

Potential of aqueous extract of *Hibiscus sabdariffa* calyces as coloring agent in three pediatric oral pharmaceutical formulations

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ABSTRACT

Aqueous extracts of Roselle or *Hibiscus sabdariffa* L calyces have characteristic intense red colouration due to the presence of anthocyanins which could be utilised as colouring agent in pharmaceutical products. The aim of the current study was to evaluate the potential of aqueous extract of *H. sabdariffa* calyces as a colouring agent in three pediatric oral pharmaceutical formulations. The colour value of *H. sabdariffa* calyx extract was determined colorimetrically at λ_{max} 540 nm to be within the BP range of ≥ 0.25 . The colour value of *H. sabdariffa* (0.26) was lower than that of amaranth (0.46), a synthetic commercial pharmaceutical colourant. *H. sabdariffa* calyx extract retained its colour value within the BP standard for up to six months. The aqueous extract of *H. sabdariffa* calyces at 33 % w/v was used as colouring agent in paracetamol syrup, diphenhydramine syrup and pediatric cough linctus and the colour stability of the formulations against temperature, light and pH were determined. *H. sabdariffa* calyx extract was less stable than amaranth to temperature, light and pH when used as a colouring agent. *H. sabdariffa* calyx extract at 33 % w/v has potential as a colouring agent in pharmaceutical formulations when buffered at pH 5.0, packaged in amber bottles and stored at low temperatures (26-37 °C).

INTRODUCTION

Colouring agents or colorants are pharmaceutical excipients employed to impart specific colour to a pharmaceutical formulation. Colouring agents are also extensively used in the food, cosmetics and textile industries to provide the preferred colouration to the products. They are employed as excipients in the manufacture of liquid (emulsions, suspensions, syrups), semi-solid (creams, lotions, ointments) and solid (sugar and film coated tablets, soft and hard gelatine capsules) pharmaceutical dosage forms. Colouring agents are added to pharmaceutical products for a variety of reasons. They enhance product appearance, aesthetic appeal and product elegance, leading to improved product acceptability by patients.

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Colorants improve product identification for the manufacturer, healthcare professional and patients. They enhance the stability of photosensitive compounds by providing protection from light (Allam and Kumar, 2011). Colouring agents also provide a measure of protection against product counterfeiting (Nyamweya and Hoag, 2008).

The colour of a product also provides an indication of product quality and freshness. Colours are obtained from either natural or synthetic sources. Natural colours are obtained from plant or animal sources. Plant colour contains flavonoids which act as pigments, imparting colour, often yellow or red to flowers, fruits and at times calyces. The suitability of a natural colourant for pharmaceutical preparations depends on factors such as colour value, stability to light, heat and pH. Colouring agents form a chemically diverse group of compounds with wide-ranging stability properties. Compared to synthetic colorants, natural colorants are less stable due to rapid breakdown, especially when formulated in liquid dosage forms. Natural colours are, however, less toxic compared to synthetic colours.

Hibiscus sabdariffa L (family: Malvaceae), also known as roselle or red sorrel is found widely distributed in West Africa, Central Africa, South East Asia and other places (Ali *et al.*, 2005). The calyces, known locally in Ghana as 'sooboro' are used in several communities and are particularly popular in Northern Ghana. In Ghana, the leaves of the plant are traditionally used in the preparation of soups while the calyces are used for medicines and soft drinks. The plant extract is known to possess several health benefits (Dokosi, 1998). Aqueous extracts of *H. sabdariffa* calyces have wound healing (Builders *et al.*, 2013), antihypertensive (Ali *et al.*, 1991; Adegunloye *et al.*, 1996; Haji-Faraji and Haji-Tarkhani, 1999; Onyenekwe *et al.*, 1999; Odigie *et al.*, 2003; Herrera-Arellano *et al.*, 2004), anticholesterol (El-Saadany *et al.*, 1991; Chen *et al.*, 2003) and other pharmacological effects.

