



POTENCY OF PARTIALLY PURIFIED ANTHOCYANIN FROM LEAF EXTRACT OF *GUIERA SENEGALENSIS* AGAINST CARBON TETRACHLORIDE – INDUCED LIPOPEROXIDATION IN RATS

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ABSTRACT

Anthocyanin was extracted and partially purified from the ethanolic leaf extract of Guiera senegalensis (GS). The recovered anthocyanin extract was found to have a concentration of 8mg/cm³ and an absorption spectrum with peak at 268nm. In order to assess the preventive action of the anthocyanin against lipoperoxidation, serum malondialdehyde (MDA) was analyzed in rats administered with 1mg/kg of the anthocyanin before induction of lipoperoxidation in the rats using carbon tetrachloride (CCl₄). Serum MDA was also analyzed in rats administered with 1 and 2 mg/kg of anthocyanin for up to seven (7) days after induction of lipoperoxidation in the rats in order to determine the curative effect of the extract. The results suggest that anthocyanin extracted from GS leaves are more effective in the cure than prevention of lipoperoxidation, and the most effective daily dose of treatment is 1mg/kg for 7 days.

Key words: *Guiera senegalensis, Anthocyanin, Potency, Lipoperoxidation*

INTRODUCTION

Medicinal plants are believed to be important sources of new chemical substances with potential therapeutic effects (Fansworth, 1987). A number of medicinal plants have been found and put into ethno-medicine by traditional healers in the management of many ailments for many years (Sofowora, 1993). However, the active principle in some of such plants are yet to be phytochemically and pharmacologically identified. This would pave way into finding new drugs of plant origin that could be used in orthodox medicine (Magaji and Yaro, 2006).

Phytochemicals are biologically active substances found in plants in small amounts, which are not established nutrients but which nevertheless seem to contribute significantly to protection against degenerative disease (Ivor, 2002). Phytochemicals are many and some of them include alkaloids, flavonoids tannins, saponins etc. Their functions and mechanism of actions may include the following among others: antioxidant activity, hormonal action, stimulation of enzymes, and interference with DNA replication and anti-bacterial properties.

Anthocyanins are water soluble pigments that may appear red, purple or blue according to pH. They belong to a parent class called flavonoids, which are synthesized via phenyl propanoid pathway. They occur in all tissues of higher plants including leaves, stems, roots, flowers and fruits. Anthocyanins are powerful antioxidants and scientific evidence for their potential health effect against cancer, aging and neurological diseases, inflammation, diabetes and bacterial infections have been provided (Neto, 2007). At the molecular level, anthocyanins from berry were

shown to turn off genes involved with proliferation, apoptosis, inflammation and angiogenesis (Neto, 2007).

Guiera senegalensis (Family: Combretaceae, commonly known as *Sabara* in Hausa) is a shrub of the savannah region of west and central Africa. Its leaves extract is used against dysentery, diarrhea, gastrointestinal pain and disorder, rheumatism and fever (Sule and Mohammed, 2006).

In order to identify the possible phytochemicals responsible for its medicinal activity, anthocyanin fraction was extracted from the ethanolic leaf extract of GS, partially purified and its potency against lipoperoxidation was determined in rats.

MATERIALS AND METHODS

Sample Collection and Preparation

The leaves of *Guiera senegalensis* used for this study were collected from a bush around Sule Tankarkar Local Government Area of Jigawa State. The plant was authenticated at the Department of Biological Sciences, Bayero University, Kano

The leaves were allowed to dry at room temperature and then pulverized into powder. The dried powdered leaves were then macerated in ethanol and allowed to stand overnight. The mixture was then filtered. The extract was then concentrated using rotary evaporator and the ethanol was recovered. The concentrated ethanolic extract was placed on boiling water bath to evaporate any ethanol that was not recovered. The ethanolic extract of *Guiera senegalensis* leaves was kept for the extraction and purification of anthocyanins.

Extraction and Purification of Anthocyanin

The modified method of Takeda *et al.* (1994) was used for the extraction and purification of the anthocyanin.

