Physicochemical Characterization, Stability and Cytotoxicity of a Blue Dye Obtained from Genipap Fruit (*Genipa americana* L.)

Running head: Blue dye from genipap fruit

Camila Verly de Miranda Sabino¹, Bárbara Janaina Paula da Silva¹, Danielle Lima Bezerra de Menezes², Felipe Moura Araújo da Silva³, Tatiane Pereira de Souza¹, Hector Henrique Ferreira Koolen⁴, Ádley Antonini Neves de Lima⁵ and Emerson Silva Lima⁶

¹Faculty of Pharmaceutical Sciences, Federal University of Amazonas, Av. Gen. Rodrigo Otavio, 6200, CEP 69077-000, Manaus, AM, Brazil

²Faculty of Pharmacy, Federal University of Rio Grande do Norte, Av. Sen. Salgado Filho, 3000, CEP 59078-970, Natal, RN, Brazil

³Institute of Exact Sciences, Federal University of Amazonas, Av. Gen. Rodrigo Otavio, 6200, CEP 69077-000, Manaus, AM, Brazil

⁴Metabolomics and Mass Spectrometry Research Group, University of Amazon State, Av. Carvalho Leal, 1777, CEP 69050-010, Manaus, AM, Brazil

Received: 20 May 2020
Accepted: 4 March 2021

**SUMMARY**

Research background. The current commercial scenario indicates an increase in the demand for natural dyes. Compared to synthetic dyes, natural ones have the advantage of being sustainable, making them of great interest for the food and cosmetic industries. The development of new natural dyes is necessary, as well as the carrying out of complementary research regarding the existing ones.

Experimental approach. The present study aimed to characterize the chemical and physicochemical characteristics of the dehydrated endocarp of the genipap (*Genipa americana*) fruit, as well as performing the relevant stability and cytotoxicity tests. The chemical characterization was performed by LC/MS/MS analyses. The stability studies were carried out by spectrophotometry and cytotoxicity assays using cell culture and fluorometric methods.

Results and conclusions. After dehydration and milling of the fruit's endocarp, a powder was obtained, which with 20 % water was used to extract the dye. Five compounds were elucidated using
HPLC-MS and confirmed the presence of the geniposide as its main compound. Via the X-ray diffraction test and electron microscopy analysis, it was possible to describe the powder obtained as being amorphous and of porous structure with a variable size, respectively. The thermogravimetric analysis indicated a maximum loss of 61% mass after exposure to a temperature range of 240 °C to 760 °C. The obtained blue dye showed to be stable in the absence of light, at room temperature and presented neutral pH. In the cytotoxicity assay, 95.05±1.33 % of viable human fibroblast were observed after exposure to this dye. The genipap fruit can be a viable alternative for the obtention of natural blue dye, since it is easy to obtain and has very low toxicity for food, pharmaceutical or cosmetic industries.

Novelty and scientific contribution. This study demonstrates for the first time the physicochemical and biological properties of a natural blue dye from G. americana fruit.

**Key words:** Genipa americana, genipap fruit, natural dye, geniposide

**INTRODUCTION**

Natural dyes are distinguished by their biocompatibility, which makes them an alternative to the widely-marketed synthetic dyes (1,2). The growing commercial demand for natural substances, which have sustainable methods of production, has aroused interest in the search for new raw materials and/or improvement in the techniques used to extract these dyes (3,2).

The genipap (Genipa americana L.) is an angiosperma which belongs to the family Rubiaceae, of the order Gentianalis and is native to Central and South America (4). The fruit, when in its immature stage, is rich in a colorless iridoid called genipin. This substance acquires high reactive potential with amine groups when exposed to oxygen, resulting in the formation of an intense blue pigment (3). Genipin is easy to extract because it presents good solubility in water and hydroalcoholic solutions, which has contributed to its historically widespread use by indigenous peoples as a dye of utensils and for body pigmentation (5,6).

