African Journal of Pharmacy and Pharmacology Vol. 4(6). pp. 304-313, June 2010
Available online http://www.academicjournals.org/ajpp
ISSN 1996-0816 ©2010 Academic Journals

Full Length Research Paper

Assessment of the intrinsic and stability properties of the freeze-dried and formulated extract of *Hibiscus sabdariffa* Linn. (Malvaceae)

Philip F. Builders[1]*, Chukwuemeka R. Ezeobi[1], Florence D. Tarfa[2] and Modupe I. Builders[3]

[1]Department of Pharmaceutical Technology and Raw Materials Development, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.
[2]Department of Medicinal Chemistry and Quality Control, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.
[3]Department of Pharmacology, University of Jos, Jos, Nigeria.

Accepted 21st May, 2010

Water extract of the calyces of *Hibiscus sabdariffa* Linn (Malvaceae) is used widely as a food additive and refreshing drink with proven medicinal benefits, which are attributed to its phytochemical constituents. Relevant physicochemical and stability studies have been carried out on the freeze-dried and formulated samples of aqueous extract of the calyces of *H. sabdariffa*. The phytochemical constituents, moisture sorption characteristics and the effect of extract concentration, light and pH on the color of the extract were determined. The stability of the anthocyanins contained in the extract and the thermal characteristics of the freeze-dried extract were also evaluated. The dye solution which has hot-pink color and a pH of 2.1 ± 0.6 contained flavonoids, glycosides, sterols, balsams, phenols, monosaccharides, free reducing and combined reducing sugars. The extract solution also showed colour and light transmittance responsiveness to changes in pH. Fourier transform infrared (FTIR) - spectra and the diffraction scanning thermograms show the degradation effect of light on the extract. The dry samples of the extract showed higher photo-stability relative to the solutions. The isothermal moisture sorption profile of the powdered freeze-dried extract and the formulated granules showed characteristic sigmoidal curves corresponding to Type 2 and 5 isotherms respectively. The aqueous extract of the calyces of *H. sabdariffa* generally showed high light and pH sensitivity and positive tests for the presence of some active secondary plant metabolites that are probably responsible for its claimed health benefits.

Key words: *Hibiscus sabdariffa*, physicochemical properties, stability properties, formulation properties.

INTRODUCTION

There has been renewed interest in the use of natural products as medicines, especially those from plant origin. This is because of their multifunctionality and diverse applications and low side effects (Wang and Jiao, 2000). Many of the identified pharmacologically active plant species are also used as food or condiments in delicacies in different communities where they are found (Igarashi et al., 2000). The family Malvaceae contains several species with such folkloric uses.

The family Malvaceae, which is also known as Mallow family, is a family of Class Magnoliopsida, flowering plants. In this family, there are more than 100 genera and about 1500 species. Most, species in this family are herbs or shrubs excluding several tree species. *Hibiscus sabdarrifar* (Linn) belongs to the Malvaceae family (Wiki directory, 2010).

H. sabdariffa is an annual dicotyledonous shrub, which grows to a height of about two meters. It has yellow or reddish flower and its leaves have three to five lobules

*Corresponding author. E-mail: philsonsky @yahoo.com. Tel: +2348035874698.

(George and Roger, 1991). Although native to India and Malaysia, *H. sabdariffa* is also widely available and must have being carried to Africa in early times (Fasoyiro et al., 2005). Many parts of the plant are of value with the leaves, seeds and calyces widely used as either food or drug (Aliyu, 2000; Parkouda et al., 2008).

Many phytochemical constituents and diverse medicinal activities have been attributed to this plant. In the Ayurvedic literature of India, different parts of the plant are recommended as remedy for ailments such as hypertension, pyrexia and liver disorders. In some other traditions, the plant is used as antidote to poisonous chemicals (acids, alkali, pesticides) and venomous mushrooms (Chifundera et al., 1994). *H. sabdariffa* contains higher amount of ascorbic acid compared to orange and mango (Wong, et al., 2002). It is also rich in riboflavin, niacin, calcium and iron (Qi et al., 2005; Babalola et al., 2000).

The water extract of the red flowered specie of *H. sabdariffa* is widely used in the preparation of fruit drinks because of its unique and appealing characteristic color and flavor (Fasoyiro et al., 2005). In the United States and Germany, it is used in the processing of juices, jellies, jams, beverages and ice cream (Leung, 1980). In Nigeria, the flavored water extract is popular and widely consumed as a refreshing tea and "soft drink" called 'zobo'. This beverage is widely consumed without any reported adverse effects. The sensitivity of the dye solution to environmental factors such as temperature, light and oxygen has limited their commercial production and use as colorant (Kanner et al., 2001).

The claimed medicinal benefits of *H. sabdariffa* can be attributed to the presence of anthocyaninins, which are the colored product of the flavonoid pathway (Niloufer et al., 2007). The anthocyanins contained in *H. sabdariffa* have been found to possess antioxidant activity, which offer protection against atherosclerosis and cancer (Meyer et al., 1997; Satue-Gracia et al., 1997; Takeoka et al., 1997). They are also linked with liver-protective and cholesterol activity enhancement. The antioxidant potential has been shown to have many times more activity than common antioxidants such as ascorbate (Wang et al., 1997). The anthocyanins identified in the calyces of the red colored specie of *H. sabdariffa* include delphinidin-3-sambubioside, cyaniding-3-sambubioside and delphinidin-3-glucose (Hong and Wrostlad, 1990). A number of environmental factors such as pH, heat and some food additives affect the stability of anthocyanins from many plant sources (Attoe and von Elbe, 1981). However, there are no reports on the effect of light on the anthocyanins contained in the aqueous extract of *H. sabdariffa*. Evaluation of the physicochemical and stability properties of the aqueous extract will be a valuable tool for storing and processing the extract in stable formulations. The objective of this work therefore is to evaluate relevant physicochemical and stability properties of the freeze-dried and granulated formulation of the aqueous extract of the calyces of *H. sabdariffa.*

MATERIALS AND METHODS

Materials

Dry calyces of *H. sabdariffa* were obtained from an open market in Abuja, Nigeria. Other materials include: buffer tablets (pH 4, 9.2, 10) (Fisher, USA); sodium chloride, magnesium chloride, tannic acid and ferric chloride (Sigma, Germany), potassium dihydrogen phosphate (May and Baker, England), sodium hydroxide, potassium thiocyanate, potassium chloride and calcium chloride (BDH, London) and sucrose (Nigerian sugar company, Nigeria).