Fifty grammes (50g) of the ethanolic leaf extract of a *Guiera senegalensis* was dissolved in 170cm³ of a mixture of formic acid, ethanol and distilled water (1:10:9). The mixture was transferred into a separating funnel and washed three times with equal volumes of ethyl acetate to remove flavones. The third volume of the ethylacetate that was added and the extract were mixed thoroughly in the separating funnel and left overnight. The ethyl acetate-free layer containing the partially purified anthocyanin was thereafter obtained.

The ethyl acetate-free extract (5cm³) and 50cm³ of 5% neutral lead acetate solution were mixed and kept at 4°C for 48 hours to ensure complete precipitation of anthocyanin. The reddish – brown supernatant (90cm³) was discarded. The precipitate was resuspended in the remaining supernatant and transferred to test tubes. The content of the test tubes was thereafter centrifuged at 4800rpm for 5 minutes. A reddish – brown supernatant and a dark precipitate were obtained and the supernatant was discarded. Some quantity (5cm³) of 0.50% H₂SO₄ was added to the precipitate to remove lead as sulphate (PbSO₄) and the precipitate was simultaneously resolubilized to give a reddish-brown solution. The mixture was filtered to remove the PbSO₄ and the filtrate was made up to 39cm³ with distilled water. Portion of the filtrate (2cm³) was evaporated to dryness in a pre-weighed beaker to estimate the amount of partially purified anthocyanin obtained; absolute ethanol (1.95cm³) was added to the remaining 37cm³ of the diluted filtrate to obtain a solution of the anthocyanins in 5% aqueous ethanol, and then transferred into a brown bottle to avoid possible effect of sunlight and kept at – 20°C until required.

The solution of the partially purified anthocyanin was analyzed with uv-visible spectrophotometer to obtain its absorption spectrum at the wavelength range of 200 to 600 nm.

$$\frac{\text{Absorbance of test sample}}{\text{Absorbance of standard}} \times \text{Concentration of Standard}$$

Statistical Analysis

Student's t test was used to analyse the results of the study.

RESULTS

The concentration of the recovered partially purified anthocyanin solution was found to be 8gm/cm³. Figure 1 shows the absorption spectrum of the recovered anthocyanin solution, which has a maximum absorption peak at 268nm.

Changes in plasma malondialdehyde (MDA) level induced by CC1₄ 24 hours after administration, and the effect of prior treatment with 1mg/kg partially

Treatment of Rats

White albino rats (Wister strain) with an average weight of 92g, obtained from the Department of Veterinary Medicine, Ahmadu Bell University Zaria, were used for the study. They were divided into seven groups of 4 rats each.

Rats in group I (normal control) were not treated with either anthocyanin or carbon tetrachloride (CC1₄). Group II rats (test control) were treated with CC1₄ alone at a single dose of 100mg/kg of CC1₄ in vegetable oil (1:50 v/v) subcutaneously. Group III rats were pre-treated with anthocyanin 1 mg/kg orally daily for three (3) days and 100mg/kg/CC1₄ was administered subcutaneously at the end of the third day. Group IV rats were pre-treated with a single dose of 100mg/kg CC1₄ and 24 hours later with 1 mg/kg dose of anthocyanin daily for 3 days. Group V rats were pre-treated with a single dose of 100mg/kg CC1₄ and 24 hours later 1mg/kg dose of anthocyanin daily for 7 days. Group VI rats were pre-treated with a single dose of 100mg/kg CC1₄ and 24 hours later 2mg/kg dose of anthocyanin daily for 3 days. Group VII rats were pre-treated with a single dose of 100mg/kg CC1₄ and 24 hours later with 2mg/kg dose of anthocyanin daily for 7 days. All the rats were allowed access to food and water *ad libitum* during the period of the experiment.

The experimental animals were sacrificed and blood samples were collected at the end of each treatment. Blood samples collected were centrifuged at 500rpm for 10 minutes and plasma samples were obtained for malondialdehyde (MDA) determination.