H. sabdariffa calyces contain high amounts of organic acids, namely: citric acid, malic acid, tartaric acid and hibiscus protocatechuic acid (Kerharo 1971; Khafaga and Koch, 1980a; Tseng *et al.*, 1996). The acid content of the calyces increases during growth but decreases when it reaches maturity or ripens. The aqueous extract of *H. sabdariffa* calyces has a very rich red pigmentation due to the presence of anthocyanins and the colour properties has been the subject of intense scientific investigations (Ali *et al.*, 2005; Cisse *et al.*, 2011; Salazer-Gonzalez *et al.*, 2012; Aishah *et al.*, 2013). Anthocyanins, which are flavonoids, are water-soluble natural pigments (Aishah *et al.*, 2013). Anthocyanins found in the calyces of *H. sabdariffa* contain delphinidin-3-sambubioside, cyanidin-3-sambubioside, delphinidin-3-monoglucoside and cyanidin-3-monoglucoside (Shibata and Furukawa, 1969; Du and Francis, 1973; Khafaga and Koch, 1980b). The anthocyanin content of *H. sabdariffa* in five strains of the plant reportedly reached 1.7 % to 2.5 % of the dry weight during calyx growth (Khafaga and Koch, 1980b).

The peculiar intense red pigmentation of *H. sabdariffa* calyx extract as well as its antioxidant properties has been exploited for various applications in the food industry and medical research. Abubakar *et al.* (2012) demonstrated that *H. sabdariffa* calyx extract could be used for fungal staining as it was successfully employed to stain both fungal mycelia and fungal sporangia. The corolla of *H. sabdariffa* was recently used as an acid-base indicator for acid-base titration (Nuryanti *et al.*, 2013). *H. sabdariffa* calyx extracts has been studied extensively as a food colourant (Esselen and Sammy, 1975; Duangmal *et al.*, 2008; Aloba and Offonry, 2009) and found to be a suitable replacement for various artificial colorants. Several *in vivo* studies in experimental animals have shown the extract to be virtually non-toxic (Onyenekwe *et al.*, 1999; Orisakwe *et al.*, 2004; Akindahunsi and Olaleye, 2003). There is however little information in the literature about the potential use of *H. sabdariffa* aqueous extracts as a pharmaceutical colourant.

This aim of the current study was to evaluate the suitability of aqueous extracts of *H. sabdariffa* calyces as potential colourant in three pediatric oral pharmaceutical products, namely; paracetamol syrup, diphenhydramine syrup and cough linctus.

MATERIALS AND METHODS

Materials

Dried samples of *H. sabdariffa* calyces were obtained from the Kumasi central market and authenticated at the Forestry Commission, Kumasi; Horticulture Department, KNUST, and the Crops Research Institute, Fumesua, Ghana. Ethanol (96 %), methanol, sodium benzoate, sodium citrate and paracetamol powder BP were supplied by U.K. Chemicals Ltd, Kumasi, Ghana. Sodium metabisulphite, disodium hydrogen orthophosphate BP, sodium dihydrogen orthophosphate BP, orthophosphoric acid BP, glacial acetic acid, sodium hydroxide, potassium dihydrogen orthophosphate, sulphuric acid, hydrochloric acid, propylene glycol, anise oil, citric acid, diphenhydramine crystals, ammonium chloride, sucrose and compound hydroxybenzoate solution were obtained from the chemical store, Department of Pharmaceutics, KNUST, Kumasi, Ghana. Water for preparation was freshly prepared and used.

Determination of foreign matter

Two hundred grammes of dried *H. sabdariffa* calyces were spread out in a thin layer. The material was inspected with the naked eyes to detect any foreign matter such as moulds, insects and any undesirable plant parts. All foreign materials were separated and weighed and the percentage of foreign matter was calculated and recorded.

