Bead milling liberates the antioxidant properties of nanosized tubers of *Vernonia guineensis* Benth. (Asteraceae)

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Abstract

Background

Vernonia guineensis Benth. (Asteraceae), locally known as 'African ginseng', is an herbaceous plant with various therapeutic properties, sold by herbalists, and used in several traditional African preparations. Nanosizing has the capability to potentiate those preparations in their pharmacological properties. Premilling and extensive grinding using a planetary ball mill were used to reduce the size of *V. guineensis* tubers towards antioxidant studies.

Results

Water was used as an environmental friendly, cost effective solvent and dispersant to generate a nanocolloidal suspension of *V. guineensis* tubers. Size and size distribution were determined via photon correlation spectroscopy at room temperature which allows discussion on stability by Zeta potential and polydispersity index. Phytochemical screening shows presence of alkaloids, coumarins, polyphenol, saponins, tanins, terpenes, and anthraquinones. The distribution curve in water shows a polydispersed system with large hydrodynamic particles of size close to 1000 nm and a Z-average of 484.5 nm. The preparation separate in two phases with polydispersity index 0.217 for the supernatant and 0.543 for the suspension. In the supernatant and suspension, the particles zeta potential were − 12.3 mV and − 13.7mV respectively. The Mastersizer analysis indicates that there are smaller particles in volume in the supernatant than in the suspension. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity shows an increase in antioxidant activity, compared to that of ascorbic acid, in the nanoformulated state.

Conclusions

These findings allow us to conclude on the potential of size reduction when compared to solvent extraction in pharmacologic preparations.

Background

Oxidative stress is implicated in the pathogenesis of many chronic and neurodegenerative diseases. (Sharifi-Rad et al., 2020). The imbalance in the redox balance between oxidants and antioxidants in favor of the oxidants is oxidative stress. Oxidants promote oxidation reactions to stabilize the immune system. (Podkowinska and Formanowicz, 2020; Sies 1997). Medicinal plants play an essential role in the developing world being sometimes the only remedies to treat or cure diseases (Kuete and Efferth, 2010). Sustainable development can be achieved in the context of limited resources if cost-effective, environmentally friendly, and high-performance plant materials can be obtained. Nanomaterials, due to their high surface-to-volume ratio, hold promise in human life applications such as agriculture, energy storage, biotechnology, or biomedicine (Khalid et al., 2020). The implication of nanotechnology in pharmaceuticals is set to continue to increase rapidly and gain worldwide attention when aiming to reduce toxicity, adverse effects, and improving release and stability towards increased safety and efficacy (Raval et al., 2019). Planetary ball mill processing is an environmentally friendly top-down method that optimizes nanostructure production (Ijaz *et al.*, 2019). In a ball mill, the container with the sample rotates on its axis (angular velocity ω) while the supporting main disk revolves in the opposite direction (angular velocity Ω), thus creating a centrifugal force. This centrifugal force causes the balls to move in a certain direction and hit the wall of the container and with each other, leading to crushing and milling via impact effect attrition (Loh et al., 2015; Ramachandraiah and Chin 2016). Ball-milled samples therefore contain smaller particles with an increased contact area and improved absorption, distribution, and bioavailability (Shahbaziniaz et al., 2013). The bridging of nanotechnology and nature can be achieved by sizing plant materials toward greater potential. The principle of nanostructuring materials to enhance their potential is easy to apply, and is mainly based on the Noyes-Whitney equation, one of the main equations in biopharmacy (Eq. 1).

$$dc / dt = D \quad A \quad (c_s - c_o) / h \quad (1)$$

where *dc* / *dt* is the dissolution rate, *D is* the diffusion coefficient, *A is* the total surface area of the particles, c_s the saturation solubility of the active ingredient, *co is* the concentration of the dissolved active ingredient in the solvent, and *h is* the diffusional distance (Griffin et al., 2017).

Therefore, increasing the total surface increases the rate of dissolution, and the saturation solubility increases as a result of a higher dissolution pressure and decreases of the diffusional distance. Eventually, nanosizing increases the overall velocity of dissolution and improves the bioactivity of poorly soluble active ingredients (Griffin et al., 2017).

