

## Evaluation of the Antioxidant Activity of *Amaranthus spinosus* Linn. by Non-Enzymatic Haemoglycosylation

(Penilaian Aktiviti Antioksidan *Amaranthus spinosus* Linn., dengan Haemoglikosilasi Tanpa Enzim)

B.S. ASHOK KUMAR\*, K. LAKSHMAN, K.N. JAYAVEERA, SALEEMULLA KHAN, B. MANOJ & V.B. NARAYAN SWAMY

### ABSTRACT

*Amaranthus spinosus* Linn. complete plant material was extracted successively by petroleum ether, chloroform, methanol, and water. All the extracts were subjected to in vitro non-enzymatic haemo-glycosylation method, i.e. an oxidation reaction. The degree of haemoglycosylation in the presence of different extracts of *Amaranthus spinosus* were measured colorimetrically at 520 nm. The preventive effect of haemoglobin glycosylation at the two concentration; 0.5 and 1 mg/mL was estimated as follows: pet. ether; 13.1%, 16.4%, chloroform; 5.7%, 12%, methanol: 36.91%, 56.07% and aqueous: 22.2%, 31.01 %, respectively. The  $\alpha$ -tocopheral (Vitamin E) was used as standard.

**Keywords:** *Amaranthus spinosus*; chloroform; haemoglobin; haemoglycosylation; vitamin E

### ABSTRAK

Bahan tumbuhan *Amaranthus spinosus* Linn. diekstrakkan secara berturut-turut dengan eter petroleum, kloroform, metanol dan air. Kesemua ekstrak telah ditindakan secara in vitro dengan kaedah glikosilasi tanpa enzyme ke atas haemoglobin, satu tindak balas pengoksidaan. Darjah haemoglikosilasi dengan kehadiran ekstrak *Amaranthus spinosus* yang berlainan telah diukur secara kolorimetrik pada 520 nm. Kesan pencegahan ke atas glikosilasi haemoglobin pada dua kepekatan, 0.5 dan 1 mg/mL, telah dianggarkan seperti berikut: eter pet. 13.1% dan 16.4%; kloroform 5.7% dan 12%; metanol 36.91% dan 56.07%; dan akuas 22.2% dan 31.01% masing-masing.  $\alpha$ -tokoferal (vitamin E) telah digunakan sebagai pawai.

**Kata kunci:** *Amaranthus spinosus*; haemoglikosilasi; kloroform; haemoglobin; vitamin E

### INTRODUCTION

Haemo glycosylation refers to the covalent bonding of blood glucose to the red blood cells. Normally, only a small percentage of blood glucose, usually between 4.5 and 6%, is covalently linked to the red blood cells in haemoglobin of the non diabetes population. Glycosylated haemoglobin is recommended for cheking blood sugar control in people who might be pre-diabetic and monitoring blood sugar control in patients with more elevated levels, termed diabetes mellitus. For a single blood sample, it provides far more revealing information on glycemic behavior than a fasting blood sugar value. That being said, fasting blood sugar tests are crucial in making treatment decision. The American diabetes association guidelines are similar to others in advising that the glycosylated haemoglobin test performed at least two times a year in patient with diabetes (American Diabetes Association 2007).

*Amaranthus spinosus* Linn. (Amaranthaceae) commonly known as Spiny amaranth or Pig weed, is an annual or perennial herb, native to tropical America and

found throughout India as a weed in cultivated as well as fallow lands (Anon. 1988). Though whole plant is used as laxative (D'ymock 1976; Varier's 1996), the root are regarded as highly specific for colic by Hindu physicians (Sivarajan & Balachandran 1994) and in Madagascar they are considered as laxative (Kirtikar & Basu 1987). Traditionally boiled leaves and roots of *Amaranthus spinosus* are given to children as laxative. However the drug is also used traditionally as diuretic, antidiabetic, antipyretic, anti-snake venom, antileprotic, and anti-gonorrhoeal (Kirtikar & Basu 1987; Varier's 1996). In Malaysia, *Amaranthus spinosus* is used as an expectorant and to relieve breathing in acute bronchitis. Some tribes in India apply *A. spinosus* to induce abortion (Grubben & Denton 2004). The *A. spinosus* is reported for its anti-inflammatory properties (Olumayokun et al. 2004). Effect on hematology (Olufemi et al. 2003), immunomodulatory activity (Tatiya et al. 2007). Antiandrogenic activity (Murgan et al. 1993a), anthelmintic properties (Assiak et al. 2002) and effect on biochemical changes in epididymis (Murgan et al. 1993b).

The main aim of the study was to investigate antioxidant activity of different extracts of *A. spinosus* by *in vitro* non-enzymatic haemo-glycosylation method.

## MATERIALS AND METHODS

### CHEMICALS

Haemoglobin (Nice Chemicals Pvt. Ltd., Cochin), glucose and  $\alpha$ -tocopherol were procured from Merck, Mumbai. Ascorbic acid and gentamycin were obtained from Biokem internationals Pvt. Ltd., Bangalore and Nicholas Piramal India Ltd., Pithampur. All other reagents and solvents used were of analytical grade.

