

Research Article

## Natural colourant from *Saccharum officinarum* L. peels: An assessment of its antioxidant and antibacterial activities and its stability as colourant in paracetamol syrup

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### Abstract

A colorant is a dye that imparts colour to food, drinks, pharmaceuticals and cosmetics. The synthetic colourants have been used frequently for this purpose and have been reported to have adverse effects on human health. *Saccharum officinarum* L. (sugarcane) belongs to the Poaceae family and its purple-brownish peel is considered as potential source of colourant. The aim of this study was to evaluate the stability of the peel colourant in paracetamol syrup and investigate its phytochemical, antioxidant and antibacterial activities. The powder peel was evaluated for macroscopic, physicochemical and phytochemical properties. The colorant was extracted by maceration in methanol. The organoleptic evaluation revealed purple-brownish colour, odourless and tasteless powder. The moisture content, total ash, acid insoluble ash, alcohol and water-soluble extractive values were  $0.867 \pm 0.288\%$ ,  $3.01 \pm 0.054\%$ ,  $1.37 \pm 0.02\%$ ,  $7.58 \pm 0.29\%$  and  $8.35 \pm 1.46\%$  respectively. The phytochemical screening has revealed the presence of saponins, flavonoids, tannins, glycosides, steroids and triterpenoids. The colourant demonstrated antioxidant activity in the DPPH and  $H_2O_2$  assays with  $IC_{50}$  values of 4.456 and 2.864  $\mu\text{g/mL}$  respectively. The zone of inhibition of growth between 15 to 60 mg/mL was found to be 1-7 mm in *Escherichia coli* (Gram-negative) and *Streptococcus pneumoniae* (Gram-positive). The ciprofloxacin (standard) at 2 mg/mL had a zone of inhibition of 29-32 mm in the test organisms. The study has revealed that the stability of the colourant in the paracetamol syrup formulation and provided preliminary evidence for the presence of phytochemicals that possess antibacterial and antioxidant activities in the extracted natural colorant.

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## 1. Introduction

In recent years, the demand for natural colorants has increased due to consumer preferences for eco-friendly and sustainable products [1]. The use of

synthetic colorants in various industries has raised concerns due to their adverse health effects [2]. Natural colorants, derived from plant sources are



viable alternative [3]. Sugarcane is one of the world's most cultivated crops and presents an unexplored opportunity for extracting natural colorants from its abundant peel [4].

Colourants (colour additives) are dyes, pigments, or other substances that impart colour to foods, drinks pharmaceuticals and cosmetics [5]. The colorants are pigments of natural or synthetic origin added to pharmaceutical products and nutritional supplements for commercial, psychological and practical purposes. Many coloured tablets, capsules, syrups and multivitamin supplements are appealing to children because of their appearance [6].

The synthetic dyes are organic substances and are the most frequently used in pharmaceutical preparations, because of their higher colouring power, stability and low production cost compared to natural dyes [7]. The synthetic dyes interact with the human body and are therefore, so they are potential health risks, especially when they are consumed excessively [8, 9]. Studies have shown that excessive consumption of these synthetic colourants may lead to neurotoxicity, genotoxicity and carcinogenicity [10].

On the other hand, plant-derived colorants have been considered safe and do not present the toxicity associated with synthetic dyes which is considered to be a great advantage. In addition, plant-derived colourants have been reported to possess inherent beneficial biological activities (antioxidant and antimicrobial) besides their usage as colourants. Examples of these dyes include: chlorophyll, annatto red orange, caramel, curcumin, and betalains [11,12]. Antimicrobial resistance has threatened human health, and new antimicrobial agents are desperately needed [13]. Oxidative stress due to excess free radical generation results in damages of DNA, protein, and lipids, contributing to the pathogenesis of several diseases such as cancer, cardiovascular diseases, neurodegenerative disorders, and inflammatory diseases which are strongly associated with the harmful effects of free radicals [14].

