Preparation and Characterizationof Natural Food Dye (Red Powder)Extracted from *Amarathus spinosus* Linn. (Hin- nu-nwe-su-pauk)

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Abstract

The aim of this research is to extract food dye (red powder)from *Amaranthusspinosus* L. (Hin-nu-new-su-pauk).*Amaranthusspinosus* L. (Hin-nu-new-su-pauk), also known as prickly amaranth. The whole plant of air dried samples was used to prepare red food dye and to determine the phyto constituents. It was observed that, α-amino acid, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins and starch were present. Heavy metals can be toxic for humans when they are not metabolized by the body and accumulate in the soft tissue. Therefore the prepared red food dye sample (water extract) was analysed by AAS to determine constituent of heavy metals and characterized by UV and FT-IR spectroscopic methods. The colour stability of prepared food dye sample (water extract) from *Amaranthusspinosus* L. (Hin-nu-new-su-pauk) was determined by variation of pH and temperature. The red colourof food dye sample has not changed at various temperature and pH. According to the experimental results, water extract, red food dye may be applied for food and drink.

Keywords : *Amaranthusspinosus* L. (Hin-nu-new-su-pauk), food dye,pH, temperature

Introduction

The present work is to extract red food dye powder from the whole plant of *Amaranthusspinosus* L. (Hin-nu-new-su-pauk). This plant can be applied food dye as well as medicine. It is astringent, diaphoretic, diuretic, emollient and febrifuge. It is used in the treatment of menorrhagia, gonorrhea, eczema and colic. Plant, especially the young leaves, can be used as vegetable(Agarwel *et al*., 2007).Natural dye colour can be obtained from natural sources such as fruit, leaves, flowers, seeds, roots and bark of trees and some dried insects, and mineral. Natural colourants are also called pigments and comprise the colour compounds formed in living or dead cells of plants, animals, fungi, or microorganisms, including organic compounds isolated from cells and structurally modified to alter stability, solubility or colour intensity (May Chan Thar Oo, 2008). Chlorophyll is essential in the process of photosynthesis and oxygen transportation would not be possible without hemoglobin (Pearson, 1981). Melanins act as protective screen in humans and other vertebrates while other pigments have pharmacological activity against cancer and cardiovascular disease. The color of food

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is an integral part of our culture and enjoyment of life, nearly civilizations such as the Romans recognized that people "eat with their eyes" as well as their palate. Color variation in foods throughout the seasons and the effects of food processing and storage often require that manufacturers add color to certain foods to meet consumer expectations.

Figure 1 The plants of *Amaranthus spinosus* Linn.

Botanical Aspect of *Amarathusspinosus*linn.(Hin- nu-nwe-su-pauk)

Family	:	Amaranthaceae
Genus	:	*Amaranthus*
Species	:	*A.Spinosus*
Botanical name	:	*AmarathusSpinosus* Linn.
Commomname	:	Spiny amaranth, prickly amaranth, thorny amaranth, Needle Burr, Spincy Pigweed.
Myanmar name	:	Hin-nu-nwe-su-pauk
Part used	:	the whole plant

Amaranthusspinosus occurs in tropical and subtropical regions, including the whole of Southeast Asia, often gregariously and as a weed. It is sometimes found in temperate zones as well. It has been suggested that spiny amaranth originates from low land tropical south and Central America.

Materials and Methods

The chemicals used in the research were from British Drug House Chemical Limited (BDH), Poole, England; "E.MERCK, Darmstadt,Germany,St.Louis,USA".

The chemicals had been used it was received unless otherwise stated. It consists of conventional lab ware, glass ware and modern equipments. They are cited in each experiment. The followings were some of the instruments used in the experiments in this study. Balance (Potellers Balance), pH(Jenway 4330, Lab quip, England), FT-IR Spectrometer(Genesis Spectrometer, Austria) ,UV-visible Spectrophotometer(UV-vis 190-700,Shimadzu,Japan) and Atomic Absorption Spectrophotometer(AA-6300 SHIMADZU).

