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Comparative spectral analysis of the effect of ethanolic fruit, leaf and root extract of *Solanum macrocarpon* on normal human haemoglobin

Eleazar Chukwuemeka Anorue[1*], Chiemekam Samuel Ezechukwu[2] and Edmund Chidiebere Mbegbu[3]
[1]Medical Unit, Department of Biochemistry, University of Nigeria Nsukka, Enugu State, Nigeria
[2]Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria
[3]Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria
eleazar.anorue.02464@unn.edu.ng

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Abstract

Solanum macrocarpon a plant of the family Solanaceae has been suggested to contain some pharmacological properties that could be useful in the treatment and management of several diseases. However, certain studies have reported the toxic effect of different parts of this plant thereby raising concern about its use in phyto-therapy. Sequel to this, this study was conducted to investigate the effect of different parts of this plant on normal human haemoglobin by spectrophotometry, so as to ascertain the safety of this plant as a potential therapeutic agent. Ten groups consisting of the control and test groups (100 µl, 200 µl and 300 µl of 25 mg/ml fruit, leaf and root extract of Solanum macrocarpon respectively) were used for the study. It was found that the fruit and the root did not cause any toxic effect on haemoglobin whereas the leaf caused significant dose-dependent oxidation of the human haemoglobin especially at higher doses. Therefore, caution should be taken in the use of the leaf extract of Solanum macrocarpon as a therapeutic agent.

Keywords: Haemoglobin, oxidation, phytotherapy, solanum macrocarpon.

Introduction

Solanum macrocarpon is of the family of Solanaceae and originated from West Africa but now with over 1000 species distributed in other parts of the continent[1]. Phytochemical analyses of fruits and leaves of *S. macrocarpon* showed high concentrations of phenols, flavonoids and alkaloids[2-5]. Several studies have corroborated the interesting biological and medicinal properties of *S. macrocarpon*, which have been largely attributed to their phytochemical constituents. However, there is paucity of information on the potentials of the root extract as most of the studies on the plant have focused on the fruit and leaves. Traditionally, the root of *S. macrocarpon* is believed to be used in the treatment of gastro-esophageal reflux disease, constipation and dyspepsia[6]. *S. macrocarpon* fruit has been associated with laxative, anthelminthic, hypotensive, antioxidant, hepatoprotective and hypolipidemic properties[7-11]. Komlaga *et al.*[8] suggested that the leaves could be used for the management ofthroat problems, Bukenya and Bonsu[12] reported that the leaves could also be used to treat stomach problems. Emeka and Joyce[13] in their study concluded that *Solanum macrocarpon* leaf- supplemented diets may help in the treatment and management of benign prostatic hyperplasia. Despite the numerous reported beneficial uses of the plant, there are doubts about the safety of other parts of the plants, with the fear that they may be toxic to human health especially at high doses[14,15].

Since *Solanum macrocarpon* might contain some phytochemical constituent that may be toxic to human health as suggested by several literatures[14,15], there is tendency for these phyto-toxins to oxidize the haemoglobin in the red blood cells as they are been carried to their different target organ[16-19].

Therefore, this study investigated the effect of fruit, leaf and root extract of *S. macrocarpon* on normal human haemoglobin by spectral analysis, so as to ascertain its safety for phytotherapy.

Materials and methods

Plant Material: The plant was grown and harvested from the agricultural farm of the University of Nigeria, Nsukka. The plant was identified by Mr. A. Ozioko at the Bioresources Development and Conservation Program (BDCP) Research Centre, Nsukka, Enugu State, Nigeria.

Blood sample: After the collection of ethical clearance from the Ethical board of faculty of biological science, University of Nigeria, blood sample was collected from a human volunteer (a non-smoking male student of the University of Nigeria, Nsukka aged 27 years), who was confirmed to be of genotype Hb AA at Renaissance Hospital, Nsukka, Nigeria. 5ml of whole blood was collected by venipunture into a Venoject tube containing ethylene diamine tetra acetic acid (EDTA) as an anticoagulant and kept at 4˚C until use.