Analysis of Serum MDA

The method adopted for the MDA analysis was that of Hunter *et al.* (1963) as modified by Gutteridge and Wilkins (1982). Blood plasma (0.2cm³) was put in a test tube containing 3cm³ glacial acetic acid and 3cm³ of 1% thiobarbituric acid (TBA) in 2% NaOH. The mixture was placed in boiling water bath for 15 minutes, allowed to cool and the absorbance of the pink colored product and MDA standard (2.5umol/L) was read at 540nm. The plasma level of MDA was calculated using the following:

purified anthocyanin extract and the effect of administering 1mg/kg and 2mg/kg anthocyanin extract 24 hours after CC1₄ administration are presented in Table 1. Significant increase in the level of plasma MDA was observed in the CC1₄ – treated, anthocyanin-free rats (p<0.05) compared to control. Significant (p<0.05) decrease in plasma MDA level in group III rats was also observed due to prior treatment with 1mg/kg anthocyanin extract compared to test control. Significant (p<0.05) decrease in plasma MDA level was also observed in rats treated with both 1 and 2mg/kg anthocyanin extract for 3 and 7 days respectively, compared to test control.

DISCUSSION

In this study, anthocyanin fraction was isolated and partially purified from the ethanolic extract of *Guiera senegalensis* leaves and the concentration of the recovered anthocyanin fraction was found to be 8mg/cm³ which is relatively high compared with reported anthocyanin concentration of 0.37mg/cm³ recovered from red reddish (Ganapathi *et al.*, 2008). Even though the two plants are of different family, this has shown the high content of the phytochemical in GS leaves. The spectrophotometric analysis of the recovered anthocyanin extract showed that it has a maximum absorption peak at 268nm, which is lower than the absorption peak (530nm) of anthocyanin extract from red cabbage (Frederick, 2006). This could be due to the high concentration of the recovered anthocyanin solution. This is in agreement with the report of previous workers that the wavelength of maximum absorption of anthocyanin solutions decreases with increase in concentration (Van Buren *et al.*, 2002).

Plasma malondialdehyde (MDA) level was used as an index of CCl₄ induced oxidative stress. The effect of isolated and partially purified anthocyanin extract recovered from the ethanolic extract of *Guiera senegalensis* leaves was studied to determine both its preventive and curative effect against the CCl₄ induced oxidative stress.

Plasma MDA level was found to be significantly (p<0.05) increased in rats treated with CCl₄ alone when compared with CCl₄-free, extract-free rats. This indicated that the CCl₄ treatment induced lipoperoxidation in the treated rats. Treatment of rats with 1mg/kg partially purified anthocyanin extract for 3 days before CCl₄ treatment caused significant (p<0.05) decrease in plasma MDA level compared with rats treated with CCl₄ alone. This therefore, indicates some level of prevention of lipoperoxidation by the anthocyanin extract in rats treated.

Significant (p<0.05) decrease in plasma MDA level was observed in rats treated with 1 and 2mg/kg for 3 and 7 days respectively, when compared with CCl₄ treated anthocyanin – free rats. Significant (p<0.05) decrease in plasma MDA was also observed in rats treated with 1 mg/kg anthocyanin for 7 days than those rats treated with the same dose for 3

days. Group VI rats were treated with 2mg/kg anthocyanin extract for 3 days and their plasma MDA level was significantly higher (p<0.05) than that of group IV rats, which were treated with 1mg/kg anthocyanin for the same duration. This indicates that anthocyanin treatment is not dose dependent. However, extending the treatment of rats with 2mg/kg for 7 days showed a significant (p<0.05) decrease in plasma MDA level than those rats treated with the same dose for 3 days.

One of the various mechanisms by which carbon tetrachloride induces tissue damage is that a trichloromethyl radical (*CCl₃) is produced from carbon tetrachloride by reductive dechlorination (Brattin *et al.*,1985; Obi and Uneh, 2003), The *CCl₃ radical produced abstracts a hydrogen atom from polyunsaturated fatty acids to form chloroform and a lipid radical. The lipid radical then reacts with molecular oxygen to initiate lipoperoxidation, which is thought to ultimately cause the cytotoxic reponse (Sipes *et al.*, 1977; Recknagel, 1983). The mechanism described here suggests an underlying process of oxidation, and anthocyanins had been reported to possess antioxidant action (Obi and Uneh, 2003). The results obtained from this study, therefore, demonstrates that anthocyanin from ethanolic leaf extract of *Guiera senegalensis* is an excellent antagonist of CCl₄ – induced lipoperoxidation.