*Saccharum officinarum* L. (Sugarcane) is a tropical plant of the Poaceae family and one of the most important economic crops grown worldwide mostly for its sucrose-rich stalks. Besides *S. officinarum*, there to four other species of the genus *Saccharum* and their hybrids have been used for sugar production. These include:

*S. barberi* ('Indian cane'), *S. robustum* ('thin cane'), *S. sinense* ('Chinese cane') and *S. spontaneum* ('wild cane') which is used for hybridisation purposes. The stability of colorants is greatly affected by environmental factors such as light, temperature, relative humidity and atmospheric oxygen [4,15].

Sugarcane peel, which is an industrial waste, has attracted interest due to its potential use as a colourant and the rich phytochemicals present in the colourants that could serve as potential antioxidant and antibacterial agents. Therefore, this study seeks to investigate the potential application of *S. officinarum* peel pigment as natural colouring agent in paracetamol syrup and to investigate the antioxidant and antibacterial activity of the colourant.

## 2. Materials and methods

### 2.1. Collection and identification

*S. officinarum* was collected in Sokoto, Nigeria in February 2022 from Kasuwan Daji located in Sokoto metropolis, Sokoto State. The plant was authenticated by Dr. H.E. Mshelia, Department of Pharmacognosy and Ethnomedicine, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. A voucher specimen (number PCG/UDUS/POAC/0009) was deposited at the Herbarium of the Department for future reference.

### 2.2. Preparation of plant material

The topmost purplish coloured peels were carefully obtained by scraping gently with the aid of a sharp knife. They were then shade dried and pulverized using wooden pestle and mortar. The powdered sample was packed and stored in aluminium foil for further use.

### 2.3. Macroscopic evaluation of *S. officinarum*

The organoleptic evaluation of the powdered peel of *S. officinarum* was conducted for the odour, taste, colour and texture [16].

### 2.4. Physicochemical evaluation

The procedure described by Lih et al. [17] was used for the determination of moisture content, total ash, acid insoluble ash, hexane, alcohol, and water-soluble extractive values. The experiments were conducted in triplicate.

### 2.5. Selection of suitable solvent for extraction of colourant

The selection of the best solvent for the extraction of

colourant was done in organic solvents. The powder peel (1.0 g) was macerated separately for 24 h in each 10 mL of n-hexane, acetone, ethyl acetate, 70% ethanol, water, absolute methanol and 70% methanol. The colourants were filtered and then dried. The percentage yield of each extract was calculated and based on this; the solvent with the highest percentage yield was selected for the extraction of colourant.

$Percentage\ yield = \frac{Mass\ of\ plant\ extract\ (g)}{Initial\ mass\ of\ plant\ (g)} \times 100$

#### 2.6. Solubility test of colourant in cold distilled water and ethanol

Each colourant (0.1 g) was transferred into two separate beakers (50 mL) A and B, to which 20 mL of cold distilled water and ethanol were added respectively, at room temperature and stirred for 20 min. The samples were observed for solubility.

#### 2.7. pH test

The colourant dissolved in distilled water was tested for acidity or alkalinity using a pH meter, according to the method described by Heniegal et al. [18].

#### 2.8. Colourant extraction and fractionation

The powder peel (50 g) was extracted by maceration in 150 mL of methanol for 24 h and filtered. The residue was washed with 50 mL of methanol and the filtrate obtained was combined with the initial filtrate and then placed in a low temperature air-drying oven for 72 h at 30°C to dry. The percentage yield was then calculated. The colourant (1 g) was fractionated in hexane and ethyl acetate to obtain their respective fractions.

#### 2.9. Qualitative phytochemical test

The protocol outlined by Halilu et al. [19] was used to screen for tannins, flavonoids, saponins, cardiac glycosides, steroids, alkaloids and terpenoids in *S. officinarum* peel colourants.