Chemicals and reagents: Sephadex G-150 (Thomas Scientific, New Jersey, U.S.A), Sodium Chloride (Panhong Chemical Company, China), Methylated Spirit (Coral Health Care, Hyderbad, India), Potassium Phosphate Salts (Panhong

Chemical Company, China), Sodium Hydroxide (Panhong Chemical Company, China), Concentrated Hydrochloric acid (Panhong Chemical Company, China), Distilled Water (Energy Centre, University of Nigeria, Nsukka).

Preparation of Extracts: The fruits, leaves and roots were washed, cut into pieces for easy drying at room temperature. After drying the plant materials were ground into fine powder and used to prepare the extracts. A total of 250g each of the powdered fruit, leaf and root samples was soaked in 70% ethanol (500 ml) for 48 hours, stirring at intervals. Afterwards, the mixtures were filtered using a muslin cloth. The filtrates were subsequently evaporated to dryness at 40^{o}C in a hot air oven to obtain the extract, which was then stored at 4^{o}C in a refrigerator prior to use[20]. During use, 1.5g of the crude extract were reconstituted in 60ml of distilled water to obtain a concentration of 25 mg/ml.

Preparation of normal saline: In a clean 250ml beaker, 1g of NaCl was dissolved in 70ml of distilled water[21, 22]. After mixing to homogeneity, the mixture was then made up to 100 ml with distilled water taking caution while topping up in order to avoid error due to parallax. The resultant solution was then transferred to a clean reagent bottle and labeled.

Preparation of potassium phosphate buffer: 10mM potassium phosphate buffer (pH 7.6) was used for this research. The buffer was prepared using Henderson- Hassel Balch equation[23].

Preparation of haemoglobin: Haemoglobin (Hb) was prepared according to the method given by Meng F. and Alayash, A. I.[24]. Briefly, 5ml of the whole blood sample was washed thrice with normal saline and spun at 3000 rpm for 30 minutes using a Legend X1R centrifuge (LABSCO, Germany). After centrifugation, the supernatant was aspirated and the pellet was diluted 3 folds with distilled water, rocked gently at room temperature for 15 minutes was and kept in an ice bath for 3 hours. The lysate was then centrifuged at 5,000 rpm for 50 minutes and was loaded onto a well packed Sephadex G-150 Fast-flow column (Bed dimension 3×40cm, 300 ml), which was equilibrated with 3 column volume 10mM potassium phosphate buffer of pH 7.6. Hb AA was eluted at 4°C with a linear gradient of 25–100% of the buffer in 2 column volumes. The column was eluted at a flow rate of 2.5ml/min and the effluent was monitored at 541, 576 and 630nm. Hb AA was collected and was stored at −18°C for future use.

Spectroscopy: For spectrophotometry, stock solution of the haemoglobin and buffer were mixed in the ratio 3:1 with different pipette for sample and reference. Ultraviolet-visible (UV visible) Spectrophotometer (Jenway, United Kingdom) was used for the analysis.

Experimental design: Group 1: 800µl of Oxyhaemoglobin (Normal Control).
Group 2: 100 µl of 25 mg/ml *Solanum macrocarpon* fruit + 800 µl Oxyhaemoglobin
Group 3: 200 µl of 25 mg/ml *Solanum macrocarpon* fruit + 800 µl Oxyhemoglobin
Group 4: 300 µl of 25 mg/ml *Solanum macrocarpon* fruit + 800 µl Oxyhemoglobin
Group 5: 100 µl of 25 mg/ml *Solanum macrocarpon* leaf + 800 µl Oxyhemoglobin
Group 6: 200 µl of 25 mg/ml *Solanum macrocarpon* leaf + 800 µl Oxyhemoglobin
Group 7: 300 µl of 25 mg/ml *Solanum macrocarpon* leaf + 800 µl Oxyhemoglobin
Group 8: 100 µl of 25 mg/ml *Solanum macrocarpon* root + 800 µl Oxyhaemoglobin
Group 9: 200 µl of 25 mg/ml *Solanum macrocarpon* root + 800 µl Oxyhaemoglobin
Group 10: 300 µl of 25 mg/ml *Solanum macrocarpon* root + 800 µl Oxyhaemoglobin