Obtaining particles of uniform size distribution and their study can be quite challenging. Dynamic light scattering (DLS), also referred to as photon correlation spectroscopy (PCS) method, using a Zetasizer instrument can help to determine the sample's size distribution and stability in colloidal suspension (Raval et al., 2019). DLS measures the random movement of particles as a result of the interaction with the solvent molecules that surround them. It also obtains the diffusion rate of the particles and calculates the hydrodynamic size of the particles (Lim et al., 2013).

Vernonia guineensis Benth., locally known as 'African ginseng', is an herbaceous plant distributed throughout Africa, mainly from Mali to West Cameroon (Burkill, 1985). Its therapeutic properties are similar to those of the Asian plant "Panax ginseng". Herbalists use and sell whole tubers of this plant, which resemble carrots. (Toyang et al., 2012; Wouamba et al., 2020a). The species was found to possess antimicrobial, antiplasmodial, antiproliferative / anticancer, and anthelmintic effects (Toyang et al., 2013a; Toyang et al., 2013b; Toyang et al., 2013c). Ethnobotanical surveys reveal that leaves of *V. guineensis* are used macerated in West Africa in the treatment of malaria, hypertension, diabetes, childhood diseases, typhoid fever conditions, prostatic diseases, and during pregnancy (Noumi, 2010). A new ceramide named vernoguinamide and fifteen already known compounds, including three anthraquinones, physion, erythroglaucin and emodin, three triterpenoids, hop-17(21)-en-3*β*-yl acetate, lupeol and betulinic acid, six steroids, vernoguinoside A, vernoguinoside, *β*-sitosterol 3-*O*-*β*-D-glucoside, stigmasterol 3-*O*-*β*-D-glucoside, stigmasterol and *β*-sitosterol and three fatty acid derivatives, tetracosanoic acid, tricosanic acid and arachidic acid glycerol ester where isolated from its roots (Wouamba et al., 2020b).

It has been reported previously that nanosuspensions of *Solanum incanum* and *Pterocarpus erinocaeus* retain a considerable antimicrobial and nematicidal activity (Griffin et al., 2016). Additionally, the antifungal activity of *Cynomorium coccineum* L. against Candida albicans has been investigated (Griffin et al., 2017). The antiradical potential was investigated by rendering the plant material into small sized particles in the presence of surfactants using *Salvia officinalis* L, *Laurus nobilis* L., *Nigella sativa* L., *Vitis vinifera* L., *Angelica sinensis* Diels, *Ziziphus jujube* Mill., *Lycium chinense* Mill., *Jasminum* L. *Argania spinosa* L. (Abraham et al., 2020) *Silybum marianum*, *Elettaria cardamomum* and *Coriandrum sativum* (Jahan et al., 2016). Therefore, the objective of this study was to liberate the antioxidant properties of the medicinal plant *V. guineensis* Benth. (Asteraceae) by bead-milling and in the absence of surfactants to strike against oxidative stress which leads to chronic and degenerative illnesses.

Methods

Plant collection

The plants used in this study are well known for their virtues in traditional medicine. The tubers of *V. guineensis* Benth. (Asteraceae) shown in Fig. 1 were collected in September 2019 from Dogmoa village, Nkongsamba Arrondissement, Moungo Department, Littoral Region Cameroon. They were authenticated by Eric Ngansop Tchatchouang, botanist at the National Herbarium, Yaounde, in comparison with a voucher specimen previously deposited N° 4869/SRFK.

Extraction

The tubers of *V. guineensis* Benth. were cut, dried for 3 days at 25°C and pulverized. The powders were stored cold for further analysis. The aqueous extracts were obtained by maceration of 450 g of *V. guineensis* tuber powder in 4500 mL of water at room temperature (25 ^{o}C) for 2 days. The macerate obtained was filtered on Whatman paper Nr 4 and concentrated in a water bath at 60°C to the crude dry extract, which was stored at 4 ^{o}C for further use. The yield was obtained using formula 2.

Yield (%) = (Mass of extract / Mass of plant material) x 100 (2)

Phytochemical screening

Qualitative phytochemical screening of the extract was performed to identify the main groups of chemical constituents present using the following established colour reactions.