#### 2.10. Antioxidant studies

##### 2.10.1. Qualitative and quantitative test using DPPH Assay

The method [17] was followed where 10 mg/mL (w/v) stock solution of the colourant was prepared followed by two-fold serial dilution to obtain 5, 2.5, 1.25, and 0.625 µg/mL concentrations. The DPPH solution (1.0 mL) was added to the colourants and incubate for 30 min in the dark at room temperature. The ascorbic

acid (standard) was prepared in a similar manner. The absorbance was measured at 517 nm using a UV spectrophotometer and the results were expressed as percentage inhibition and IC<sub>50</sub> values.

##### 2.10.2. Hydrogen peroxide assay

The ability of *S. officinarum* colourant to scavenge hydrogen peroxide was estimated according to the previously reported method [20], with minor modifications. A solution of hydrogen peroxide (43 mM) was prepared in 10x Dulbecco's phosphate buffered saline (DPBS) (1M pH 7.4). Different concentrations of the plant extract (10 µg/mL) were prepared in 4mL of ethanol which hydrogen peroxide solution (0.6 mL, 43 mM) was added to each test tube, then allowed to incubate in a dark place for 10 min and recorded the absorbance at 230 nm against a blank solution containing ethanol without hydrogen peroxide. Ascorbic acid was used as the standard.

#### 2.11. Antibacterial studies

The method was outlined by Puja et al. [21] for the assessment of the antibacterial studies.

##### 2.11.1. Preparation of plant extracts and antibiotic discs

The stock solutions containing 120 mg/mL (w/v) of each n-hexane, ethyl acetate (fractions) and methanol (colourant) were prepared. Two-fold serial dilutions were used to obtain 60, 30 and 15 mg/mL sample concentrations. Paper discs of diameter 6 mm were soaked in different concentrations of the colourant.

##### 2.11.2. Preparation of nutrient agar medium

The agar was prepared according to the manufacturer's specifications where a conical flask was sterilized by boiling in a water bath for about 45 min. The nutrient agar (7.0 g) was dissolved in 250 mL of distilled water in a sterilized conical flask. The conical flask was corked and the agar was allowed to soak for 10 min followed by sterilization and heating over a Bunsen burner until boiling was attained. It was then cooled to about 37 °C and 25 mL of the agar solution was poured into sterile disposable petri dishes and allowed to gel for 12 h.

##### 2.11.3. Inoculation of microorganisms

A primary inoculum was first made on the media and then the organism (inoculum) was spread on the whole Petri dish by streaking. This procedure was used for transferring the organisms (*Escherichia coli* and *Streptococcus pneumoniae*) separately into their

culture media. The antimicrobial discs were placed on the inoculated organisms (at the centre of the media) using sterile forceps. The petri-dishes were labelled according to the different microorganisms and concentrations of their antibiotic discs. The organisms were then incubated at 37 °C for 24 h. Their zones of inhibition in mm were determined using a ruler [22].

### 2.12. Formulation of paracetamol (acetaminophen) syrup

Sucrose (20 g) was weighed to prepare 30 mL syrup BP. A freshly boiled distilled water was cooled and then added to make up volume to 100 mL. The mixture was boiled and then cooled. Paracetamol powder (5 g) was dissolved in 20 mL of absolute ethanol, followed by addition of 20 mL propylene glycol. The syrup was added to the mixture and stirred. Sodium benzoate (1 g) was added as a preservative. The mixture was filled into a bottle and labelled. 0.6 mL of *S. officinarum* colourant (20% and 40%) was measured and added to the paracetamol syrup with stirring [1]. Four (4) batches of paracetamol syrups were prepared: batch S1 was coloured with amaranth, S2 with 20% *S. officinarum* colourant, S3 with 40 % *S. officinarum* colourant and the S4 was left uncoloured (Table 1).

