In vitro Erythrocyte Haemolysis Inhibition Properties of *Senna singueana* Extracts

Mebrahtom Gebrelibanos
Department of Pharmacy, Course and Research Unit of Pharmacognosy, College of Health Sciences, Mekelle University, P.O. Box 1871, Mekelle, Ethiopia (mebrahomgs@yahoo.com)

ABSTRACT

Both leaves and bark of *Senna singueana* (Del.) Lock(Fabaceae) are traditionally used, in some parts of Ethiopia, for the treatment of a form of skin cancer. Also the inner bark of the plant is chewed fresh to soothe stomach spasm and smoke from the wood and bark is used as smoke baths. The objective of this work was to study the antioxidant properties, of this plant using an erythrocyte haemolysis inhibition assay, because one way to justify the traditional anticancer uses can be through the concept of antioxidant effect. Crude extracts from leaves and bark of *S. singueana* were prepared by maceration with 80% methanol. Fractions were prepared from both leaves and bark using solvents of different polarity (diethyl ether, chloroform, and 80% methanol). The 80% methanol fraction was partitioned using ethyl acetate. These crude extracts and solvent fractions were then evaluated for their capacity to inhibit H_2O_2induced erythrocyte haemolysis. Also to evaluate the presence of any intrinsic haemolysis inducing constituents such as saponins, foaming index determination and preliminary haemolysis activity determination tests were performed on extracts from the bark and leaves of the plant. The results revealed that the ethyl acetate solvent fraction from the bark of *S. singueana* exhibited concentration dependent erythrocyte haemolysis inhibitory activity, with an IC_{50} value of 233 μg/ml. However, the other solvent fractions and crude extracts did not show meaningful haemolysis inhibitory activity. The results of the foaming index determination and preliminary haemolysis activity determination tests showed the presence of saponins; but, none of the dilutions of the extracts showed any visible haemolysis activity compared to H_2O_2, used as a control. The results of the ethyl acetate solvent fraction from the bark of the plant could indicate the possible presence of constituents that can inhibit erythrocyte haemolysis, which in turn, could be due to lipid peroxidation inhibitory activity.

Key words: *Senna singueana*, Extracts, Haemolysis Inhibition, Antioxidant.

1. INTRODUCTION

Senna singueana (Del.) Lock (Syn: *Cassia singueana* Del., *Cassia goratensis* Fresen., *Cassia sinqueana* Del., *Cassia zanzibarensis* Vatke) (Fabaceae) is one of the 18 senna species found in Ehiopia. Several species of senna have important medicinal properties and are used in both traditional and modern medicine (Hedberg and Edwards, 1989). *S. singueana* has many medicinal uses throughout Africa (Kawanga, 2007). In some parts of Ethiopia, the leaves as well as the bark of the plant are traditionally used for the treatment of a form of skin cancer locally called 'Minshiro Nekersa' (Abate, 1989). Other applications of the plant, in Ethiopia; is that the inner bark is chewed fresh to soothe stomach spasm and smoke from its wood and bark is used