Preparation of crude calyces

One kilogram of *H. sabdariffa* calyces were washed lightly in water to avoid losing the colour. Excess water was blotted from the sample with a clean towel, and dried in a hot air oven at 30°C for 4 h. The calyces were blended into moderately fine powder with a Waring® Commercial Heavy Duty blender and stored in a black polythene bag for extraction.

Extraction of pigments by cold maceration

Five hundred grammes of powdered *H. sabdariffa* calyces were weighed into a large beaker and 1 L of 70 % ethanol was added and stirred. The beaker was covered and left to macerate for 5 days at room temperature, with occasional stirring. The liquid was decanted and filtered through Whatman No. 1 filter paper. The marc was pressed, filtered and added to the first filtrate. The resultant filtrate was placed in an evaporating dish on a water bath and evaporated to a thick syrupy mass. This was placed in a desiccator for 10 days to dry up after which the dry mass was weighed and the percentage yield was calculated and recorded. The dry mass was placed in a beaker and covered with a black polythene bag to avoid discoloration.

Aqueous extraction of pigments

Five different concentrations of *H. sabdariffa* powdered calyces, namely: 5, 8.5, 17, 25 and 33 % w/v were prepared by infusing the calyces in boiling water for 30 min and then strained. The extracts were filtered using Whatman No.1 filter paper. After

cooling, 1 % v/v compound hydroxybenzoate solution was added as preservative. Sodium metabisulphite (0.1 % w/v) was also added as antioxidant, to help retain the bright red colour for a longer period. Each extract was stored in 125 ml amber bottles and labelled appropriately.

Approximate solubility determination

To 20 ml of cold water in a beaker, was added 0.5 g of the solid extract of *H. sabdariffa* calyces obtained by cold maceration. It was stirred and allowed to stand for 20 min. This procedure was repeated using 96 % ethanol and chloroform. The same procedure was repeated for amaranth powder. The approximate solubility in the three solvents were observed and recorded.

pH determination

Two grammes of the solid extract of *H. sabdariffa* calyces obtained by cold maceration were added to 20 ml of water in a volumetric flask. The mixture was shaken for 3 min and filtered using Whatman No.1 filter paper. The pH of the filtrate was determined with a pH meter (Mettler Delta 350) at 26 °C.

Insoluble matter (%)

One gramme of solid extract of *H. sabdariffa* calyces and amaranth powder were individually dissolved in 100 ml of boiling water and allowed to cool. The solutions were filtered and any residue, washed with water until the last washing was practically colourless. The residues were dried at 105 °C for 2 h in a hot air oven. The weights of each residue were recorded and the percentage insoluble matter calculated.

Total ash

Three grammes of powdered *H. sabdariffa* calyces was incinerated in a tarred silica dish, cooled and weighed. The percentage of ash was then calculated. For amaranth powder, 1g was used according to BP specifications.

Acid insoluble ash

The ash for each sample was boiled for 5 min with 25 ml of 2 M HCl. The insoluble matter was collected in a sintered glass crucible and washed with hot water. It was then ignited to a dull redness and weighed. The percentage of acid-insoluble ash was calculated with reference to the air dried material.

Moisture content

Two grammes of powdered *H. sabdariffa* calyces and amaranth powder were individually weighed out and dried to constant weight at 100 °C. The loss in weight was calculated as a percentage.

Determination of colour value

Sixty millilitres of phosphate buffer pH 8.0 was added to 500 mg of moderately fine powdered *H. sabdariffa* calyces and heated on a water bath for 30 min. It was cooled and sufficient

phosphate buffer added to produce 100 ml and then filtered. Five millimeters of the filtrate was diluted to 100 ml with phosphate buffer pH 8.0. The absorbance of the resultant solution was determined colorimetrically (Sherwood Colorimeter 257, Sherwood Scientific Ltd, UK) at 540 nm at time 0, 3, 6 and 9 months. The same procedure was repeated using amaranth powder.

Preparation of pediatric formulations

Paracetamol syrup BP, Diphenhydramine syrup BP and Pediatric cough linctus BP were freshly prepared without the addition of any colouring agent and were used for subsequent colour stability tests.