### 2.13. Stability studies

#### 2.13.1 Drug stability test

Accelerated stability was carried out based on the ICH guidelines of 75% relative humidity at 40°C and was maintained in a humidity chamber [1]. From the formulations 1 and 2; 2 mL of paracetamol syrup which is equivalent to 48 mg of paracetamol was transferred into a 100 mL volumetric flask. Sodium hydroxide (70 mL of 0.01 M) was added and shaken for 15 min and the volume was made up to 100 mL with 0.01 M sodium hydroxide. The solution (1 mL) was measured and transferred into another 100 mL volumetric flask. The volume was made up to 100 mL with 0.01 M sodium hydroxide and mixed well. The absorbance was measured at 315 nm weekly for 4 weeks taking 0.01M sodium hydroxide as a blank. The content of paracetamol syrup was calculated using the standard calibration curve [23].

#### 2.13.2. Light stability test

Two sets of amber and plain coloured bottles were filled with 15 mL of paracetamol syrup. 0.3 mL of 20% and 40% *S. officinarum* extracts were added to each set

of bottles to colour the syrup, respectively. The bottles were observed after being exposed to sunlight and the absorbances were measured at 315 nm and recorded periodically at 48 h interval for 2 weeks. The results were then recorded [24].

**Table 1.** Formulation of paracetamol syrup

Excipient	Standard	S1	S2	S3	S4
Paracetamol	5.0	+	+	+	+
Ethanol	20.0	+	+	+	+
Propylene glycol	20.0	+	+	+	+
Syrup B.P.	30.0	+	+	+	+
Sodium benzoate	0.2	+	+	+	+
Red Dye	0.2	+	-	-	-
<i>S. officinarum</i> 20%	5.0	+	-	-	-
<i>S. officinarum</i> 40%	5.0	-	+	+	+
Water to 100 mL	100	+	+	+	+

Key: (+) indicates the presence of colourant, (-) indicates absence of colourant, S1= syrup prepared with 20% *S. officinarum* colourant; S2= syrup prepared with 40% *S. officinarum* colourant; S3= syrup prepared with amaranth (standard colourant); S4= plain syrup (without colourant).

#### 2.13.3. Temperature stability test

Two sets of plain coloured bottles were filled with 15 mL of paracetamol syrup. To each set of bottles, 0.3 mL of 20% and 40% *S. officinarum* extracts were added as colourants to the syrup, respectively. The bottles were placed in a hot air oven at 37 °C and 52 °C. The absorbances were recorded by taking 1.0 mL of the formulation and making it up to 100 mL with distilled water. Each sample was analysed at 48 h intervals for 14 days and the results were recorded using a UV spectrophotometer [1].

## 3. Results and discussion

### 3.1. Macroscopy

The macroscopic evaluation revealed the characteristic features of *S. officinarum* (Table 2, Figs. 1a, b, c). The organoleptic and physicochemical assessments of plant based medicinal products are basic requirements in drug research. WHO [16], stated that herbal products should pass through a standardized set of guidelines for establishing identity and purity to screen for pharmacological activity. The results of the organoleptic evaluation of the powdered sample of *S. officinarum* showed brownish colour, tasteless, odourless with a coarse texture.

**Table 2.** Macroscopic features of *S. officinarum* peel

Parameters	Result
Colour	Brownish
Odour	Odourless
Taste	Tasteless
Texture	Fine powder



**a**



**b**



**c**

**Figure 1.** (a) *S. officinarum* stem  
(b) *S. officinarum* peel,  
(c) *S. officinarum* powdered stem.

### 3.2. Physicochemical examination

The results of the physicochemical evaluation of the powder peel are presented in Table 3. The moisture content was within the acceptable limit which indicated that the powder was properly

**Table 3.** Parameters of *S. officinarum* peel's powder

Parameters	Result (% Mean ± S.D)
Moisture content	0.87±0.02
Total ash content	3.01±0.05
Acid insoluble ash	1.37±0.01
n-hexane soluble extractive	0.48±0.08
Alcohol soluble extractive	7.58±0.28
Water soluble extractive	8.35±0.46

stored from humidity. The total ash and the acid insoluble values have been found to be low indicating that the sugarcane peel was not adulterated with sand and siliceous materials. Ash value is the residue

remaining after incineration of plant material which simply represents the inorganic salts that naturally occur in crude drugs or as a form of adulteration. Extractive values aid in determining the quantity of active constituents as well as selecting a suitable extraction solvent [16]. The extracting solvent which gave the highest yield of colourant was 70% methanol (Table 4).