for purposes of smoke baths to containers of milk and milk products. Scientific reports indicate that the plant has anthelminthic properties (Kawanga, 2007), antiprotozoal activity against cestodes of *Hymenolepis diminuta(*Mølgaard et al., 2001), antiplasmodial, antinociceptive, antipyretic (Adzu et al., 2003), in *vivo* antioxidant and hepatoprotective properties (Ottu et al., 2011), *in vitro*free radical scavenging activity (Gebrelibanos et al., 2007), enzyme inhibition acivities (strong- acetylcholinestrase and carboxylesterase inhibitory activities and weak gluthation-S-transferase and xanthine oxidase inhibitory activities) (Bangou et al., 2011), antiulcer effects(Ode and Asuzu, 2011)and reduce both gastric free-HCl and total acids (Ode and Onakpa, 2010).Hydrochloric acid (HCl) exists in two forms in the gastric juice; as a free HCl and as HCl bound up with acidic proteins and other acidic compounds of the gastric juice. The total acidity of the gastric juice is the sum of the free acidity (the free HCl) and the bonded acidity; and80% methanol extract from leaves of the plant reduced both free and total acids (Ode and Onakpa, 2010). It is also reported that the plant is used as food and fodder. Its leaves, pods and seeds are fed to livestock. As food item, pods are edible raw or cooked, whereas leaves are eaten as vegetable (ICRAF, 2004).For instance, the leaves are eaten as a cooked vegetable in Malawi and Tanzania, but elsewhere they are considered poisonous (Kawanga, 2007). There has been growing interest in natural antioxidants from plant origin due to their promising biological capacities to protect the human body from free radicals, retard the progression of many chronic diseases and lipid oxidative rancidity in foods (Yuana et al., 2005). The plant is reported to contain antraquinones, quinoids, sterols, alkaloids, terpenes, saponins, phenols, tannins (Adzu et al., 2003), flavonoids, glycosides and carbohydrates (Adeyanju et al., 2011). Isolated constituents of this plant include: the anthraquinones - chrysophanol, physcion and 7-methylphyscion; cassiamin A, a dimmer of chrysophanol, (ICRAF, 2011); four tetrahydroanthracene derivatives from the root - torosdchrysone, germichrysone, singueanol-I and singueanol-II (Endo and Naoki, 1980); the pentacyclic triterpene lupeol, and the sterols - campesterol, β-sitosterol and stigmasterol. The leaves contain the flavonoid leucopelargonidin, which has dyeing properties (Kawanga, 2007). Thus, both traditional and scientific report claim indicate that the plant possesses a number of medicinal uses and can be a potential phyto-drug of multiple medicinal values and the objective of this work is to provide scientific evidence related to antioxidant properties of the plant, since it can be a potential source of antioxidant based therapies.

2. MATERIALS AND METHODS

2.1. Solvents and chemicals

Disodium hydrogen orthophosphate anhydrous (Na_2HPO_4), chloroform (TECHNO PHARMACHEM, India), sodium dihydrogen phosphate dihydrate (NaH_2PO_4 .$2H_2O$), sodium chloride (National High Corporation, China), sodium hydroxide, (Rahmsi diagnostics, India), sodium citrate (Blulux Laboratories, India), ethylacetate (Nice Laboratory reagent), hydrogen peroxide (30%)(ABRON chemicals) diethyl ether (Sigma-Aldrich chemicals); ethanol and methanol (BDH Chemicals Ltd, England) have all been used as received.

2.2. Plant material

The leaves and bark of *Senna singueana* (Del.) Lock (Fabaceae) were collected from North-west Tigray, Northern Ethiopia; and the plant material had already been authenticated in the National Herbarium, Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia. The photograph of *S. singueana*which was taken from the Ethiopian flora is shown in figure 1.

Figure 1. Photograph of *Senna singueana* (Del.) Lock (Fabaceae) from the Ethiopian flora.

2.3. Methodology

2.3.1. Extraction and fractionation

2.3.1.1. Extraction

Air-dried and powdered leaves (100 g) and bark (50 g) were extracted by maceration using 80% methanol to prepare crude extracts. The extracts were collected, filtered, concentrated under reduced pressure, and dried in a vacuum oven at a temperature of 35^{o}C. The dried extracts were then transferred into vials and stored at room temperature for further use.

2.3.1.2. Solvent fractionation

Solvent fractions were prepared from air-dried powdered leaves (300 g) and bark (200 g) by maceration with different solvents of increasing polarity starting from diethyl ether, chloroform and then 80% methanol. The 80% methanol fraction was further partitioned using ethyl acetate. The different fractions were concentrated under reduced pressure and dried in a vacuum oven at a temperature not exceeding 35^{o}C. The dried fractions were then transferred into vials and stored at room temperature for further use. The fractionation procedures adopted for the leaves and bark of *S. singueana*are shown in figure 2.