Sample collection

The whole plant of *Amaranthus spinosus* Linn. (Hin-nu-new-su-pauk), used in this study was collected from Mayan gone Township, Yangon Region. The whole plant was washed with water to be clean and dried at room temperature for two

weeks. Then it was cut into small pieces and made into powder by using grinding machine. Then, the dry powdered sample was stored in air-tight container to prevent moisture changes and contamination.

Preliminary Phytochemical Tests on the Whole Plant of *Amaranthusspinosus* Linn.(Hin-nu-nwe-su-pauk)

A few grams of dried powder sample was subjected to the tests of of alkaloids, glycosides, carbohydrates, -amino acids, flavonoids, steroids, terpenoids, phenolic compounds, reducing sugars, saponins and tannins(Trease and Evans,1980; M- Tin Wa,1972; Vogel, 1966).

Preparation of Food Dye Sample (red powder) by using Water

Air dried powder sample (50 g) was placed into a round bottomed flask and added 1000 mL distilled water. Then the mixture is heated about (2 hr) and filtered with filter paper. The filtrate was then evaporated until the red powder was obtained. This powder was placed in the oven and dried at 110 °C for 2 hr. Then, the resultantdye powder (red) was achieved and stored in airtight container

Characterization of prepared samples

Fourier-transform infrared spectroscopy (FTIR) measurements had performed using 8400 SHIMADZU, Japan FTIR spectrometer. UV spectra of extracted dye sample (red) was recorded by using a Perkin Elmer (Lambda 25) UV-Visible spectrophotometer at Universities' Research Center University of Yangon.

Determination of Trace Elements by Atomic Absorption Spectrophotometry

5.0 g of sample was ashed in a pre-weighed porcelain crucible by allowing firstly smoking off the fat without burning. The crucible was placed in the furnace at 550°C overnight till a white ash of constant weight was obtained. About 0.1 g of ash sample was accurately weighed and dissolved in 2 mL of concentrated hydrochloric acid. The resulting solution of ash sample was evaporated to dryness and dissolved in 6 cm^3 of 25 % HCl solution (volume by volume) followed by centrifugation. The centrifuged solution was decanted and the clear solution was made up to 100 cm^3 with deionized water. The resultant solution (10 mL) was pipetted accurately and made up to 100 mL with deionized water again. The sample solution prepared was now ready for analysis of trace elements by AAS.

Determination of Colour Stability of Food Dye Sample (red powder) with Temperatures

50 mL of dye solution was placed into 150 mL of conical flask and heated in oven. The temperature was controlled at 30, 40, 50, 60, 70 and 80°C. The colour change with various temperatures were recorded by photos .

Determination of Colour Stability of Extracted Food Dye Samples (red powder) with pH of Solution

50 mL of dye solution was placed in to 100 mL of plastic cup. The pH of the solution was varied from 1 to 12 by adding 1% HCl and 1% NaOH. The colour changes of solutions were recorded by photos .

Utilization of Extracted Dye Samples in the Preparation of Jelly and Bread

The extracted dyes samples (red powder) were added in the preparation of jelly and bread as additives.

Results and Discussion

Phytochemical Examination of *Amaranthusspinosus* Linn. (the whole plant)

Preliminary phytochemical screening was performed in order to know different types of compounds present in *Amaranthusspinosus* Linn. The results of phytochemical tests are summarized in Table 1. Phytochemical test in the *Amaranthusspinosus* Linn. (the whole plant) indicated the presence of alkaloids, glycosides, carbohydrates, -amino acids, flavonoids, steroids, terpenoids, phenolic compounds, reducing sugars, saponins and the absence of tannins.

Table 1. Results of Preliminary Phytochemical Tests on *Amaranthusspinosus* Linn.