Method

Extraction

A 300 g quantity of dry calyces of *H. sabdariffa* was rinsed with two 500 ml portions of distilled water to remove dust and adhering dirt. This was then transferred into 2 L of hot distilled water maintained at 80°C for 45 min. The extracted dye solution was strained off and the exhausted calyces rinsed with another 1 L of hot (80°C) distilled water. The extract was freeze-dried and pulverised with a glass pestle and mortar before passing through a 250 µm mesh sieve (US Standard sieve, USA). The powdered extract was stored in a chamber of activated desiccator for 48 h and then transferred into an air tight, amber coloured screw capped bottle.

Formulation of granules

Granules were prepared by the massing and screening method (Kunle et al., 2003). The powdered freeze dried extract was massed by using syrup BP (BP, 2004) as the binder. The wet mass was hand screened through a 0.6 mm mesh sieve and then dried at 50°C for 6 h in a hot air oven. The dry granules were then stored in an air tight, amber coloured screw capped bottle.

Phytochemical analysis

Phytochemical analysis for bioactive constituents was undertaken using standard qualitative methods (Jack and Okorosaye-Orubite, 2008). Flavonoids was detected by adding 3 ml of the extract to 1ml sodium hydroxide and observed for yellow coloration. Glycoside was detected by adding 10 ml of 50% sulphuric acid to 1 ml of the extract in a test tube. The mixture was heated in a boiling water-bath for 15 min. 10 ml of fehling's solution was added and the mixture was heated and observed for brick red precipitate. Alkaloids was detected by adding 1 ml of hydrochloric acid to 3 ml of the extract in a test tube. The mixture was heated for 20 min, cooled and filtered. 2 drops of Wagner's reagent was added to 1 ml of the filtrate and observed for reddish brown precipitate. Saponins were detected by using the frothing test: 2 ml of the extract in a test tube was vigorously shaken for 2 min and observed for persistent foaming.

Moisture sorption characteristics

Quantities of the freeze-dried extract of *H. sabdariffa,* sucrose powder and the granulated formulation of *H. sabdariffa*/sucrose were placed in 100 ml Petri dishes in an activated desiccating chamber at 25°C for one week to remove any residual moisture. The moisture sorption isotherms of these were then determined by the gravimetric method (Beristain et al., 2006). 1 g was placed in an aluminum foil and put in a desiccator with a gauze holding tray containing either distilled water or saturated solution of different

salts to provide the required relative humidity (RH), (water 100%, potassium chloride 84%, sodium chloride 75%, potassium thiocynate 47% and calcium chloride 31%). The samples were weighed at 12 h intervals until equilibrium was attained. The equilibrium moisture sorption (EMS) was evaluated using Equation 1 (Lin and Chen, 2005).

EMS = M_e/M_d x 100

Where M_e is the amount of moisture sorbed at equilibrium and M_d is the dry weight of the material. The profile of percentage weight gain vs relative humidity was then evaluated for each sample.

Effect of pH on the colour of dye solution

The effect of pH on the color of the dye solution was investigated by altering the pH of a 0.5 %w/v dye solution from acidic through neutral to basic by dropwise addition of either 0.1 N HCl or 0.1 N NaOH solution. The color of the dye solution was assessed by cross matching with a standard color chart.

Effect of pH on transmittance of dye solution

Different concentrations (0.02, 0.04, 0.12 mg/ml) of the solutions of the freeze-dried extract of *H. sabdariffa* were prepared in different buffer solutions (pH 1, 2.5, 4, 7 and 9.2). The transmittance of the solutions was determined at a predetermined λ_{max} of 600 nm. A curve of transmittance vs concentration was plotted.

***Stability* of *H. sabdariffa* dye solution**

Samples of the powdered freeze-dried extract, formulated granules and 0.5 %w/v solutions of the freeze-dried extract were stored in a photostability chamber (Vindon Scientific, Oldham England). 10 ml volume of each of 0.5% w/v solutions of the freeze-dried granules and formulated extract was prepared daily, and the transmittance of each solution was determined at the predetermined λ_{max}. This was done daily for 7 days.

Effect of light on the stability

A 0.1% w/v solution of *H. sabdarriffa* was prepared and the tannin content was determined (Muller-Harvey and McAllan, 1992). The solution was then stored in photostability chamber under a fluorescent light at 33°C. The tannin content was determined by photometric method using a standard curve prepared with tannic acid.

***Effect of storage on the pH* of *H. sabdariffa* solution**

The pH of 0.125 %w/v solution of the freeze-dried extract and formulated granules was determined. Each of the solutions was then divided into two portions with one portion stored in a refrigerator (5°C) and the other at room temperature (27°C) and the pH monitored periodically for 7 days.