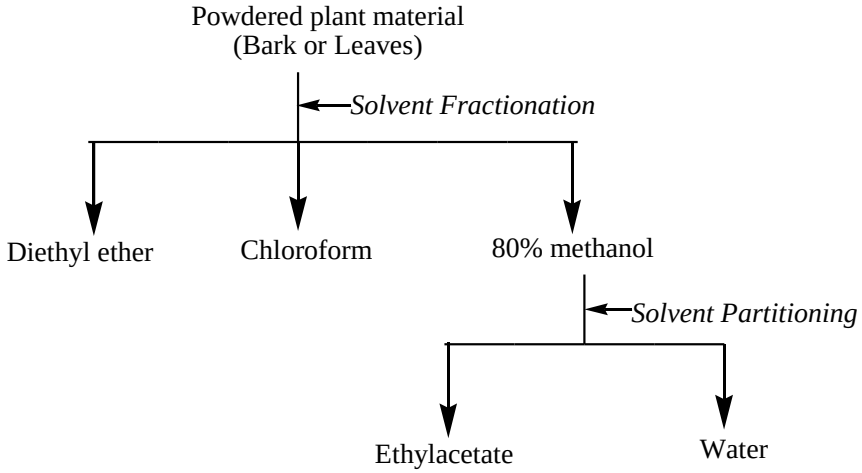

Figure 2. Flow chart followed for solvent fractionation and partitioning of the leaves and bark of *Senna singueana.*

2.3.2. Measuring inhibition of induced lipid per oxidation

Several *in vitro* antioxidant capacity assays that evaluate lipid oxidation have been designed (Chirinos et al., 2008). A number of approaches used to test antioxidants in foods and biological systems consist of: (1) oxidizing a lipid or lipoprotein substrate under standard conditions (Frankel and Meyer, 2000) and (2) assessing the activity by various methods to determine the degree of inhibition of oxidation given by an antioxidant (Frankel and Meyer, 2000; Chirinos et al, 2008). Different models have been employed to detect and understand both the effects of reactive oxidizing species and the activity of natural and synthetic scavengers. Due to their ready accessibility, ease of preparation, abundance of polyunsaturated fatty acids and membrane proteins, and wealth of available information, as well as the high cellular oxygen and hemoglobin concentrations, erythrocytes are excellent model for the study of biomembrane toxicity *in vitro* and have been extensively adopted (Hseu et al., 2008; Singh and Rajini, 2008).

Accordingly, the erythrocyte haemolysis inhibition assay method was adopted in this work, to evaluate H_2O_2 - induced erythrocyte haemolysis inhibition properties of extracts and fractions of *S. singueana*.

2.3.2.1. Preparation of erythrocytes

Blood samples (80 ml) were collected from male goat of weight 65 Kg in citrated container. The blood was collected from the animals while being slaughtered in a restaurant. 10ml of the collected blood samples were centrifuged (CENTRIFUGE, TD3, China) at 30 rpm for 10 minutes and erythrocytes were separated from the plasma and were washed three times by centrifugation (30 rpm, 10 min) with 10 ml of 10 mM phosphate buffer saline (PBS) at pH 7.4 (prepared by mixing 10 mM of NaH_2PO4 and Na_2HPO4, and 125 mM of NaCl in 1 L of distilled water). The supernatant was carefully removed with each wash. Washed erythrocytes were stored at 4^oC and used within 6 h for the haemolysis assay.

2.3.2.2. In vitro inhibition of goat erythrocyte haemolysis assay

The procedure described by Barreira et al. (2008) was adopted with minor modification (eg. the type of haemolysis inducer) to evaluate the inhibition of erythrocyte haemolysis by the different sample extracts and solvent fractions of bark and leaves of *Senna singueana* (Del.) Lock (Fabaceae). The erythrocyte haemolysis was performed with H_2O_2 as free radical initiator. 50 μl of sample extracts and solvent fractions with different concentrations (25–125 μg in PBS, pH 7.4) were added to 100 μl of 10% (v/v) suspension of erythrocytes in PBS. To this, 100 μl of 100 μM H_2O_2 (in PBS, pH7.4) was added. The reaction mixture was incubated at 37^oC for 3 h and was occasionally and gently shaken while being incubated. The reaction mixture was diluted with 8 ml of PBS and centrifuged at 30 rpm for 10 min. The absorbance of the resulting supernatant was measured at 540 nm by spectrophotometer (JENWAY 6305 UV/Vis.) to determine the extent of haemolysis inhibition. Likewise, the erythrocytes were treated with 100 μM H_2O_2 and without inhibitors (sample extracts or solvent fractions) to obtain a complete haemolysis and with 50 μl of inhibitors and without inducer (H_2O_2) to observe if the sample extracts or solvent fractions have any haemolysis effect. The haemolysis caused by 100 μM H_2O_2 was taken as 100% haemolysis; and the percentage haemolysis inhibition was calculated by the equation

$$\textit{\% haemolysis inhibition} = [(A_{H2O2} - A_{Sample}) / A_{H2O2}] \times 100$$

The concentration required for 50% inhibition of haemolysis (IC_{50}) was calculated from the plot of percentage haemolysis inhibition against extract concentration.

