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STABILITY OF *HIBISCUS SABDARIFFA* COLOURANT IN PARACETAMOL SYRUP FORMULATION

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ABSTRACT

Synthetic colourants currently in use in pharmaceutical formulations are only relatively safe as several studies have shown that they cause hypersensitivity and allergic reactions and many have even been banned due to their risk of carcinogenicity. This study was carried out to determine the stability of *H. sabdariffa* as colourants in paracetamol syrup formulation. The extract of the plants was extracted using ethanol. Physicochemical tests were carried out on the extract using standard method. Formulated Paracetamol syrup was coloured using a solution of 20 %w/v and 40 %w/v *H. sabdariffa* extract with amaranth as standard. Accelerated stability tests was carried out based on ICH guidelines at 75 % relative humidity and 40 ^{o}C. The absorbance for the drug stability tests was taken at 257nm. The results of the drug stability tests showed a general reduction in the concentration of paracetamol in the formulated syrups over time. There was generally a reduction in colorant concentration in formulated paracetamol syrups over time when exposed to light but with slightly higher concentration in amber coloured bottles as compared to plain bottles. Results of temperature stability tests showed a decrease in concentration of the colorant at 37 ^{o}C and 52 ^{0}C. In conclusion, the *H. sabdariffa* is suitable as colorants in paracetamol syrup formulations but their stability is affected by light and also slightly by temperature.

KEYWORDS: **Colorant, *Hibiscus sabdariffa*, Light, Paracetamol, Syrup and Temperature.**

INTRODUCTION

Colouring agents or colourants are pharmaceutical excipients added to medicines to mask/conceal an unpleasant visual appearance or to increase the acceptability of medicines to patients [1]. A lot of coloured tablets, capsules, syrups and multivitamin supplements are attracted by children precisely because of their appearance [2]. In the past, the major colourant used in pharmaceutical formulation are synthetic colourants. Several of these synthetic colorants have been banned because they cause allergy-like symptoms [3]. Researches have demonstrated that synthetic food colourants have some toxicological effects such as carcinogenicity, hypersensitivity reactions and behavioral effects [4]. Tartrazine, quinoline yellow, sunset yellow, carmoisine, ponceau 4R, amaranth, brilliant black BN and allura red have not been recommended for children's consumption and was subsequently banned due to their linkage with behavioral problems in children and other allergic rreaction they produce [5-6]. Also, erythrosine (FD&C red #3) has been delisted in the list of acceptable colourant in USA since 1990, following the studies in rats that

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suggested that it was carcinogenic [5]. Research have shown that administration of synthetic food colorants decreased the percentage of high-density lipoprotein cholesterol, glutathione secretion, superoxide dismutase plasma immune-system and increased plasma lipid lipoprotein blood glucose, plasma urea and creatinine in male albino rats [7].

Recently, there is global shift towards the use of natural colourants in food, pharmaceutical and personal care industries due to their comparative advantage such as safety and ecofriendliness [1,8]. Natural colourants are not just nontoxic, but they are rich in nutrients and substances that are of immense benefit to health [1]. The health benefits of the natural colourants ranges from antioxidant, antimutagenic, anti-inflammatory, antineoplastic and antiartheritic effects [9,4]. Natural colourants have been classified based on their structure as chlorophylls, carotenoids, flavonoids, quinonoids, coumarins, indigoids, curcuminoids and betalains [8]. The majority of natural food colourants are carotenoids, flavanoids, anthocyanidins, chlorophyll and crocin, which are extracted from several horticultural plants [1]. Other natural colourants from plants include: Bixin derived from *Bixa Orellana*, caramel derived from sugarcane, curcumin obtained from *Curcuma longa* [10], betacyanin/ betalains derived from Caryophyllales [11-12].

Hibiscus sabdariffa is one of the most common flowering plant grown worldwide. There are more than 300 species of *Hibiscus* around the world. *Hibiscus sabdariffa* has high nutritional potential mostly leaves, calyx parts and seeds [13]. The plant is thought to be native to Asia (India to Malaysia) or Tropical Africa. The plant is widely grown in tropics like Caribbean, Central America, India, Africa, Brazil, Australia, Hawaii, Florida and Philippines as a home garden crop [14]. *Hibiscus sabdariffa* have been considered as a promising possible alternative colourant or an adjuvant in pharmaceutical formulation due its favourable health benefit. However, to the best of our knowledge its application as colourant in liquid preparation such as paracetamol for paediatric formulation has not been evaluated. Therefore, the study is designed to determine the stability of *Hibiscus sabdariffa* as colourant in paracetamol syrup formulations.