Temperature stability tests

For each pediatric formulation eight amber-coloured bottles were filled with 100 ml of the product. Four of these were coloured with 1 ml of 33 % w/v *H. sabdariffa* calyx aqueous extract and the other four were coloured with 1ml of 1% w/v amaranth solution. Each sample was appropriately labelled and exposed to the following temperatures: 26 °C, 37 °C and 52 °C in a hot air oven. To serve as control, eight amber-coloured bottles were filled with 100 ml distilled water; four of them being coloured with 1 ml of 33 % w/v *H. sabdariffa* extract and the other four with 1 ml of 1 % w/v amaranth solution. The absorbance of each sample was determined colorimetrically at 540 nm, bi-weekly, over a 4-month period.

Light stability tests

Light stability tests were carried out on the three pediatric formulations prepared. Each formulation was coloured with 1 ml of 33 % w/v *H. sabdariffa* calyx extract by placing 100 ml of each formulation in plain glass bottles (to admit light) and then in amber-coloured bottles to serve as control. The samples were observed at room temperature (26 °C) over a 4-month period and the absorbance of each sample was determined colorimetrically at 540 nm, bi-weekly, over 4 months.

pH stability tests

pH stability tests were carried out by measuring the pH change in the three pediatric formulations coloured with 1 ml of 33 % w/v *H. sabdariffa* calyx extract over a 4-month period. A control group, comprising pediatric syrups coloured with 1 ml of 1 % w/v amaranth solution were also tested. Each pediatric formulation coloured with *H. sabdariffa* extract was prepared again using buffers at pH 2.5, 3.0, 4.0, 4.9, 5.4, 6.5 and 7.0 and the pH of maximum stability determined (Frimpong, 2008). The stability of the formulations buffered at pH 5 was analyzed over a 4-month period at bi-weekly intervals, using a colorimeter at 540 nm.

Statistical analyses

Statistical analyses on the stability data obtained from the three formulations coloured with *H. sabdariffa* extract or amaranth were determined using chi-square test followed by Tukey's

comparison test. Differences between samples were deemed significant when $p < 0.05$.

RESULTS ND DISCUSSION

Table 1 presents some physicochemical properties of *H. sabdariffa* calyx extract compared to that of amaranth. Crude *H. sabdariffa* contained 1.2 % foreign matter and the aqueous extract was acidic in character in contrast with amaranth which was basic. Macroscopic and other physico-chemical properties of *H. sabdariffa* calyces conformed to standards reported in the literature while those for amaranth powder conformed to BP standards. Both amaranth powder and *H. sabdariffa* calyx extract were readily soluble in cold water and could therefore be suitable as colouring agents for water soluble drugs. The solid extract of *H. sabdariffa* was soluble in alcohol, whilst amaranth powder was only partially soluble. Both amaranth and the extract of *H. sabdariffa* calyx were slightly soluble in chloroform.

Aqueous extracts of *H. sabdariffa* calyces were used as source of natural pigments for colouring three pediatric oral medicines because of their peculiar intense red colouration due to the presence of anthocyanins. A 33% w/v aqueous extract of *H. sabdariffa* powdered calyces produced the requisite colour intensity and was found suitable for colouring the pediatric formulations. Aqueous extracts of *H. sabdariffa* calyces of concentration 5-25 % w/v had low colour intensity and were deemed unsuitable for use as colorants. Amaranth powder is highly refined hence the lower concentration needed for colouring products compared to aqueous extracts of *H. sabdariffa* powdered calyces which are in the crude state. The initial bright-red colour of the pigment extracted from *H. sabdariffa* calyces kept changing with time till it had turned brown after storage at room temperature for three months. The extract should therefore be protected from light and inclusion of antioxidants is recommended to keep the red colour of the pigment for a longer period.