2.3.3. Determination of foaming index

The procedure described by WHO (1998) with minor modification (eg. 14cm test tube height was used instead of 16cm), was used to determine the foam index of the aqueous decoctions of the bark and leaves of *Senna singueana* (Del.) Lock (Fabaceae). Powdered bark and leaves of *S. singueana*, each 1 g, were transferred into 250 ml conical flasks containing 100 ml of boiling water; and then boiled at moderate temperature for 30 minutes on a water bath. It was then cooled, filtered and transferred into a 100 ml volumetric flask and the volume adjusted with distilled water. The filtrate of each decoction was poured into stoppered test-tubes (height 14 cm, diameter 1 cm) in successive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml, and the volume of the liquid in each tube adjusted to 10 ml using distilled water. The tubes were stoppered and shaken in a lengthwise motion for 15 seconds, two shakes per second; and allowed to stand for 15 minutes. The height of the foam in each test tube was then measured. The results were assessed as follows: If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100, if a height of foam of 1 cm is measured in any tube, the volume of the plant material decoction in this tube (*a*) is used to determine the index, and if the height of the foam is more than 1 cm in every tube, the foaming index is over 1000. The foaming index was then calculated using the following formula: 1000/a**,** where *a* = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

2.3.4. Determination of haemolysis activity- Preliminary test

The procedure described by WHO (1998) was used to perform preliminary haemolysis activity test on the aqueous decoctions of the bark and leaves of *Senna singueana*. A serial dilution of the bark and leaves of *S. singueana*extracts with phosphate buffer (pH 7.4) and blood suspension (2%) (Prepared by taking 1 ml of citrated blood into a 50 ml volumetric flask containing phosphate buffer pH 7.4 and the volume adjusted carefully) were prepared using four test tubes as shown in the table 1 below. Similarly, dilutions of H_2O_2 (initial concentrations of 0.1M and 1mM were taken) were made using phosphate buffer, pH 7.4.

As soon as the tubes had been prepared, they were gently inverted to mix them, avoiding the formation of foam. The tubes were shaken again after a 30-minute interval and allowed to stand

for 6 hours at room temperature. The tubes were then examined to check for any haemolysis changes and to record the dilution at which total haemolysis has occurred.

Table1. Determination of haemolysis activity: serial dilution for the preliminary test.

Samples	***Tube no.***			
	1	2	3	4
Plant material extract (ml)	0.10	0.20	0.50	1.00
Phosphate buffer pH 7.4 TS (ml)	0.90	0.80	0.50	-
Blood suspension (2%) (ml)	1.00	1.00	1.00	1.00

3. RESULTS

3.1. Extraction and fractionation

The percentage yields (w/w) of the different crude extracts and solvent fractions are summarized in table 2.

Table 2. Percentage yields (w/w) of total extracts and solvent fractions of leaves and bark of *Senna singueana*

Plant part	***Percentage yield (w/w)***			
	Crude extracts	***Fractions***		
		Diethyl ether	***Chloroform***	***80% Methanol***
Bark	20.5	0.82	0.41	15.6
Leaf	19.45	3.31	1.51	13.64

3.2. Measuring inhibition of induced lipid peroxidation

3.2.1. In vitro erythrocyte haemolysis inhibition assay

Results of H_2O_2 - induced erythrocyte haemolysis inhibition properties of *S. singueana*extracts and fractions are summarized in figures 3and 4 below.

3.3. Determination of the foaming index

The foaming index determination test results of the aqueous decoctions of the bark and leaves of *S. singueana* are shown in table 3. The foaming index calculation results indicate that the foam index for the aqueous decoctions of leaves was less than 100 and that of the bark was about 170.

Table 3. Foam index determination results.