METERIALS AND METHODS

Materials

All materials used were of analytical grade. The flower calyx of *H. Sabdarrifa* were obtained from Sokoto in July, 2017 and was identified by a Pharmacognosist in the Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto. Voucher specimen of the plant was prepared and assigned voucher number PCG/UDUS/MALV/0001. The Voucher specimen was deposited at the herbarium of the Department for reference.

Soxhlet extraction

The dried powdered calyx of *Hibiscus Sabdariffa* (100 g) was transferred into a double clothed thimble and extracted using the Soxhlet apparatus, ethanol was used as the solvent. The extraction lasted for 3h. The liquid extract collected was concentrated in an oven to dryness at 40 ^{o}C. Thereafter, it was further kept in a desiccator for complete dryness. The percentage yield was calculated and the dried extract was stored in a tight container for further study.

Physicochemical tests on the extract

Solubility test

The solubility of *Hibiscus sabdariffa* extract was determined in n-hexane, ethanol and water according to BP (2012) specifications.

Test for acidity and alkalinity

The pH of the extract of *Hibiscus sabdariffa* was determined according to the method described by [15].

Determination of moisture content, test for total ash and acid insoluble ash

The experiments were conducted on the powdered extract using the methods described previously [16].

Stability studies

Drug stability

To four sets of formulated paracetamol syrups, one was coloured with amaranth, the second with 20 % *H. Sabdariffa* extract, the third with 40 % *H. Sabdariffa* extract and the fourth left uncoloured (plain). Accelerated stability was carried out based on ICH guidelines of 75 % relative humidity at 40 ^{o}C and was maintained using a humidity chamber. The content of paracetamol was determined as follows. Syrup (2 mL) equivalent to 48mg of paracetamol was transferred into a 100 mL volumetric flask. A 70mL of 0.01M NaOH was added, mixed for 15 min and was made up to the 100 mL mark with 0.01M NaOH. 1mL of the solution was taken using a

pipette into a 100 mL volumetric flask. The volume was made up to the 100 mL mark with 0.01M NaOH and then mixed well. The absorbance was then measured at 257 nm and 0.01M NaOH was taken as blank. The content of PCM was calculated taking 715 as the value of A (1%, 1cm) at the maximum at 257nm (concentration of sample=0.00048%).

Light stability tests

Two sets of amber and plain coloured bottles were filled with 15 mL of paracetamol syrup. To each set of bottles, 0.3 mL of 20% and40% *H. Sabdariffa* extract were used to colour the syrup respectively. The bottles were exposed to light and the absorbance's were recorded by taking 1 mL of the formulation and making it up to 100 mL with distilled water. The results obtained were recorded.

Temperature stability tests

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% *H. Sabdariffa* extract were used to colour the syrup respectively. The bottles were exposed to temperatures of 37^{o}C and52^{o}C and the absorbance were recorded by taking 1 mL of the formulation and making it up to 100 mL with distilled water. The results obtained were recorded.

Statistical analysis

The data obtained were analysed using SPSS version 20. Results were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Table 1 shows a yield of 37 % of the extracts, and that the extract is dull red in colour with a low tinctural strength as compared to amaranth. The total ash of 4.8 %, water insoluble matter of 7.8 % which could be attributed to inefficiency during filtration and a pH of 2.89 (acidic) which would pose problems for formulation with basic drugs or substances.

Table 2 shows the solubility of the extracted colorant, as expected, it is soluble in ethanol and also highly water soluble due to increase solvent polarity of water, but with less polar solvent like hexane, it is slightly soluble. There was no significant difference in the degradation of paracetamol in all the formulated syrups as seen in Fig. 1. This also could be attributed to the fact that the *H. Sabdariffa* colorant was not responsible for the degradation of paracetamol over time since the syrup without colorant showed similar pattern in reduction of paracetamol concentration.