Due to the increase in demand for natural dyes, technological studies, such as those performed by Neri-Numa et al. (3), which evaluated the extraction of bioactive compounds from the genipap fruit, and those of Brauch et al. (7), which studied the obtaining and use of the dye from this fruit, are necessary in order to gain better knowledge of this raw material and its constituents, as well as defining it as a safe source. Therefore, the present study had the objective of physicochemically characterizing the dehydrated endocarp of the fruit, and performing *in vitro* cytotoxicity tests of the liquid dye, as well as evaluating its stability under changes in its storage conditions.
MATERIALS AND METHODS

**Dye extraction**

The immature fruits were obtained at the headquarters of the Brazilian Agricultural Research Corporation (EMBRAPA) located on the AM 10 Highway (Km 28) (2° 52' 51.3'' S 59° 57' 25.8'' W), state of Amazonas, Brazil. This occurred in the period from July to September 2016. The study was registered in the National Genetic Heritage and Associated Traditional Knowledge Management System (8) under the number A3965C3. The fruits were washed in running water, followed by the removal and desiccation of the endocarp in an oven at 45 °C for 3 days. This material was processed in a knife mill until a powder was obtained, which was then screened (9). The extractive process variables, plant material concentration:solvent (5, 10 and 20 %) and extractant liquid (ethanol, water and ethanol:water (φ =1:1) were tested. The solutions were macerated for 7 days, and the absorbances were monitored using a spectrophotometer (T70 UV/VIS, PG Instruments, Vietnam) (λ= 590nm) on days 0, 4 and 7 after extraction. A liquid blue dye was obtained, which was used for cytotoxicity and stability tests.

**HPLC-MS/MS analysis**

All chemical analyses were performed on an HPLC-MS/MS system consisting of a liquid chromatography system (Accela, Thermo, Waltham, MA, USA) coupled to a triple quadrupole mass spectrometer TSQ Quantum Access equipped with an electrospray ionization source ESI) operating in positive mode. A Phenomenex Luna-C18 column (5.0 μm, 4.6 mm i.d., 150 mm) (Torrance, CA, USA) was used to achieve the chromatographic separation using the binary mobile phase. Solvent A was water and solvent B methanol (Tedia, Mexico City, Mexico). Gradient elution was performed at 35 °C from 0-15 min, 10-80 % (V/V) B at a flow rate of 0.7 mL/min. The temperature of the autosampler was maintained at 25 °C and the injection volume was 15 μL. The ESI source parameters were previously optimized as follows: voltage of the ionization source, 4.7 kV; main gas pressure, 1,2 x 10^6 Pa ; auxiliary gas pressure, 5 x 10^5 Pa; scanning gas pressure, 0 Pa; capillary temperature, 200 °C; transfer capillary voltage, 36 V; voltage of the lenses, 120 V; microscans rate, 4 ms; maximum injection time, 100,000 ms. Argon (Praxair, Danbury, CT, USA) was used as collision gas, where collision energies ranged from 15 to 35 %. Attempts to identify the compounds present in the fruit endocarp were performed by manual interpretation of MS/MS spectral data and comparison with previously published data (10).

**Fourier transform infrared (FTIR)**
The resulting powder from the dehydrated endocarp was characterized by infrared spectroscopy using Prestige-21 IR equipment (Shimadzu Corporation, Kyoto, Japan) with Attenuated Total Reflectance (FTIR-ATR) equipment. The analysis was performed in the region of 700 to 4000 cm\(^{-1}\), with 20 scans and resolution of 4 cm\(^{-1}\).

**X-ray diffraction (XRD)**

The powder diffraction profile resulting from the dehydrated endocarp was characterized in a Bruker D2 Phaser apparatus (Karlsruhe, Germany) using CuK\(\alpha\) radiation (\(\lambda = 1.54\ \text{Å}\)) with a Ni filter, with a 0.02° step, 10 mA, voltage of 30 kV, and the use of a Lynxeye detector.

**Scanning electron microscopy (SEM)**

Scanning Electron Microscopy was performed for morphological analysis of the obtained powder, which was placed under double carbon tape and analyzed using a Hitachi Tabletop Microscope TM-3000 (Tokyo, Japan) with a minimum magnification of 200\(\times\) and maximum of 1.0 Kx at a voltage of 15 kV.

**Differential scanning calorimetry**

Differential scanning calorimetry (DSC) measurements were performed on Q20 DSC cell (TA instrument, Tokyo, Japan) using a hermetically-sealed aluminum crucible. Approximately 4 mg of powder were used for all experiments under a dynamic nitrogen atmosphere (50 mL/min) at a distinct heating rate (2.5, 5.0 and 10 °C/min) in the temperature range of 25 to 500 °C. The temperature and heat flow of the DSC instrument were calibrated with indium (melting point = 157.5 °C and \(\Delta H = 26.7 \ J/g\)).

