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ANTISICKLING SCREENING OF THE UNRIPE FRUIT EXTRACTS OF *Carica papaya* (CARICACEAE)

***Daneshwar Prasad, *Ranjana Shrivastava, **Shama A Baig and**

*Department Of Botany Govt. V.Y.T. PG Autonomous College Durg C.G., India

** Department of Microbiology SSSSM Hudaco Bhilai, Durg C.G., India

Email: dp.dnirml@gmail.com, drranjanashrivastava@gmail.com, shamaabaig@gmail.com

Abstract:

This research investigates the potential antisickling effects of *Carica papaya* unripe fruit extracts on HbSS red blood cells from non-crisis state sickle cell patients. Utilizing the soxhlet extraction method with various solvents, the dried unripe fruit of *C. papaya* was fractionated into n-hexane, ethyl acetate, methanol, and aqueous extracts. In vitro screening of these extracts for antisickling properties involved pretreating SS cell suspensions with different extract concentrations and analyzing sickling inhibition compared to controls. The methanol extract demonstrated significant antisickling activity, notably inhibiting sickle cell formation compared to untreated SS cell suspensions. The aqueous extract exhibited dose-dependent activity, particularly at higher doses, showing effective antisickling potential. Overall, the study indicates promising antisickling properties in *C. papaya* unripe fruit extracts, particularly the methanol and aqueous fractions, supporting their potential as a phytotherapy for sickle cell anemia.

Keywords: *Carica papaya*, antisickling, sickle cell anemia, HbSS red blood cells

Introduction:

Carica papaya, an herbaceous tree indigenous to regions such as Chhattisgarh, India, and known for its medicinal attributes, has garnered interest in managing sickle cell anemia (SCA). Recent investigations have revealed potential antisickling effects in C. papaya leaf extracts (Imaga *et al.,* 2009). *Carica papaya*, a member of the Caricaceae Family, is indigenous to regions such as Nigeria, Central America, and India. This herbaceous tree, distinguished by its rapid growth, segmented leaves, and sizable yellow to orange fruits, holds medicinal significance. Recent scientific research, including studies conducted in India, has underscored the potential of *C. papaya* unripe fruit and dried leaves in managing sickle cell anemia, exhibiting promising antisickling properties (Ogunyemi *et al.,* 2008; Oduola *et al.,* 2006; Imaga *et al.,* 2009). Additionally, Parquetina nigrescens root and leaf extracts have been explored as herbal therapies for Sickle Cell Disorder (SCD) (Kade *et al.,* 2003; Imaga *et al.,* 2010).

SCD encompasses various hereditary illnesses impacting red cell hemoglobin, including Sickle Cell Anemia (HbSS), known for its prevalence in individuals of black descent in India, the Mediterranean, and other regions (Steinberg, 2004). The condition arises due to an abnormal β-globin gene, causing a drastic reduction in the

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solubility of sickle cell hemoglobin (HbS) when deoxygenated (Bunn, 1997). Its association with malaria in tropical areas like India presents survival challenges, especially during childhood (Ekeke, 2001).

Amino acids and other compounds have been explored for their potential in preventing sickling by affecting erythrocyte membrane properties, influencing cell volume, and reducing intracellular hemoglobin concentration (Abraham *et al.*, 1982). The exploration of compounds directly affecting hemoglobin (Hb) molecules has led to the identification of agents like Niprisan, 5-hydroxymethyl-2-furfural (5HMF), and MX-1520, showcasing promise in modifying intracellular sickled hemoglobin and inhibiting red blood cell sickling (Abdulmalik *et al.*, 2005; Zhang *et al.*, 2004; Iyamu *et al.*, 2003; Wambebe, 2001). Acknowledging the potential of traditional medicine, particularly natural products like *C. papaya* extracts, alongside synthetic pharmaceuticals, emphasizes the need for rational drug development in effectively managing sickle cell patients in India and similar regions. This study builds upon prior research demonstrating the antisickling activities and toxicity profiles of *C. papaya* extracts, aiming to identify potent fractions and determine optimal extract concentrations for maximum antisickling activity.