No.	Tests	Extract	Test reagents	Observation	Remark
1.	Alkaloids	1% HCl	Mayer Dragendroff reagent Wagner	Yellow ppt White ppt Deep blue ppt	+
2.	-amino acids	H_2O	Ninhydrin reagent	Purple	+
3.	Carbohydrates	H_2O	10% -Naphthol, conc: H_2SO_4	Red ring	+
4.	Flavonoids	EtOH	Mg ribbon, conc: HCl	Pink	+
5.	Glycosides	H_2O	10% lead acetate solution	White ppt	+
6.	Phenolic compounds	H_2O	1% $FeCl_3$and $K_3Fe(CN)_6$	Deep blue solution	+
7.	Reducing sugars	DilH_2SO_4	Benedict's solution	Reddish brown color	+
8.	Saponins	H_2O	Distilled water	Frothing	+
9.	Tannins	EtOH	2% NaCl, 1%Gelatin solution	No ppt	-
10.	Steroids	PE	Acetic anhydride, conc: H_2SO_4	Green colour	+
11.	Terpenoids	Chloro -form	Acetic anhydride, conc: H_2SO_4	Pink colour	+

+ present, - absent

FTIR analysis

Figure 2 shows FTIR spectra of extracted dye sample (red powder). In the FTIR spectra of sample, 765 $cm^{-1}$and 781 cm^{-1} were N-H banding and bending of CH of aromatic appear at 825 $cm^{-1}$the band at nearly 1200cm^{-1} show C-O-C stretching. The absorption band nearly 1600 cm^{-1} to 1780 cm^{-1} of sample (red powder)show C=C stretching (aromatic), C=O stretching, -NH_2 and -CH(CH_2, CH_3) banding. The absorption band of 1300-1400 cm^{-1} of red dye sample shows the presence of nitrate group. The bands appears at nearly 3400 cm^{-1} of stretching vibration of OH and>C=N-H (Silverstein and Terence, 1991).

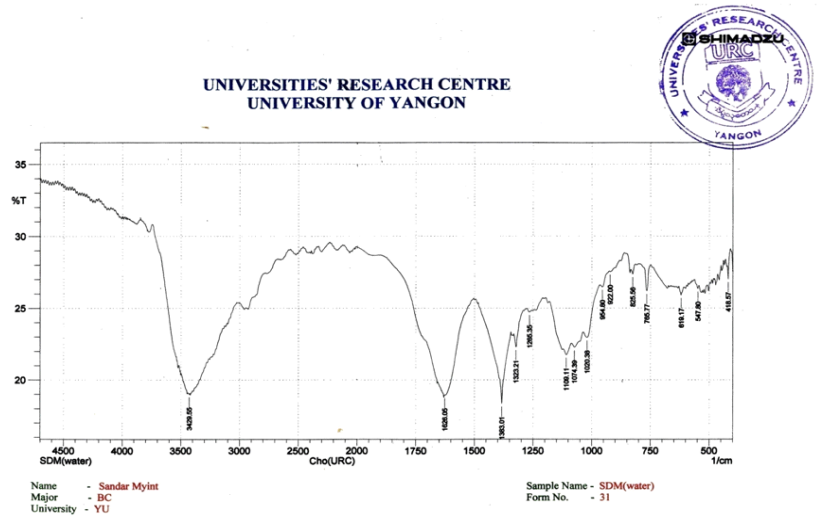

Figure 2.FTIR spectrum of food dye sample (red powder) from *Amaranthusspinosus* Linn.

UV analysis

Ultraviolet spectrum of food dye sample (red powder) of *Amaranthusspinosus* Linn.is described in Figure 3. The maximum wavelength ($_{max}$) values and possible transitions are summarized in Table 2. The $_{max}$was found to be 262,272 and 289 nm.