### COLLECTION OF PLANT AND PREPARATION OF EXTRACT

The fresh plant of *A. spinosus* was collected from B. Kotha Kota, Chittor district (AP), India, was authenticated by B.K.Venkatesh, Department of Botany, Government First Grade College, Chickballapur, Karnataka (INDIA). A voucher specimen (SKVCP 11) was deposited in the college herbarium. The leaves were separated from plant, dried and coarsely powdered. The coarse powder (100 g) was successively extracted with solvents (Pet ether, chloroform, methanol and water) by soxhlet apparatus. The different extracts were concentrated to dryness in vacuum.

### PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening of pet ether, chloroform, methanol and water extracts was carried out. Quantification of flavonoids, phenols and tannins were carried out (Kokate 1986).

### ANTIOXIDANT ACTIVITY STUDY BY NON ENZYMATIC HAEMOGLYCOSYLATION

The antioxidant activity of different extracts was investigated by estimating the degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically. Haemoglobin, 60 mg/100mL in 0.01 M phosphate buffer (pH 7.4) was incubated in the presence of 2 g/100 mL concentration of glucose for 72 hours in order to find out the best condition for haemoglobin glycosylation. The assay was performed by adding 1 mL of glucose solution, 1 mL of haemoglobin solution and 1 mL of gentamycin (20 mg/100 mL), in 0.01 M phosphate buffer (pH 7.4). The mixture was incubated in dark at room temperature for 72 hours. The degree of glycosylation of haemoglobin in the presence of different concentration of extracts and their absence, were measured colorimetrically at 520 nm (Yadav et al. 2000).

## RESULTS AND DISCUSSION

### PHYTOCHEMICAL ANALYSIS

Alkaloids, cardiac glycosides, flavonoids, tannins, amino acids, carbohydrates and phenolic compounds were present in preliminary phytochemical screening.

### ANTIOXIDANT ACTIVITY STUDY BY NON ENZYMATIC HAEMOGLYCOSYLATION

Percentage inhibition of haemoglobin glycosylation was measured at two concentrations of all the extracts of *A. spinosus* (0.5 and 1 mg/mL) and results are showed in Table 1. Methanol extract showed more antioxidant activity than petroleum ether, chloroform, and aqueous extract. By phytochemical analysis it was found to contain flavonoids, steroids, terpenoids, saponins, anthraquinone glycosides, volatile oils and amino acids. Flavonoids was found to have antioxidant properties (Asgary et al. 1999). Since glycosylation of protein is an oxidation reaction, antioxidants should be able to prevent this reaction.  $\alpha$ -tocopherol (vitamin E) was used as a standard antioxidant compound.

TABLE 1: The antioxidant activity of different extracts of *A. spinosus linn*

S.No	Samples	Conc. (mg/ml)	% of Scavenging
1	Pet.ether	0.5	13.1±0.2
		1	16.4±0.41
2	Chloroform	0.5	5.7±0.28
		1	12.0±0.32
3	Methanol	0.5	36.19±0.1
		1	56.07±0.2
4	Aqueous	0.5	22.12±0.26
		1	31.01±0.14
5	$\alpha$ -Tocopherol	0.5	61±0.10
		1	86.68±0.16

The activities were compared with those of  $\alpha$ -Tocopherol, values are mean  $\pm$ SEM of three observations

Glycosylated haemoglobin is a form of haemoglobin used primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic pathway by haemoglobin normal exposure to high plasma levels of glucose. Glycation of haemoglobin has been implicated in nephropathy and

retinopathy in diabetes mellitus. Monitoring the Hb<sub>A1C</sub> in diabetic patients may improve treatment. In the normal 120 day life span of the red blood cell, glucose molecules join haemoglobin, forming glycated haemoglobin. In individuals with poorly controlled diabetes, increase in the quantities of these glycated haemoglobins are noted. The antioxidant activity of the extracts was concentration dependent. *Amaranthus spinosus* contains flavonoids like rutin and quercetin (Ashok Kumar et al. 2008). It has been reported that rutin and quercetin showed the inhibition of haemoglycosylation (Asgary et al. 1999) as maximum 42% and 52%, respectively.

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- B.S. Ashok Kumar, B Manoj & V.B. Narayan Swamy  
Department of Pharmacognosy  
Sri K.V.College of Pharmacy  
Chickballapur-562101  
Karnataka, India
- K. Lakshman  
Department of Pharmacognosy  
PES College of Pharmacy  
Bangalore, Karnataka India
- K.N. Jayaveera  
Department of Chemistry  
Jawaharlal Nehru Technological University of College of Engineering  
Anantapur  
Andhra Pradesh, India
- Saleemulla Khan  
Department of Pharmacognosy  
Manipal college of pharmaceutical Sciences  
Manipal, Karnataka, India

\*Corresponding author; email: ashok4vani@gmail.com

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