to treat a variety of ailments, there is little evidence available about the plant’s possible anti-sickling capabilities. However, a recent ethnobotanical study found that the herbs are traditionally utilized in the treatment of SCD [13]. This provides some validity for the plant’s ethnomedicinal applications in the prevention and management of SCD. The present investigation is being carried out with the objective of determining the anti-sickling activity of seeds of *Sorghum bicolor* and, more specifically, to determine the solvent fraction that possesses the most potent anti-sickling activity.

2. Materials and methods

2.1 Sample collection and extract preparation

Flower buds of *Sorghum bicolor* were purchased from the nearby market, dried in the shade, and ground up into a fine powder. 500g of this powder was macerated in 80% ethanol for seven days in giant amber bottles before being filtered. Through the use of a vacuum evaporator operating at lower pressure, the filtrate was concentrated. The resulting filtrates were further separated using butanol, ethyl acetate, and n-hexane in turn. The n-hexane, ethyl acetate, and butanol fractions were concentrated using the vacuum evaporator [14-15]. The individual fractions were then serially diluted with normal saline (0.9% NaCl) to yield 250 mg/ml, which was employed in the anti-sickling study.

Figure 1: *Sorghum bicolor* plant and seeds

2.2 Phytochemical Evaluation

Preliminary phytochemical screening was performed on the crude extracts to investigate the presence of alkaloids, saponins, polyphenols, flavonoids, tannins, anthocyanins, terpenes and steroids according to standard protocol described by [16-17].

2.3 Blood Collection and Sample Preparation/Biological material

The homozygote HbS/HbS (SS) blood sample, used to evaluate the biological function was collected from patients at Pt. Jawaharlal Nehru Memorial Medical College in Raipur. All anti-sickling investigations were conducted using freshly collected blood. To validate their SS nature, the aforementioned blood samples underwent hemoglobin electrophoresis on cellulose acetate gel at pH 8.5. They were found to be SS blood and were then stored in a refrigerator at 4°C [18].

2.4 Anti-sickling activity assay (*In vitro* induction of sickling)

5 ml blood samples obtained from patients were centrifuged at 5,000 rpm for 10 min in saline thrice to obtain the RBC which were then resuspended in normal saline and used for the analysis according to the method described [19]. After mixing 100 µL of SS blood cell

suspensions with 100 µL of 2% sodium metabisulphite solution, the mixture was incubated for 30 minutes at 37°C. A microscopically examined SS erythrocyte sickling was performed.

The number of cell and the percentage of sickling cells were calculated using the formula:

(%) Sickling = Number of sickling cells × 100/total cells ………………..[20]

The anti-sickling activity of various *Sorghum bicolor* extracts was determined *In vitro* using a saline solution. To perform the experiment, 100 µL of SS-RBC pre-incubated with 2% $Na_2S_2O_5$ was mixed with 100 µL of extract solution at a final concentration of 250µg/mL. Each mixture was incubated at 37°C for 2 hours (the period required to achieve maximal sickling). Following incubation, 10µL of the mixture was 100 times diluted. A drop of each sample was analyzed under a light microscope, and sickled and total cells were counted over five distinct fields of view on the slide. For the negative control, the extract-containing solution was replaced with a saline solution. The percentage of sickling was estimated.

3. Result and Discussion

3.1 Phytochemical screening

The phytochemical screening of the plant's crude extracts revealed significant information about its potential medicinal properties. Several significant polyphenols, such as flavonoids, quinones, and tannins, were found in the analysis. These chemicals have been extensively studied for their antioxidant qualities, which contribute to the overall therapeutic benefits of plant. Furthermore, the presence of saponins, alkaloids, and terpenes supports the plant’s use in traditional medicine, as these compounds have a wide range of biological implications including anti-bacterial, anti-inflammatory, and analgesic properties. Interestingly, the absence of sterols points to a distinct phytochemical profile that might set it apart from other plants with related therapeutic uses. The existence of secondary metabolites, including the polyphenols described earlier and other substances, gives the plant’s therapeutic uses a scientific support, especially when it comes to preventing erythrocyte sickling. Inhibiting erythrocyte sickling is a critical step in the therapy of sickle cell anemia, as it can reduce symptoms and avoid consequences. Amino acids, anthocyanins, and organic acids are intriguing possibilities for the anti-sickling activity. These metabolites not only have strong antioxidant properties, but they also show *in vitro* efficacy against erythrocyte sickling, making them important components in the plant's medicinal arsenal. The combined presence of these bioactive chemicals highlights the plant's potential as an alternative healthcare option for sickle cell anemia and other associated disorders (Table 1) [21-22].

