HEMOLYSIS OF EQUINE ERYTHROCYTES FROM EXPOSURE TO LEAF EXTRACTS OBTAINED FROM VARIOUS BEGONIA L. SPECIES

Halyna Tkachenko¹
Lyudmyla Buyun²
Marlena Witaszek¹
Paweł Pażontka-Lipiński¹
Zbigniew Osadowski¹

¹Department of Zoology and Animal Physiology
Institute of Biology and Environmental Protection,
Pomeranian University in Słupsk, Poland
Arciszewskiego Str. 22B, 76-200 Słupsk, Poland
e-mail: tkachenko@apsl.edu.pl, biology.apsl@gmail.com

²M. Gryshko National Botanical Garden,
National Academy of Science of Ukraine, Kyiv, Ukraine

ABSTRACT

The main goal of current study aimed at assessment of percentage haemolysis of equine erythrocytes induced by treatment with extracts of various species from Begonia genus was determined to exemplify their further potential development and use as drug against metabolic diseases in medicine and veterinary. The leaves of Begonia plants, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanical Garden (NBG), National Academy of Science of Ukraine. The leaves of thirty plant species, i.e. Begonia foliosa Kunth, B. psilophylla Irmsch., B. convolvulacea (Klotzsch) A.DC., B. ulmifolia Willd., B. rex Putz., B. thiemei C.DC., B. manicata Brongn., B. solimutata L.B.Sm. & Washsh., B. arborescens var. oxyphylla (A.DC.) S.F.Sm., B. mexicana G. Karst. ex Fotsch, Begonia × credneri F.Haage & E.Schmidt, B. sanguinea Raddi, B. olbia Kerch., B. goegoensis N.E.Br., B. imperialis var. smaragdina Lem., B. epipsila Brade, B. pustulata Liebm., B. heracleifolia var. nigricans Hook.f., Begonia × erythrophylla Hérincq, B. aconitifolia A.DC., B. peltata Otto & Dietr., B. nelumbiifolia Cham. & Schldrl., B. subvillosa Klotzsch, B. oxyphylla A.DC., B. masoniana Irmsch. ex Ziesenh, B. cucullata Willd., B. angularis Raddi, B. glabra Aubl., B. boisiana Gagnep., B. venosa Skan ex Hook.f.
were sampled for study. Our study demonstrated that among 30 species of *Begonia* genus, the most species of plants investigated possessed anti-hemolitic activity. The results of these biological assays demonstrated that compounds present in *B. glabra*, *B. aconitifolia*, *B. sanguinea*, *B. thiemei*, *B. masoniana*, *B. × credneri*, *B. oxyphylla*, *B. subvillosa*, *B. ulmifolia*, *B. conconvulaceae* can cause the prevention of methemoglobin formation and reduce hemolysis, while *B. erythrophylla*, *B. psilophylla* and *B. arborescens var. oxyphylla* extracts can induce formation of methemoglobin and hemolysis in healthy equine blood. Extracts from leaves of *B. foliosa*, *B. rex*, *B. solimutata*, *B. mexicana*, *B. goegoensis*, *B. imperialis var. smaragdina*, *B. pustulata*, *B. peltata*, *B. cucullata*, *B. angularis*, *B. boissiana*, *B. venosa* exhibited the decrease of percentage hemolysis of equine erythrocytes, but these alterations were non-significant. The extensive use of plants from this genus by the local people in treating various types of diseases and disorders might therefore be justified by their antioxidant activities against oxidative stress and hemolysis, which are known to be responsible for causing various metabolic states and diseases. The results also indicate that scientific studies carried out on plants of *Begonia* genus having traditional claims of effectiveness might warrant fruitful results in medicine and veterinary practice.

**Key words:** *Begonia*, *leaves*, *extracts*, *antioxidant activity*, *equine erythrocytes*, *hemolysis*

*Begonia* is now considered to be one of the five largest genera of vascular plants with approximately 1500 species currently recognized (Frodin 2004, Hoover et al. 2004).

The genus has a near pantropical distribution being absent only from the Australian tropical forest. There are centers of diversity in both the Neotropics and mainland Asia but the genus is relatively species poor in Africa. The African species, despite being relatively small in number show the greatest morphological diversity (Neal et al. 2006). The recent phylogenetic studies of *Begoniaceae* have indicate that the most basal *Begonia* species are African, from which both Asian and American *Begonia* species are derived (Forrest and Hollingsworth 2003, Harrison et al. 2016). Species radiation in the South America, South-East Asia and Africa, has generated many examples of parallel evolution, for example in leaf form, plant architecture, inflorescence arrangement and drought tolerance, reproductive systems, including dichogamy (Neale et al. 2006, Twyford et al. 2014).