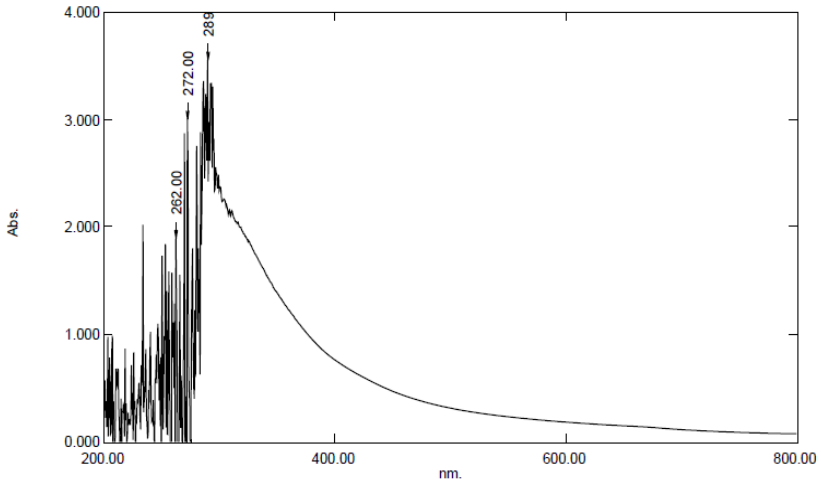

Figure 3. UV visible spectrum of food dye sample (red powder) of *Amaranthusspinosus*Linn.

Table 2. Maximum Wavelengths of Red Food Dye Sample (red powder) Isolated from the whole plant of *Amaranthusspiosus* Linn.

Wavelength (nm)	Transition
262	$\pi \rightarrow \pi^*$
272	$\pi \rightarrow \pi^*$
289	$\pi \rightarrow \pi^*$

Heavy Metal Concentration in *Amaranthusspinosus* Linn.

Toxicity of heavy metals can occur at levels just above naturally occurring back ground levels, meaning that consumption of food with a high heavy metal concentration can cause acute or chronic poisoning.

Table 3.Heavy Metal in *Amaranthusspinosus* Linn.

Heavy metal	FDA permitted value (ppm)	Observedvalue (ppm)
Hg	< 1	1.6
Pb	< 10	1.409
Cd	< 20	Not detected
As	< 3	2.744

Effect of Temperature on the Colourof Red Food Dye Sample

The colour of some dye may be changed when the temperature is changed. Therefore, the colour stability of extracted red food dye from *Amaranthusspinosus* Linn. was studied by changing the temperature from 30° to 80°C. The colour of this sample at various temperatures is shown in Figure 4.The colour of food dye samples is stable at low temperatures as well as elevated temperature. Therefore the colour of these dye samples is not temperature sensitive.

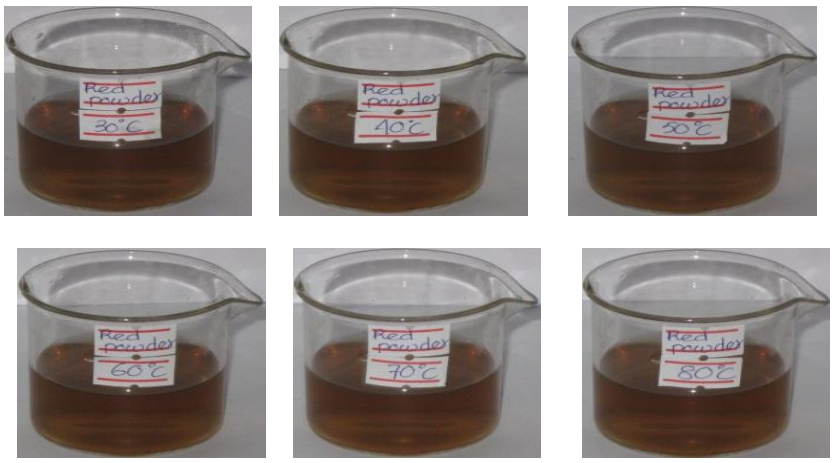

Figure 4. The stability of the colour of red food dye sample as a function of temperature

Effetc of pH on the Colour of Extracted Food Dye Samples

The original pH of food dye sample (red powder) was 6.6. The pH value of food dye solution was adjusted with 1% HCl and 1% NaOHfrom 1 to 12. It was found that the colour of food dye sample does not changed between pH 2 to 7. At higher alkaline medium (pH 10-12) the colour of extracted food dye (red powder) became deeper and highest acidic medium (pH 1) the colour of solution became paler. Therefore, these dye samples are not pH sensitive.