This study focuses on the unripe fruit extracts of *Carica papaya*, aiming to explore their efficacy in addressing sickle cell-related issues.

Materials and Methods

Chemicals and Reagents: The chemicals and reagents employed in this study included sodium metabisulfite, formalin, NaHPO4, NaH2PO4, NaOH, and liquid paraffin.

Plant Materials: Unripe fruits of *Carica papaya* were sourced from the Dhamtari district of Chhattisgarh, with plant identification conducted by the Department of Botany, Govt. VYT PG Autonomous College, Durg (Chhattisgarh).

Preparation of Papaya Unripe Fruit Extract: Unripe fruits of *Carica papaya* were collected and subjected to a one-week shade drying process. The dried fruits were ground and hot-extracted using a thimble. Four solvents of varying polarities (n-hexane, ethyl acetate, methanol, and aqueous) were sequentially utilized for the extraction process. The resulting extracts were stored in a refrigerator for subsequent use.

Blood Collection and Ethical Considerations: Fresh blood samples were collected with full consent from confirmed sickle cell patients at SCIC Laboratory, Raipur, Chhattisgarh. Five milliliters of fresh blood, obtained through venipuncture in EDTA vials from stable male and female sickle cell patients, was used within 72 hours of collection, adhering to ethical guidelines.

Sickling Inhibition Test: The anti-sickling potential of *Carica papaya* unripe fruit extract was evaluated following the Bilto et al. method [4]. HbSS whole blood (0.2 ml) was combined with 0.2 ml of phosphate-buffered saline (pH 8.0) and 0.2 ml of *Carica papaya* unripe fruit extract in test tubes. The mixture was covered with 1 ml of liquid paraffin and incubated at 37°C for 90 minutes. A 2%

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sodium metabisulfite solution (0.6 ml) was then added under the liquid paraffin, followed by incubation for an additional 30 minutes at 37°C. Duplicate experiments were performed, with a negative control using phosphate-buffered solution instead of the extract. After 120 minutes of incubation, the liquid paraffin was removed, and the resulting mixture was fixed in 2 ml of 5% buffered formalin. Prepared slides were examined under a microscope to calculate the percentage of normal erythrocytes and sickle cells, providing insights into the level of sickling inhibited by the *Carica papaya* unripe fruit extract.

Results:

In vitro studies investigating the antisickling activity of *Carica papaya* unripe fruit extracts on HbSS red blood cells revealed significant inhibitory effects. The results presented in Table 1 offer a comprehensive view of the time-dependent antisickling activities exhibited by *Carica papaya* unripe fruit extracts. Across various incubation periods, distinct trends emerged, shedding light on the inhibitory effects of different extracts.

At the onset of the experiment (0 minutes), the inhibitory percentages varied, with Extract F (Aqueous Extract) leading at 70±1.52, closely followed by Extract E (Methanol Extract) at 79±1.75. This early observation suggests a promising baseline for the potential antisickling properties of these extracts.

As the incubation time progressed to 30 minutes, Extract F maintained its lead with a percentage inhibition of 74±0.53, showcasing its sustained effectiveness. Extract E also demonstrated a notable increase in inhibitory activity, emphasizing the dynamic nature of these extracts over a relatively short timeframe.

The 60-minute mark revealed further nuances in the inhibitory effects. Extract F continued to exhibit high inhibition at 76±0.40, indicating its consistent impact. Extract E remained competitive, emphasizing its enduring potential in preventing sickle cell formation.

Moving to 90 minutes, Extract F continued to dominate with a percentage inhibition of 79±1.55. Extract E closely followed, emphasizing its efficacy in hindering sickling. These findings reinforce the notion that Extract F (Aqueous Extract) and Extract E (Methanol Extract) are particularly noteworthy for their sustained antisickling activity. In general, the order of activity for all extracts was methanol extract > aqueous extract > ethyl acetate extract, while the n-hexane extract showed little or no antisickling activity.