Table 2 shows the comparative colour value of *H. sabdariffa* calyx extract and amaranth powder studied over a 9-month period. The BP standard for colour value is "not less than 0.25". Both *H. sabdariffa* extract and amaranth passed the test for colour value. However, amaranth powder had a higher colour value almost twice that of *H. sabdariffa* extract. *H. sabdariffa* maintained its colour value within the BP standard for 6 months but fell below the BP standard on the 9th month. Amaranth powder however, retained its colour value within the BP standard even after the nine month period. *H. sabdariffa* calyx extract could probably be used to colour pediatric medicines for not more than six months as the colour intensity deteriorated after that period. The loss of colour over time may be due to oxidation which could be prevented by the addition of 0.1 % sodium metabisulphite, an antioxidant.

Anthocyanins are responsible for the intense red colouration of *H. sabdariffa* calyx extracts but are unstable during processing and storage conditions (Aishah *et al.*, 2013). The stability and chemical structure of the colour pigment can be

influenced by factors such as pH, storage temperature, light, oxygen, enzymes, organic acids, sugars, proteins, ascorbic acid, metallic ions and co-pigmentation (Rein, 2005; Patras *et al.*, 2010; Cavalcanti *et al.*, 2011). *H. sabdariffa* calyx extracts were therefore evaluated for their stability to temperature, light and pH when employed as colorants in the three pediatric medicines. The colour intensity of the formulations due to the anthocyanin content in the extract was measured colorimetrically at wavelength of maximum absorption (λ_{max}) of 540 nm.

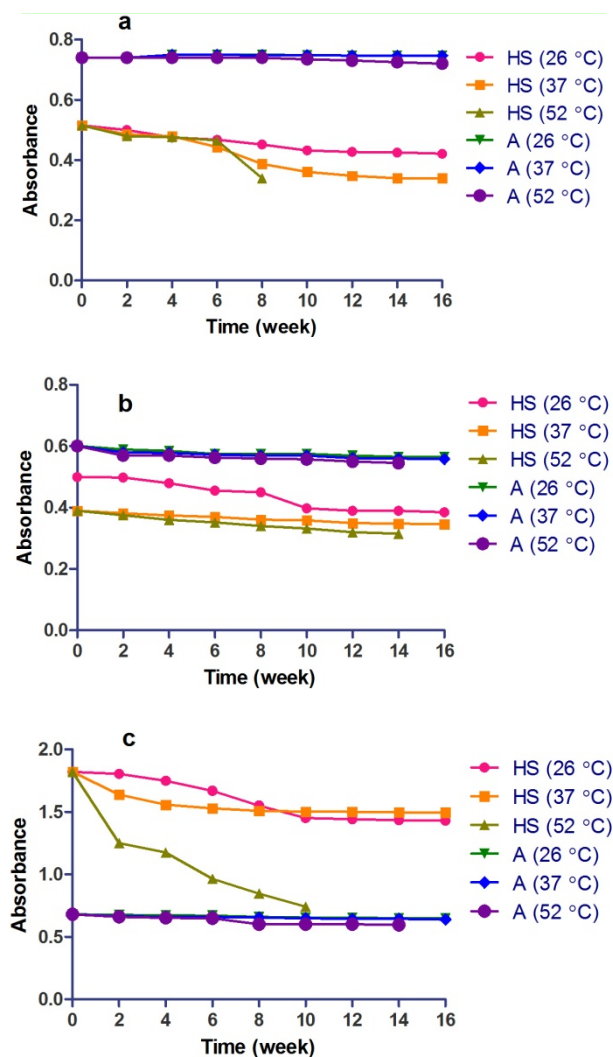


Fig. 1: Effect of temperature on absorbance of a) paracetamol syrup b) diphenhydramine syrup and c) pediatric cough linctus coloured with *Hibiscus sabdariffa* (HS) extract or amaranth (A)