**Table 4.** Mass and percentage yield of extracts

Solvent	Colour	Mass of extract (g)	Yield (%)
n-hexane	Yellowish	0.02	2
Acetone	Yellowish	0.02	2
Ethyl acetate	Yellowish	0.01	1
Ethanol 70%	Dark brown	0.13	13
Water	Brown	0.10	10
Methanol	Light brown	0.11	11
Methanol 70%	Pink	0.20	20

### 3.3. Colourant extraction and phytochemical screening

The colourant obtained by extracting the *S. officinarum* peel using methanol was purplish in colour (Fig. 2) and the percentage yield was 36%.



**Figure 2.** *S. officinarum* peel colourant extracted in methanol.

The mass of the extracts obtained from the successive extraction revealed 0.32 g (n-hexane) and 0.46 g (ethyl acetate). The preliminary phytochemical screening of *S. officinarum* colourant revealed the presence of secondary metabolites such as flavonoids, tannins, steroids, triterpenoids and saponins (Table 5). Uchenna et al., [24] also reported the presence of these secondary metabolites and attributed their presence to the antibacterial activity demonstrated by the ethanol extract of the stem bark.

**Table 5.** Phytochemical screening of *S. officinarum* Linn. peel's methanol extract

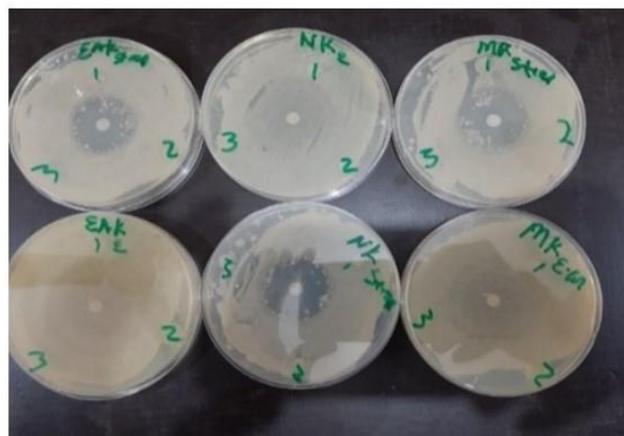
Secondary metabolite/Test	n- Hexane		Ethyl acetate	Colourant
	fraction	fraction		
<b>Phenolic compounds</b>	-	+	+	+
Ferric chloride				
<b>Flavonoids</b>	-	+	+	+
KOH test				
<b>Tannins</b>	-	+	+	+
Lead acetate				
<b>Alkaloids</b>	-	-	-	-
Mayer's				
Dragendorff's				
Hager's				
<b>Steroids/Triterpenoids</b>	+	+	+	+
Salkowski's				
Liebermann-Burchard's				
<b>Saponins</b>	-	-	-	+
Frothing test				

### 3.4. Antimicrobial activity

This colourant demonstrated significant antibacterial activity against *E. coli* and *S. pneumoniae*. The lowest zone of inhibition was observed for *S. pneumoniae* in response to the n-hexane fraction, therefore, this minimal antibacterial activity may be due to the resistance by the organism. The antibacterial activity exhibited by the colourant was concentration dependent (Table 6 and Fig. 3). The ethyl acetate fraction showed dose-dependent antibacterial activity against *E. coli* with the highest zone of inhibition at the highest concentration, therefore, the extract has strong activity toward gram (-) bacteria; while the lowest concentration showed no activity toward gram (+) bacteria; *S. pneumoniae* which suggests some degree of resistance by the organism. Therefore, higher concentration is needed to inhibit growth. The highest zone of inhibition was observed in *E. coli* in response to the ethyl acetate fraction, but an increase in the concentration of this fraction did not increase the threshold of activity towards *E. coli* and *S. pneumoniae* with the same zone of inhibition observed at 30 and 15 mg/mL of the ethyl acetate fraction. The colourant demonstrated minimal dose dependent activity against *S. pneumoniae* and *E. coli*.