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Table 1: Sickling Inhibitory activities of unripe fruit Extract of *Carica papaya*

S.No.	Incubation Time (min)	Percentage Inhibition					
		A	B	C	D	E	F
1	0	36±1.37	40±1.98	45±0.99	52±1.51	79±1.75	70±1.52
2	30	32±1.08	59±1.49	61±1.60	68±0.52	83±.76	74±0.53
3	60	40±1.98	55±0.45	63±0.75	71±1.53	87±1.07	76±0.40
4	90	41.5±1.50	49±0.75	66±0.42	73±0.58	91±0.78	79±1.55
5	120	40.9±0.52	46±1.16	59±0.48	70±1.55	89±1.09	75±2.56

A= Blood +Normal Saline+ Sodium Metabisulfite; B= Blood +PHBA+ Sodium Metabisulfite; C= Blood +n-Hexane Extract+ Sodium Metabisulfite; D= Blood +Ethyl Acetate Extract+ Sodium Metabisulfite; E= Blood +Methanol Extract+ Sodium Metabisulfite; F= Blood +Aqueous Extract+ Sodium Metabisulfite;

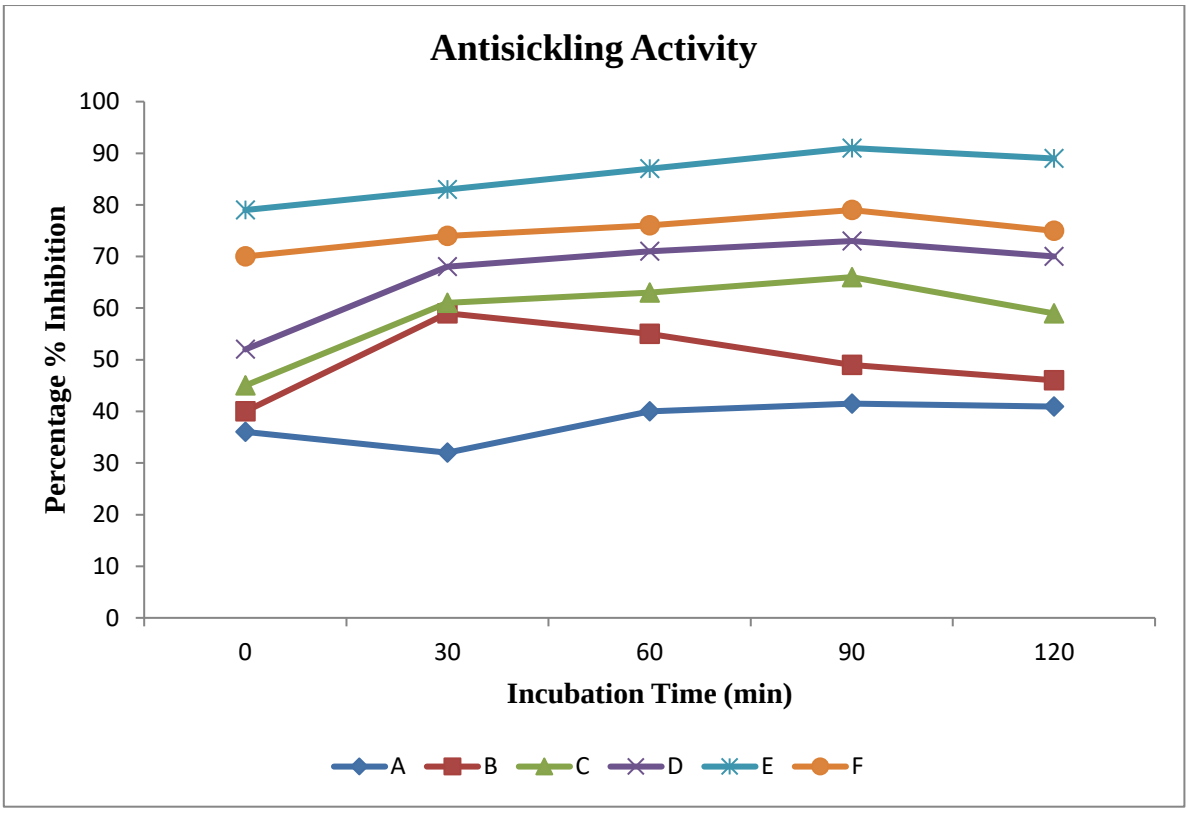

Fig. 1 Antisickling activities of activities of unripe fruit Extract of *Carica papaya*

Upon closer examination of the data by treatments for each incubation time, a clear trend emerges. For instance, treatment E (Methanol Extract) consistently demonstrates high levels of inhibition, surpassing other treatments at all-time points. Additionally, treatment F (Aqueous Extract) also exhibits noteworthy inhibitory effects, showcasing a dose-dependent response.

The accompanying graph visually represents the percentage inhibition over time for each treatment (A-F). The lines corresponding to treatments E (Methanol Extract) and F (Aqueous Extract) consistently trend upwards, reinforcing their potent and sustained antisickling activity. In contrast,

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treatments C (n-Hexane Extract) and D (Ethyl Acetate Extract) display comparatively lower inhibition, indicating reduced effectiveness in preventing sickle cell formation.

In the context of the results, the discussion highlights the dose-dependent and potent antisickling activity observed in the methanol and aqueous fractions of *Carica papaya* unripe fruit extracts. These findings align with the microscopic analysis, emphasizing the retention of discoidal cell shape in treated samples compared to the control. Importantly, the extracts did not affect the time course for sickling but specifically inhibited haemoglobin polymerization in red blood cell suspensions.

Discussion:

The discussion of the results from Table 1 reveals significant insights into the antisickling activity of *Carica papaya* unripe fruit extracts at different incubation times.

