

## Short Communication

# Antisickling agent in an extract of unripe pawpaw (*Carica papaya*): Is it real?

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**Investigations into antisickling and reversal of sickling activities of an aqueous extract of unripe pawpaw (*Carica papaya*) were carried out on blood from sickle cell patients (Haemoglobin SS, HbSS) using 2% sodium metabisulphite in a sickling test. The minimum concentration of the extract that achieved maximum antisickling *in vitro* and the fraction of the extract where the antisickling agent resides were determined. Our findings confirmed both antisickling and reversal of sickling activities of the extract. It was established that 1.0 g of unripe pawpaw in 1.0 ml of physiological saline was the minimum concentration that achieved maximum antisickling. Solvent partitioning of the extract with ethyl acetate and butanol revealed that the antisickling agent in the extract of unripe pawpaw resides in the ethyl acetate fraction as this fraction prevented sickling of Hb SS red cells and reversed sickled Hb SS red cells in 2% sodium metabisulphite whereas the butanol and aqueous fractions had none of these properties. We concluded that extract of unripe pawpaw really has antisickling agent and that this antisickling agent lies in the ethyl acetate fraction of the extract.**

**Key words:** Antisickling agent reversal activities, extract, unripe pawpaw, *Carica papaya*, solvent partitioning, ethyl acetate fraction.

## INTRODUCTION

The *Carica papaya* (pawpaw) is a member of the small family "Caricaceae" allied to the "passifloraceae". Its main medicinal use is as a digestive agent; it is prescribed for people who have difficulty digesting protein and is used to break up blood clots after surgery, which is due to the presence of enzyme papain in the plant's latex. The latex from the trunk of the tree is also applied externally to speed the healing of wounds, ulcers, boils and warts. The seed is used to expel worm and the flower may be taken in an infusion to induce menstruation (Reed, 1976; Morton, 1977; Duke, 1984b). It has also been that repor-

ted that annonaceous acetogenins derived from the extracts of the twigs of the pawpaw tree may be good chemotherapeutic agents for cancer as these compounds inhibit enzymes necessary for metabolic processes in tumour cells (Rupprecht et al., 1986; Hui et al., 1989a; Hui et al., 1989b; Zhao et al., 1992; Zhao et al., 1995; Reiser et al., 1992).

The complications posed by sickle cell disease is life threatening. A lot of efforts had been made and are still being made to get treatment for sickle cell disease, especially drugs that will prevent sickling of red cells that usually precipitate crisis. Benzoic acid derivatives had been shown to be the active compounds in an antisickling extract from the root of *Fagara xanthoxyloides* (Sofowora and Isaac, 1971). Charache (1974) reported that benzylo-

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xy and phenoxy acids, the most potent being 3, 4 dichlorobenzyloxy acetic acid, prevent sickling by interacting with the haemoglobin molecule to produce an antigelling effect. In their own work, Adesanya et al. (1988) reported that extract of the bark of *Adansonia digitata* possesses reversal of sickling properties but no antisickling activities. Thomas and Ajani (1987) established both antisickling and reversal of sickling activities of an extract of unripe pawpaw. Ripe pawpaw was reported to possess none of the properties. It has been observed that aqueous extracts of the stem bark and leaves of *Khaya senegalensis* exhibited a strong antisickling activity, the active ingredient being a rearranged limonoid (Fall et al., 1991). Ogodu et al. (2000) reported antisickling properties of aqueous methanol extract of the seeds of *Cajanus cajan*.

In the past some plants have been used traditionally as a remedy for one ailment or the other which were proved by scientific methods not to possess properties relating to what they were used for. Although antisickling and reversal of sickling activities of an extract of unripe pawpaw has been reported, the minimum concentration of the extract that achieved maximum antisickling *in vitro* and the fractions of the extract that possess antisickling agent was not reported. Hence this study is designed to evaluate the previous report and then to determine the minimum concentration of the extract of unripe pawpaw that possesses maximum antisickling activities *in vitro* as well as locate the fraction or fractions of the extract where the antisickling agent resides.