Phytochemicals have been suggested to be responsible for the antimicrobial activity of some

plant extracts [25]. Flavonoids have been reported to possess many useful properties including antimicrobial activity. Some of the proposed mechanisms for the antibacterial activity of flavonoids include inhibition of nucleic acid synthesis, cytoplasmic membrane function and energy metabolism [22, 26]. Saponins have also been reported to possess antimicrobial effects which may be responsible for their antibacterial activity against *E. coli* and *S. pneumoniae* [26].

**Figure 3.** Zones of inhibition of n- hexane fraction, ethyl acetate fraction and methanol extract.

### 3.5. Qualitative and quantitative determination of antioxidant activity using DPPH and hydrogen peroxide assays

The colourant showed a yellow spot against a purple background on the TLC plate after staining with the DPPH solution. This revealed the preliminary presence of free radical scavenging compounds in the colourant (Fig. 4).

The quantitative analysis of antioxidant activity using the DPPH and Hydrogen peroxide scavenging assays (Table 7), indicated that colourant had better antioxidant activity in  $H_2O_2$  than the ascorbic acid. The antioxidant activity of colourant has been attributed to flavonoids and tannins [27]. The colourant exhibited antioxidant activity in the DPPH free radical scavenging assay with an  $IC_{50}$  of 4.456  $\mu\text{g/mL}$  while that of ascorbic acid was 2.86  $\mu\text{g/mL}$ . The colourant in  $H_2O_2$  assay had  $IC_{50}$  of 2.864  $\mu\text{g/mL}$  and the ascorbic acid was 3.93  $\mu\text{g/mL}$ . Flavonoids and tannins are strong antioxidants due to the presence of phenolic groups [27, 28].

**Table 6.** Antibacterial activity of the test samples

Bacteria	Conc. mg/mL	Zone of Inhibition (mm)			
		n-Hexane fraction	Ethyl acetate fraction	Colourant	Ciprofloxacin (2 mg/mL)
<i>E. coli</i>	15	1.0	5.0	1.0	32.0
	30	2.0	5.0	1.0	32.0
	60	5.0	7.0	2.0	32.0
<i>S. pneumoniae</i>	15	0	3.0	3.0	29.0
	30	3.0	3.0	4.0	29.0
	60	4.0	5.0	5.0	29.0

**Table 7.** Percentage inhibition and IC<sub>50</sub> of DPPH and hydrogen peroxide scavenging activity

Conc. (µg/mL)	DPPH Assay		H <sub>2</sub> O <sub>2</sub> Assay	
	Colourant	Ascorbic acid	Colourant	Ascorbic acid
10	83.09	96.37	96.37	80.00
5	85.39	98.98	98.98	81.49
2.5	95.02	99.19	99.19	98.23
1.25	95.24	98.46	98.46	98.69
0.625	91.51	91.72	91.72	99.02
IC <sub>50</sub> (µg/mL)	<b>4.456</b>	<b>2.864</b>	<b>2.864</b>	<b>3.934</b>

**Figure 4.** TLC profile of the extract, after sprayed with DPPH.

### 3.6. Colorant solubility and pH test

The extraction of natural colorants from the sugarcane peels offers a sustainable alternative to artificial dye/colorants. The colourant was soluble in both ethanol and water. The pH of the water extract was found to be acidic with pH of 4.44. pH measurement is very important for the preparation of oral liquid formulations, as it affects the solubility, absorption, activity, biological tolerability and stability of the active pharmaceutical ingredients [29].