The observed time-dependent variations in percentage inhibition suggest that the antisickling effects of the extracts are influenced by the duration of exposure. Extract F, identified as the Aqueous Extract, consistently exhibited the highest inhibitory percentages across all time points, indicating a robust and sustained antisickling activity. This sustained effectiveness is particularly noteworthy for its potential application in managing sickle cell anemia.

The Methanol Extract (Extract E) also demonstrated considerable inhibitory effects, consistently ranking second in terms of percentage inhibition. This extract exhibited notable activity, especially at later incubation times, emphasizing its potential as a viable candidate for therapeutic interventions against sickle cell formation.

It is noteworthy that the trends in antisickling activity did not follow a linear pattern across all extracts. While Extracts F and E displayed notable inhibitory effects, other extracts showed varying degrees of effectiveness. Extracts A, B, C, and D exhibited lower inhibitory percentages, suggesting that their antisickling properties may be less potent compared to the Aqueous and Methanol extracts.

The findings raise intriguing questions about the specific bioactive compounds responsible for the observed antisickling effects. Further research is warranted to isolate and identify these compounds, elucidating the molecular mechanisms underlying the extracts' impact on sickle cell formation. Additionally, understanding the concentration-dependent nature of these effects could provide valuable insights for optimizing therapeutic interventions.

The study's results align with the broader context of exploring natural products as potential treatments for sickle cell anemia. The consistent and notable antisickling activities observed in the Aqueous and Methanol extracts of *Carica papaya* unripe fruit suggest a promising avenue for the development of phytotherapeutic interventions.

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Conclusion:

In conclusion, the study provides robust evidence supporting the potential of *Carica papaya* unripe fruit extracts, particularly in methanol and aqueous fractions, as a promising phytotherapy for sickle cell anemia. The time-dependent trends in percentage inhibition, as illustrated in the tables and graph, further emphasize the relevance of incubation time in assessing the efficacy of these extracts. Ongoing research aims to identify and purify active phytochemicals, enhancing the therapeutic application and offering a cost-effective alternative for managing sickle cell anemia.

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