The effect of light on the formulated syrups (Figs. 2 and 3), showed a reduction in colorant effectiveness and concentration over time. This could be due to the reduction of anthocyanin pigments which are a major constituent of *H. sabdariffa* extract and other flowering plants [17]. Figs. 4 and 5 show the effect of temperature on the formulated syrups. There was a general reduction in concentration of the colorant concentration over time with increase in temperature. Generally, temperature, pH, light oxygen, metals, organic acids, sugars, ascorbic acid, enzymes, sulfur dioxide, co-pigmentation and interactions with food components may affect both the structure and stability of anthocyanin's [14].

CONCLUSION

The physicochemical properties and the stability study *H. Sabdariffa* extract has shown a promising potential as an alternative colourant for paediatric formulation after careful selection of storage conditions. The extract was soluble in water; therefore, it is important to mention here that the extraction can be done using water instead of ethanol because of the likely residual effect of ethanol in the extract.

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Table 1: Physicochemical tests of *H. sabdariffa* extract

Results	***H. sabdariffa***
Percentage yield (%)	37.0
Water insoluble matter (%)	7.8
Total Ash	4.8
Percentage moisture content (%)	14.8
Colour	Dull red
pH	2.89

Table 2: Solubility of *H. sabdariffa* extract in various solvents

Substance	Solvent	Results
H. sabdariffa extract	Water	Highly soluble
H. sabdariffa extract	Ethanol	Very soluble
H. sabdariffa extract	Hexane	Slightly soluble

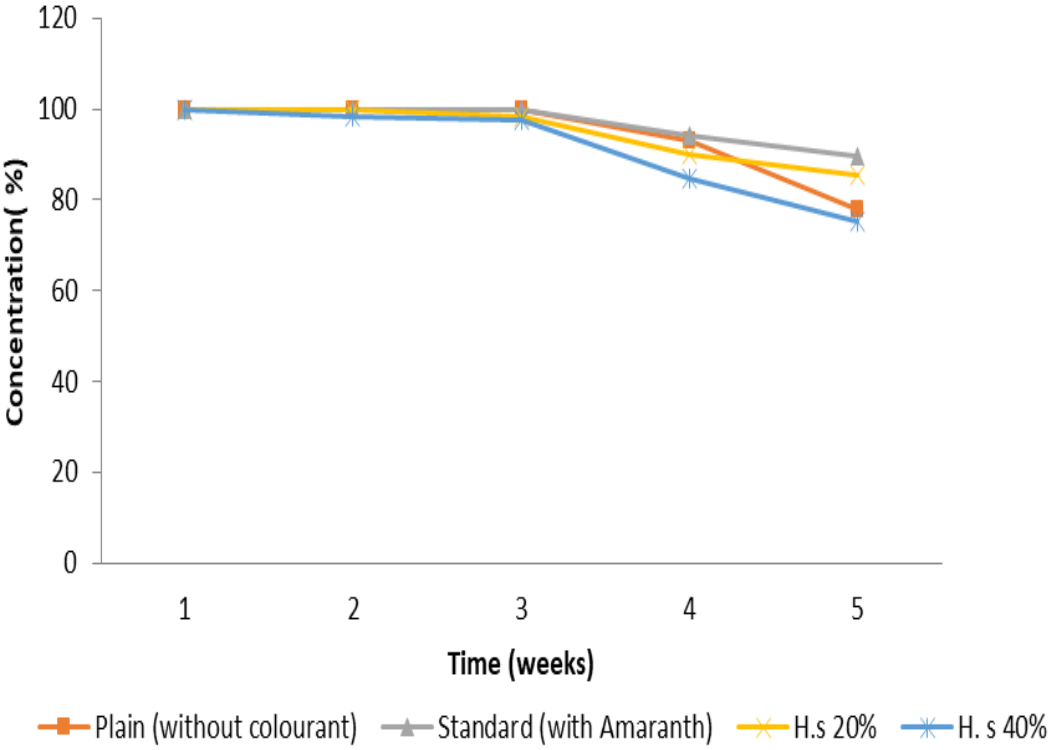

Figure 1: The Accelerated drug stability on formulated paracetamol syrup.

