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Stability Studies of *Daucus carota* (Apiaceae) Extracts as Colourant in Paracetamol Syrup Formulation

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ABSTRACT

Background: Colouring agents are pharmaceutical excipients used in imparting a distinctive appearance on pharmaceutical dosage forms thereby making them appealing, appetizing and distinctive. The stability of colourants is significantly affected by environmental factors such as light, temperature, relative humidity and atmospheric oxygen.

The study was carried out to determine the stability of *Daucus carota* (carrot) extracted pigment which was used as a colourant in paracetamol syrup formulation**.**

Method: The dried plant material was macerated using ethanol as the extractive solvent. Paracetamol syrup was formulated using formula from the British Pharmacopoeia (BP), and the extracted pigment (20% and 40%) was added as the colourant and stored in both plain and amber coloured bottles and subsequently compared to amaranth, a synthetic colourant which was used as the standard. The effect of light and temperature at 37°C and 52°C on the colourant was also determined over a period of 14 days.

Results: All coloured formulations used for drug stability studies showed a significant decrease in concentration of the colourant over the study period. The result obtained from light stability studies showed better stability when stored in amber bottles than in plain bottles for all colourants however, there was a gradual decrease in the concentration of *D. Carota* and amaranth with increase in temperature.

Conclusion: The stability and physicochemical properties of *Daucus carota* extract have shown its potential as a natural colourant in the formulation of liquid pharmaceutical dosage forms.

1. Introduction

As defined by United States Food and Drug Administration (US FDA), colour additive is any dye, pigment, or other substance that has the ability to give colour to a food, drug and cosmetic. Colour additives are important components of many products, making them attractive, appealing, appetizing and informative[1,2]. A colourant acts as a code which allows us to identify a particular product on sight. It is usually used in small concentration in many dosage forms[3,4].

Colourants are mostly used to give pharmaceutical dosage forms a distinctive appearance. Tablets, capsules (hard gelatin, soft gelatin), liquid oral preparations, dental pastes,

ointments and other medicinal preparations all require colourants to make them look appealing and aesthetic to the consumers especially children[5,6]. The purpose of colouring differs depending on the formulation. Colouring of pharmaceutical products may be necessary to improve their visual appearance, increase the stability, formulate standard preparations, or for the identification of a specific formulation. Natural colourants are less toxic compared to synthetic colourants[7,8].

Carrot (*Daucus carota* L.) belongs to the family Apiaceae and is very rich in carotene which is a precursor of vitamin A, ascorbic acid, vitamins, protein, fat, carbohydrates, sugars, and fibre[9]. Carrots contain a high content of carotenoids and have a unique flavour due to the terpenoids and polyacetylenes. Carrots are one of the most important root vegetables because they are rich in bioactive elements like carotenoids and dietary fibres, as well as a variety of other constituents that are beneficial to the health of humans[10]. The use of carrots and their products has increased drastically over the years as it is recognized as a valuable source of natural antioxidants with anti-cancer properties[11]. Carrots are very rich in mostly red and orange pigments which when extracted can serve as a natural colourant in the manufacture of various pharmaceutical dosage forms[11].

Colouring agents are commonly used in almost all kinds of pharmaceutical dosage forms. The rationale for the use of colouring agents in pharmaceutical products can be therapeutic or aesthetic[8]. Previous studies have shown that natural colourants are more stable and safer than the synthetic ones, hence there is the need to extract pigments from natural sources that can be of commercial value to the pharmaceutical industry[12]. Therefore, the aim of this study was to extract natural pigments from *Daucus carota* (carrot) and determine its stability in paracetamol syrup formulation**.**

2. Materials and Methods

2.1 Materials

Carrot (*Daucus carota*) was obtained from Sokoto central market, paracetamol pure sample was obtained as a gift from Edo Pharmaceuticals Limited, Nigeria. Ethanol and Propylene were obtained from Sigma Aldrich, Germany.

2.2 Methods

2.2.1 Collection, identification and preparation of *Daucus carota*

The carrot (2 kg) were obtained from Sokoto central market which is located between latitude 6°120 and 5°180 North of equator and longitude 13°20 and 13°80 East of Greenwich[13]. The plant was authenticated at Department of Pharmacognosy and Ethnomedicine, the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto by Dr. H.E. Mshelia. A voucher specimen with number: PCG/UDUS/APIA/001 was prepared and deposited at the herbarium of the department for future reference. The carrots were then sliced and dried, first under the sun for a day and then in an oven at 80°C

Table 1.0. Formulation parameters for paracetamol syrup

Excipients	F1	F2	F3
Paracetamol (%w/w)	02.4	02.4	02.4
Ethanol (%v/w)	10.0	10.0	10.0
Propylene glycol (%v/w)	10.0	10.0	10.0
Syrup B.P(%v/w)	27.5	27.5	27.5
Sodium benzoate (%v/w)	0.1	0.1	0.1
Amaranth (%)	-	-	2.0
D. carota 40%	2.0	-	-
D. carota 20%	-	2.0	-
water to 100 mL	100	100	100

Key: F1= formulation coloured with amaranth, F2 and F3= formulation coloured with 20 and 40% *D. carota* respectively.