Many begonias have been in cultivation for at least 600 years (Neal et al. 2006). Its leaves, flowers, and roots are used in diverse ailments in traditional and folklore remedies. For instance, *Begonia floccifera* is used to increase the body vigor, to increase the body weight and fleeting pain in the limbs and joints. The juice of the fresh leaves is given to the young babies for proper development of teeth and bones. It also arrests the gum and teeth diseases. The juice of the leaves mixed with honey is taken in as a tonic and they believe that is having the rejuvenation capacity (Ariharan et al. 2012). The juice of *B. malabarica* Lamk is used along with honey for blood purification. It is given in for fever to reduce the body temperature and it is taken as a general health tonic. The leaf juice mixed with ginger is taken for treating
anemia (Ariharan et al. 2012). The leaves are used for the treatment of respiratory infections, diarrhea, blood cancer and skin diseases (Nisha et al. 2009). The juice of *B. picta* is taken to relieve headache and also consumed in treatment of peptic ulcer. The paste is applied to stop bleeding from cuts and wound and is applied externally on ringworm and scabies. The root juice is used as eyes wash to treat conjunctivitis. The whole plant is feed to sterile animals to help them conceive. Whole plant is used as appetizer and juice of leaves is given to relieve the fever. Plant decoction is used in colic and dyspepsia. Paste of young shoot also taken for respiratory tract infections. *B. picta* also called patherchattha in India is used in dysentery and mouth ulcer. Juice of *B. picta* was used as slight venom. Other species of *Begonia*, i.e. *B. nepalensis* is used as anthelmintic in Nepal (Nisha Shrestha et al. 2016).

Moreover, the leaves of *Begonia* species are used for the treatment of cancer and possess the anti-HIV activity (Wu et al. 2004). Some of the plants of the *Begonia* genus were previously reported for their antimicrobial activities (Ramesh et al. 2002; Maridass 2009; Dan-Ping et al. 2012, Jeeva et al. 2012; Indrakumar et al. 2014; Amutha, Sreedevikumari 2016; Nisha Shrestha et al. 2016).

In our previous study (Tkachenko et al. 2016, Buyun et al. 2017), we have assessed the anti-*Escherichia coli* activity of the ethanolic extracts from the leaves of *Begonia* species, i.e. *B. solimutata* L.B. Sm. & Wassh., *B. goegoensis* N.E.Br., *B. foliosa* Kunth, *Begonia × bunchii* L.H. Bailey (syn. *Begonia × erythropylla* Hérincq), *B. thiemei* C.DC., *B. peltata* Otto & Dietr., *B. heracleifolia* Cham. & Schltdl., *B. dregei* Otto & Dietr., *B. mexicana* G. Karst. ex Fotsch. In our study, ethanolic extracts obtained from leaves of *Begonia* species had average activity against *E. coli*. The inhibition zone diameter for *B. solimutata* was 14 mm, 11.5 mm for *B. goegoensis*, 13 mm for *B. foliosa*, 13.5 mm for *Begonia × bunchii*, 15 mm for *B. thiemei*, 19 mm for *B. peltata*, 12 mm for *B. heracleifolia*, 11.5 mm for *B. dregei*, and 16 mm for *B. mexicana*. The highest antimicrobial effect was recorded for *B. peltata*, *B. mexicana*, and *B. thiemei*. The most antimicrobially effective plant against *E. coli* was *B. peltata*, being highly active with the ethanolic extract (inhibition zone diameter 19 mm). The obtained results highlighted the interesting antimicrobial potency of various *Begonia* species and provided scientific basis for the traditional use of these plants in the treatment of microbial infections (Tkachenko et al. 2016; Buyun et al. 2017). Moreover, the highly active antimicrobial effects of various *Begonia* species against *Candida albicans* and *Pseudomonas aeruginosa* isolates are worthy of highlighting (Buyun et al. 2016; Tkachenko et al. 2017).

Though many model systems are frequently used to study the biochemical alterations under the condition of oxidative stress including the tissues from various parts of the body, erythrocytes, as the most common type of blood cells, get superiority amongst them (Pandey, Rizvi, 2010). Red blood cell along with its membrane has always been an important medium for the study due to the important role it plays in varied physiological and metabolic processes (Karabulut et al. 2009).

Equine erythrocytes are more sensitive to oxidant-induced damage due to the use of inefficient mechanisms to correct and protect against oxidative damage, i.e. methemoglobin formation, alteration of aggregation, and reduction of cellular deformability (Baskurt, Meiselman 1999). Therefore, the high susceptibility of equine erythrocytes to oxidant damage, and the resulting hemorheologic alterations, may
have important consequences for tissue perfusion and cardiovascular adequacy in horses (Baskurt, Meiselman 1999; Walter et al. 2014). Oxidants typically damage erythrocytes by oxidizing the heme iron in hemoglobin, reactive sulphydryls, or unsaturated lipids in the membranes. The oxidation of the heme iron in hemoglobin to the ferric (Fe\(^{3+}\)) state generates methemoglobin, which is incapable of transporting oxygen. Methemoglobin can be enzymatically reduced back to the functional ferrous (Fe\(^{2+}\)) state, primarily by nicotinamide adenine dinucleotide (NADH)-dependent methemoglobin reductase (Wright et al. 1999; Walter et al. 2014). In our numerous studies, oxidative stress biomarkers, as well as osmotic, peroxide and acid resistance of erythrocytes were used as informative indices for assessment of exercise-induced alterations and physiological state of well-trained athletes and horses involved in recreational horseback ridings (Andriichuk et al. 2012-2016; Andriichuk, Tkachenko 2017; Tkachenko et al. 2016; Pażontka-Lipińska et al. 2016, 2017). Exercise has variable effects on the hematological parameters, depending on exercise duration and intensity (short-term high intensity or maximal exercise and long-term low intensity or submaximal prolonged exercise), fitness and training levels and environmental conditions (Andriichuk et al. 2012-2016; Andriichuk, Tkachenko 2017; Tkachenko et al. 2016; Pażontka-Lipińska et al. 2016, 2017).