Fourier transform infrared - spectra (FT-IR)

The Fourier transform infrared (FTIR) spectra were acquired on a NICOLET IR 100 (Themo Electro Corporation, USA). Spectra over a range of 4000 - 400 cm^{-1}, with threshold of 1.303, sensitivity of 50 and resolution of 2 cm^{-1} range were recorded on KBr pellets (1 mg of extract powder per 400 mg of KBr). Spectra scan to determine the effect of light on the freeze-dried extract of *H. sabdariffa* was also carried out.

Differential scanning calorimetry (DSC)

Thermal studies were carried out on a differential scanning calorimeter (DSC 204 F1, Phoenix NETZSCH, Germany) equipped with a thermal analysis system. Indium (156.8 °C) was used as the internal standard. Samples of approximately 1 mg of the freshly prepared freeze-dried extract before and after exposure to fluorescent light in a photostability chamber for 7 days were placed in an aluminum pan (25 µl) and covered with a perforated lid respectively. Dry nitrogen was used as the purge gas (purge 20 $mlmin^{-1}$). The probes were heated from a start temperature of 25 to 500°C at a rate of 10 °C min^{-1}. The thermal characteristics were evaluated with the Proteus analysis software (Builders et al., 2008).

RESULTS AND DISCUSIONS

Phytochemical screening

In many places, *H. sabdariffa* is cultivated mainly as a food crop. However, it has also been used as an important medicinal plant because of the presence of some biologically active components with identified pharmacological benefits (Qi et al., 2005). The phytochemical screening of the water extract of the calyces of *H. sabdariffa* showed the presence of flavonoids, glycolsides, sterols, balsams, monosaccharides, free reducing and combined reducing sugars (Table 1). Flavonoids refer to a class of plant secondary metabolites, which are synthesized by the phenylpropanoid metabolic pathway. Anthocaynins are the final products of the series of enzymatic modification of the pathway. This group of compounds is responsible for the yellow, red or blue pigmentation in many flowers and fruits (Irani and Grotewold, 2006). These bioactive agents have some of the strongest physiological effects of any plant compound probably because of their strong antioxidant properties. The flavonoids inside the human body have little or no direct antioxidant value because they are poorly absorbed (less than 5%) by the human body (Lotito and Frei, 2006). When flavonoids are absorbed they are quickly metabolized and excreted from the body. The huge increase in antioxidant capacity of blood seen after the consumption of flavonoid-rich foods is not caused directly by the flavonoids themselves, but due to the increased uric acid levels that result from degradation of flavonoids from the body (Krishnaiah et al., 2009). Consumption of large dietary supplements or large quantities of the tea or drink prepared with high concentrations of the aqueous extract of *H. sabdariffa* provides no extra benefit and may pose some risks, however they have relatively low toxicity compared to other active plant compounds (Wang and Jiao, 2000). Like other natural sources of flavonoids the aqueous extract of the calyces *H. sabdariffa* will induce the production of Phase II

Table 1. Phytochemical constituent of the aqueous extract of *H. sabdarriffa.*

Compound	Remark
Alkaloids	-
Saponin	-
Tannin	+
Glycosides	+
Anthraquinones	-
Flavanoids	+
Phenols	+
Sterols	+
Balsams	+
Monosaccharides	+
Free reducing sugar	+
Combined reducing sugar	+

Key: - Absence; + Presence.

Figure 1. Color profile of *H. sabdarriffa* aqueous extract solution at different pHs

enzymes that help eliminate mutagens and carcino-gens, and therefore may be of value in cancer prevention and probably also induce mechanisms that could kill cancer cells and inhibit tumor invasion. In addition, the antihypertensive benefits of flavonoids have being attributed to other mechanism other than the antioxidant property (Ali et al., 2005; Andriambeloson, 1998).

The aqueous extract of the calyces of *H. sabdariffa* or drinks made with it have a characteristic dry and puckery feeling in the mouth when consumed. These properties are due to the astringent property that is characteristic of tannins. The tannin present in the calyces is also the product of enzymatic pathway during the formation of the anthocyanins (Niloufer et al., 2007).

Presence of phenol and glycosides (Table 1), like that of the flavonoids are due to the polyphenolics produced during the anthocynanins enzymatic pathway (Elliot, 1992). Generally, glycosides are a group of compounds in which a sugar part is bound to a nonsugar part of the molecules. Thus, the positive test observed for phenols, flavonoids and sterols in the freeze-dried extract of the calyces of *H. sabdarriffa* may also be responsible for the positive test for glycosides (Sofowora, 1993; Trease and Evans, 1989). The sugar moiety may consist of a single sugar group (monosaccharide) or several sugar groups (oligosaccharide) (Table 1). This is probably responsible for the observed positive test for monosaccharide, free reducing sugar and combined reducing sugar obtained in the extract (Trease and Evans, 1989).

Effect of pH change on extract solution color

The aqueous extract of the calyces of *H. sabdariffa* is acidic (Fasoyiro et al., 2005; Wong et al., 2002) with a 0.5 % w/v aqueous solution of the freeze-dried extract characterized by a hot-pink color and a pH of 2.1 ± 0.6 (Table 1). The low pH of the aqueous extract has been attributed to the presence of a number of organic acids: oxalic, tartaric, malic and succinic acids (Wong et al., 2002). When the pH of the extract solution was altered from acid through neutral to basic, the solution showed characteristic changes in color as shown in Figure 1. The color changes that were observed with change in the pH of the dye solution are consistent with the presence of anthocyanins (Laleh et al., 2006). On adjusting the solution to pH 1, the solution changed to red due to the protonation of the pigments resulting from pH-induced condensation of the anthocyanins. At alkaline pH (7 and above), the colors of the solutions gradually faded turning colorless due to the formation of unstable intermediate peroxide complexes (Lopez-Serrano and Ros-Barcelo, 1999).