Table 1: Phytochemical screening of crude extract of *S. aromaticum* buds

S. No.	Phytochemicals	Presence in crude extract	Remarks
1.	**Alkaloids**	+	Known for various biological activities.
2.	**Flavonoids**	+	Contributes to the plant's medicinal properties.
3.	**Quinones**	+	Contributes to the plant's medicinal properties.
4.	**Saponins**	+	May justify the plant's medicinal use.
5.	**Sterols**	-	Not detected in the plant.
6.	**Tannins**	+	May contribute to the plant's medicinal properties.
7.	**Terpenes**	+	Contributes to the plant's medicinal properties.

3.2 Anti-sickling activity of different fractions from S. aromaticum bud

The image below shows different micrographs of SS blood alone and SS blood in the presence of various extracts.

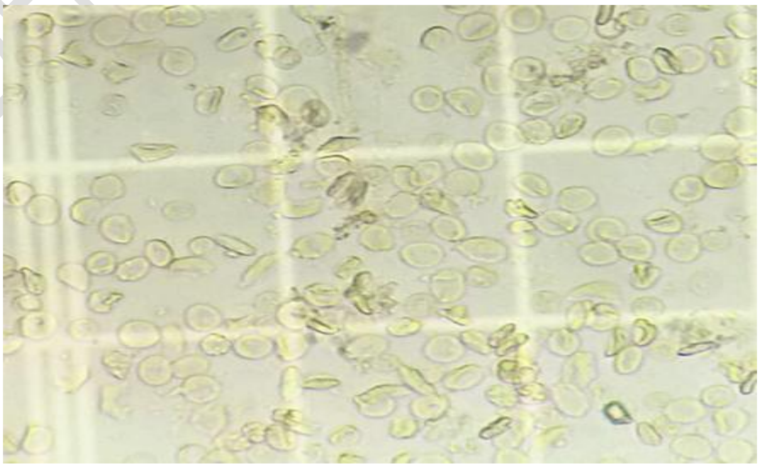

Figure 2: sickle-red blood cells alone in a NaCl 0.9% solution (control)