Most active plants are toxic at high doses and it is therefore important to investigate the preliminary toxicity of plant extracts. Toxicological study is essential in order to determine dosage of the plant extracts which will not be lethal to the body when administered (Chitemerere, Mukanganyama 2014). Extracts of various plants can induced methemoglobin formation and resulted in hemolysis. The \textit{in vitro} assays used in many studies (Boyer et al. 2002; Walter et al. 2014) provide a useful diagnostic method for the rapid identification of oxidizing agents from different sources. Therefore, in the present study, the percentage haemolysis of equine erythrocytes induced by treatment with extracts of various species from \textit{Begonia} genus are determined to exemplify their further potential development and use as drug against metabolic diseases in medicine and veterinary. Our current scientific project undertaken in the frameship of cooperation programme between Institute of Biology and Environmental Protection (Pomeranian University in Słupsk, Poland) and M.M. Gryshko National Botanical Gardens of National Academy of Sciences of Ukraine, directed to assessment of medicinal properties of tropical plants has encompassed some tropical mega-diverse genera, including genus \textit{Begonia} with a near pantropical distribution.

**MATERIALS AND METHODS**


**Preparation of Plant Extracts.** Freshly leaves were washed, weighted, crushed, and homogenized in 0.1M sterile phosphate buffer saline solution (pH 7.4) (in proportion 1:19, w/w) at room temperature.

**Horses.** Eighteen healthy adult horses from central Pomeranian region in Poland (village Strzelinko, N54°30'48.0" E16°57'44.9"), aged 8.9±1.3 years old, including 6 Hucul pony, 5 Thoroughbred horses, 2 Anglo-Arabian horses and 5 horses of unknown breed, were used in this study. All horses participated in recreational horseback riding. Horses were housed in individual boxes, with feeding (hay and oat) provided twice a day, at 08.00 and 18.00 h, and water available *ad libitum*. All horses were thoroughly examined clinically and screened for hematological, biochemical and vital parameters, which were within reference ranges. The females were non-pregnant.

**Collection of blood samples.** Blood was drawn from jugular veins of the animals in the morning, 90 minutes after feeding, while the horses were in the stables (between 8:30 and 10 AM). Blood was stored into tubes with sodium citrate and held on the ice until centrifugation at 3000 rpm for 5 min. The plasma was removed. Pellet of blood was washed three times in sterile 4 mM phosphate buffer (pH 7.4). Erythrocytes aliquots were used in study.

**Anti-hemolytic activity assay.** To evaluate the extracts’ potential to cause hemolysis in equine erythrocytes, a hemolysis assay based on the spectro-photometric measurement of hemoglobin in the supernatant was performed. Anti-hemolytic activity was assessed by following the spectrophotometric method (Kamyshnikov 2004). The pellet of blood was re-suspended in sterile 4 mM phosphate buffer (pH 7.4). A volume of 0.1 ml of the various extracts were added to 1.9 ml of clean equine erythrocytes. After incubation the mixture at 37°C for 60 min with continuous stirring, it was centrifuged at 3000 rpm for 5 min. The assay mixture contained 0.25 mL of erythrocytes suspension and 4.5 mL 4 mM phosphate buffer (pH 7.4). The mixture was heated at 37°C for 15 min. The absorbance of the obtained solution was measured at 540 nm. The mixture was centrifuged at 3,000·rpm for 10 min. Absorbance of mixture contained erythrocytes and distilled water was determined as 100% (blank). The degree of hemolysis in every test tubes (%) was calculated in accordance to the absorbance of the blank. For positive and negative control (distilled water and phosphate buffer saline, respectively) were used. Percent hemolysis was determined relative to the absorbance measured in the supernatant of erythrocytes treated with distilled water, which should cause complete hemolysis.

**Statistical analysis.** Statistical analysis of the data obtained was performed by employing mean ± standard error of the mean (S.E.M.). All variables were tested for normal distribution using the Kolmogorov-Smirnov test (p > 0.05). In order to find significant differences (significance level, p < 0.05) between groups, Kruskal-Wallis test by ranks was applied to the data (Zar 1999). All statistical analyses were performed using STATISTICA 8.0 software (StatSoft, Poland).
RESULTS