Alkaloids test: 1 mg of extract was stirred with 6 mL 1% HCl on a water bath for 5 min and filtered. 1 mL of filtrate was mixed with 1 mL of Dragendorff's reagent (potassium bismuth iodide solution). The appearance of an orange-red precipitate indicated the presence of alkaloids (Banso and Adeyemo, 2006).

Quinones test: A yellow coloured precipitate which appeared after the addition of sample plant extract with concentrated HCl confirmed the presence of quinones (Ismail et al., 2017).

Saponins test: 10 mL of distilled water were added to 5 mL of the extract in a test tube. The mixture was subsequently stirred for 20 s and allowed to stand for 15 min. The presence of saponins was confirmed by the appearance of a persistent foam of height greater than 1 cm (Harborne, 1998).

Flavonoids test: A few drops of concentrated HCl and 2–3 magnesium chips were added to 5 mL of the extract in a test tube. The release of heat and the appearance of a pink-orange or purplish colour indicated the presence of flavonoids (Harborne, 1998).

Polyphenols test: A drop of alcoholic solution of 2% ferric chloride ($FeCl_3$) was added to 2 mL of the extract. The appearance of a blue or violet complex confirmed the presence of polyphenols (Yeo et al., 2011).

Coumarin test: 3 mL of 10% NaOH was added to 2 mL of aqueous plant extract. The appearance of a yellow colour confirmed the presence of coumarins (Ismail et al., 2017).

Steroids and terpenoids test: The crude extract (about 100 mg) was thoroughly mixed with chloroform (2 mL) followed by the addition of concentrated H_2SO_4 (2 mL) along the wall of the test tube formation of a reddish-brown coloration at the interface indicated the presence of terpenoids (Ayoola et al., 2008).

Tannin test: 0.5 g of extract was stirred with distilled water (10 mL) and filtered. A few drops of 5% $FeCl_3$ were then added to the filtrate. The appearance of blue-black or green coloration or precipitate affirmed the presence of tannins (Banso and Adeyemo, 2006).

Determination of Total phenolic content

The total phenol content was determined by the Folin-Ciocalteu method. A volume of 23 µL (1 mg/mL) of each extract was mixed with 1817 µL of distilled water, 115 µL of the Folin-Ciocalteu reagent (1: 10 dilution, v/v; Merck KGaA, Darmstadt, Germany) and 345 µL of sodium carbonate solution (15%). The mixture was incubated at room temperature in the dark for 2 h and the absorbance was recorded at 765 nm on a Cary50 Bio UV/VIS spectrophotometer (Varian Australia Pty Ltd., Mulgrave, Australia). Gallic acid (Thermo Scientific, Waltham, MA, USA) was used as the reference standard and the results were expressed as milligram equivalents of gallic acid per gram of dry extract (mg EAA/g ES) (Singleton et al., 1999).

Determination of flavonoid content

To determine the flavonoid content, a volume of 1 mL of extract was added to 1 mL of 2% aluminium chloride in methanol. The mixture was placed in the dark for 10 min and then the absorbance was recorded at 430 nm. The results were expressed as mg of quercetin equivalent per 1 g of dry weight of the samples. These concentrations were determined by referring to the calibration curve performed with quercetin prepared in methanol at 200 µg /mL (Hegazy and Ibrahium, 2012).

Nanosizing and size determination of *V. guineensis* tubers

The sample was nanosized according to the method described in literature and modified by using wet-milled samples (Darkal et al., 2021; Vaso et al., 2021).

DPPH radical scavenging capacity

The percentage of DPPH radical scavenging was calculated using formula 3:

% scavenging activity = [Absorbance (control) - Absorbance (extract)] /Absorbance (control) × 100 (3)

The percentage obtained for every concentration was plotted as a graph using GraphPad Prism software (version 8.0.1 for Windows, GraphPad Software, San Diego, California USA)

Statistical analysis

The descriptive statistics and the comparison of mean values were performed with GraphPadPrism software (version 9.1.1). Data were proven for normal distribution and variance homogeneity with the Shapiro Wilk and Levene test, respectively. Multiple comparisons were subsequently performed with one-way ANOVA and two-way ANOVA with Tukey correction. *P*-values < 0.05 were considered statistically significant.