		Height of foam of the different volumes									
Volume (ml)		1	2	3	4	5	6	7	8	9	10
Sample	Bark	0.1	0.3	0.5	0.6	0.8	1.2	1.8	2.1	2.5	2.5
	Leaf	0	0.3	0.3	0.5	0.6	0.6	0.6	0.7	0.8	0.8

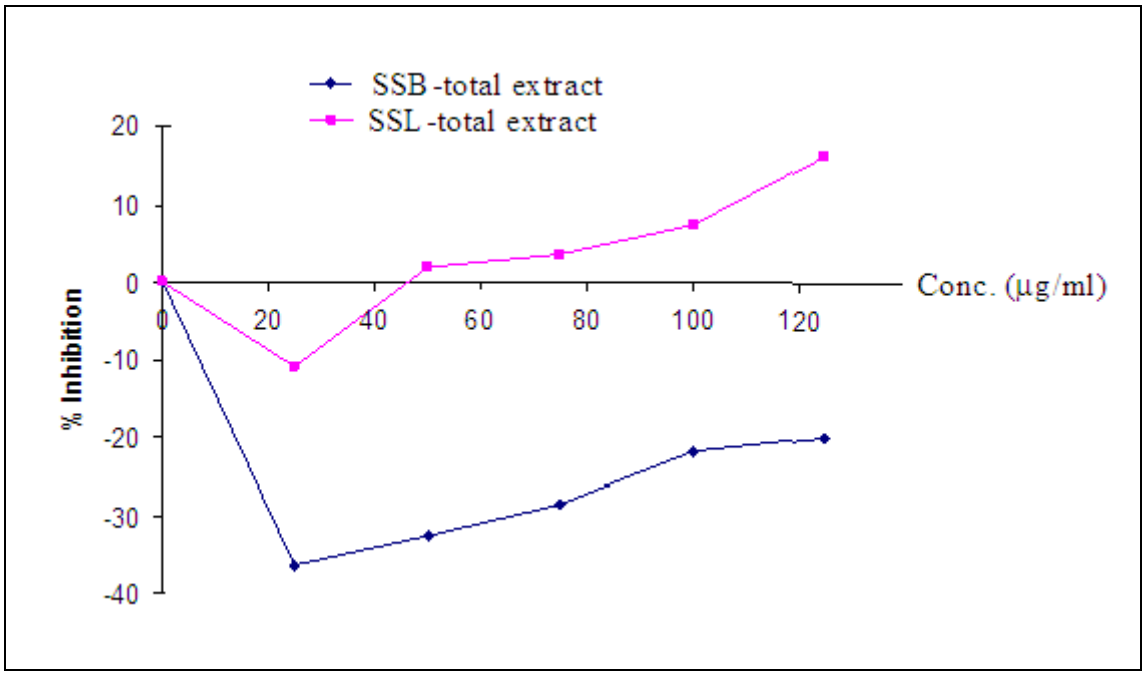

Figure 3. Haemolysis inhibition activities of the 80% methanolic leaf and bark extracts of *Senna singueana* (SSL: leaf extract; SSB: bark extract).