Results and discussion

Characterization of the absorption spectrum of unreacted haemoglobin revealed two distinct absorbance maxima (namely β and α band) at 541nm and 576nm with the absorbance values of 0.262 and 0.249 respectively (Figure-1). With this as a baseline, the effect of ethanolic fruit, leaf and root extract of *Solanum macrocarpon* on normal human oxyhaemoglobin were determined. The absorption spectrum of 100 µlof 25 mg/ml *Solanum macrocarpon* fruit showed absorbance values of 0.273 and 0.289 at the absorbance maxima of oxyhaemaeglobin (576 nm and 541nm) respectively compared to the unreacted haemoglobin; that of 200 µl of 25 mg/ml *Solanum macrocarpon* fruit showed absorbance values 0.284 and 0.300 at the absorbance maxima of oxyhaemaeglobin (576nm and 541nm) respectively compared to the unreacted haemoglobin whereas the absorption spectrum of 300µl of 25 mg/ml *Solanum macrocarpon* fruit showed absorbance values of 0.273 and 0.289 at the absorbance maxima of oxyhaemaeglobin (576 nm and 541nm) respectively compared to the unreacted haemoglobin (Figure-2-4). It has been shown that the magnitude of reduction in the absorbance maxima of oxyhaemoglobin is proportional to the degree of it oxidation and reduction in concentration[25,26]. This finding reveals that the different concentrations of *Solanum macrocarpon* fruit used did not cause any oxidative effect on human oxyhaemoglobin neither did it reduced oxyhaemoglobin concentration rather the increase in the absorbance maxiama as indicated by the increased absorbance values is informative that the fruit extract may aid in improving oxyhaemoglobin concentration[26,27].

Furthermore, it may suggest that *Solanum macrocarpon* fruit do not possess any oxidative properties or reductive effect on human oxyhaemoglobin concentration and therefore has no inimical effect on human haemoglobin. Although no spectral analysis on the effect of *Solanum macrocarpon* fruit extract on haemoglobin has been done, this finding is consistent with the

work of other researchers who have studied on the biochemical effects of *Solanum macrocarpon* fruit extracts on haematological parameters. For instance, Mbegbu et al.[4] reported that *Solanum macrocarpon* fruit extract did not have any adverse effects on haemoglobin of experimental rats who were administered the fruit extract for 3 weeks but rather there was a noticeable increase in the haemoglobin concentration on the third week of the treatment. Sodipo et al.[16,17] also reported that the aqueous fruit extract did not cause reduction in haemoglobin concentration of triton-induced hyperlipidaemic rats but rather significantly increased it. Similarly, Duru et al.[27] did not observed any reduction in the haemoglobin of rats on adding powdered *S. macrocarpon* fruit into their feed. Haemoglobin is the oxygen carry protein in most vertebrates, reduction in the concentration of haemoglobin causes low oxygen delivery to the tissues which leads to tissue hypoxia and in serious cases death[4]. Also, low concentration of haemoglobin in the blood causes anaemia[4]. The non-reductive spectral effect of *Solanum macrocarpon* fruits extract on human haemoglobin concentration suggests that the fruits extract cannot impair the oxygen delivery ability of the protein neither can it induce anaemic conditions in man[4]. Possibly, the non-oxidative spectra effect of *Solanum macrocarpon* fruits extract on human haemoglobin could be due to its high possession of antioxidants properties such as phenols, flavonoid, saponins and ascorbic acids[6,28].

On the other hand**,** the absorption spectrum of 100µl of 25mg/ml *Solanum macrocarpon* leaf extract as shown in Figure-5 shows a loss of the 576nm and 541nm absorption peaks as described by the absence of the arrows at 576nm and 541nm and absorbance values of 0.245 and 0.262 at the two characteristic absorbance maxima of oxyhaemoglobin respectively compared to the unreacted haemoglobin. This signifies onset of oxidation of the oxyhaemoglobin[26]. Also absorption spectrum of 200µl and 300µl of 25mg/ml *Solanum macrocarpon* leaf extract showed a total loss of the two characteristic absorbance maxima of oxyhaemoglobin (Figure-6 and 7).