The pink color of the solution persisted in different

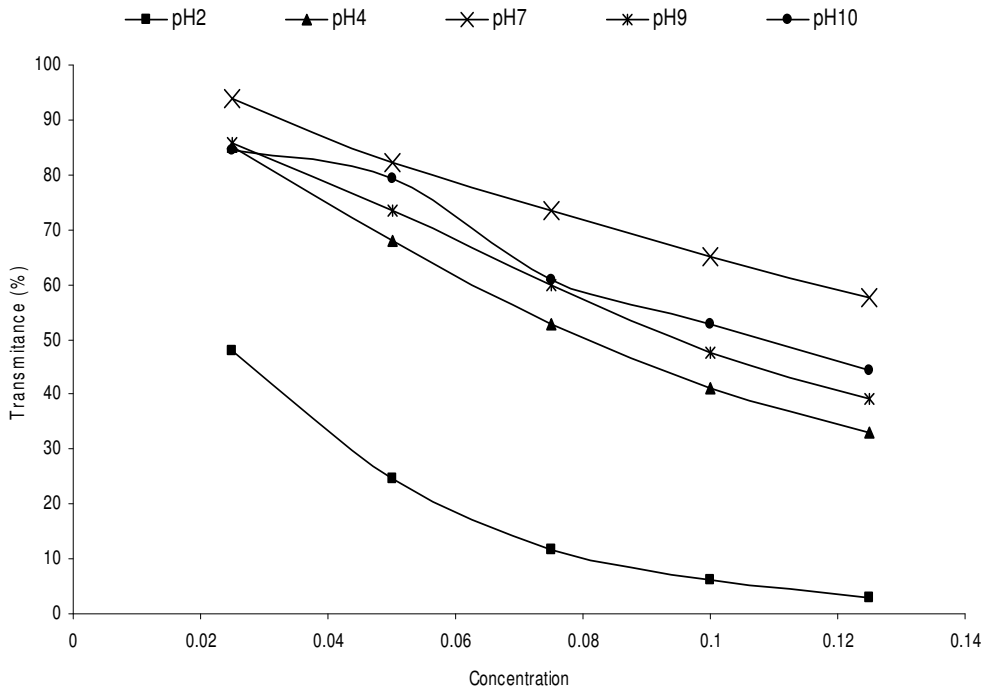


Figure 2. Effect of pH on light transmission of H. sabdariffa solution

shades until pH 5. At pH 5, the dye solution changed to a color coinciding with mystyrose on the color chart. At pH9 to pH 11, the solution was olive while pH 12 and 13 were olive drab.

At the lower pH values (1 to 4) the colored complexes formed were more stable than those formed at the higher pH values (pH 5-13). The color changes at pH 5 and above could be due to the deprotonation of the coloured pigments. Thus, the extent of deprotonation increased with decrease in pH of the solution. Though the characteristic instability of the colour of the dye solution to changes in pH makes it unsuitable for use as a colorant in formulations, this property however could be used as an indicator to monitor changes in pH of certain preparations.

Effect of light on the color of the dye solution

The intensity of the colour was determined by evaluating the transmittance of light through the solution. As is characteristic of dyes, the transmittance increased with increase in the dye concentration (Figure 2). Figure 3 shows the pH sensitivity of the light transmittance through the solution. The pH of least sensitivity to transmittance with change in concentration was observed at pH 2 while the highest sensitivity was observed at pH 7. The differences in light transmittance due to changes in pH of the solution indicate the variation in the absorption-transmittance of light of different wavelengths by the coloured pigments of the solution.

Effect of light on the color stability of formulated and unformulated freeze-dried extract solution

Anthocyanins are highly photosensitive compounds (Attoe and Von Elbe, 2006). The exposure of the dye solution of the extract of the calyces of *H. sabdariffa* to fluorescent light was evaluated in order to determine the color stability of the freeze-dried extract and the formulated extract sample to light. The stability of the freeze-dried powder and the formulated granules and their solutions were comparatively evaluated. The photo- stability profiles were evaluated by assessing the changes in light transmittance with time on exposure to light (Figure 4). The dry solid samples of the formulated and the unformulated freeze-dried extract exhibited higher photo-stability as compared to their solutions. There was no significant difference ($p < 0.05$) in the photostability of the solutions prepared with the dry freeze dried sample and the formulated samples. There was increase in transmittance with aging of the solutions when exposed to light, an indication of degradation of the anthocyanins especially as it coincides with the fading of the color of the solution. Photodegradation may be an important degradation pathway of the coloured pigments of the aqueous extract of *H. sadariffar*. The fading of the dye color as assessed by increase in light transmittance was lower in the solutions containing sucrose (Figure 4). Sucrose has being