Results

Extraction and phytochemical study

An extract of pasty appearance with 9.2% yield was obtained. The secondary metabolites contained in *V. guineensis* tubers aqueous extract are recorded in Table 1. *V. guineensis* extract contains alkaloids, coumarins, polyphenols, saponins, terpenes and anthraquinones. Flavonoids are absent from the aqueous extract.

Table 1
Results of the screening performed on the aqueous extract of *V. guineensis* tubers.

Secondary metabolites test	**Alkaloids**	**Coumarins**	**Flavonoïdes**	**Polyphenols**	**Saponins**	**Tanins**	**Terpenes**	**Anthraquinones**
Aqueous extract of *V. guineensis* tubers (AEQVG)	+	+	-	+	+	+	+	+
Caption: + = presence; - = absence; AEVG = aqueous extract of *V. guineensis* tubers								
Total phenol and flavonoid content								

The contents of total phenols and flavonoids in the extracts of *V. guineensis* are presented in Table 2. They are determined graphically by linear regression of the standard curve of gallic acid for total phenols and quercetin for flavonoids. In carrot like tuber of *V. guineensis*, a polyphenol content of 28.43 mg EAA/g ES and a zero flavonoid content are obtained.

Table 2
Total phenol and flavonoid content of the aqueous extract

Aqueous extract solution at 1 mg/ml	**TPC in mg EAG/g extract**	**TFC in mg EQ/g dry extract**
Tuber of *V. guineensis*	28.43	0.00

The calibration curve for gallic acid is shown in Supplement 1a and the calibration curve for quercetin in 1b. TPC total phenol content, TFC total flavonoids content

Nanoformulation and dispersion studies

The width parameter known as the polydispersity index (PdI) (formula 2) is defined as the standard deviation (σ) of the particle diameter distribution divided by the mean particle diameter (a).

PdI=(σ/2a)2 (2)

The polydispersity index (PdI) measures the uniformity of a sample. It is dimensionless and the value ranges from 0 to 0.05 for a monodispersed sample and is larger than 0.08 for midrange polydispersity. Above 0.7, the sample is polydispersed and is not suitable for DLS. The Z-average and PdI are important characterization parameters, as they give the size and size distribution.

Having identified the secondary metabolites present in the plant extract, the nanoformulation was obtained via ball milling and high pressure dispersion. The zeta nanosizer was employed to determine the size and Zeta potential of the particles. Water was used as the dispersing medium. The distribution curve shows a polydispersed system with large particles of size close to 1000 nm and a Z-average of 484.5 nm. The dispersed system (water-metabolites dissolved in aqueous media-plant particles) forms a colloidal dispersion with a supernatant liquid containing smaller molecules which agglomerate in a suspension to heavier particles. Polydispersity indices below 0.7 have been obtained for both the supernatant (0.217) and the suspension (0.543) (see Fig. 2). In the supernatant and suspension, the particles are negatively charged with a potential of -12.3 mV and − 13.7mV, respectively.

In the supernatant and suspension, the particles are negatively charged with a potential of -12.3 mV and − 13.7mV, respectively.

The size distribution by volume of suspension and the supernatant of *V. guineensis* is presented in Fig. 3. The results of the duplicate analysis report the quantities Dv(0.1), Dv(0.5), and Dv(0.9) which correspond to the diameters below which 10, 50 and 90% of the volume of the particles are located, respectively. Dv [4,3] corresponds to the average diameter in volume.

DPPH scavenging study

The percentages of DPPH scavenging at different concentrations for *V. guineensis* tuber extracts, their nanosized particles and ascorbic acid are presented in Fig. 4. The potential to inhibit free radical formation is not significative at low concentrations of 0.025 and 0.025 mg/mL concentration of plant extract and nanoparticles generated from the organic material. The difference in scavenging activity is not significative between the reference drug ascorbic acid at 0.25 and 2.5 mg/mL. At 0.25 mg/mL and 2.5 mg/mL the scavenging activity of nanosized *V. guinensis* tuber is significatively higher than extract ($P = 0.0116$) and positive control ascorbic acid ($P < 0.0001$).