The hemolysis assay demonstrated that the extracts of *B. erythrophylla* induced the greatest percent hemolysis of the equine erythrocytes, 122%, respectively, than the *B. psilophylla* and *B. arborescens* var. *oxyphylla* extracts, 78% and 56.5%, respectively, compared to phosphate buffer saline control. The extracts of *B. masoniana*, *B. × credneri*, *B. oxyphylla*, *B. subvillosa*, *B. ulmifolia*, *B. conconvulaceae* exhibited the inverse pattern, and induced hemolysis at approximately half the extent of the phosphate buffer saline control (36.7%, 35.9%, 32.4%, 30.4%, 29.6%, 25.7%, respectively). The most potent effect was demonstrated by the *B. glabra*, *B. aconitifolia*, *B. sanguinea*, and *B. thiemei* extracts, which caused decrease of erythrocytes’ hemolysis compared to phosphate buffer saline control (48.3%, 43%, 41.3%, 39.8%, respectively). The extracts of the *B. glabra* caused minimal hemolysis, significantly reduced from the phosphate buffer saline control (Fig. 1). Extracts from leaves of *B. foliosa*, *B. rex*, *B. solimutata*, *B. mexicana*, *B. goegoensis*, *B. imperialis* var. *smaragdina*, *B. pustulata*, *B. peltata*, *B. cucullata*, *B. angularis*, *B. boisiana*, *B. venosa* exhibited the decrease of percentage hemolysis of equine erythrocytes, but these alterations were non-significant (Fig. 1).

![Graph showing percentage of hemolysis for various species of Begonia genus compared to control (ultrapure water)](image)

Fig. 1. Percentage hemolysis of equine erythrocytes induced by treatment with extracts of various species from *Begonia* genus with compared to treatment with ultrapure water (control). The data was analyzed using one-way analysis on ranks (ANOVA) using Kruskal-Wallis test by ranks. P value < 0.05 was considered as significant (n = 18).
The results of these biological assays demonstrated that compounds present in *B. glabra*, *B. aconitifolia*, *B. sanguinea*, *B. thiemei*, *B. masoniana*, *B. × credneri*, *B. oxyphylla*, *B. subvillosa*, *B. ulmifolia*, *B. conconvulacea* can cause the prevention of formation of methemoglobin and reduce of hemolysis, while *B. erythrophylla*, *B. psilophylla* and *B. arborescens* var. *oxyphylla* extracts can facilitate formation of methemoglobin and hemolysis in healthy equine blood.

**DISCUSSION**

As antioxidant therapy is gaining importance in the treatment of several metabolic disorders, where free radicals are implicated, scientifically developmental programs aimed at investigating the medicinal properties of plants for their potent antioxidant properties are relevant and perspective (Auddy et al. 2013; Eshwarappa et al. 2015). In these lines, the antioxidant potential of aqueous extracts obtained from leaves of various *Begonia* species was evaluated by determining of percent of hemolyzed equine erythrocytes after incubation with plant extracts. In this screening, *B. convolvulacea, B. ulmifolia, B. thiemei, B. × credneri, B. sanguinea, B. aconitifolia, B. subvillosa, B. oxyphylla, B. masoniana, B. glabra* leaf extracts possessed significant antioxidant activity to reduce of erythrocytes' hemolysis (fig. 1).

Phytochemical constituents in *Begonia* species are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal, and anticancer (Suresh, Nagarajan 2009). All secondary metabolite components displayed antioxidant and antimicrobial properties through different biological mechanisms (Hossain, Nagooru 2011). Variation in the chemical profile of extracts could influence their biological activities. Therefore, it was important to know the chemical composition of extracts to correlate with their antioxidant activities. A study conducted by Kalpanadevi and Mohan (2012) has shown that the extracts of *B. malabarica* and *B. floccifera* contain higher quantities of phenolic compounds, which exhibit antioxidant and free radical scavenging activity. *In vitro* assay systems confirm *B. malabarica* and *B. floccifera* whole plants as natural antioxidants. The phenolics and flavonoids could be the reason for its antioxidant activity. The preliminary phytochemical studies revealed the presence of flavone, sterol, triterpene in hexane, chloroform and methanol extracts; phenol in chloroform and methanol extracts of *B. malabarica* and quinone, saponin, tannin and starch in methanol extract. All the extracts did not answer for alkaloid (Ramesh et al. 2002). Preliminary phytochemical screening of *B. floccifera* and *B. malabarica* conducted by Ariharan and co-workers (2012) showed the presence of a number of bioactive constituents, e.g. vitamin C. The antimicrobial activity could be due to the presence of this phytoconstituent. The contents of flavonoids (including glycosides of quercetin and kaempferol), anthocyanins and ascorbic acid in overground part of plants of 7 species and cultivars of *Begonia* genus (*B. Bahiensis* A.DC., *B. Bowerae* Ziesenh., *B. Carolineifolia* Regel, *B. Fischeri* Schrank, *B. Heracleifolia* Cham. & Schltdl., *B. ‘Erythrophylla’, B. ‘Helen Teupel’*) were determined by Karpova and co-workers (2009). The contents of flavonoids were 24-650 mg% of dry weight, including glycosides of quercetin – 3-76 mg%. Kaempferol glycosides was detected only in species of section
Gireoudia (1.2-5.7 mg%). The contents of anthocyanins were between 60 and 157 mg%, ascorbic acid – 5-43 mg% of fresh weight. Studied plants of Begonia can be considered as the sources of biologically active compounds with antioxidant and antimicrobial activity (Karpova et al. 2009). The results of the phytochemical screening of the methanolic flower extracts of B. floccifera revealed the presence of phenol, tannins, xanthoproteins, steroids, tannins, phytoestersols, triterpenoids, sapogenins, coumarins and carbohydrates (Jeeva, Marimuthu Antonisamy 2012).