2.2.7 Temperature Stability Test

Formulation F1 – F3 were labelled appropriately and placed in a hot air oven at 37°C and 52°C. Each sample was analyzed at 48 h intervals for 14 days by taking 1 mL each of paracetamol syrup in the bottle and made up to 100 mL with distilled water. The absorbance was taken and recorded with the use of a UV spectrophotometer (UV-1800, Shimadzu, Japan).

2.2.8 Light stability test

Formulation F1 – F3 were prepared both in amber and plain bottles. They were observed after exposure to sunlight for 2 weeks and analyzed periodically using a colorimeter (Shimadzu, Japan) at 48 h intervals for a period of two weeks.

2.2.9 Drug stability test

For formulation F1 –F3, 2 ml of paracetamol syrup equivalent to 48 mg of paracetamol was accurately transferred into a 100 mL volumetric flask. 70 mL of 0.01 M sodium hydroxide was added and shaken for 15 min and the volume was made up to 100 ml with 0.01 M sodium hydroxide, 1 mL of the solution was again taken 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 257 nm weekly for 4 weeks taking 0.01M sodium hydroxide as blank. The content of paracetamol syrup was calculated from the standard calibration curve.

3. Results

The physicochemical properties of DC crude containing 0.25% of foreign matter, 2.0 %w/w insoluble matter, 6.20%w/w total ash, 2.33%w/w acid insoluble ash and 8.0%w/w moisture content was quantified, the extract was shown to be soluble in cold water

Table 2.0: Physicochemical properties of *Daucus carota* extract

Parameters	***D. carota***
Foreign matter (%)	0.25
Yield (%)	2.00
Colour	Yellow
pH	5.60
Solubility in cold water (4^{o}C)	Soluble
Solubility in Alcohol	Soluble
Insoluble matter (%w/w)	2.00
Total ash (%w/w)	6.20
Acid insoluble ash (%w/w)	2.33
Moisture content (%w/w)	8.00

3.1 Light stability test of Paracetamol syrup formulation containing DC ethanolic extract

Paracetamol syrup coloured with DC ethanolic extract showed better stability when stored in amber coloured bottles than in plain bottles (P>0.05). The concentration of the colourants was stable over a period of time, and it began to decrease after 14 days. However, the concentration of the synthetic colourant (amaranth) in the amber coloured bottle remained stable even after 14 days[15]. The concentration of the synthetic and natural colourants in the paracetamol syrup stored in plain bottles decreased after 14 days on exposure to light[16]. There was significant difference in the concentration of both the natural and synthetic colourants stored in amber coloured and plain bottles (Figure 1 A& B).