Discussion

Plants are a source of secondary metabolites and are used for pharmaceutical or medicinal purposes. Nanoformulation is a way to potentiate the therapeutic power of plants. Thus, *V. guineensis* tubers have been extracted in water in order to perform a phytochemical screening. The pasty appearance can be due to the presence of residual water maintained in the material or the presence of lipids. The yield of 9.2% is higher than that obtained by Toyang and coworkers (8.7%) after a double extraction in dichloromethane (1 kg of *V. guineensis* tuber powder, 4 L of dichloromethane) of the plant collected in the North West Region of Cameroon in 2009 (Toyang et al., 2012). *V. guineensis* tubers contains alkaloids, coumarins, polyphenols, saponins, terpenes and anthraquinones secondary metabolites. Flavonoids are absent from the aqueous extract. Polyphenols are secondary metabolites of plants and are ubiquitous in the plant kingdom and are generally involved in the defense against ultraviolet light or invasion by pathogens (Mukherjee, 2019). They are recognized for their antioxidant properties, important for reducing oxidative stress, which is related to the pathophysiology of multiple diseases, such as neurodegenerative diseases, inflammation, cancer, cardiovascular health, type 2 diabetes, or obesity (García-Pérez et al., 2017; Hannah et al., 2018). Polyphenols are commonly subdivided into tannins, lignins, flavonoids, and non-flavonoids (Jideani et al., 2021) and are found in carrot extracts such as tuber *V. guineensis*.

No flavonoids during phytochemical screening has been observed in previous report, for example when *Trichoscypha acuminata* Engl (Anacardiaceae) stem bark is used (Mbosso Teinkela *et al.*, 2023). This observation is corroborated by the phytochemical screening (Table 1). The variability in polyphenol content and antioxidant activity are generally related: - to the section of the

plant used *i.e.* generative part (flowers, seeds) > leaves > root > stem (for flowering and fruiting stages)) (Feduraev et al., 2019); - to the time period (Feduraev et al., 2019); - to plant species (Sarfaraz et al., 2021); - to climate, geographical variations and environment (Kabtni et al., 2020).

The distribution curve of *V. guinensis* shows a polydispersed system. Particles combine with each other because of Brownian movement, which favours the formation of colloidal-size particles. The interest in using water as a dispersing medium is beneficial for several reasons: its availability in low-cost resource-limited regions, nature conservation considerations, religious acceptance, and its use in traditional medicine preparations. It should be emphasized that no surfactants, such as Plantacare intended for medicinal applications, agriculture or polymers (chitosan, collagen, gelatine, hyaluronic acid, and PLGA), solid lipids (cholesterol, palmitic acid and stearic acid), and proteins (milk protein, nisin, and zein) used in food processing have been employed as part of this investigation (Griffin et al., 2016; Griffin et al., 2017; Miyazawa et al., 2021). The dispersed system (water-metabolites dissolved in aqueous media-plant particles) forms a colloidal dispersion with a supernatant liquid containing smaller molecules which agglomerate in a suspension to heavier particles. The dispersed system shows a tendency to agglomerate due to hydrophilic and hydrophobic interactions and van der Waals forces. Hartmann and colleagues have previously noted the difficulty in preparing stock solutions of manufactured nanoparticles in solution (Hartmann et al., 2015).

Additionally, particle size and uniformity play an important role in drug formulation. The average size obtained is of the same order of magnitude as after using different milling instruments for the production of drug nanoparticles (< 500 nm) (Loh et al., 2015). Thus a maximum dispersion can be obtained in well diluted solutions.

Ideally, the particle size distribution should be uniform (PdI = 0) (Danaei et al., 2018). Indeed, particle size, PdI and Zeta potential of nanoformulated systems are the main physicochemical factors influencing endocytosis-dependent cellular uptake, material transport into cells, and cellular absorption (Sadat et al., 2016). The application of an electric field and the movement of nanoparticles (electrophoretic mobility) in a sample is measured by laser Doppler velocimetry as part of Zeta potential measurements. (Clogston and Patri, 2011; Borthagaray et al., 2018). The Zeta potential is used to study the interactions between colloids and electrolytes of the same or opposite charge (Rajpoot *et al*., 2019).