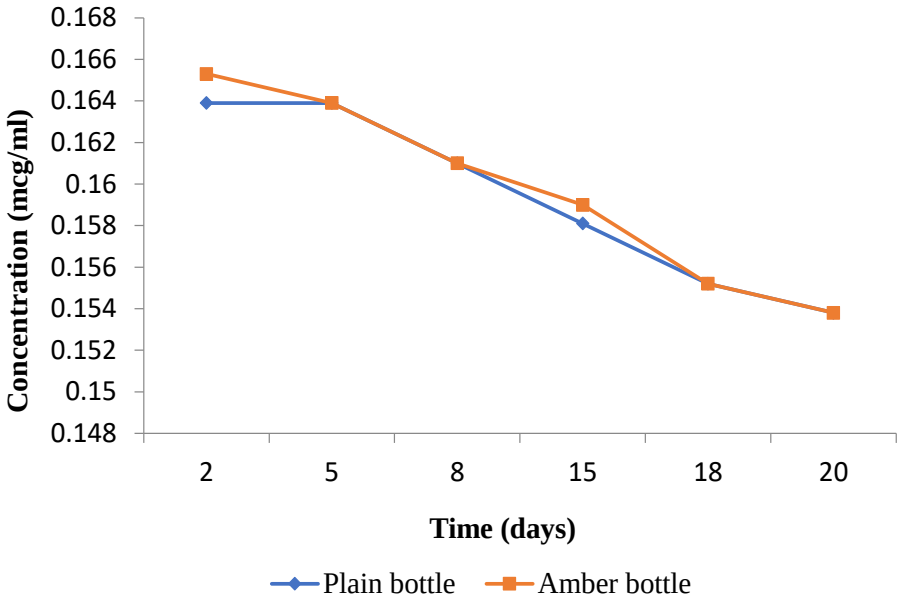

Figure 2: The effect of light on paracetamol syrup coloured with 20%w/v *H. Sabdariffa* extract.

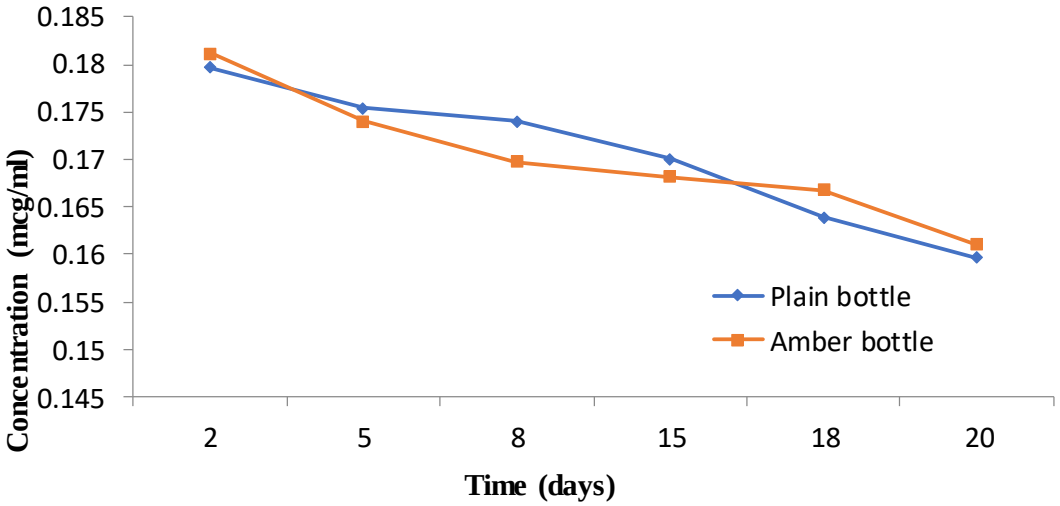

Figure 3: The effect of light on paracetamol syrup coloured with 40%w/v *H. Sabdariffa* extract.