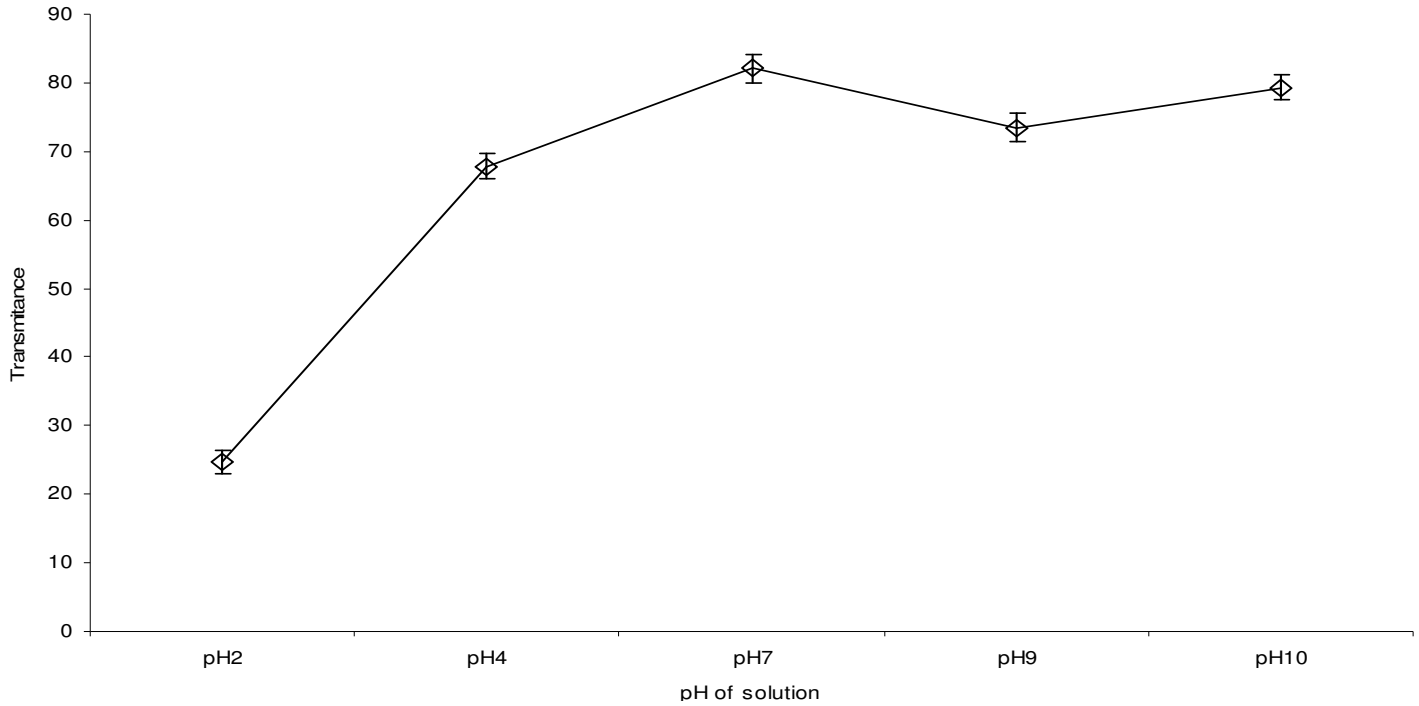


Figure 3. Effect of *H. sabdarrifa* dye solution on light transmittance.

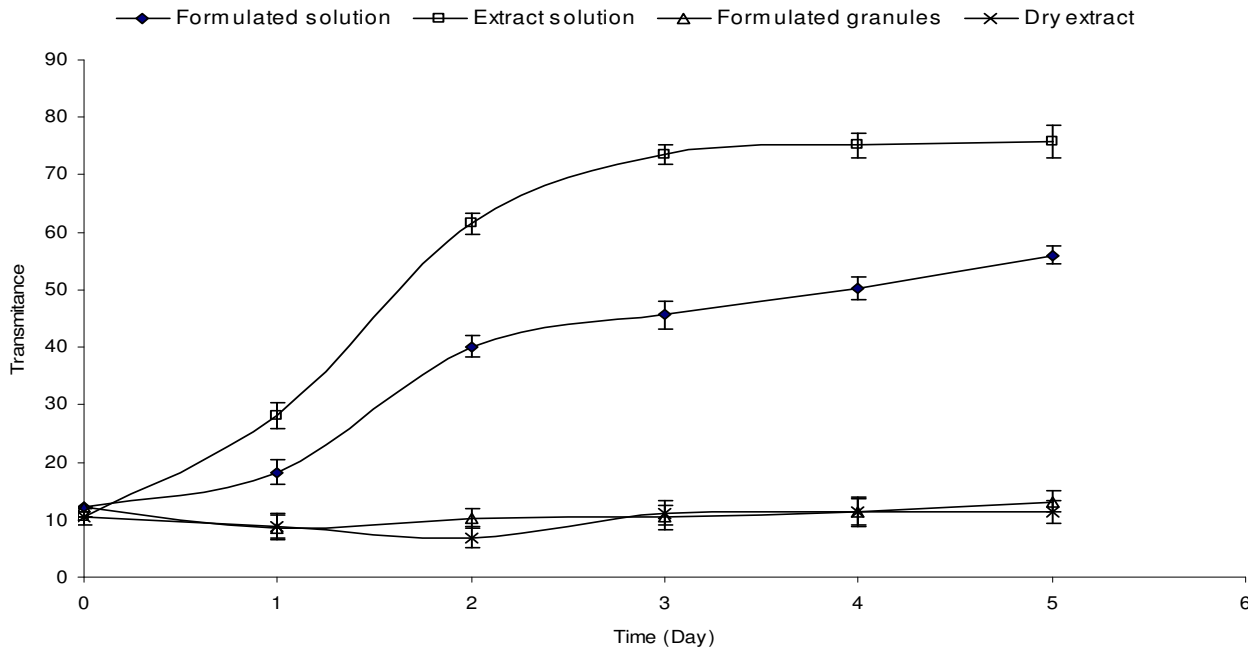


Figure 4. The effect of light on the transmittance of dry and solubilised and unformulated freeze-dried extract of *H. sabdariffa*.

reported to protect anthocyanins from photodegradation by inhibiting degradative enzymes and steric interference due to condensation reactions (Wang and Xu, 2007; Attoe and Von Elbe, 2006).