Nanoparticles with a Zeta potential between − 10 and + 10 mV are considered approximately neutral, while nanoparticles with Zeta potentials greater than + 30 mV or less than − 30 mV are considered strongly cationic and strongly anionic, respectively (Clogston and Patri, 2011). The Stern layer is composed of a stratum of strongly bound ions on the surface of neutrally charged nanoparticles in an ionic solution (Clogston and Patri, 2011). Thus, the nanoparticles produced from *V. guineensis* have a low negative charge and therefore a medium flocculation capacity. The manufacture and stabilization of this plant nanosuspension consequently will require additional input of dispersion-like sonication.

The Mastersizer analysis indicates that there are smaller particles in volume in the supernatant than in the suspension. These large particles represent less than 10% of the amount of nanoparticles in this suspension. The nanoparticles present in the supernatant of all groups of percentage are significatively smaller than those in the corresponding suspension. The study of the dynamic aggregation may require measurement of the nanoparticle's formation in function of time.

The DPPH assay is commonly used to evaluate the free radical scavenging potential of an antioxidant molecule and is considered one of the standard and easy colourimetric methods for evaluating the antioxidant properties of pure compounds. Nanoparticles produced by nanoscale size reduction offer improved solubility, enhanced molecule transport and, consequently, bioavailability and biological activity. In the case of cosmetics, the release kinetics on the lipid/aqueous surface of the skin are improved thanks to the new technologies. (Griffin et al., 2017). *The V. guineensis* nanoparticles trapped significantly more DPPH radicals compared to ascorbic acid and crude extract. The percentage of scavenging is concentration dependent; properties of the actual drugs. At 0.025 mg/mL the nanoparticles and the extract activities differ insignificantly. At 2.5 mg mL^{-1} the antiradical activity of the nanoparticles is significatively different compared to the crude extracts. This experiment shows the positive impact of reducing the size of plant nanoparticles on the antioxidant power. The *V. guineensis* nanoparticle solution with nanocolloidal sizes obtained has a higher antioxidant power than the plant solution. The size reduction would therefore be beneficial for the fight against diseases related to oxidative stress

Conclusions

The tubers of *Vernonia guineensis* Benth. (Asteraceae) were reduced to nanocolloidal sizes by ball milling. A phytochemical study confirmed the main classes of secondary metabolites contained in the plant. The treatment with water as dispersion medium led rapidly to two phases in the experimental conditions: a supernatant and a suspension due to aggregation of the plant nanoparticles. A substantial increase in antioxidant activity, compared to ascorbic acid, is obtained in the nanoformulated state, allowing the potential of size reduction for the formulation of plant-based drugs. The method can find usability to increase the potential of local traditional based preparations.

Abbreviations

DPPH: 2,2-diphenyl-1-picrylhydrazyl

DLS: Dynamic Light Scattering

PCS: Photon Correlation Spectroscopy

PdI: Polydispersity Index

Declarations

Ethics approval and consent to participate

Study was approved by the Institutional Ethic Committee for Research on Human Life the University of Douala Nr 2210 CEI-Udo/02/2020/T

Consent for publication

NA

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests

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Authors Contributions

Siegfried Didier Dibong and Muhammad Jawad Nasim nanosized characterized the particles. Siegfried Didier Dibong and Yossa Djomaha Ludrice Dorence conducted and analysed the biological studies. Gisele Etame Loe, Siegfried Didier Dibong, Claus

Jacob contributed by supervising the studies at their respective institutes. Francois Eya'ane Meva, Yossa Djomaha Ludrice Dorence, Muhammad Jawad Nasim and Ntoumba Agnes Antoinette drafted the manuscript. The latter includes work, text, and figures which were prepared jointly by all authors. Mvogo Ottou Patrice Brice and Yossa Djomaha Ludrice Dorence contributed to the study by collecting and identifying the plants.