The total loss of the two characteristic absorbance maxima of oxyhaemoglobin as seen in Figure-6 and 7 signifies significant oxidation of oxyhaemoglobin, importantly a close observation of the absorption spectra of 200 µland 300µl of 25 mg/ml *Solanum macrocarpon* leaf extract shows that the oxidation of 300µl of 25 mg/ml *Solanum macrocarpon* leaf extract is spectra-wise more significant than that of 200µl of 25 mg/ml *Solanum macrocarpon* leaf extract. The result reveals that *Solanum macrocarpon* leaf extract possesses phytochemical properties that could oxidatively damage human haemoglobin, interestingly, this oxidative potentials of *Solanum macrocarpon* leaf extract is dose-dependent as can be revealed by the spectra differences in the three concentrations of *Solanum macrocarpon* leaf extract used. The severity of the oxidative damage to the oxyhaemoglobin worsens with increased concentration of the leaf extract. This corroborates the report of Oboh *et al.*[14] who reported adverse haemolytic properties of the leaf extract especially at high doses. Also, Owolabi *et al.*[15] reported that the leaf extract had dose-dependent deleterious effect on brain tissues of experimental rats with specific effects such as alteration of cerebral histoarchitecture and alteration of the morphologies of the cells at high doses. Contrarily, the toxicity of the leaf extract especially at high doses does not corroborate the work of other researchers who reported it as having anti-oxidant potentials[29]. The potential toxicity of *Solanum macrocarpon* leaf could be due to very high level of phytochemical oxidants like glycoalkaloids[15]. Oxidation of haemoglobin has been shown to produce free radicals which could be deleterious to the cells ranging from heme breakdown, release of the heme iron, haemolysis, formation of Heinz bodies, loss of membrane integrity, and other degenerative changes in the erythrocyte[30,31]. More so, excess production of free radicals has been implicated in other pathophysiologic conditions in the body such as diabetes, neurodegeneratives diseases, kidney diseases, cardiovascular diseases and the likes[31]. Therefore, caution should be taken in the use of *Solanum macrocarpon* leaf in folklore or ethno medicine.

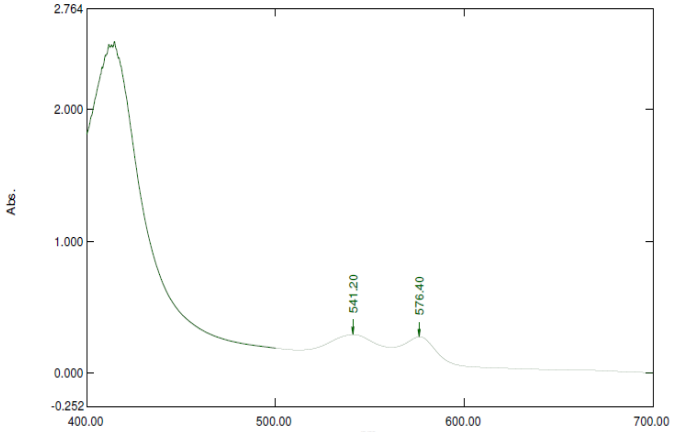

Figure-1: Absorption spectral of 800µl of oxyhaemoglobin (16 mM) in 10mM phosphate buffer, pH 7.6 after 3 hours incubation at 25˚C.

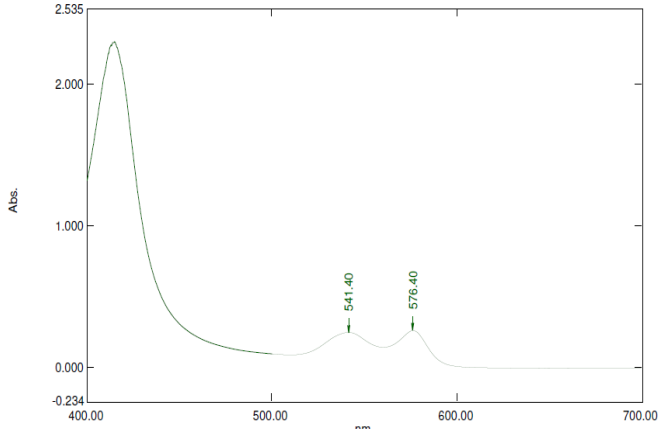

Figure-2: Spectral changes after reacting 800µl of oxyhaemoglobin (16mM) in 10mM phosphate buffer, pH 7.6 with 100µl of 25mg/ml *Solanum macrocarpon* fruit for 3 hour at 25˚C, compared to unreacted oxyhemoglobin.