Moisture sorption characteristic

Moisture sorption isotherm shows the equilibrium amount of water sorbed onto a solid as a function of steady state

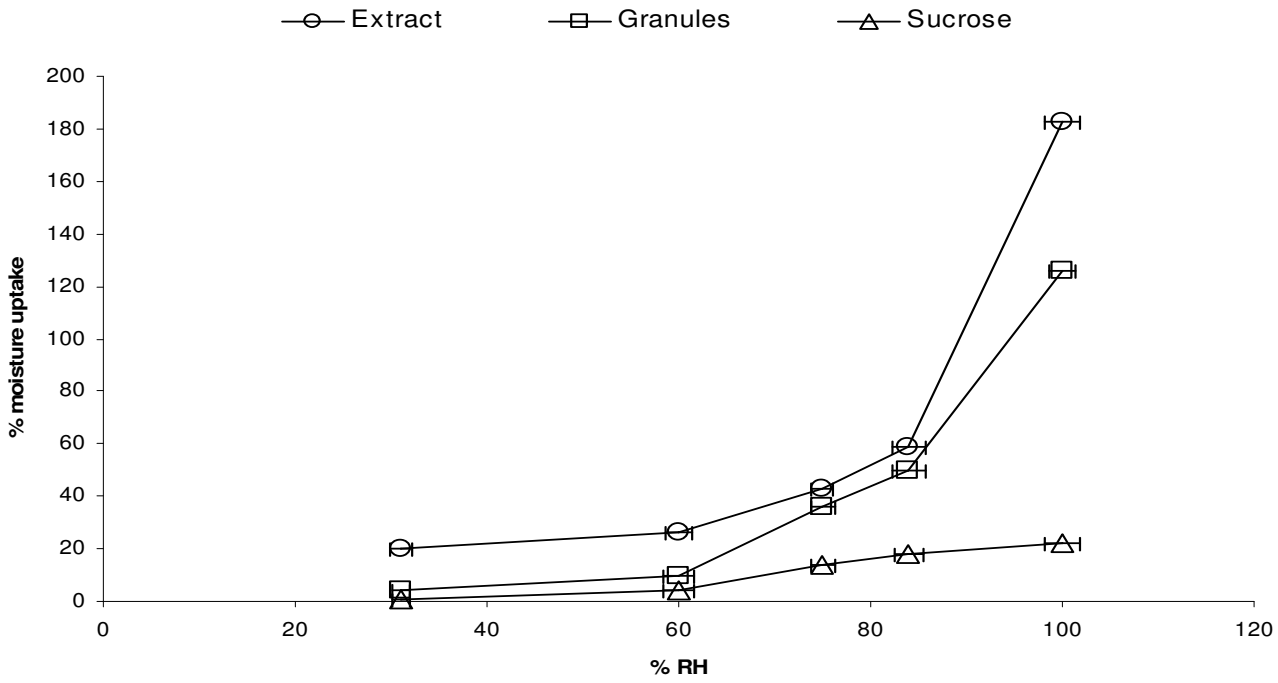


Figure 5. Moisture uptake profile of freeze-dried extract of *H. sabdarriffa*, formulated granules and sucrose.

vapour pressure at a constant temperature (Roos, 1995; Oyelade et al., 2008). Water sorption property is important in predicting the physical state of materials under various conditions, because most structural transformations and phase transitions are significantly affected by water (Roos, 1995). The interaction of the extract and its formulations with water molecules can affect chemical stability as well as physical and mechanical properties. The importance of moisture on the solid-state stability of bioactive agents has been extensively documented (Swaminathan and Kildsig, 2001). The moisture sorption properties of the freeze-dried extract, sucrose-freeze dried extract granules and sucrose were evaluated to determine the physical changes in terms of amount of moisture sorbed at different RH. The moisture uptake profiles are presented in Figure 5. Curve 1 and 2 coincides with the Type 2 isotherm, which are sigmoidal curves normally associated with monolayer-multilayer uptake on the non-porous or macroporous surface of a powder. The powdered freeze-dried extract and the formulated granules have macroporous particles that permit fast uptake of water molecules by capillary action and surface water interactions (Oyelade et al., 2008; Bell and Labuza, 2000). The profile shows a significant ($p < 0.05$) uptake at low RH followed by a small adsorption at intermediate RH and a high uptake at high RH which is indicative of a well-formed monolayer. Curve 3 indicates a Type 5 isotherm, which is characterized by a weak adsorbent–adsorbate interaction and shows a low uptake at low RH and a high adsorption at higher RH (Sing et al., 1985; Rouquerol et al., 1999).

The freeze-dried and the formulated freeze-dried extracts show a high degree of hygroscopicity as their moisture uptake were greater than 50% of their dry weight after storage for less than one week at RH of less than 90% (Callahan et al., 1982).

The phenomenon of deliquescence is important in pharmaceutical systems because the exposure of solids to high RH results in the formation of a liquid phase where chemical reactions may be accelerated or physical changes catalysed (Hancock and Shamblin, 1998). The deliquescence occurred in the extract and granulated extract-sucrose granulated formulation at RH above 60 and at 100% RH for sucrose. The deliquescence of the materials results from the dissolution of the particles by water adsorbed at the surface to form saturated solution at high RH (Van Campen et al., 1983; Kontny and Zografi, 1995).