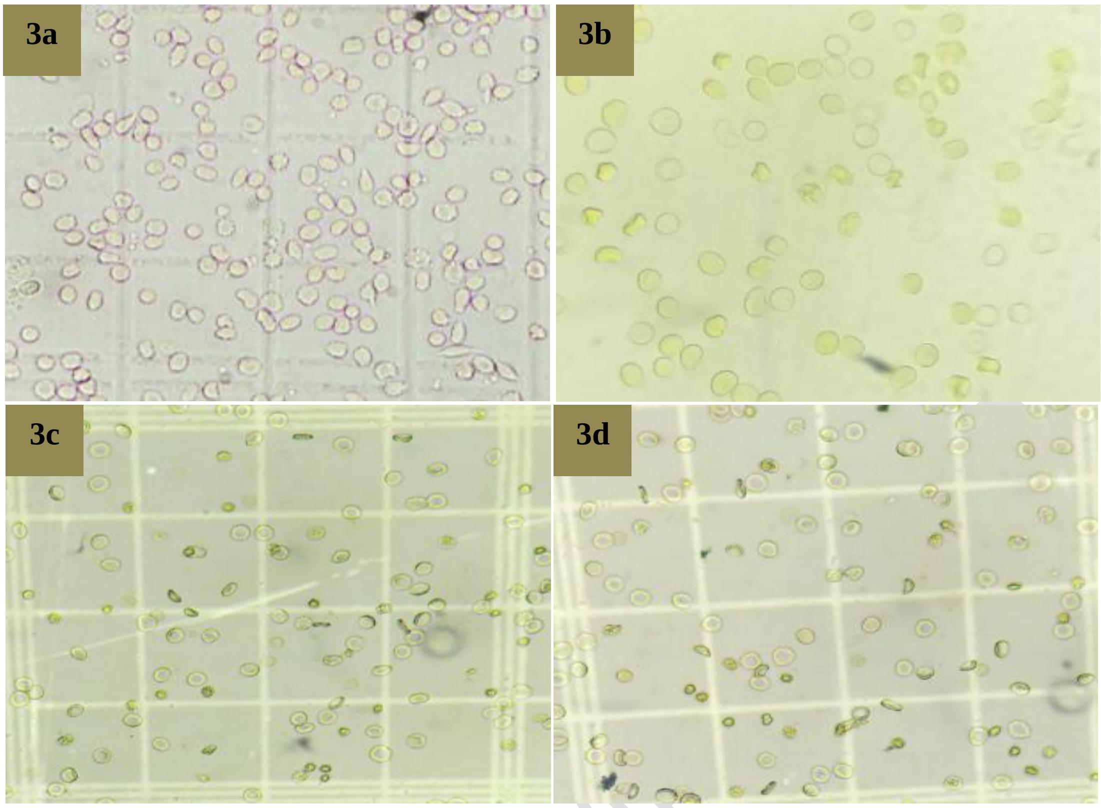

Fig. 2 depicts sickle-red blood cells alone in a 0.9% NaCl solution (control). Figures 3a, 3b, 3c, and 3d show the morphology of SS blood erythrocytes in the presence of n-hexane (Fig. 3a), ethyl acetate (Fig. 3b), butanol (Fig. 3c), and water (Fig. 3d) soluble fractions of S. aromaticum.

Fig. 2 demonstrates that in hypoxic conditions, all red blood cells (RBCs) adopt a sickle shape, confirming the SS nature of the blood samples tested (control). When sickle erythrocytes are mixed with n-hexane, ethyl acetate, butanol, and water-soluble fractions (Fig. 3a-3d) in the identical conditions of investigation, the majority of them have a distinct morphology; they return to the biconcave normal form. The treated SS RBCs showed significant resemblance to normal blood value. The ethyl acetate and butanol fractions demonstrated the strongest anti-sickling activity. In comparison to the other extract fractions, the hexane and water fractions had least anti-sickling activity. The abundance of secondary metabolites may be responsible for this characteristic. This corresponds to a normalization rate of 58.49% for n-hexane fraction, 84.37 % for ethyl acetate fraction, 72.36% for butanol and 67.24% for the water extract (Table 2). These results show that the ethyl acetate fraction is more active than the other fraction. Would grant them the ability to obstruct the cellular processes that are responsible for the sickled cells' normalization and subsequent restoration of their distinctive biconcave shape. This supports the use of *Sorghum bicolor* in traditional medicine by demonstrating that its buds have anti-sickling properties.

Other investigations have found anti-sickling properties in various plant extracts. For example, Seck *et al.* (2015) evaluated the anti-sickling activity of numerous medicinal plants and discovered that extracts from plants such as *J. gossypiifolia, J. secunda and P. nigrescens* had significant anti-sickling effects, which were attributable to their high polyphenolic content. The normalization rates in these investigations were comparable to those reported in the current study, indicating the importance of polyphenols and other secondary metabolites in sickling prevention [23]. However, it is noteworthy that the n-hexane fraction in the current investigation, which had a lower normalization rate of 58.49%, outperformed the hexane fraction in several previous experiments, such as studies by Egunyomi *et al.* (2017), in which hexane extracts shown poor anti-sickling effect. This discrepancy could be explained by changes in plant species, extraction procedures, and experimental conditions, emphasizing the significance of standardizing methodologies for comparative purposes [24]. Furthermore, similar results were observed in a recent study where the ethyl acetate fraction displayed higher anti-sickling activity [25-27].