**Thermogravimetry (TG)/differential thermal analysis (DTA)**

Thermal analysis by thermogravimetry was performed on TGA-60H thermocouple (Shimadzu Corporation, Kyoto, Japan). The platinum crucibles were used with approximately 4 mg of the obtained powder under a dynamic atmosphere of N\(_2\) (50 mL min\(^{-1}\)) at a heating rate of 10 °C. min\(^{-1}\) in the temperature range from 25 to 600 °C. Data were analyzed using TA-60WS software (11).

**Moisture content**

*G. americana* dye powder (1 g) was weighed and analyzed in the Moisture Determination Scale, model M5 THERMO (BEL Engineering, Milano, Italy) at a constant temperature of 100 °C.
Stability Study of the blue dye

The liquid dye, obtained by ethanol/water (φ=1:1) extraction process, was followed by vacuum filtration. In order to suppress microbial growth, a 0.3 % of potassium sorbate (Sigma-Aldrich, St. Louis, MO, USA) was used as the preservative. The stability test consisted of observing the behavior of the dye in the presence of changes in temperature (2-8, 22-27 and 42-47 °C) and pH (pH= 4, 7 and 10), in addition to assessing the interference of light during the study period. Absorbance monitoring was performed using a T70 UV/VIS spectrophotometer (PG Instruments, Vietnam) (λ = 590 nm) in the first day (D0), after thirty (D30), sixty (D60) and ninety days (D90) of incubation under different experimental conditions.

Evaluation of in vitro cytotoxicity

The evaluation of the cytotoxic activity of the liquid dye was performed on fibroblast (MRC-5) cells strains using the Alamar Blue® assay (Sigma-Aldrich, St. Louis, MO, USA) according to methodology described by Ahmed et al. (12). Cells were obtained from the Rio de Janeiro Cell Bank, cultivated in Dulbecco's Modified Eagle's medium (Sigma-Aldrich, St. Louis, MO, USA) at 37 °C, with 5 % of CO₂ and plated at 0.5·10⁴ cells/well in 96-well microplates. To determine the IC₅₀ values (cytotoxicity index causing 50 % cell death), the cells were treated with the dye at the concentrations of 100, 50, 25, 12, and 6.25 μg/mL. Dimethyl Sulfoxide (Sigma-Aldrich, St. Louis, MO, USA) was used as a control. Subsequently, 10 μL of the 0.4 % Alamar blue solution was added to all wells of the treated plate for a period of 24 h. After 3 h of incubation, microplates were analyzed using the fluorescence mode (540 nm exchange filter and 585 nm emission filter) of a DTX800 microplate reader (Beckman and Coulter, Vienna, Austria).

Statistical analysis

The data obtained in this study are presented as the mean value±standard deviation (S.D.) and analyzed statistically using the GraphPad Prism software (13). Difference among the groups was compared using two-way analysis of variance (ANOVA) followed by Tukey’s test and considered significant at p≤0.05.

RESULTS AND DISCUSSION

Processing of G. americana fruit

The process was started with 20 kg of the whole green genipap fruit. Due to the higher deposition of genipin in the endocarp of the fruit (14), this part was used in the extractive process,
which resulted in 5.65 Kg of endocarp. In order to facilitate the packing and to control the microbial growth in this material, the desiccation and then milling was performed, which in turn resulted in 2.5 kg of a raw powder. After sieving, it was classified as a coarse powder according to the Brazilian Pharmacopoeia \(8\), based on retention of 50% of powder in a mean sieve diameter of 0.925 mm. According to Fonseca et al. \(15\), coarse powder is better for extraction of plant drugs, since very fine powders can compromise this process.

**Analysis of the extraction process**

Among the solvents tested in the extraction of the blue dye from the endocarp of the dehydrated fruit, water had a better extraction potential in association with the 20% concentration (Fig. 1). Renhe et al. \(16\) performed the same procedure with water and ethanol solvents. However, extraction with hexane was not possible, leading to the conclusion that the color precursor compound is of polar origin. Water and ethanol are often recommended for the preparation of extracts due to their polarities \(17\). The water has a higher polarity than ethanol, and may justify better extraction of the dye \(18\). Genipin is present in the immature genipap fruit and is responsible for the formation of the blue color through the reaction with amino acids in the presence of oxygen \(19\). The extractive process performed by Neves et al. \(14\) resulted in bluish tones, which corroborate the findings of this study. Statistical analysis of the results showed that only the proportion of genipap powder was significant, confirming that a higher proportion of genipap powder and water as extracting liquid are the best parameters in order to obtain the blue pigment.