Fourier transform infrared - spectra (FT-IR)

The infra-red (IR) spectrum of a given compound is always unique and characteristic. IR spectroscopy is a quick and relatively cheap technique for identifying compounds (Sherman, 1997). The IR spectra of the *H. sabdariffa* when stored in the dark and when exposed to light are presented in Figure 6. The IR spectra of the freeze dried extract stored in the dark and under fluorescent light were carried out to identify the effect of light on the stability of the freeze-dried extract of the calyces of *H. sabdariffa*. The spectra of the freshly freeze-dried

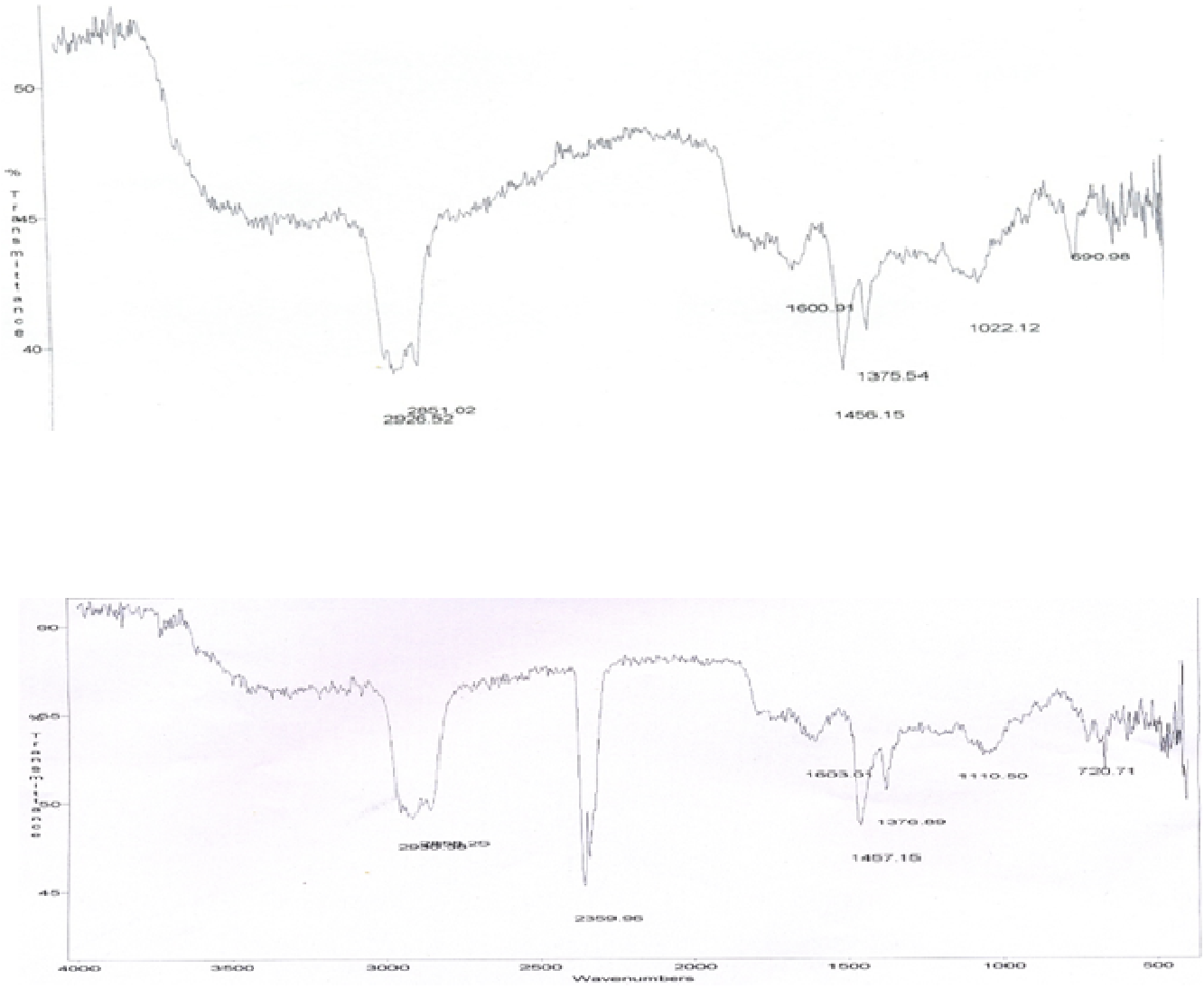
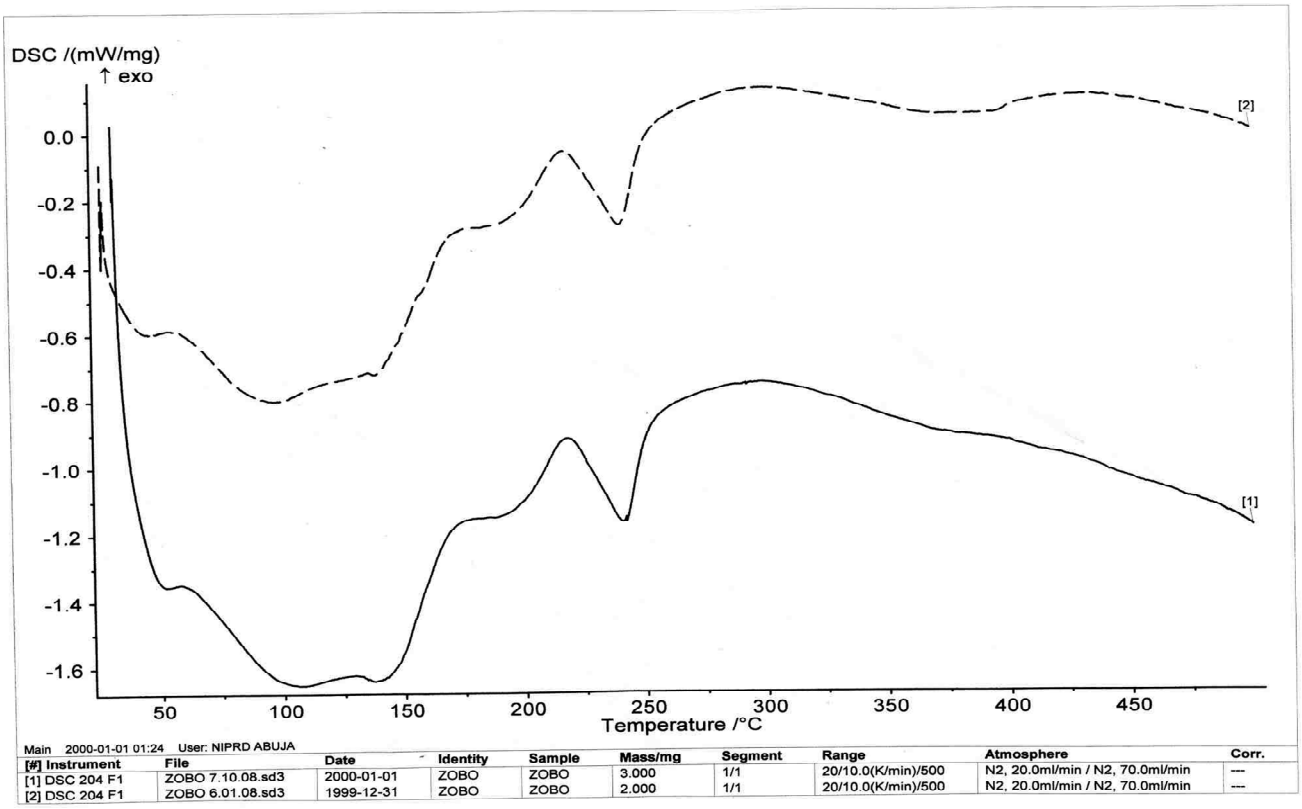