Table 2: Normalization rate (%) of examined fractions with their effectiveness

Fraction	Anti-sickling activity	Normalization rate (%)	Remarks
n-Hexane	Least Efficacious	58.49%	Lower normalization rate compared to other fractions.
Ethyl Acetate	Most Efficacious	84.37%	Highest normalization rate, indicating strong anti-sickling properties.
Butanol	Highly Efficacious	72.36%	High normalization rate, second only to ethyl acetate fraction.
Water extract	Less Efficacious	67.24%	Moderate normalization rate, better than hexane but less than others.

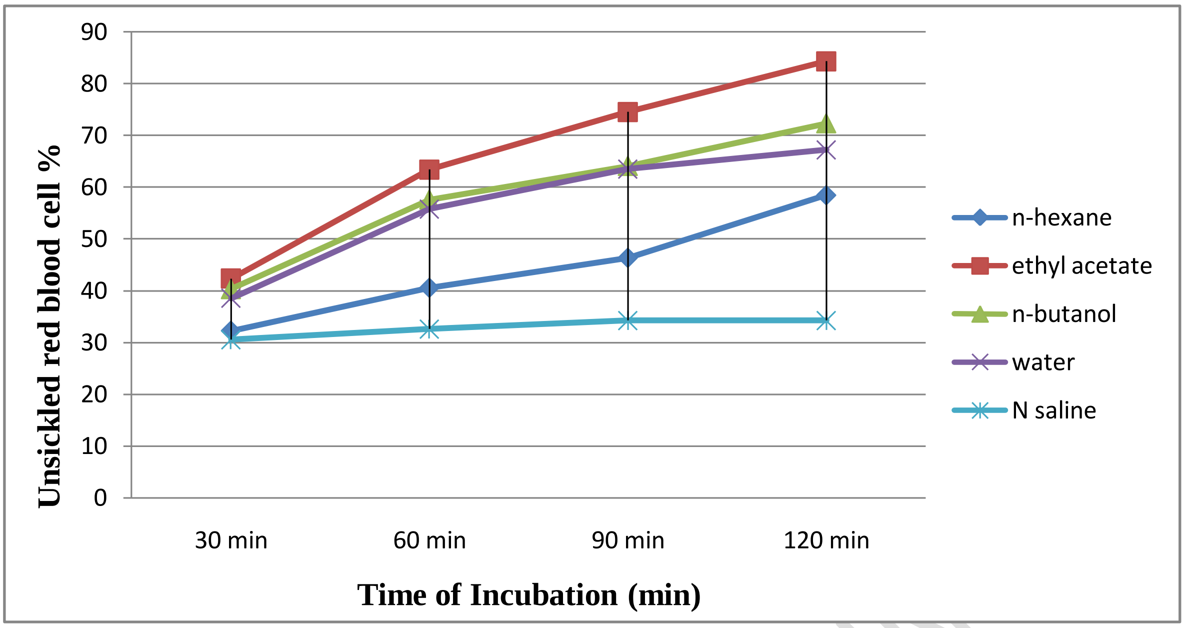

Figure 4: Percentage reversal of sickling by normal saline and partitioned fraction of *Sorghum bicolor* at 250 mg/ml

4. Conclusion

The present study showed the phytochemical composition and in vitro anti-sickling characteristics of extracts from *Sorghum bicolor* seeds. The results validated the traditional usage of the plant in the treatment of sickle cell disease, showing that the active metabolites in these extracts efficiently reduced hypoxia-induced sickling of red blood cells. These findings support the potential of *Sorghum bicolor* as a traditional solution to sickling disease and are consistent with earlier research. Building on these promising results, additional research is being conducted to profile the active components in the extract. This entails identifying and characterizing the particular chemicals responsible for the anti-sickling properties. Purification of these fractions is critical for understanding the molecular mechanisms by which the metabolites exert their therapeutic effect. Identifying the essential bioactive chemicals will also help develop more particular and effective treatments for sickle cell disease. Overall, this study provides strong evidence to support the traditional therapeutic usage of *Sorghum bicolor* in the treatment of sickle cell disease. The findings call for additional investigation and validation of its therapeutic potential, which could lead to the development of new phytotherapeutic drugs. Future studies should isolate and study the bioactivities compounds responsible for the potential antisickling in this plant.

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Conflict of interest

The authors declare that they have no any conflict of interest in this study.

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