\[\text{[ Insert Fig. 1]}\]

**Chemical characterization of the dye**

The chemical characterization of the dye was made firstly by injection of the genipin standard in the LC/MS/MS system, resulting in a chromatogram with a time retention peak at 9 min and 98 s (Fig. 2a). Subsequently, the endocarp powder of the fruit was analyzed and this presented a higher retention peak at 8 min and 14 s and other at 9 min and 98 s (Fig. 2b). Using structural elucidation, it was possible to identify five compounds present in the \(G.\) *americana* powder, which presented expressive peaks of retention in the HPLC chromatogram (Fig. 2a). After analyzing these results, it was verified that the largest fraction of genipin present in the fruit is in its glycosylated form, the geniposide (Fig. 2c). According to Bentes et al. \(6\), genipin is an aglycone, resulting from the hydrolysis of the geniposide, through the enzyme β-glucosidase.
In order to identify the iridoids compounds present in the endocarp of the immature genipap fruit, Bentes and Mercadante (20) identified the prevalence of geniposide in relation to the others, followed by geniposide, gardenoside, shanzhisid and genipin. However, the study of the extraction of bioactive compounds by Náthia-Neves et al. (21) indicated a higher content of genipin in the endocarp and geniposide in the fruit’s mesocarp after changes in the variables of the extractive process.

[Insert Fig. 2]

**Thermogravimetric analysis of the dye from G. americana**

The thermogravimetric analysis of the dye from *G. americana* showed an initial endothermic event in the range of 27-95 °C, with a loss of mass equivalent to 6.5 % related to the water loss previously observed by the dye moisture determination analysis (Fig. 3a). This phenomenon was also observed in the thermal characterization of the *Dicksonia sellowiana* extract performed by Malucelli et al. (22). Up to about 160 °C, the dye showed no significant mass variation, however, in the range between 163-220 °C, there was a mass loss equivalent to 19 %, indicated by the peak in the DTG curve at 211 °C when mass varied more at a faster rate. From 330 °C onwards, a continuous mass loss of 40 % was observed.

The DSC curve showed a marked endothermic event between 18 and 110 °C (Tonset 21 °C, Tpeak 75 °C, Tendset 102 °C, ΔH =52.39 J/g), which may be related to the loss of volatile constituents of the sample (Fig. 3b); in this case, the loss of water that was also observed in the thermogravimetric analysis. The decomposition process was progressive with increasing temperature, starting at approximately 133 °C. According to Fernandes et al. (23), degradation products of plant extracts can present different thermal behavior due to factors such as loss of volatile components, sample heating rate and the presence of impurities that can directly interfere in the enthalpy of the obtained peak.

**Infrared and X-ray diffraction analysis of the dye from G. Americana**

FTIR analysis was performed to determine the functional groups present in the endocarp (Fig. 3c). The spectra obtained from the powder analysis showed bands around 3337 and 2946 cm⁻¹ which correspond to the stretching of the -OH binding present in alcohols and polyphenols, whereas at 2834 cm⁻¹ it refers to the elongation of the aliphatic CH which can be attributed to the organic nature of the compounds present in the endocarp. In 1645 bands, related to the C=O double bond of the aldehyde group -COOCH₃ or the C=C conjugated, carboxyl group are observed. The band at 1449 cm⁻¹, corresponds to deformation of the binding of the methoxy groups of genipin. The approximate region
between 1112 and 1019 cm\(^{-1}\) corresponds to the sugar absorption bands related to the C-OH bond of the C-O-C group and the deformation of the hydroxyl CH\(_2\)-OH group present in the geniposide. The results obtained are in agreement with that observed by Kumar et al. (24) in a study with the extract from the fruit of G. americana.

The X-ray diffraction analysis was performed to determine the degree of crystallinity of the obtained powder. Post-crystalline powders are characterized by a well-defined melting point and three-dimensional structure capable of refracting X-rays (25). Contrastingly, amorphous powders consist of randomly oriented molecules and diffract X-rays in all directions resulting in the typical "halo" pattern, that is, the absence of crystalline reflections. The diffractogram of the powder of the endocarp is shown in Fig. 3d, in which a totally amorphous diffraction profile is observed, that is, there are no crystalline reflections in the diffractogram, confirming that it is a powder without any character or crystalline nature. This characteristic directly impacts the solubility of the powder, since amorphous particles are more easily solubilized in polar solvents, due to the fact that the particles present more interaction points with these solvents, by the random distribution of the molecules, favoring the wettability. The study by Gallo et al. (26) showed that the dried extract of Rhamnus purshiana also presented an amorphous profile.