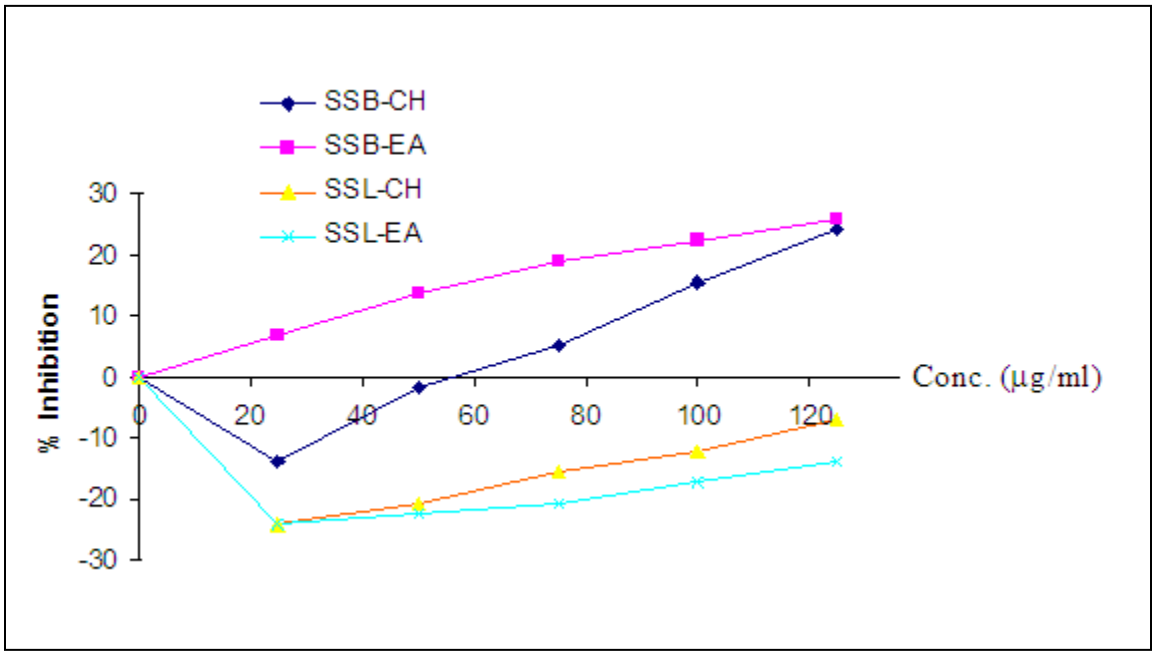

Figure 4. Haemolysis inhibition activities of the various solvent fractions of the bark (SSB-CH: chloroform, SSB-EA: ethyl acetate) and leaf (SSL-CH: chloroform; SSL-EA: ethyl acetate) of *Senna singueana.*

3.4. Determination of haemolysis activity- Preliminary test

None of the dilutions of the extracts showed any visible hemolysis activity while the dilutions of H_2O_2 exhibited significant hemolysis activity; that is the erythrocyte suspension turned to transparent from the initial red color.

4. DISCUSSION

4.1. Extraction and fractionation

As can be seen in table 2, the percentage yield of 80% solvent has highest value, in both the leaves and bark, compared to the diethyl ether and chloroform fractions. As hydroalcoholic solvents generally possess optimum solubility characteristics for initial extraction (Mukherejee, 2002) and since water and alcohol are the most commonly used solvents to prepare traditional formulations (Pootakham, 2005), 80% methanol was used in the preparation of total extracts from the leaves and bark of the plant. Because, the relatively non polar solvent fractions (diethyl ether and chloroform) possessed lower percentage yield than 80% methanol fraction, further partitioning using ethyl acetate was made on the 80% methanol fraction. Of course, previous works indicated that *S. singueana* constituents of intermediate polarity showed radical scavenging activities and that test sample extracts showed higher solubility in organic solvents like methanol and ethyl acetate than in non polar solvents or polar solvents like water (Gebrelibanos et al., 2007). Hence, further fractionation which was carried out to locate the fraction(s) that contain active constituents was made on the 80% methanol fraction using ethyl acetate.

4.2. Measuring inhibition of induced lipid per oxidation

4.2.1. In vitro erythrocyte haemolysis inhibition assay

The assay principle of this experiment is that: (1) hydrogen peroxide, which crosses the red blood cell (RBC) membrane and acts on the intracellular moiety, forms ferryl radical or hydroxyl radical by interacting with hemoglobin and initiates a series of reactions, resulting in RBC lysis (haemolysis) (Blasa, et al, 2007). (2) Haemolysis is then determined by measuring released hemoglobin into the supernatant of the induced samples using a spectrophotometer at 540 nm and represented on the basis of the maximum absorbance (100%) in the aliquots of erythrocytes completely haemolysed (Shiva Shankar Reddy et al., 2007). When measured accordingly, all tested sample extracts and fractions from S. *singueana* exhibited concentration dependent

activity as shown in figures 3 and 4. However, except for SSB-EA, the other test sample extracts partially or fully exhibited percentage inhibition values below zero (control). The IC_{50} value of SSB-EA was calculated to be 233 μg/ml. Since the most characteristic property of saponins is their ability to cause haemolysis (WHO, 1998), and since their presence in S. *singueana* has been reported (Adzu et al., 2003), one can expect that the percentage inhibition values below zero could arise from the haemolysis effect of constituents such as saponins in the different extracts and fractions. Thus, foaming index determination test and preliminary test to determine the haemolysis activity of sample extracts was performed.