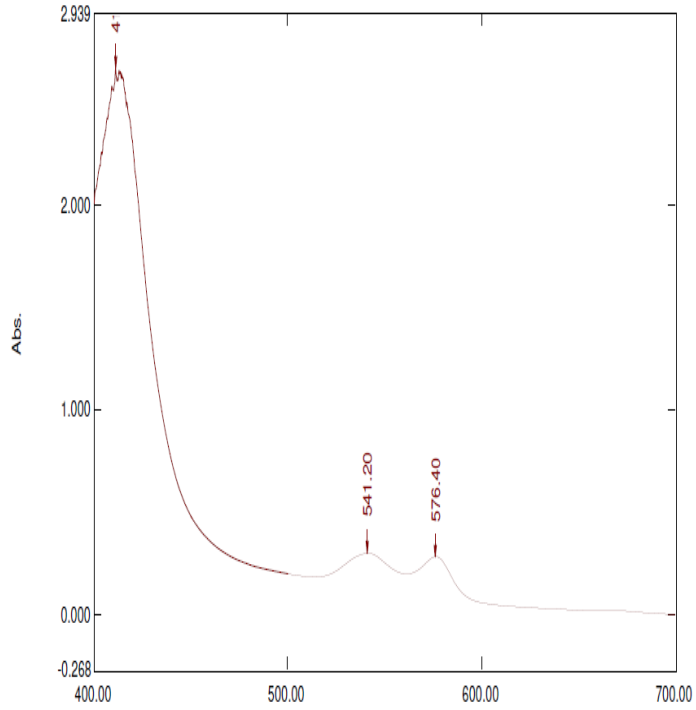

Figure-3: Spectral changes after reacting 800µl of oxyhaemoglobin (16mM) in 10mM phosphate buffer, pH 7.6 with 200µl of 25mg/ml *Solanum macrocarpon* for 3 hours at 25˚C, compared to unreacted oxyhemoglobin.

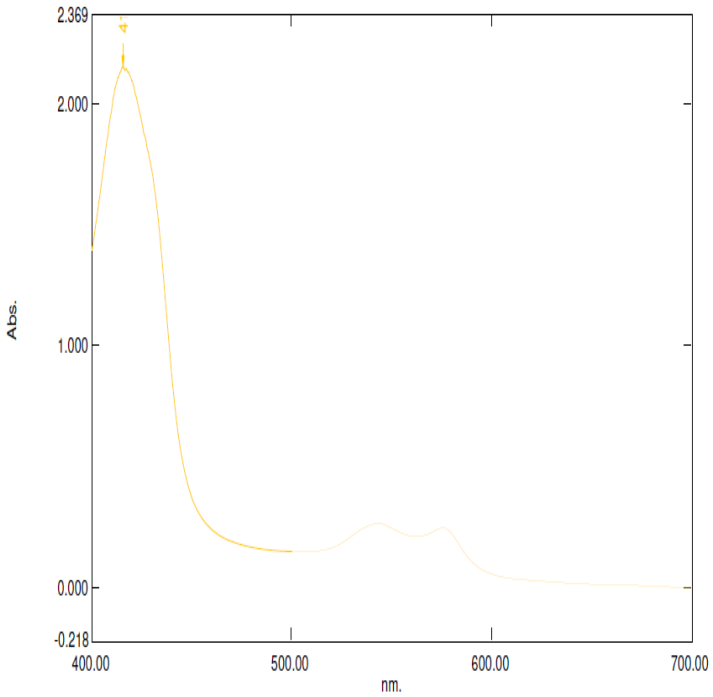

Figure-5: Spectral changes after reacting 800µl of oxyhaemoglobin (16mM) in 10mM phosphate buffer, pH 7.6 with 100µl of 25mg/ml *Solanum macrocarpon* leaf for 3 hours at 25˚C, compared to unreacted oxyhemoglobin.