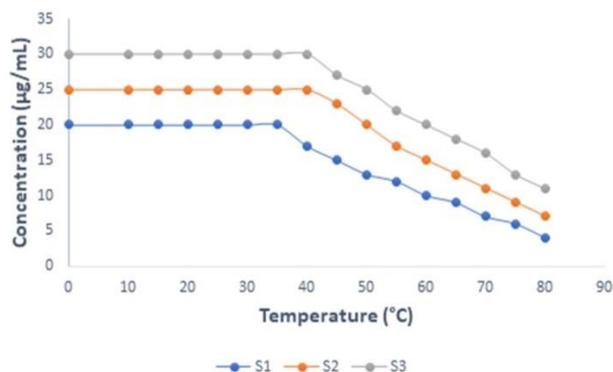
### 3.7. Stability Studies

#### 3.7.1. Temperature stability test

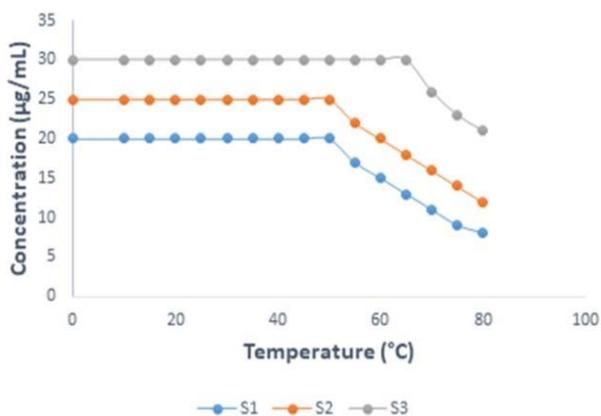
The heat stability of the colourants was studied after subjecting the prepared paracetamol syrups to varying temperatures (37 °C and 52 °C). There was a log-linear decrease in the colourant as a function of temperature and amaranth (standard colourant) was more stable to heat compared to *S. officinarum* colourant ( $P < 0.05$ ). The paracetamol syrups prepared with *S. officinarum* colourant were stable at 37 °C and an increase in the temperature to 52 °C led to a corresponding decrease in the concentration of the colourant. However, the syrup prepared with amaranth (standard colourant) was stable to heat at 52 °C as the concentration remained constant over a period of 14 days (Fig. 4). This may be due to the presence of lycopene which imparts colour to amaranth and has been reported to be stable at a temperature as high as 70 °C and only degrades at temperatures above 100 °C [9].

The concentration of paracetamol syrups prepared with *S. officinarum* and stored in amber coloured bottles was stable at 37 °C and decreased when the temperature was increased to 52 °C. This result is similar to those of previous studies by Acho *et al.*, [1]. However, the syrups prepared with amaranth as

colourants were stable to heat at 52 °C as the concentration remained constant over a period of 14 days (Figs. 5 and 6). Similar findings were reported by Halilu et al. [5].



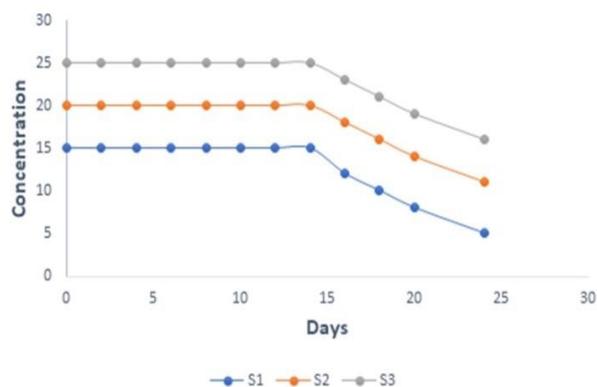
**Figure 5.** Effect of temperature at 37 °C on paracetamol syrups prepared with *S. officinarum* and amaranth. S1= syrup prepared with 20% *S. officinarum* colourant, S2= syrup prepared with 40% *S. officinarum* colourant, S3= syrup prepared with amaranth (standard colourant).