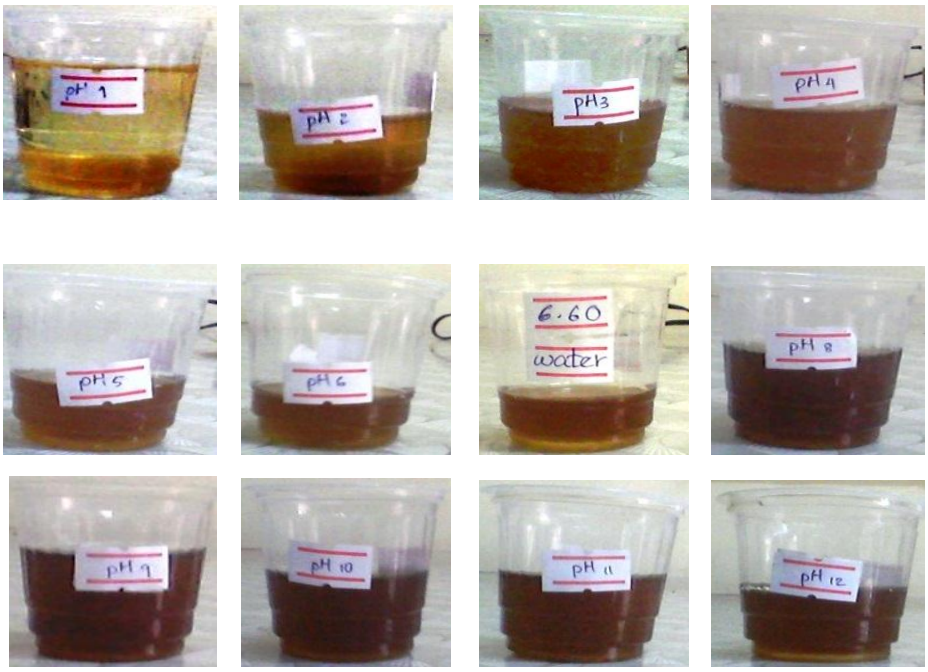

Figure 5. Effect of pH on the colour of food dye sample (red powder)

Application of Extracted Red Food Dye Sample in the Preparation of Jelly and Bread

Food dye samples (red powder) from *Amaranthusspinosus* Linn. were used as additive for dying jelly and bread. The taste and smell of the product foods were not distinct with and without dye samples but the attractive colour were obtained when using dye samples see in Figure 6.

Figure 6. Application of food dye samples (red powder) of *Amaranthusspinosus* Linn. in (a) jelly and (b) bread

Conclusion

From the overall assessment of present work, the following inferences can be deduced. It was found that, the presence of alkaloids, glycosides, carbohydrates, -amino acids,flavonoids, steroids, terpenoids, phenolic compounds, reducing sugars, saponins and the absence of tannins. Extracted red food dyes from *Amaranthusspinosus* Linn were determined by AAS. Mercury (Hg) 1.6, lead (Pb) 1.409 and arsenic (As) 2.744 part pre million (ppm) were found in *Amaranthusspinosus* Linn .and cadimium was not found in this sample. The observed amount of all there metals are in the range of permitted levels of FDA except mercury which are slightly higher than the permitted level. The red food dye sample (water extract) was characterized by UV and FT-IR spectroscopic methods. The prepared dye sample (water extract) from *Amaranthusspinosus*L. (Hin-nu-new-su-pauk) was determined by variation of pH and temperature. The colour of water extract, red food

dye was found to be original colour in the pH 6.6.The colour of extract dye sample was found to be paler in the pH 1 and more intense in the pH range 10 to 12. The colour of water extract dye sample has not changed in the pH range of 2 to 7. Variation of temperatures have not effected on the colour of dye sample. According to overall results, this red food dye is cheaper and no toxic for human. Therefore, this red food dye sample may be used for food and drink.

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