4.2.2. Determination of foaming index and haemolysis activity

Despite the presence of saponins, as indicated in the results of the foaming index determination tests, none of the dilutions of the extracts showed any visible haemolysis activity in the preliminary haemolysis activity determination tests when compared with H_2O_2. These results don't support the above expectation that constituents such as saponins, in the test samples, might have induced haemolysis of erythrocytes and hence produced a percentage inhibition less than the control. Such controversy might not be surprising when one considers the complex nature of both test extracts and the reaction mixture of the assay. The expected complex interactions in the reaction mixture, in turn, may render difficulty for definite explanations about these contradicting observations. Of course, the *in vitro* effects of a phytochemical should be appreciated as supporting evidence only, since the *in vivo* effects may be complicated by a plethora of chemical, physical and physiological factors (Issa et al., 2006). In addition, the validity of some of the currently used antioxidant test methods may be questioned because the assay models do not take into account the complexity of antioxidant actions (Frankel and Meyer, 2000). Yet, even though the overall complexity of interactions in the reaction mixture of the assay may render difficulty to make definite explanations, the presence of constituents that can inhibit erythrocyte haemolysis in the plant can be evidenced from the results of the SSB-EA test sample. Indeed, It is reported that the plant contains the sterolscampesterol, β-sitosterol and stigmasterol (Kawanga, 2007), which in turn are reported to demonstrate antioxidant effect against lipid peroxidation (Conforti et al., 2008). In addition, the concentration dependent activity of the total extracts solvent fractions other than SSB-EA could also indicate the presence of constituents that can inhibit erythrocyte haemolysis but are masked by the overall complexity of interactions, and hence their activity is not significant.

5. CONCLUSION

The ethyl acetate solvent fraction from the bark of *S. singueana* exhibited concentration dependent erythrocyte haemolysis inhibitory activity indicating that the plant contains constituents that can inhibit erythrocyte haemolysis. This in turn could possibly be due to inhibition of lipid peroxidation. Also, despite the presence of saponins, no observable induction of haemolysis was observed in any of the tested samples. Thus, further studies are suggested to provide additional evidence that strengthens the claim that the plant can be a potential source of antioxidant based therapies.

6. ACKNOWLEDGMENT

This study was financially supported by the ***NORAD II project***, Mekelle University, College of Health Sciences. Halefom G/yohanes is gratefully acknowledged for his active technical help, while collecting blood samples and in some laboratory activities, my acknowledgement go also to Dagim Ali for his invaluable feedback and comment on this final manuscript.

7. REFERENCES

Abate, G. 1989.*Etse debdabe: Ethiopian traditional medicine*. Department of Biology, Science Faculty, Addis Ababa University, Addis Ababa, 175p.

Adeyanju O., Olutayo, Michael A & Khan IZ. 2011. Preliminary phytochemical and antimicrobial screening of the leaf extract of *Cassia singueana* Del. *African Journal of Pure and Applied Chemistry*, **5(4)**: 65 - 68.

Adzu, B., Abbah, J., Vongtau, H & Gamanie, K. 2003. Studies on the use of *Cassia singueana* in malaria ethnopharmacy. *Journal of Ethnopharmacology*, **88**:261–267.

Bangou, M.J., Kiendrebeogo, M., Compaore, M., Coulibaly, A.Y., Meda N-T.R., Abarca, N.A., Zeba, B., Millogo-Rasolodimby, J & Nacoulma, G. 2011. Enzyme Inhibition Effect and Polyphenolic Content of Medicinal Plant Extracts from Burkina Faso. *Journal of Biological sciences*, **11(1)**: 31-38.

Barreira, J.C.M., Ferreira, I.C.F.R.,OliveiraM. B. P.P & Pereira. J.A. 2008. Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. *Food Chemistry*, **107**: 1106 - 1113.

Blasa, M., Candiracci, M., Accorsi, A., Piacentini, M.P & Piatti,E. 2007. Honey flavonoids as protection agents against oxidative damage to human red blood cells. *Food Chemistry*, **104**: 1635-1640.

Chirinos, R., Campos, D., Warnier, M., Pedreschi, R., Jean-Francois Rees & Larondelle, Y. 2008. Antioxidant properties of mashua (Tropaeolum tuberosum) phenolic extracts against oxidative damage using biological in vitro assays. *Food Chemistry,* **111**: 98-105.

Conforti, F., Sosa, S., Marrelli, M., Menichini, F., Statti, G.A., Uzunov, D., Tubaro, A., Menichini, F & Loggia, R.D. 2008. *In vivo* anti-inflammatory and *in vitro* antioxidant activities of Mediterranean dietary plants. *Journal of Ethnopharmacology*, **116**: 144-151.

Endo, M& Naoki, H. 1980. Antimicrobial and antispasmodic tetrahydroanthracenes from Cassia singueana.*Tetrahedron*, **36(17)**:2449-2452.