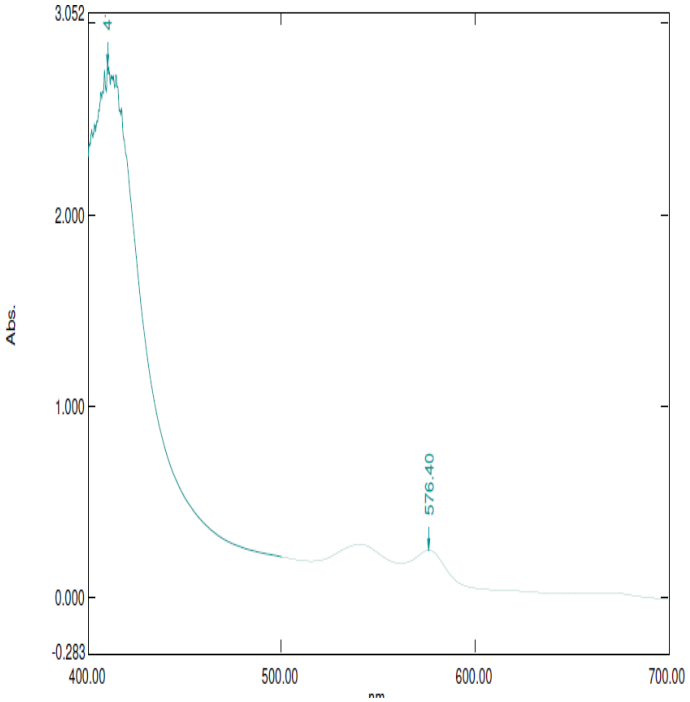

Figure-4: Spectral changes after reacting 800µl of oxyhaemoglobin (16mM) in 10mM phosphate buffer, pH 7.6 with 300µl of 25mg/ml *Solanum macrocarpon* fruit for 3 hours at 25˚C, compared to unreacted oxyhemoglobin.

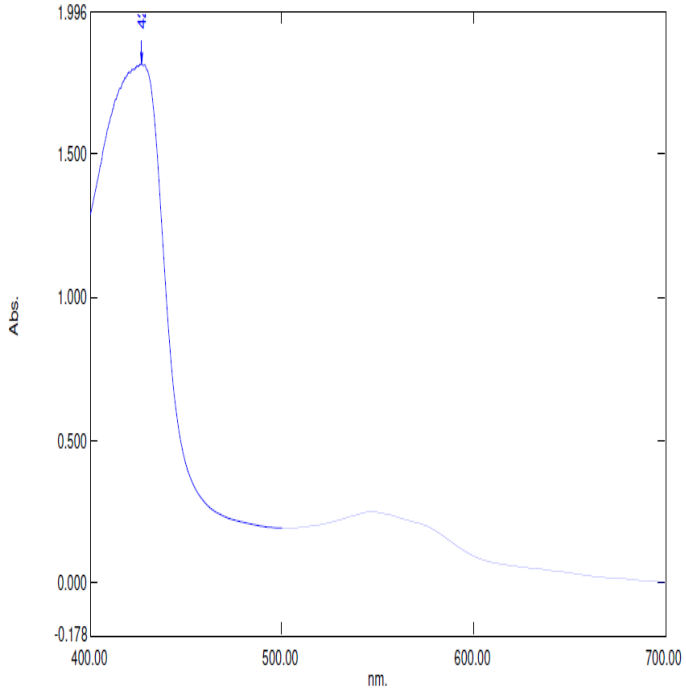

Figure-6: Spectral changes after reacting 800µl of oxyhaemoglobin (16mM) in 10mM phosphate buffer, pH 7.6 with 200µl of 25mg/ml *Solanum macrocarpon* leaf for 3 hours at 25˚C, compared to unreacted oxyhaemoglobin.