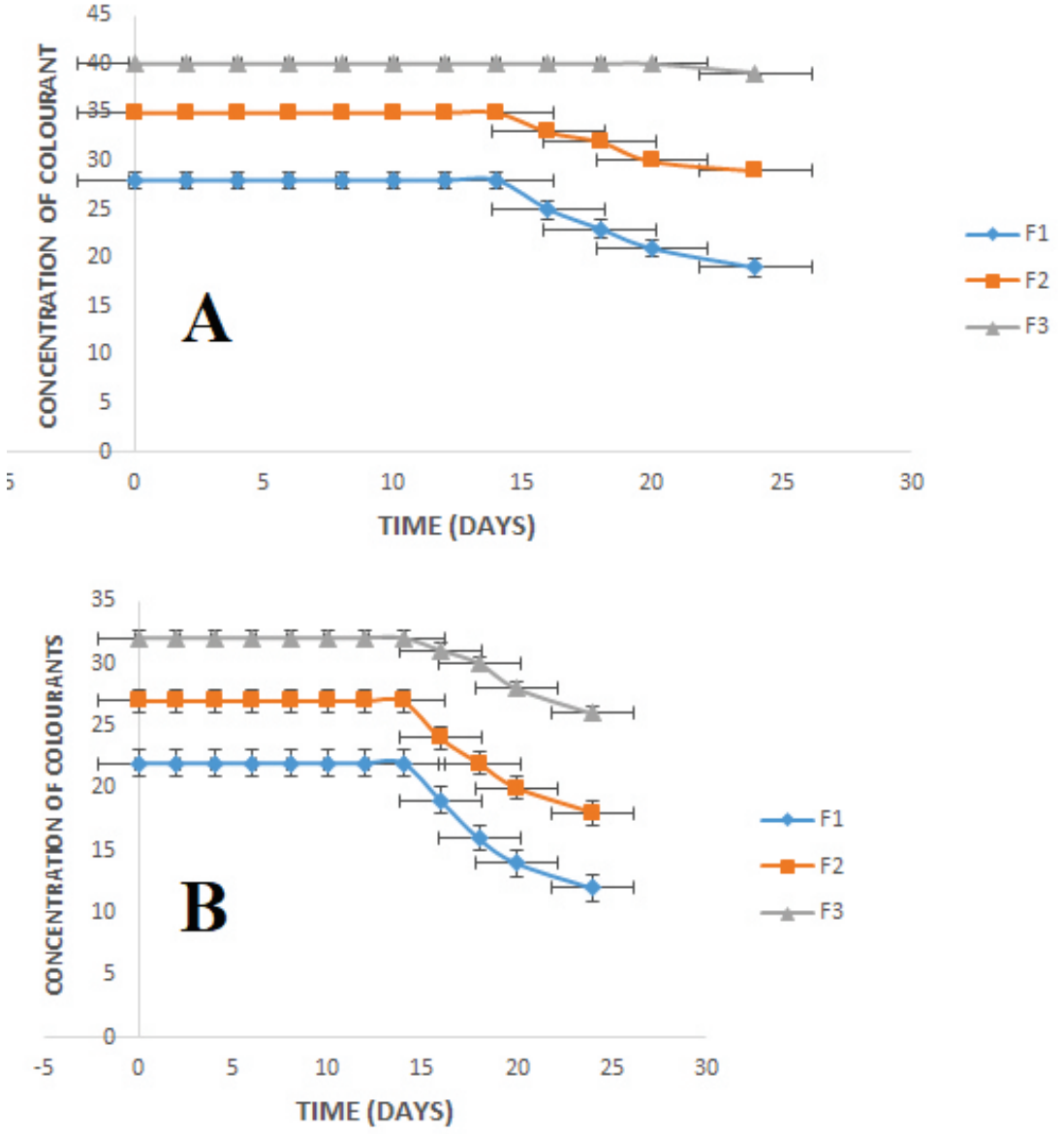

Figure 1 (A) Effect of light on the concentration of DC extract in paracetamol syrups formulated in amber coloured bottles (B) Effect of light on the concentration of DC extract in paracetamol syrups stored in plain bottles.

3.2 Results of temperature stability test

Paracetamol syrup formulations coloured with DC stored in amber coloured bottle showed stable concentration at 37°C and when the temperature was increased to 52°C the concentration decreased. However, the formulations coloured with amaranth was stable to heat at 52°C as the concentration remained constant over a period of 14 days (Figure 2A& B). This is due to the fact that lycopene responsible for the colour is stable at a temperature as high as 70°C and only degrades at temperatures above 100°C[17,18].

Temperature stability of the colourants was studied after subjecting the samples to various temperatures and heating time conditions. The log-linear decrease of the colourant as a function of temperature is illustrated in Figure 2C which shows a decrease in the concentration of the colourants as a function of temperature. The estimated kinetic parameters are summarized in Table 3.