Friedelin, epi-friedelinol, β-sitosterol, luteolin, quercetin and β-sitosterol-3-β-d-glucopyranoside were reported from the leaves of B. malabarica. Begonanline, nantoinamide, and methyl-(S)-glycerate were isolated from B. nantoensis. Cucurbitacin B, E, I and dihydrocucurbitacin isolated from B. nantoensis were reported to have cytotoxicity in cancer cell lines. Three new compounds begonanline, nantoinamide and methyl (S)-glycerate, as well as forty-four known compounds have been isolated and characterized from the rhizomes of B. nantoensis by Wu and co-workers (2004). Among them, cucurbitacin B, dihydrocucurbitacin B, cucurbitacin E, dihydrocucurbitacin E, cucurbitacin I and (-)-auranamide showed cytotoxicity against four human cancer cell lines. 3β,22α-Dihydroxyolean-12-en-29-oic acid, indole-3-carboxylic acid, 5,7-dihydroxychromone, and (-)-catechin demonstrated significant activity against HIV replication in H9 lymphocyte cells (Wu et al. 2004). The methanol and chloroform extracts of B. malabarica were shown to have antibacterial activity against Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Vibrio parahaemolyticus, and Chromobacterium violaceum (Ramesh et al. 2002). Pandikumar and co-workers (2009) supported the use of B. malabarica for the treatment of diabetes. Fractionation of this extract may yield novel prototypes to manage diabetes mellitus.

The antioxidant activities of various plant extracts are often explained by their total phenolic and flavonoid contents. Results of Sultana and co-workers (2007) showed that the methanol extract possessed significant activity in releasing most of the secondary metabolites from leaves of Begonia species. This may be due to the fact that phenolic and flavonoid compounds are often extracted in higher amounts by using polar solvents (Sultana et al. 2007). Phenolic antioxidants are products of secondary metabolism in plants, and their antioxidant activity is mainly due to their redox properties and chemical structure, which can play an important role in chelating transitional metals and scavenging reactive oxygen species (ROS). Similarly, the mechanisms of action of flavonoids are also through scavenging or chelating processes (Eshwarappa et al. 2015).

Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, anti-inflammatory, and vasodilating actions (Pietta 2000; Rice-Evans 2001; Benavente-Garcia, Castillo 2008). Many epidemiological studies have shown that regular flavonoid intake is associated with a reduced risk of cardiovascular diseases. In coronary heart disease, the protective effects of flavonoids include mainly antithrombotic, anti-ischemic, anti-oxidant, and vasorelaxant. It is suggested that flavonoids decrease the risk of coronary heart disease by three major actions: improving coronary vasodilatation, decreasing the ability of platelets in the blood to clot, and preventing low-density lipoproteins (LDLs) from oxidizing (Benavente-Garcia, Castillo 2008). However, most interest has been devoted to the
antioxidant activity of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals. Most ingested flavonoids are extensively degraded to various phenolic acids, some of which still possess a radical-scavenging ability (Pietta 2000). The reducing properties of flavonoids might contribute to redox regulation in cells, independently of their antioxidant properties, and thus might protect against cell ageing, for example, by working together with the intracellular reductant network (Rice-Evans 2001). It should also be emphasized that both the absorbed flavonoids and their metabolites may display an in vivo antioxidant activity, which is evidenced experimentally by the increase of the plasma antioxidant status, the sparing effect on vitamin E of erythrocyte membranes and low-density lipoproteins, and the preservation of erythrocyte membrane polyunsaturated fatty acids (Pietta 2000).

In addition, compounds such as flavonoids, which contain hydroxyl functional groups, are responsible for the antioxidant effects of plants (Eshwarappa et al. 2015). On the other hand, flavonoids cannot only be considered purely as antioxidants, since under certain reaction conditions they can also display pro-oxidant activity. This unexpected behaviour could explain, in part, the observed toxicity of some flavonoids in vivo (Kessler et al. 2003). Our results are consistent with the general agreement on the decrease for free radical scavenging activity for B. psilophylla, B. arborescens var. oxyphylla and B. × erythrophylla. Extracts from those species caused the hemolysis of equine erythrocytes in vitro study (fig. 1).

Compounds such as tannins and coumarins are also responsible for the antioxidant effects of Begonia species. Tannins known as the group of phenolic compounds are the significant plant secondary metabolites. Condensed tannins are also known as proanthocyanidins, the oligomeric and polymeric flavan-3-ols, which are linked through C4-C8 or C4-C6 linkages (Zhang, Lin 2008). Proanthocyanidins possess potent antioxidant capacity and possible protective effects on human health (Santos-Buelga, Scalbert 2000). Moreover, they have antioxidant properties related to their radical scavenging capacity (Ricarda Da Silva et al. 1991). Therefore, they are of great interest in nutrition and medicine against heart disease through reducing lipid oxidation. It was hypothesized that the ROS scavenging properties of proanthocyanidins may reduce the risk of cardiovascular diseases, cancer and blood clotting (Bagchi et al. 2000, Zhang, Lin 2008).