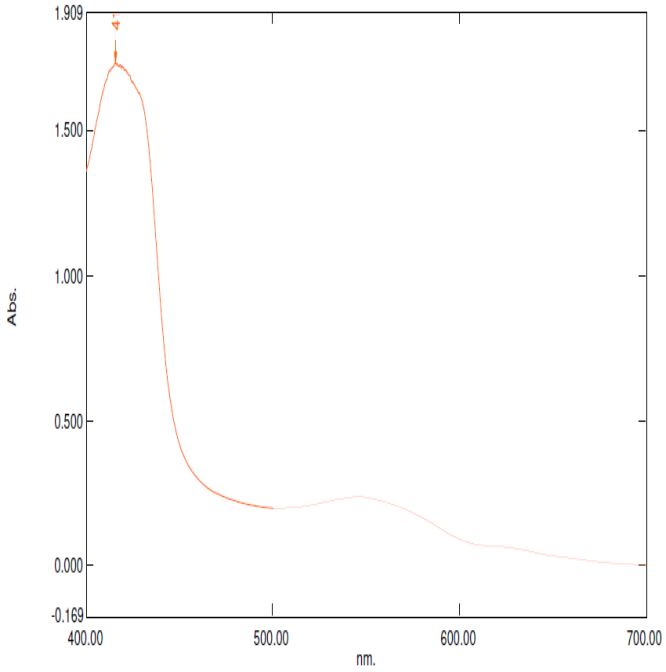

Figure-7: Spectral changes after reacting 800µl of oxyhaemoglobin (16mM) in 10mM phosphate buffer, pH 7.6 with 300µl of 25mg/ml *Solanum macrocarpon* leaf for 3 hours at 25˚C, compared to unreacted oxyhaemoglobin

Absorption spectrum of 100µl of 25mg/ml *Solanum macrocarpon* root extract shows absorbance values of 0.295 and 0.296 at the absorbance maxima of oxyhaemaeglobin (576 nm and 541nm) respectively compared to the unreacted haemoglobin; that of 200µl of 25mg/ml *Solanum macrocarpon* root extract shows absorbance values 0.302 and 0.307 at the absorbance maxima of oxyhaemaeglobin (576nm and 541nm) respectively compared to the unreacted haemoglobin where as the absorption spectrum of 300µl of 25mg/ml *Solanum macrocarpon* root extract showed absorbance values of 0.302 and 0.327 at the absorbance maxima of oxyhaemaeglobin (576 nm and 541nm) respectively compared to the unreacted haemoglobin (Figure-8-10). The result shows that *Solanum macrocarpon* root did not cause any oxidative changes on the haemoglobin neither did it reduce haemoglobin concentration as indicated by the absorbance values of the characteristic peaks of oxyhaemoglobin instead there was a dose-dependent rise in the absorbance values of the absorbance maxima of oxyhaemoglobin suggesting that the root extracts might possess properties that may aid in the increase of oxyhaemoglobin concentrations. Also, the finding suggests that *Solanum macrocarpon* root extract do not possess oxidative properties that could cause oxidative damages to human haemoglobin. Presently, there is paucity of reports on the roots extract of *Solanum macrocarpon.* But the finding of this study gives credence to its use in ethno medicine[6], therefore more studies should be done to know the potential properties of the *Solanum macrocarpon* root in order to know the best way to harness its properties for the benefit of man.

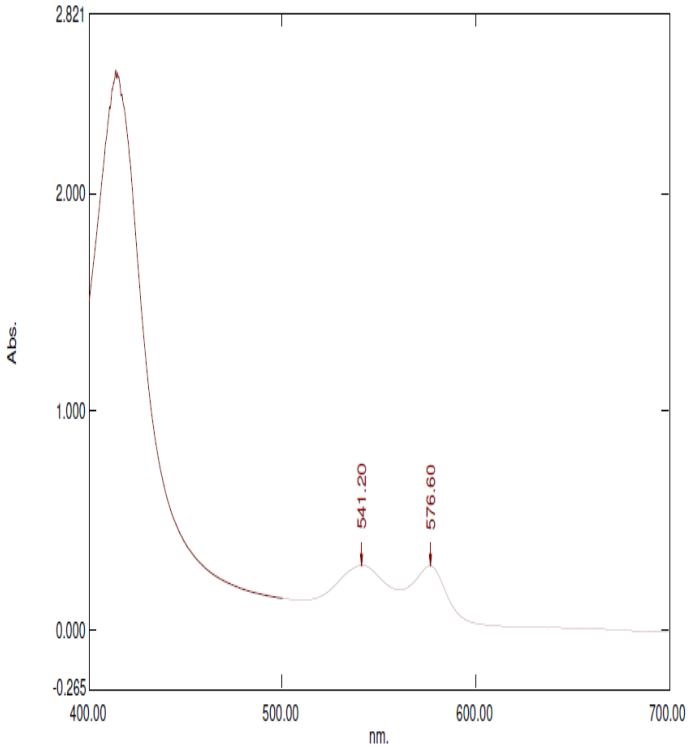

Figure-8: Spectral changes after reacting 800µl of oxyhaemoglobin (16mM) in 10mM phosphate buffer, pH 7.6 with 100µl of 25mg/ml *Solanum macrocarpon* root for 3 hours at 25˚C, compared to unreacted oxyhaemoglobin