Figure 1 presents the results of temperature stability tests performed on paracetamol syrup, diphenhydramine (pediatric) syrup and cough linctus (pediatric) coloured with *H. sabdariffa* calyx extract or amaranth. All the formulations coloured with amaranth were heat-stable as evidenced by the stability of the absorbance values over the four month period no matter the intensity of heat applied. Amaranth being a synthetic product is relatively more heat stable than natural products. For diphenhydramine syrup, colour stability of products prepared

using *H. sabdariffa* extracts at the various temperatures was very similar ($p > 0.05$) to those coloured with amaranth. At room temperature and 37 °C all the formulated products coloured with *H. sabdariffa* extract were as stable as those coloured with amaranth ($p > 0.05$) upon storage. Paracetamol syrup and cough linctus were less stable to high temperatures showing significant change ($p < 0.05$) at 52 °C. In all cases, the temperature stability of formulations coloured with *H. sabdariffa* extract were comparable to those coloured with amaranth solution at 26 °C and 37 °C proving that storage of the formulations at low temperatures was preferable.

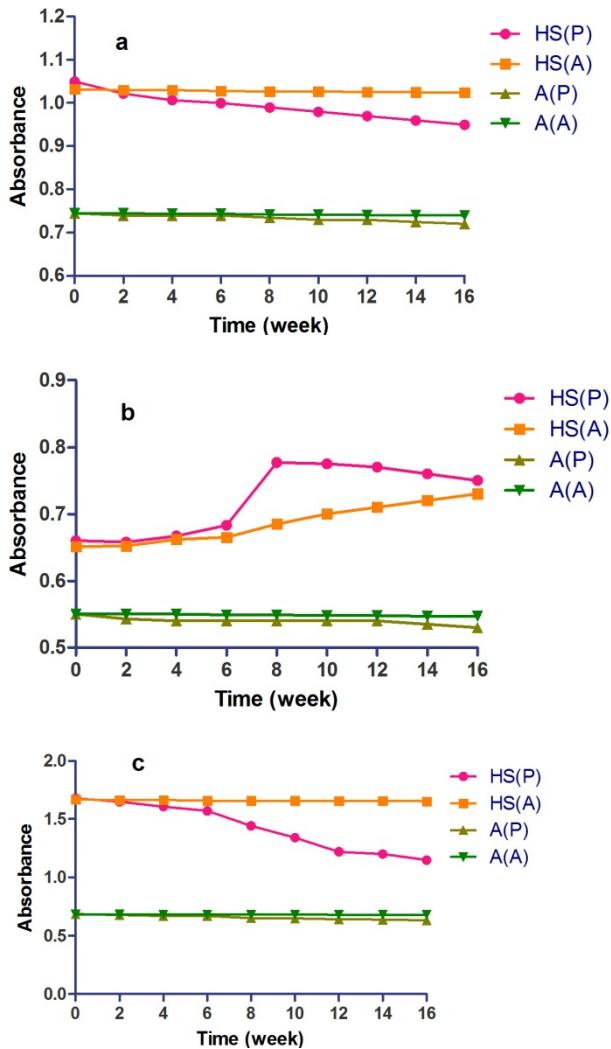


Fig. 2: Effect of light on absorbance of a) paracetamol syrup b) diphenhydramine syrup and c) pediatric cough linctus coloured with *Hibiscus sabdariffa* (HS) extract or amaranth (A) stored in plain (P) or amber (A) bottles

Figure 2 shows the effect of light on colour stability of formulations prepared with *H. sabdariffa* aqueous extract and amaranth. All the three formulations coloured with *H. sabdariffa* extract showed better colour stability when stored in amber bottles than in plain bottles. Absorbance values over time remained steady when amber bottles were used whereas fluctuations in absorbance values were noted when plain bottles were used. For products

coloured with *H. sabdariffa* extract storage in plain bottles showed much deterioration in colour on exposure to light. There was a significant colour change ($p < 0.05$) when paracetamol syrup and pediatric cough linctus were stored in amber bottles or plain bottles. However, there was no significant change in colour ($p > 0.05$) when diphenhydramine syrup coloured with *H. sabdariffa* extract was stored in amber or plain bottles. For diphenhydramine syrup stored in plain bottles, absorbance values increased initially, then stabilised from the 8th to 16th week. Thus, diphenhydramine syrup appears to have better colour stability to light than paracetamol syrup and pediatric cough linctus. The study shows that formulations coloured with *H. sabdariffa* extract are best stored in amber bottles since their stability is affected by light. On the other hand, formulations coloured with amaranth were stable to light whether placed in amber or plain bottles ($p > 0.05$) over the test period.