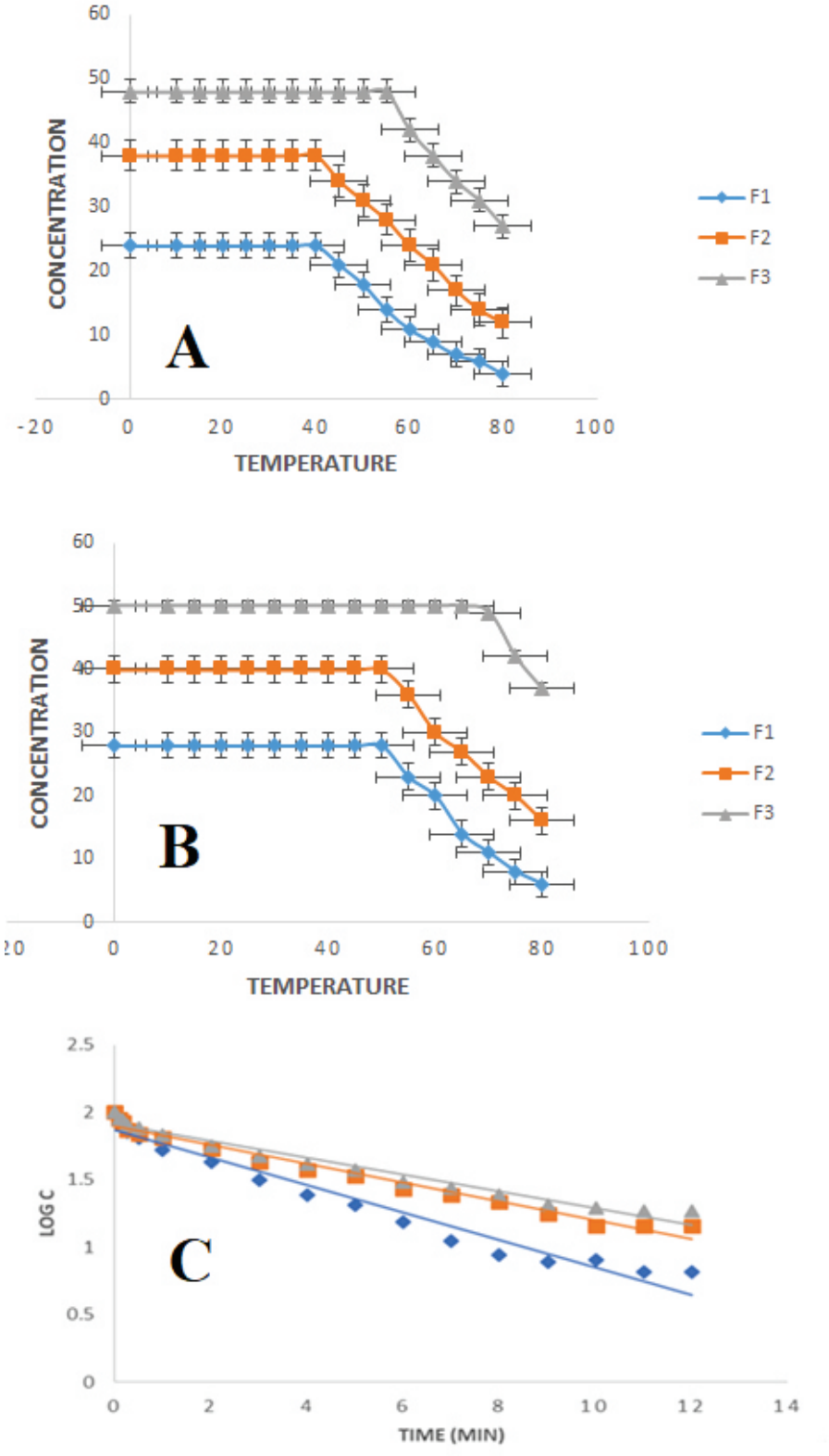

Figure 2 (A) Effect of temperature on the concentration of DC extract in paracetamol syrup stored in amber coloured bottles (B) Effect of temperature on the concentration of DC extract in paracetamol syrup stored in plain bottles (C) Thermal stability of the natural colourant under different temperature (37 and 52°C) heated for different time durations.

Table 3: Correlation coefficient and release kinetics of the colourants

Models	Zero		First		Higuchi		Korsmeyer and Peppas	
Formulation	r^2	K_0	r^2	K_1	r^2	K_H	r^2	n
F1	0.922	4.17	0.982	-0.051	0.951	19.06	0.573	0.58
F2	0.957	3.96	0.988	-0.028	0.974	17.54	0.625	0.60
F3	0.954	3.41	0.989	-0.042	0.963	18.73	0.587	0.64

4.0 Discussion

Ethanolic extract of DC was readily soluble in water making it a suitable colourant for water soluble drugs. The degree of acidity and alkalinity (pH) of the DC extract was within physiological pH (i.e. 5.0) hence less likely to cause gastrointestinal problem[5]. It was found that the percentage of insoluble matter present in *D. carota* ethanol extract was 2%w/w. This study shows that paracetamol syrup coloured with DC ethanolic extracts and amaranth are best stored in amber coloured bottles since their stability is more guaranteed when exposed to light because the amber colour protects the content of the bottle from photolysis. Formulations coloured with amaranth on the other hand, were more stable to light whether placed in amber or plain bottles over a period of time[18].

Hence, it can be deduced from the experiment that the formulations coloured with amaranth which is a synthetic colourant remained stable to heat over a long period of time because amaranth has a higher concentration of lycopene compared to the natural colourant obtained from DC extract. Paracetamol syrup formulations coloured with DC extract stored in plain bottle showed a stable concentration at 37°C

5.0 Conclusion

Natural colourants have the advantages of biosafety, easy extraction and purification and are gotten from renewable sources. The stability and physicochemical properties of *Daucus carota* extract has demonstrated its potential as a natural colourant in the formulation of liquid pharmaceutical dosage forms such as syrups, solutions and suspensions.

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