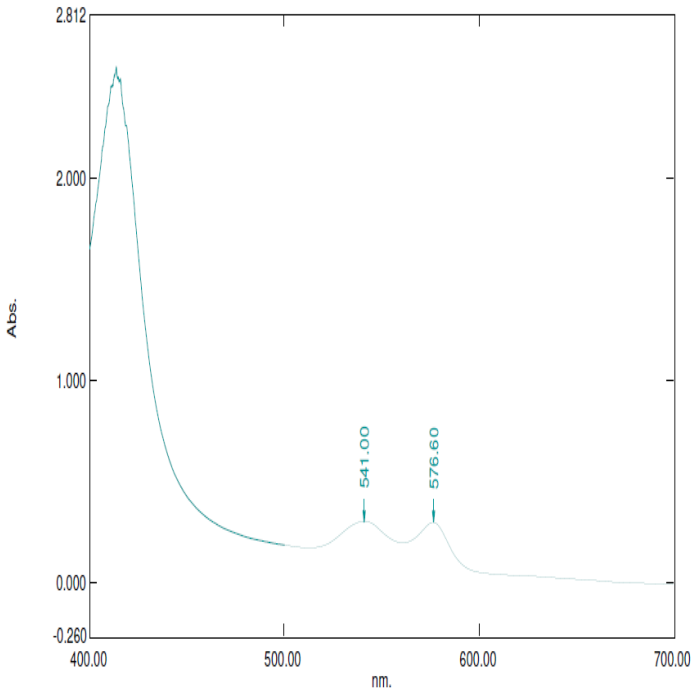

Figure-9: Spectral changes after reacting 800µl of oxyhaemoglobin (16 mM) in 10mM phosphate buffer, pH 7.6 with 200µl of 25mg/ml of *Solanum macrocarpon* root for 3 hours at 25˚C, compared to unreacted oxyhaemoglobin.

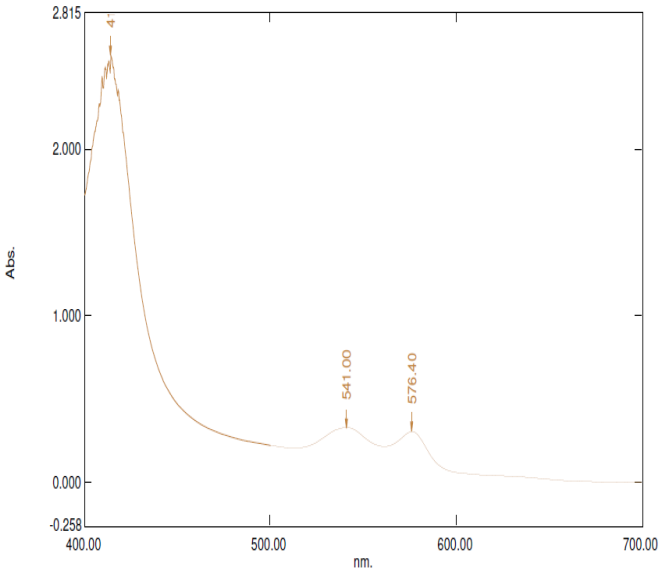

Figure-10: Spectral changes after reacting 800µl of oxyhaemoglobin (16mM) in 10mM phosphate buffer, pH 7.6 with 300µl of 25mg/ml of *Solanum macrocarpon* root for 3 hours at 25˚C, compared to unreacted oxyhaemoglobin

Conclusion

The comparative spectral analysis of the effect of fruit, leaf and root extract *Solanum macrocarpon* on human haemoglobin showed that the fruit *and* root extract of *Solanum macrocarpon* did not oxidize normal human haemoglobin whereas the leaf extract of *Solanum macrocarpon* caused dose-dependent oxidative damages to normal human haemoglobin, hence raising concern about its use in ethno-medicine.

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