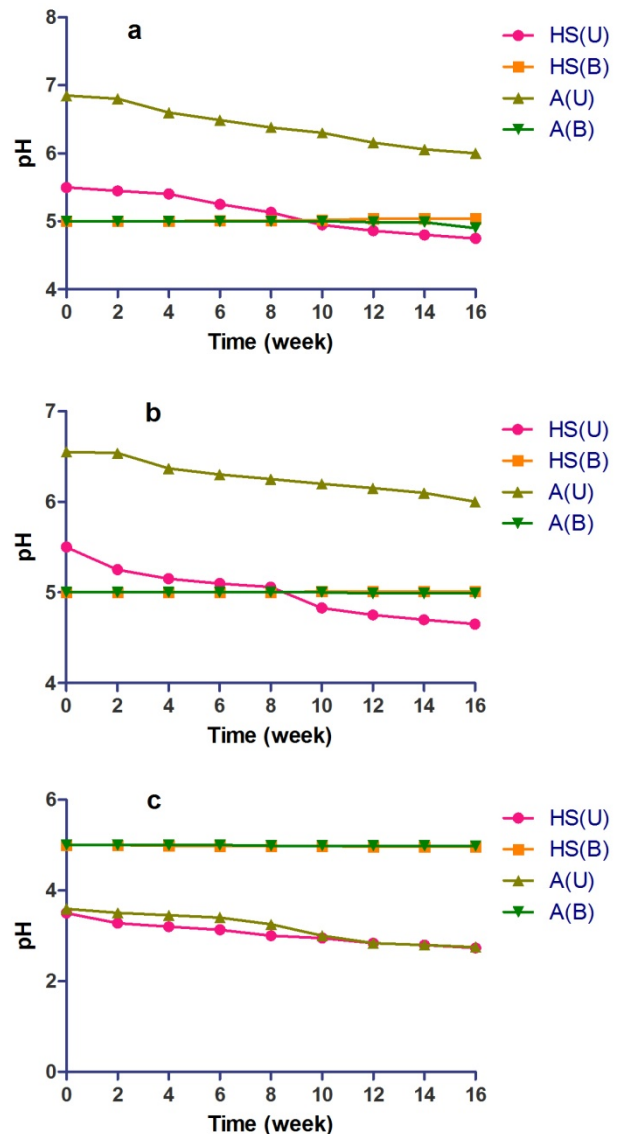


Fig. 3: Ph stability of a) paracetamol syrup b) diphenhydramine syrup and c) pediatric cough linctus buffered at pH 5.0 (B) or unbuffered (U) and coloured with *Hibiscus sabdariffa* (HS) extract or amaranth (A)

Table 1: Physicochemical properties of *H. sabdariffa* calyx extract and amaranth.

| Property | <i>Hibiscus sabdariffa</i> extract | Amaranth powder |
|----------------------------|------------------------------------|---------------------|
| Foreign matter (% w/w) | 1.2 ± 0.1 | - |
| Organoleptic/macrosopic | | |
| Colour | Purple-red | Reddish-brown |
| Taste | Acidic, astringent | Saline |
| Odour | Floral, berry-like | Near odourless |
| Nature | Hard, curled-up, not easily broken | Free-flowing powder |
| pH (10% w/v @ 26 °C) | 4.5 ± 0.1 | 10.5 ± 0.1 |
| Solubility | | |
| Cold water | Readily soluble | Readily soluble |
| Alcohol (96 %) | Soluble | Slightly soluble |
| Chloroform | Slightly soluble | Slightly soluble |
| Insoluble matter (% w/w) | 0.4 ± 0.02 | 0.2 ± 0.01 |
| Total ash (% w/w) | 10.0 ± 0.3 | 6.0 ± 0.2 |
| Acid insoluble ash (% w/w) | 1.4 ± 0.1 | 1.0 ± 0.1 |
| Moisture content (% w/w) | 11.0 ± 0.4 | 1.0 ± 0.1 |