[Insert Fig. 3]

**Scanning electron microscopy of the powder dye from G. americana**

Scanning electron microscopy (SEM) is a qualitative analysis used to observe the surface texture of solids (27), in other words, to evaluate the morphology and particle size. The dye particles presented particle agglomerates with an irregular porous surface with different sizes and shapes, which is expected from the particles of plant extracts. These characteristics are common to the surface of amorphous compounds, corroborating with what was observed via the analysis of X-ray diffraction, *i.e.* the absence of crystalline particles in the analyzed powder (Fig. 4). These characteristics may be directly related to the wettability properties of the powder (28).

[Insert Fig. 4]

**Stability of the dye from G. americana**

In the stability test, the behavior of the genipap aqueous dye was observed during temperature, pH and luminosity changes over 90 days (Figs. 5a-g). Due to this dye being of an aqueous nature, addition of an antimicrobial preservative was necessary. The preservative potassium sorbate was chosen because its use in food and cosmetics is regulated by the Brazilian National Agency for Sanitary Surveillance (ANVISA) (29,30). The temperature variations caused visual
changes in the product color at the end of the analysis. At 45 °C, the solutions turned greenish, but the temperatures of 4 °C and ambient temperature the initial coloration did not change. These results corroborate those of Cho et al. (31), who observed that at room temperature the pigments remained stable, however they lost 30% of their initial value after 140 h of exposure at 75 °C. Meanwhile, the study by Paik et al. (32) showed that, after 10 h of exposure to a temperature range of 60 °C to 90 °C, the pigments remained stable, which makes it necessary to monitor their stability for longer.

The pH variable did not significantly interfere with the stability of the dye. However, when compared to each other, neutral pH kept the dye stable for longer. Brauch et al. (7), who also evaluated the stability of the blue dye from G. americana fruit, noticed that the pH variation of the dye did not cause large changes throughout the process, demonstrating that this parameter does not significantly influence its stability. Cho et al. (31) analyzed the stability of the dye obtained from the reaction of genipin with amino acids, within the range of pH=4-12 after 200 h of incubation at 55 °C and found that the pH had no great influence, maintaining 80 % of the initial absorbance.

While evaluating the exposure of the dye to the light, it was noticed that there is a decrease of its stability when compared to samples not exposed to luminosity. Paik et al. (32) evaluated the influence of light on the blue dye obtained by the interaction of genipin with phenylalanine, and this showed a loss of stability due to exposure to intense light. Likewise, in the study by Jespersen et al. (33) a degradation of the blue dye from Gardenia occurred when exposed to light.

Evaluation of cytotoxicity of G. americana dye

MRC-5 human fibroblast was exposed to G. americana dye. In the cytotoxicity assay, the percentage of viable cells was (95.1±1.3) % at the concentration of 100 μg/mL of the dye, as shown in Fig. 6. Due to these results, the IC50 cannot be calculated, since, even in the highest concentration tested, there were no deaths in a percentage greater than 50 %. These findings corroborate the belief that natural dyes are less harmful to health because they are biocompatible and therefore do not have a significant degree of toxicity, especially when compared to synthetic dyes (33). The cytotoxicity and neurotoxicity assays performed by Ab Kadir et al. (34) in the evaluation of the natural dyes from Caulerpa lentillifera and Sargassum sp. plants showed the lack of toxicity in these raw materials, once again showing the safety of the use of natural dyes.

CONCLUSIONS
This study showed the feasibility of using genipap as a potential source for the production of a stable blue dye, which could easily be applied in the food, pharmaceutical or cosmetic industries. With the characterization of the endocarp of this fruit, it was possible to identify two major compounds (geniposide and genamid D). It was possible to describe this material as having an amorphous, porous structure, which varied in size, and had a low moisture content. The extractive process and dye stability analyses indicated the use of water as the best extractive liquid and a 20% drug-solvent concentration, in addition to establishing room temperature, neutral pH and lack of luminosity as the ideal conditions for obtaining and maintaining the stable blue pigment. The biocompatibility of the produced dye was evidenced in cytotoxicity assay, resulting in high cell viability after high concentration dye exposition. The work demonstrated that the dye from genipap fruit is a promising alternative for the substitution of synthetic dyes, since it has a sustainable production method and is not harmful to the human organism, and can thus reduce cases of allergies that are widely attributed to the use of synthetic colorants. Subsequent studies should be performed in order to show details of the applications of this dye in medicines, food or cosmetics.