Figure 6. Effect of light on the FT-IR spectra of the freeze-dried extract of calyces.
A – Stored in the dark. B– Stored in light.

sample stored protected from light served as the finger prints to identify the extract. The IR spectrum of the extract powder protected from light was different from that exposed to fluorescent light (Figure 6). The powder of the freshly freeze-dried extract protected from light is characterized by eight strong peaks as shown in spectra 6A. Spectra 6B show the changes resulting from the effect of light on the vibrational motions the molecule that compose the freeze-dried extract. The spectra of the powdered extract exposed to light showed seven prominent shifts.

The phytochemical tests indicate the presence of different compounds. Different bonds and functional groups characterize the molecules of the different compounds. The different peaks in the IR spectra indicate the various vibrations that is indicative of the different bonds in the molecules of the different compounds. There was obvious change in the IR spectra of the freeze-dried extract when exposed to light. The change could result from the loss of a functional group that corresponds to either a C≡C or C≡N triple bonds. There are no changes in peaks within the frequencies less than 1500 cm^{-1}. These correspond to the finger print region, which indicate that light did not affect the characteristic fingerprint of the extract. The changes that occurred due to exposure may be due to photo- induced dissociation leading to rearrangement or isomerization of the triple bonds of the chromophore.



[#] Instrument	File	Date	Identity	Sample	Mass/mg	Segment	Range	Atmosphere	Corr.
[1] DSC 204 F1	ZOBO 7.10.08.sd3	2000-01-01	ZOBO	ZOBO	3.000	1/1	20/10.0(K/min)/500	N2, 20.0ml/min / N2, 70.0ml/min	---
[2] DSC 204 F1	ZOBO 6.01.08.sd3	1999-12-31	ZOBO	ZOBO	2.000	1/1	20/10.0(K/min)/500	N2, 20.0ml/min / N2, 70.0ml/min	---

Figure 7. Thermograms of stored in different light conditions *H.*
(1) Fluorescent light (-----); (2) Darkness(——)

Effect of light on DSC thermogram

Differential scanning calorimetry (DSC) is a versatile technique that is based on heat flow into and out of a material as a function of temperature and/or time. Two characteristic endothermic transitions characterize the thermogram of the freeze-dried extract (Figure 7). The first transition is broad and irregular, characteristic of the complex or impure nature of the extract. The second transition is sharp and endothermic, characteristic of melting of a pure compound. Phytochemical screening of the extract indicates the presence a number of compounds that includes flavonoids, glycosides, sterols, tannins and sugars. The broad and irregular nature of the first endothermic transition may result from the merging of the different endothermic transitions characteristic of many crude plant extracts. The second sharp and endothermic transition is characteristic of the melting of a pure compound whose melting temperature is significantly far from the other compounds contained in the extract. The freeze-dried extract is moderately hygroscopic and contains a large amount of both loosely and tightly bound water. The presence of balsams that have defined glass transitions and water molecules could also contribute to broaden the first endothermic transition.

The DSC thermogram of the powdered freeze-dried samples was used to assess the effect of light on the stability of the powdered extract of *H. sabdariffa*. The empirical differences between the thermographs compared the differences in the stability of the samples in the different storage conditions (Olaniyi, 2000). The DSC thermograms of the freshly freeze-dried extract stored in the dark and under fluorescent light are shown in Figure 7. The thermogram of the dry extract stored in the dark was similar to that obtained with that stored under fluorescent light after seven days. This indicates there was no detectable degradation of the dried powder after exposure to fluorescent light.

Conclusions

Some relevant physicochemical and stability properties of the freeze-dried extract were evaluated. The phytochemical analyses showed the presence of some bioactive agents such as tannins, flavonoides and ascorbic acid which could be responsible for its attributed health benefits. The colour band obtained for the extract solution at different pHs is characteristic of anthocyanins and was highly sensitive to light as shown by UV and IR spectroscopy. However, the changes were not detectable by thermal analysis using the DSC. The granules of the extract formulated with sucrose showed less moisture

uptake and more photo-stability in comparison to the unformulated extract.

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