CONCLUSION

The results obtained, have shown that *Guiera senegalensis* leaf has high concentration of anthocyanin. In addition, the partially purified anthocyanin extract has displayed both preventive and curative effects against CCl₄-induced liver damage in the treated rats. Therefore, it can be concluded that anthocyanin extracted and partially purified from *Guiera senegalensis* ethanolic leaf extract possess antioxidant property, and that it could both prevent and cure tissue damage that may be caused by toxic chemicals and/or natural processes in the body that may generate free radicals. It can also be concluded that treatment of rats with anthocyanin extract is not dose dependent and that treatment of rats with 1mg/kg dose of anthocyanin for 7 days is the best treatment against chemically induced tissue damage in rats.

Table 1: Plasma malondialdehyde levels in rats treated with carbon tetrachloride (CCl₄) and anthocyanin (AN) extract from *Guiera senegalensis* leaves.

Groups (n =4)	Treatments	MDA level (umoldm ⁻³) Mean ± SD
I	Normal control	0.53 ± 0.006 ^b
II	Test control (Treated with 100mg/kg CCl ₄ only)	0.94 ± 0.013 ^a
III	Pre-treated with 1mg/kg AN for 3 days + 100mg/kg CCl ₄	0.69 ± 0.006 ^{a,b}
IV	Treated with 100mg/kg CCl ₄ + 1mg/kg AN for 3 days	0.84 ± 0.003 ^{a,b}
V	Treated with 100mg/kg CCl ₄ + 1mg/kg AN for 7 days	0.49 ± 0.003 ^{a,b}
VI	Treated with 100mg/kg CCl ₄ + 2 mg/kg AN for 3 days	0.86 ± 0.003 ^{a,b}
VII	Treated with 100mg/kg CCl ₄ + 2 mg/kg AN for 7 days	0.59 ± 0.003 ^{a,b}

n = Number of rats in each group

Values with superscript a are significantly different from normal control (P<0.05)

Values with superscript b are significantly different from test control (P<0.05)

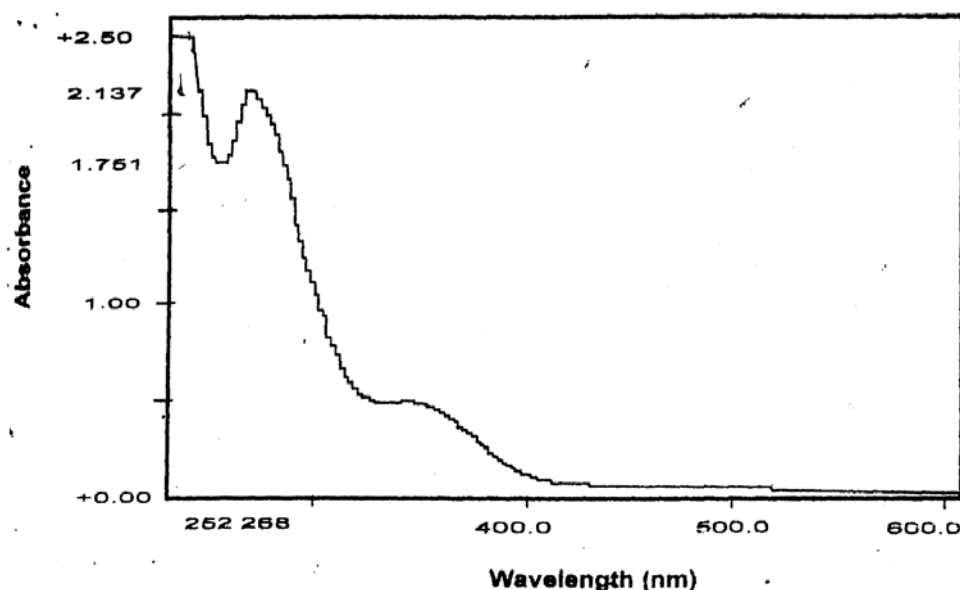


Figure 1: The Absorption of spectrum of partially purified anthocyanin (0.004g/cm³) from *Guiera senegalensis* leaves extract

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