Coumarins, a well-known class of naturally occurring compounds, display a remarkable array of biochemical and pharmacological actions, some of which suggest that certain members of this group of compounds may significantly affect the function of various mammalian cellular systems. The development of coumarins as antioxidant agents has attracted much attention in recent years. Coumarins afford an opportunity for the discovery of new antioxidants with truly novel mechanisms of action (Kostova et al. 2011). In a similar way to isomeric flavonoids, coumarins might affect the formation and scavenging of ROS and influence processes involving free radical-mediated injury. Coumarin can reduce tissue edema and inflammation. Moreover, coumarin and its 7-hydroxy-derivative inhibit prostaglandin biosynthesis, which involves fatty acid hydroperoxy intermediates. Natural products like esculetin, fraxetin, daphnetin and other related coumarin derivatives are recognized as inhibitors not only of the lipoxygenase and cyclooxygenase enzymic systems, but
also of the neutrophil-dependent superoxide anion generation (Fylaktakidou et al. 2004). Moreover, cytotoxic effects of coumarins were most extensively examined and these natural compounds have served as valuable leads for further design and synthesis of more active analogues (Kostova 2005).

The high doses of herbal remedies and dietary supplements can be toxic to cells. For instance, He and co-workers (2009) have investigated the effects of *Ginkgo biloba* leaf extract on the properties of human red blood cells in the presence and absence of amyloid peptide (Aβ25-35), peroxide and hypotonic stress. The results suggest that *G. biloba* leaf extract has a dual action, both protective and disruptive, on red blood cells, depending on whether an exogenous stress is present. *G. biloba* leaf extract has a protective role on red blood cells against Aβ- and hypotonic pressure-induced hemolysis, peroxide-induced lipid peroxidation, as well as glutathione consumption and methemoglobin formation. On the other hand, *G. biloba* leaf extract also exhibited damage to erythrocytes by increasing cell fragility, changing cellular morphology and inducing glutathione consumption and methemoglobin formation, especially when applied at high doses. These anti- and pro-oxidative activities of polyphenolic substances are thought to be involved in the dual function of *G. biloba* leaf extract (He et al. 2009).

In order to quickly identify toxin sources, 2 rapid in vitro assays were developed by Walter and co-workers (2014) to screen plant extracts for the ability to induce methemoglobin formation or cause hemolysis in healthy equine donor erythrocytes. The plant extract screening focused on 3 species of the genus *Pistacia*: *P. atlantica*, *P. terebinthus*, and *P. chinensis*, which were located in the horse pasture. Extracts of the seeds and leaves of each species induced methemoglobin formation and resulted in hemolysis, with seed extracts having greater potency. The in vitro assays used in the study of Walter and co-workers (2014) provide a useful diagnostic method for the rapid identification of oxidizing agents from unidentified sources. There is no effective treatment for oxidative erythrocyte damage in horses, making rapid identification and removal of the source essential for the prevention of poisoning. It is clear that horses must be isolated from these trees to prevent acute hemolytic anemia and death (Bozorgmanesh et al. 2015).

Horses develop severe and often fatal hemolytic anemia after ingesting dried leaves from red maple (*Acer rubrum*) trees. Toxicosis appears related to an unknown oxidant present in the dried or wilted leaves (Stair et al. 1993). The leaves of *A. rubrum*, especially when wilted in the fall, cause severe oxidative damage to equine erythrocytes, leading to potentially fatal methemoglobinemia and hemolytic anemia. Gallic acid and tannins from *A. rubrum* leaves have been implicated as the toxic compounds responsible for red maple toxicosis (Agrawal et al. 2013). Gallotannins and free gallic acid are present in *A. rubrum* leaves and can be metabolized by *Klebsiella pneumoniae* and *Enterobacter cloacae* found in the equine ileum to form pyrogallol either directly or through a gallic acid intermediate (gallotannins) (Agrawal et al. 2013). Boyer and co-workers (2002) have identified compounds in *A. rubrum* that cause hemolysis or oxidation of equine erythrocytes and also determined whether these toxins are found in other *Acer* spp. Washed erythrocytes were incubated with extracts and fractions of *Acer* spp that were separated by thin layer chromatography. Erythrocytes incubated separately with either *A. rubrum, A. saccharum,
or *A. saccharinum* extracts had increased methemoglobin formation, compared with extract-free control samples. Two *Acer* spp. fractions had toxic effects on erythrocytes *in vitro*. A major component of the *Acer* fraction that caused a significant amount of methemoglobin formation was identified as gallic acid. An amount of gallic acid equivalent to that found in *A. rubrum* extract significantly increased methemoglobin, compared with extract-free control erythrocytes, but caused less methemoglobin formation than *A. rubrum* extracts did. A potential co-oxidant, 2,3-dihydro-5,7-dihydroxy-6-methoxy-4H-pyran-4-one, was found in the *A. rubrum* extract and may have been responsible for increasing methemoglobin formation. A second *A. rubrum* fraction caused methemoglobin formation and significant hemolysis. *A. saccharum* and *A. saccharinum* extracts caused hemolysis but less than the *A. rubrum* extracts did (Boyer et al. 2002).