ACKNOWLEDGEMENTS
The authors are grateful to Central Analytica (UFAM) for the chemical analysis and CAPES, CNPq and FAPEAM for financial support.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

SUPPLEMENTARY MATERIALS
All supplementary materials are available at: www.ftb.com.hr.

ORCID ID
CVM Sabino https://orcid.org/0000-0003-4889-0690
BJP Silva https://orcid.org/0000-0002-5077-3584
DLB Menezes https://orcid.org/0000-0003-3067-6266
AAN Lima https://orcid.org/0000-0002-3798-3915
TP Souza https://orcid.org/0000-0003-1164-2191
HHF Koolen https://orcid.org/0000-0002-0181-348X
FMA Silva https://orcid.org/0000-0002-1809-1372
ES Lima https://orcid.org/0000-0002-9367-2812
AUTHORS’ CONTRIBUTIONS

CVM Sabino participated in the work conception, designing, carrying out all the experimental work, data analyses and interpretation, and writing of the manuscript. AAN Lima and DLB Menezes was involved in spectroscopy analyses. BJP Silva was involved in cell culture analyses. T.P Souza, HHF Koolen and FMA Silva was involved in chromatography analyses. E.S. Lima conceived, planned, designed, and supervised the entire work, contributed to writing and finalizing the manuscript. All authors have read and approved the final manuscript.

REFERENCES

   https://doi.org/10.4172/2329-6631.1000151

   https://doi.org/10.1007/s13659-017-0119-9

   https://doi.org/10.1016/j.tifs.2017.06.018

   http://dx.doi.org/10.1590/S0103-901390161998000100006

   https://www.arca.fiocruz.br/bitstream/icict/19149/2/1.pdf

   https://doi.org/10.1007/s13197-014-1651-9

   https://doi.org/10.1016/j.foodres.2016.08.029
Available from: https://sisgen.gov.br/


https://pdfs.semanticscholar.org/16fa/f53f06ae0f24072d6da74757ebc36c557c10.pdf

https://doi.org/10.1016/0022-1759(94)90396-4


https://doi.org/10.1016/j.foodres.2017.09.041

Available from: https://www.graphpad.com/scientific-software/prism/

https://doi.org/10.1590/S0102-695X2010005000049

http://dx.doi.org/10.1590/S0100-204X2009000600015

https://doi.org/10.3390/antiox5040045

https://doi.org/10.1016/S0021-9673(00)85732-5


25. Sharma VK, Mazumdar B. Feasibility and characterization of gummy exudates of *Cochlospermum religiosum* as pharmaceutical excipient. Ind Crop Prod. 2013;50:776–86. [https://doi.org/10.1016/j.indcrop.2013.08.004](https://doi.org/10.1016/j.indcrop.2013.08.004)


Fig. 1. Factorial response surface study of the extraction parameters analyses from Genipa americana dye.
Fig. 2. LC-MS/MS analysis of: a) *G. americana* fruit dye; b) genipine standard and c) chemical structures of compounds found mostly in the *G. americana* dye: 1=deacetyldaphylloside, 2=genameside C, 3=genameside D, 4=genoposide, 5=genipin

Fig. 3. Thermal analysis by: a) thermogravimetry and b) differential scanning calorimetry, c) spectrum resulting from Fourier transform infrared analysis and d) diffractogram of X-ray diffraction of *G. americana* dye

Fig. 4. Micrographs of the *G. americana* fruit dye particles in different magnifications: a) 200×, b) 400×, c) 600×, d) 800× and e) 1.0 Kx.

Fig. 5. Absorbance stability of the *G. americana* fruit dye: a) 20-25 °C in dark, b) 42-47 °C in dark, c) 2-8°C in dark; d) 20-25 °C exposed to white light; e) 42-47 °C exposed to white light and f) 2-8 °C exposed to white light.

Fig. 6. Cytotoxicity of the *G. americana* fruit dye in the MRC-5 fibroblast cells.
Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Fig. 1
Fig. 2
Fig. 3
Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.
Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

**Fig. 6**

![Graph showing cell viability percentage against different concentrations of substance](image-url)