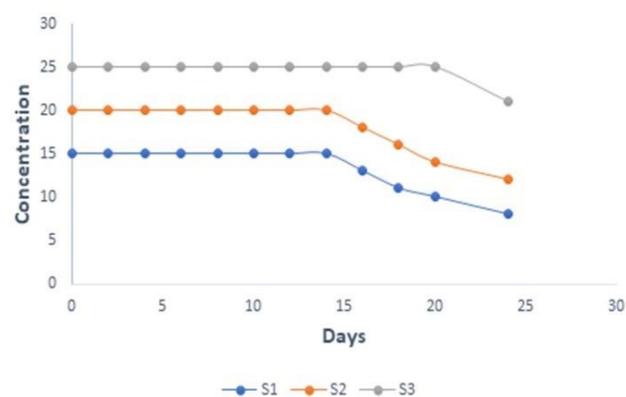
**Figure 6.** Effect of temperature at 52 °C on paracetamol syrups prepared with *S. officinarum* and amaranth.

### 3.7.2. Light stability test

The paracetamol syrups prepared with *S. officinarum* colourant extract showed a significantly higher stability when stored in amber coloured bottles compared to plain bottles ( $P < 0.05$ ). The concentration of the colourants was relatively stable for two (2) weeks, and it began to decrease afterwards due to the effect of photodegradation on the colourant. However, the concentration of amaranth in the amber coloured bottle remained stable even after 14 days (Figs. 7 and 8).



**Figure 7.** Effect of light on paracetamol syrup prepared with *S. officinarum* and amaranth.



**Figure 8.** Effect of light on paracetamol syrup prepared with *S. officinarum* and amaranth.

Amber coloured bottles have been previously reported to protect drugs from the effects of thermal photolytic degradation hence, syrups stored in amber coloured bottles demonstrated better stability to heat compared to those stored in plain bottles [1]. There was a significant difference in the concentration of both the natural and synthetic colourants stored in amber coloured and plain bottles ( $P > 0.05$ ).

The stability of natural colorants is crucial for their successful application in various industries. Understanding the degradation mechanisms and finding suitable protective strategies will prolong the shelf life and usability of these colourants. It is essential to conduct a comprehensive sustainability assessment of the extraction process, considering factors such as energy consumption, waste management, and environmental impact. Comparative studies between sugarcane peel colourants and synthetic dyes should be conducted to highlight the environmental benefits and promote the adoption of natural colourants. Due to their

sensitivity to heat, many natural colourants may degrade or lose their colour intensity. The colour may fade or alter as a result of chemical reactions caused by high temperatures. In this study, the formulations were relatively stable despite the varying temperatures. According to Mohammed *et al.*, [30], the ethanol and methanol extracts were readily soluble in water making them suitable colourant for water soluble drugs. The study also showed that the syrups kept in amber bottles were more stable when exposed to light and heat because of their slightly dark amber colour which protects the content from photolysis. For the artificial colourant, the solution showed long term stability when stored in plain and amber bottles because the synthetic dye contains a higher concentration of lycopene compared to natural colorants. Lycopene from natural sources is more sensitive to environmental effects such as light, pH, heat which may affect its stability in food and drugs compared to its synthetic form. Despite natural colourants consume more time and require longer processing times, they are much safer. After careful observation and storage selection, the physico-chemical properties and stability study of the natural sources have shown promising potential as alternative colourant for pediatric formulation [1].

#### 4. Conclusions

The physicochemical properties and the stability studies of *S. officinarum* colourant demonstrated good properties which makes it a potential alternative natural colourant for paediatric formulations. The colourant was soluble in water and also possessed antioxidant and antibacterial activities thus also acting as a preservative in the syrup. Future studies can explore methods to improve the stability of sugarcane peel colorants by encapsulation, nanoemulsions formation, or utilizing natural stabilizers.

#### Authors' contributions

Conceived and supervised the laboratory study, H.M.E.; Reviewed the manuscript, A.C.O.; Drafted the manuscript, K.H.J.; Conducted the laboratory study, K.L.S.; A.J.; K.M.I.; Analyzed the data, A.L.

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#### Availability of data and materials

All data will be made available on request according to the journal policy.

#### Conflicts of interest

The authors declare no conflicts of interest.

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