As exemplified earlier, the antioxidant activities of plant extracts are often explained by their products of secondary metabolism, mainly by total phenolic and flavonoid contents. In many study, it was observed that there was a strong correlation of antioxidant activities with that of total phenolic and flavonoid content in the plant extracts (Eshwarappa et al. 2015). For example, Chaity and co-workers (2016) also have assessed comprehensively membrane stabilizing, thrombolytic and antioxidant properties of rhizomes and fertile foliage fronds *Drynaria quercifolia* L. (*Polypodiaceae*). Crude methanol extracts and petroleum ether fractions of rhizomes and fertile foliage fronds were very promising to show membrane stabilizing anti-inflammatory potential. In membrane stabilizing assay, crude methanol extracts of rhizomes and fertile foliage fronds and their petroleum ether fractions were found to be very effective for stabilizing erythrocyte membrane in hypotonic solution. Besides, crude methanol extracts and aqueous fractions were very active for displaying thrombolytic activity. In addition, aqueous fractions and crude methanol extracts of both parts of the plant were very capable to scavenge the free radicals and reduce oxidized materials, which might be attributed to the high level of phenolic contents of the polar extractives (Chaity et al. 2016).

The study Ahmed and Rahman (2016) has revealed the medicinal capabilities of different organic fractions of *Callistemon citrinus* (Curtis) Skeels (Myrtaceae) displaying free radical scavenging, thrombolysis and membrane stabilizing anti-inflammatory potentials. The crude methanol extract showed prominent free radical scavenging activity. On the other hand, the petroleum ether fraction displayed dominant thrombolytic action. However, the chloroform soluble fraction showed the highest membrane stabilizing activity in both heat- and hypotonic solution induced hemolysis among the plant extractives. It was equivalent to the membrane stabilizing action of the standard drug, acetylsalicylic acid in the hypotonic solution induced hemolysis assay and more effective than the standard drug in heat induced hemolysis assay. Numerous flavonoids and alkaloids have been reported previously to display anti-inflammatory activity. Chloroform fraction of the leaves of *C. citrinus* was also found to contain flavonoids and alkaloids. The persuasive membrane stabilizing anti-inflammatory activity of this fraction might be related to these biosynthesized metabolites (Ahmed, Rahman 2016).

The *in vitro* antioxidant potential of *Carica papaya* leaf extract and its effect on hydrogen peroxide-induced erythrocyte damage assessed by hemolysis and lipid
Peroxidation was investigated by Okoko and Ere (2012). Preliminary investigation of the extract showed that the leaf possessed significant antioxidant and free radical scavenging abilities using in vitro models in a concentration dependent manner. The extract also reduced hydrogen peroxide induced erythrocyte hemolysis and lipid peroxidation significantly when compared with ascorbic acid. Thus, the *C. papaya* leaves possess significant bioactive potential which is attributed to the phytochemicals which act in synergy and can be exploited for pharmaceutical and nutritional purposes (Okoko, Ere 2012).

The determination of the toxicity of the plant extracts on mammalian cells and correlation with its antifungal activity is practical method for assessment of safety of the antifungal constituents of plant extracts. Mapfunde and co-workers (2016) have determined the toxicity of the antifungal constituents of *Combretum zeyheri* on mammalian cells and correlated it with antifungal activity of leaf extracts. The alkaloids, saponins and ethanol extracts were found to be non-toxic towards mouse peritoneal cells and Jurkat T cells. In the hemolysis assay, all extracts were hemolytic at varying degrees and showed their greatest hemolytic activity at the highest concentration of 5 mg/ml. The saponins were the least hemolytic, followed by the ethanol extracts and the alkaloids respectively. Although these extracts were hemolytic to some extent, they may considered safe at therapeutic concentrations since there was a large difference between the antifungal IC\textsubscript{50} and hemolysis EC\textsubscript{50} values, hence a large therapeutic window (Mapfunde et al. 2016).

**CONCLUSIONS**

In conclusion, among 30 species of *Begonia* genus, the most species of plants investigated possessed anti-hemolytic activity. The results of these biological assays demonstrated that compounds present in *B. glabra*, *B. aconitifolia*, *B. sanguinea*, *B. thiemei*, *B. masoniana*, *B. × credneri*, *B. oxyphylla*, *B. subvillosa*, *B. ulmilota*, *B. conconulacaeae* can prevent the formation of methemoglobin and reduce of hemolysis, while *B. eryrophylla*, *B. psilophylla* and *B. arborescens* var. *oxyphylla* extracts can induce formation of methemoglobin and hemolysis in healthy equine blood. Ex-prots from leaves of *B. foliosa*, *B. rex*, *B. solimutata*, *B. mexicana*, *B. goegoensis*, *B. imperialis* var. *smaragdina*, *B. pustulata*, *B. peltata*, *B. cucullata*, *B. angularis*, *B. boisiana*, *B. venosa* exhibited the decrease of percentage hemolysis of equine erythrocytes, but these alterations were non-significant.