## MATERIALS AND METHODS

### Pawpaw extract

Matured fresh unripe pawpaw fruit was plucked, peeled and the cream coloured seeds inside discarded. The pawpaw fruit was cut into pieces, and 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 and 2.0 g were weighed separately. Each was soaked in 1.0 ml of physiological saline and incubated at room temperature for 72 h. The extracts were sieved into different bottles.

### Extraction of pawpaw fruit materials

Matured unripe pawpaw was peeled and the seeds inside discarded. The fruits were extracted with 5 l of methanol at room temperature for 72 h and concentrated to dryness *in-vacuo* on a rotary evaporator to obtain the crude methanolic extract. The crude methanolic extract was dissolved in water and partitioned with ethyl acetate and n-butanol, successively. The aqueous, ethyl acetate and n-butanol fractions were collected separately in different bottles.

### Sickling test

A drop of blood from a sickle cell patient (SS) was mixed with a drop of freshly prepared 2% sodium metabisulphite on a clean slide, mixed well and cover slipped (Barbara, 1980). The cover slip

was gently pressed to remove excess mixture, the excess mixture was removed with cotton wool and the edges of the cover slip sealed with vaseline to prevent air from going in. The slide was incubated at 37°C for 30 min and then viewed under microscope. Similar slides as described above were prepared and a drop of saline extract from each of the bottles with different concentration of the extract or the aqueous, ethyl acetate and n-butanol fraction was added to each of the slides. The slides were incubated at 37°C for 30 min and viewed under microscope.

### Reversal activity of the extract

A drop of blood from a sickle cell patient (SS) and a drop of freshly prepared 2% sodium metabisulphite were mixed together on a clean glass slide and cover slipped. The edges were sealed with vaseline after gentle pressing to remove excess mixture. The slide was incubated at 37°C for 30 min and viewed under microscope.

A drop of saline extract or the aqueous, ethyl acetate and n-butanol fraction of the fruit was then added to the mixture on the slide, mixed and cover slipped. The slide was incubated at 37°C for another 30 min. The slide was viewed under microscope.

## RESULTS AND DISCUSSION

All the red cells in slides with 2% sodium metabisulphite with no extract of unripe pawpaw sickled within 30 min. In the slides containing mixture of 2% sodium metabisulphite with various concentrations of the extract, antisickling effect started manifesting in the extract obtained from 0.7 g of unripe pawpaw in 1.0 ml of saline as most of the red cells remained unsickled. The maximum antisickling effect was achieved in the extract obtained from 1.0 g of unripe pawpaw in 1.0 ml of saline as all the red cells remained unsickled. This same concentration reversed sickled red cells. In the slide with ethyl acetate fraction, no sickled cell was observed whereas all the red cells sickled in the slides with aqueous and n-butanol fractions.

From the findings of this study, we have confirmed that extract of unripe pawpaw has both antisickling and reversal of sickling properties thereby confirming the earlier reports (Thomas and Ajani, 1987). Its use as an antisickling agent by few sickle cell patients in our environment is then justified. We also established the minimum concentration of the extract of unripe pawpaw that achieved maximum antisickling to be 1.0 g of unripe pawpaw in 1.0 ml of physiological saline. This concentration also reversed sickled cells. The antisickling agent in the extract of unripe pawpaw resides in the ethyl acetate fraction of the extract as this fraction prevented sickling of Hb SS red cells whereas the aqueous and n-butanol fractions of the extract failed to prevent sickling meaning that there was no antisickling agent in these fractions.

In conclusion, it is real that the extract of unripe pawpaw possesses antisickling and reversal of sickling properties and that the antisickling agent resides in the ethyl acetate fraction. Toxicity studies using laboratory animals is being carried out and work is also in progress to purify and characterize the antisickling agent in the extract.

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