Frankel, E.N&Meyer, A.S. 2000.The problems of using one-dimensionalmethods to evaluate multifunctional food andbiological antioxidants. *J Sci Food Agric.*, **80**: 1925-1941.

Gebrelibanos, M, Asres, K & Veeresham, C. 2007. *In Vitro* Radical Scavenging Activity of the Leaf and Bark Extracts of *Senna singueana. Ethiop pharm J.,* **25(2):**77-84.

Hedberg, I & Edwards, S. 1989. Flora of Ethiopian and Eritrea. National Herbarium, Addis Ababa and Uppsala., **3**:57-59.

Hseu, Y.C., Chang, W.H., Chen, C.S., Liao, J.W., Huang, C.J., Lu, F.J., Chia, Y.C., Hseng-Kuang, Hsu., Jia-Jiuan, Wu & Hsin-Ling. Yang. 2008. Antioxidant activities of Toona Sinensis leaves extracts using different antioxidant models. *Food and Chemical Toxicology*, **46**: 105-114

ICRAF. 2011. Species information. World Agroforestry Center document repository.URL:http://www.worldagroforestrycentre.org/SEA/Products/AFDbases/AF/asp/SpeciesInfo.asp?SpID=18035 (accessed on 26-02-2011).

Issa, A.Y., Volatea, S.R & Wargovich, M. J. 2006. The role of phytochemicals in inhibition of cancer and inflammation: New directions and perspectives. *Journal of Food Composition and Analysis,* **19**: 405-419.

Kawanga, V & Bosch, C.H. 2007. Senna singueana (Delile) Lock. [Internet] Record from Protabase. In: G.H. Schmelzer and A. Gurib-Fakim (eds.), PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. < http://database.prota.org/search.htm>. Accessed 26 May 201126 May 2011.

Mølgaard, P., Nielsen, S. B., Rasmussen, D.E., Drummond, R.B., Makaza, N & Andreassen, J. 2001. Anthelmintic screening of Zimbabwean plants traditionally used against schistosomiasis. *Journal of Ethnopharmacology*, **74**: 257-264.

Mukherejee, P.K. 2002. *Quality control of herbal drugs; an approach to evaluation of botanicals.* Business Horizons pharmaceutical publishers, 389p.

Ode, O.J & Asuzu, O.V. 2011. Investigation of *cassia singueana* leaf extract for antiulcer effects using ethanol-induced gastric ulcer model in rats. *International Journal of Plant, Animal and Environmental Sciences*, **1(1)**: 1-7.

Ode, O.J & Onakpa, M.M. 2010. Evaluation of *Cassia singueana* extract on stomach HCl production and gastric emptying in rats. *International Journal of Applied Biology and Pharmaceutical Technology,* **1 (3)**: 1352-58.

Ottu, O.J., Atawodi, S.E & Onyike, E.O. 2011. Assessment of the *In Vivo* Antioxidant and Hepatoprotective Properties of *Cassia singuena* Root Methanolic Extract. Available at:http://www.abu.edu.ng/bsn/pdf/Biochemistry.pdf orwww.searchuu.com.

Pootakham, K. 2005. Bioactivity directed extraction, fractionation and isolation ofbioactive natural products from medicinal and aromaticplants; ICS-UNIDOdocument repository URL: http://www.ics.trieste.it/Documents/Downloads/df3186.pdf(accessed on 28-05-2011).

Shiva Shankar Reddy, C.S., Subramanyam, M.V.V., Vani, R & Asha Devi, S. 2007. In vitro models of oxidative stress in rat erythrocytes: Effect of antioxidant supplements. *Toxicology in Vitro*, **21**: 1355-1364.

Singh, N & Rajini, P.S. 2008. Antioxidant-mediated protective effect of potato peel extract in erythrocytes against oxidative damage. *Chemico-Biological Interactions*, **173**: 97-104.

WHO.1998. *Quality Control Methods for Medicinal Plant Materials*. World Health Organization, Geneva, 46p.

Yuana, X., Wanga, J., Yaoa, H & Chen, F. 2005. Free radical-scavenging capacity and inhibitory activity on rat erythrocyte hemolysis of feruloyl oligosaccharides from wheat bran insoluble dietary fiber. *LWT-Food Science and Technology*, **38**: 877-883.