= Not determined

Table 2: Comparative colour value of *H. sabdariffa* calyx extract and amaranth powder determined over a 9-month period .

| Colouring agent | Colour value (A.u.) | | | |
|------------------------------------|---------------------|---------------|---------------|---------------|
| | Month | | | |
| | 0 | 3 | 6 | 9 |
| <i>H. sabdariffa</i> calyx extract | 0.258 ± 0.002 | 0.258 ± 0.002 | 0.250 ± 0.001 | 0.200 ± 0.001 |
| Amaranth solution | 0.461 ± 0.002 | 0.461 ± 0.002 | 0.461 ± 0.002 | 0.461 ± 0.002 |

Table 3: Summary of stability tests undertaken on three pediatric medicines coloured with *H. sabdariffa* calyx extract and amaranth solution.

| Formulation | Temperature stability | | | Light stability | | pH stability | |
|------------------------------|-----------------------|------|------|-----------------|--------------|--------------|-------------------|
| | 26°C | 37°C | 52°C | Plain bottle | Amber bottle | Unbuffered | Buffered (pH 5.0) |
| Paracetamol syrup (HS) | P | P | F | F | P | F | P |
| Paracetamol syrup (A) | P | P | P | P | P | P | P |
| Diphenhydramine syrup (HS) | P | P | F | F | P | F | P |
| Diphenhydramine syrup (A) | P | P | P | P | P | P | P |
| Pediatric cough linctus (HS) | P | P | F | F | P | F | P |
| Pediatric cough linctus (A) | P | P | P | P | P | P | P |

HS: Product coloured with *H. sabdariffa* calyx extract

A: Product coloured with amaranth solution

P: Product passed stability test

F: Product failed stability test

Figure 3 presents the colour stability to pH of the pediatric formulations upon storage at room temperature. The pH values of formulations coloured with *H. sabdariffa* extract were lower (more acidic) than those coloured with amaranth. This is probably due to the fact that *H. sabdariffa* extract is acidic while amaranth solution is basic. In general, pH values reduced with time for both amaranth and *H. sabdariffa* extract formulations, though the level of reduction for the latter was more pronounced. The reduction in pH denotes instability of the formulations possibly due to hydrolytic degradation. There was a significant difference ($p < 0.05$) in pH stability of all the three formulations when coloured with either *H. sabdariffa* extract or amaranth. This means that pediatric formulations coloured with *H. sabdariffa* extract needs to be buffered to ensure better pH stability. The pH at which hydrolytic degradation was minimal in the three formulations was determined to be pH 5.0 (Frimpong, 2008). All formulations coloured with *H. sabdariffa* calyx extract showed optimal stability over the test period when buffered at pH 5.0. This pH is physiologically acceptable and is less likely to cause any gastrointestinal problems. On the whole, *H. sabdariffa* calyx extract being a natural product was more susceptible to deterioration on storage than amaranth, a synthetic product.

CONCLUSION

Aqueous extract of *H. sabdariffa* calyces at a concentration of 33 % w/v solution was successfully used to colour three pediatric oral formulations. Formulations coloured with the extract were susceptible to deterioration on exposure to light, pH and high temperatures. It can be concluded that the extract of *H. sabdariffa* calyces can be a good substitute for amaranth as a colouring agent for pediatric oral pharmaceutical formulations when buffered at pH 5.0 and protected against high temperatures and light.

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