The extensive use of plants from this genus by the local people in treating various types of diseases and disorders might therefore be justified by their antioxidant activities against oxidative stress and hemolysis, which are known to be responsible for causing various metabolitic states and diseases. The results also indicate that scientific studies carried out on plants of *Begonia* genus having traditional claims of effectiveness might warrant fruitful results in medicine and veterinary practice. Further studies aimed at the isolation and identification of active substances from the various species of *Begonia* genus, as well as assessed oxidative stress biomarkers and antioxidant defenses could also disclose compounds with better therapeutic value and doses. We believe that screening of all the investigated plants for other
biological activities including anti-microbial and antioxidant activities is essential and may be effective for searching the preventive agents in the pathogenesis of some metabolic diseases.

REFERENCES


**SUMMARY**

Most active plants are toxic at high doses and it is therefore important to investigate the preliminary toxicity of plant extracts. Toxicological study is essential in order to determine dosage of the plant extracts which will not be lethal to the body when administered (Chitemerere, Mukanganyama 2014). Extracts of various...
plants can induce methemoglobin formation and result in hemolysis. The in vitro assays used in many studies (Boyer et al. 2002, Walter et al. 2014) provide a useful diagnostic method for the rapid identification of oxidizing agents from different sources. Therefore, in the present study, the percentage haemolysis of equine erythrocytes induced by treatment with extracts of various species from Begonia genus are determined to exemplify their further potential development and use as drug against metabolic diseases in medicine and veterinary. The leaves of Begonia plants, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanical Garden (NBG), National Academy of Science of Ukraine. The leaves of thirty plants, i.e. Begonia foliosa Kunth, B. psilophylla Irmsch., B. convolvulacea (Klotzsch) A.DC., B. ulmifolia Willd., B. rex Putz., B. thiemii C.DC., B. manicata Brongn., B. solimutata L.B.Sm. & Wassh., B. arborescens var. oxyphylla (A.DC.) S.F.Sm., B. mexicana G. Karst. ex Fotsch, Begonia × credneri F.Haage & E.Schmidt, B. sanguinea Raddi, B. olbia Kerch., B. goegoensis N.E.Br., B. imperialis var. smaragdina Lem., B. epipsila Brade, B. pustulata Liebm., B. heracleifolia var. nigricans Hook.f., Begonia × erythrophylla Hérincq., B. aconitifolia A.DC., B. peltata Otto & Dietr., B. nelumbifolia Cham. & Schldtl., B. subvillosa Klotzsch, B. oxyphylla A.DC., B. masoniana Irmsch. ex Ziesenh., B. cucullata Willd., B. angularis Raddi, B. glabra Aubl., B. boisiana Gagnep., B. venosa Skan ex Hook.f. were sampled for study. Freshly leaves were washed, weighted, crushed, and homogenized in 0.1M sterile phosphate buffer saline solution (pH 7.4) (in proportion 1:19, w/w) at room temperature. Blood was drawn from jugular veins of the animals in the morning, 90 minutes after feeding, while the horses were in the stables (between 8:30 and 10 AM). Blood was stored into tubes with sodium citrate and held on the ice until centrifugation at 3000 rpm for 5 min. Erythrocytes aliquots were used in study. To evaluate the extracts’ potential to cause hemolysis in equine erythrocytes, a hemolysis assay based on the spectro-photometric measurement of hemoglobin in the supernatant was performed. A volume of 0.1 ml of the various extracts were added to 1.9 ml of clean equine erythrocytes. After incubation the mixture at 37°C for 60 min with continuous stirring. Anti-hemolytic activity was assessed by measuring the absorbance at 540 nm. For positive and negative control (distilled water and phosphate buffer saline, respectively) were used. Percent hemolysis was determined relative to the absorbance measured in the supernatant of erythrocytes treated with distilled water, which should cause complete hemolysis. Our study demonstrated that among 30 species of Begonia genus, the most species of plants investigated possessed anti-hemolitic activity. The results of these biological assays demonstrated that compounds present in B. glabra, B. aconitifolia, B. sanguinea, B. thiemii, B. masoniana, B. × credneri, B. oxyphylla, B. subvillosa, B. ulmifolia, B. convolvulacea can cause the prevention of formation of methemoglobin and reduce of hemolysis, while B. erythrophylla, B. psilophylla and B. arborescens var. oxyphylla extracts can formation of methemoglobin and hemolysis in healthy equine blood. Extracts from leaves of B. foliosa, B. rex, B. solimutata, B. mexicana, B. goegoensis, B. imperialis var. smaragdina, B. pustulata, B. peltata, B. cucullata, B. angularis, B. boisiana, B. venosa exhibited the decrease of percentage hemolysis of equine erythrocytes, but these alterations were non-significant. The extensive use of plants from this genus by the local people in treating various types of diseases and
disorders might therefore be justified by their antioxidant activities against oxidative
stress and hemolysis, which are known to be responsible for causing various
metabolic states and diseases. The results also indicate that scientific studies carried
out on plants of Begonia genus having traditional claims of effectiveness might
warrant fruitful results in medicine and veterinary practice. Further studies aimed at
the isolation and identification of active substances from the various species of
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