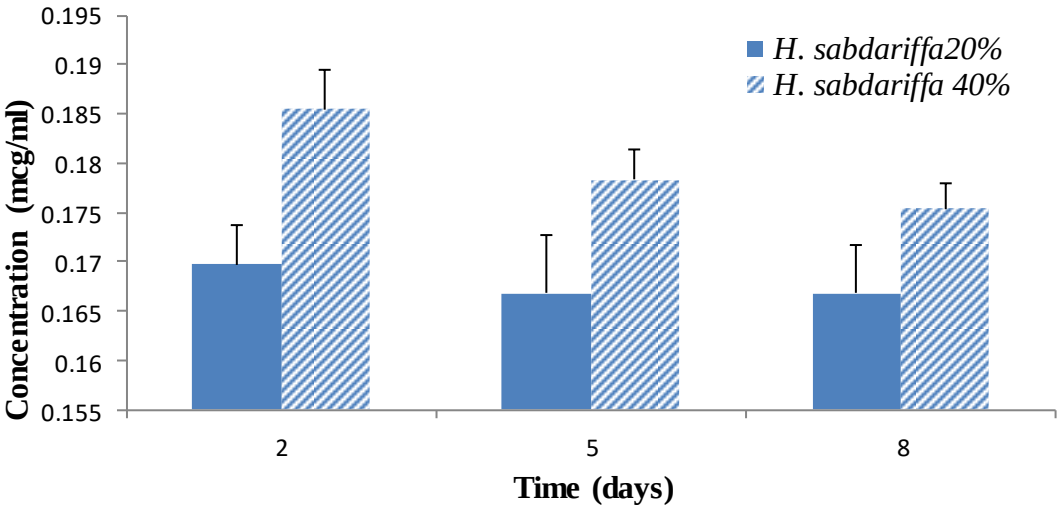

Figure 4: Graph of concentration against time showing the effect of temperature (37 °C) on the paracetamol syrup coloured with *H. Sabdariffa*.

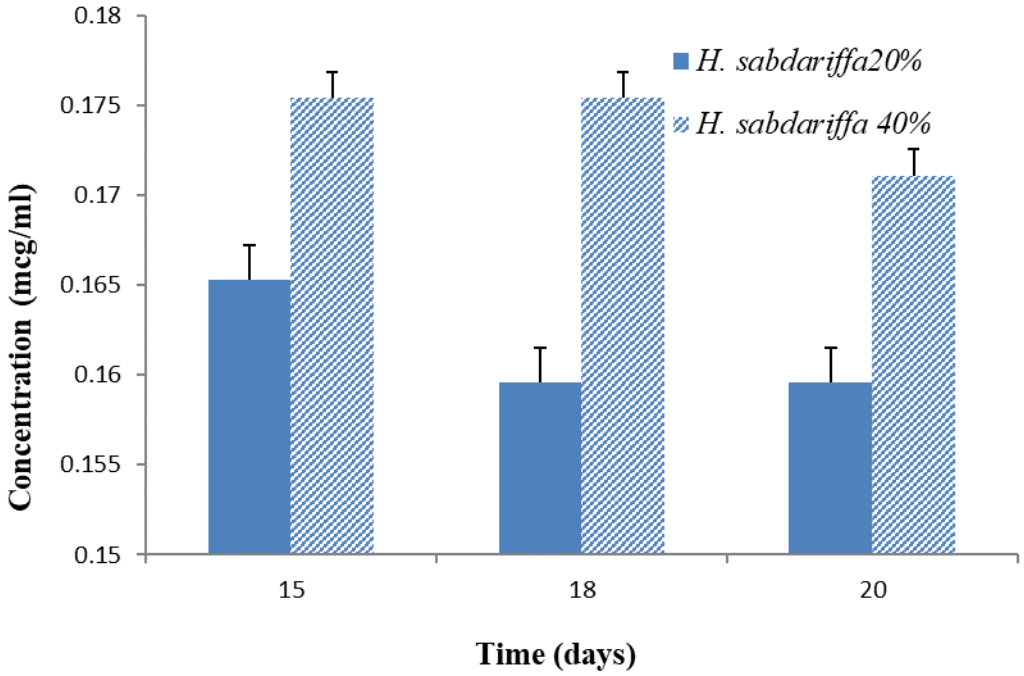

Figure 5: Graph of concentration vs. time showing the effect of temperature (52 °C) on the paracetamol syrup coloured with *H. Sabdariffa*.