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References

1. Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, Prakash Mishra A, Nigam M, El Rayess Y, El Beyrouthy M, Polito L, Iriti M, Martins N, Martorell M, Oana Docea A, Setzer WN, Calina D, Cho WC, Sharifi-Rad J. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. Front. Physiol. 2020; 11: 694. https://doi.org/10.3389/fphys.2020.00694
2. Podkowinska A and Formanowicz D. Chronic Kidney Disease as Oxidative Stress-and Inflammatory-Mediated Cardiovascular Disease Antioxidants 2020; 9: 752; doi:10.3390/antiox9080752
3. Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol. 1997;82(2):291–5. https://doi.org/10.1113/expphysiol.1997.sp004024.
4. Kuete V, Efferth T. Cameroonian medicinal plants: pharmacology and derived natural products. Front Pharmacol. 2010; 1:123. Published 2010 Oct 25. https://doi.org/10.3389/fphar.2010.00123
5. Khalid K, Tan X, Zaid HFM, Tao Y, Chew CL, Chu D-T, Lam MK, Ho Y-C, Lim JW & Wei LC Advanced in developmental organic and inorganic nanomaterial: a review, Bioengineered, 2020; 11:1: 328–355, https://doi.org/10.1080/21655979.2020.1736240
6. Raval N, Maheshwari R, Kalyane D, Youngren-Ortiz SR, Chougule MB, Tekade RK. Chapter 10 - Importance of Physicochemical Characterization of Nanoparticles in Pharmaceutical Product Development, Academic Press, Basic Fundamentals of Drug Delivery, Advances in Pharmaceutical Product Development and Research, 2019: 369–400. https://doi.org/10.1016/B978-0-12-817909-3.00010-8
7. Ijaz I, Gilani E, Nazir A & Bukhari A (2020) Detail review on chemical, physical and green synthesis, classification, characterizations and applications of nanoparticles, Green Chemistry Letters and Reviews, 2019; 13(3): 223–245, https://doi.org/10.1080/17518253.2020.1802517
8. Loh ZH, Samanta AK, Heng PWS (2015) Overview of milling techniques for improving the solubility of poorly water-soluble drugs Asian Journal of Pharmaceutical Sciences, 2015; 10(4): 255–274. https://doi.org/10.1016/j.ajps.2014.12.006.
9. Ramachandraiah K, Chin KB, Evaluation of ball-milling time on the physicochemical and antioxidant properties of persimmon by-products powder, Innovative Food Science & Emerging Technologies 2016; 37: 115–124, https://doi.org/10.1016/j.ifset.2016.08.005.
10. Shahbaziniaz M, Foroutan SM, Bolourchian N. Dissolution rate enhancement of clarithromycin using ternary ground mixtures: nanocrystal formation. Iran J Pharm Res. 2013; 12(4): 587–598. PMID: 24523739; PMCID: PMC3920702.
11. Griffin S, Masood MI, Nasim MJ, Sarfraz M, Ebokaiwe AP, Schäfer KH, Keck CM, Jacob C. Natural Nanoparticles: A Particular Matter Inspired by Nature. Antioxidants (Basel). 2017; 7(1): 3 https://doi.org/10.3390/antiox7010003..
12. Lim J, Yeap SP, Che HX, Low SC. Characterization of magnetic nanoparticle by dynamic light scattering. Nanoscale Res Lett. 2013; 8: 381. https://doi.org/10.1186/1556-276X-8-381
13. Burkill BM 'The Useful Plants of West Tropical Africa', Roy. Bot. Gardens, 1985; 1.

14. Toyang NJ, Wabo HK, Ateh EN, Davis H, Tane P, Kimbu SF, Sondengam LB, Bryant J. In vitro anti-prostate cancer and ex vivo antiangiogenic activity of Vernonia guineensis Benth. (Asteraceae) tuber extracts. Journal of Ethnopharmacology, 2012; 141(3): 866–871. https://doi.org/10.1016/j.jep.2012.03.021.
15. Wouamba CNS, Mouthé Happi G, Nguiam Pouofo M, Tchamgoue J, Jouda JB, Longo F, Ndjakou Lenta B, Sewald N, Fogue Kouam S. Antibacterial Flavonoids and Other Compounds from the Aerial Parts of Vernonia guineensis Benth. (Asteraceae). Chem Biodivers. 2020a;17(9):e2000296. https://doi.org/10.1002/cbdv.202000296.
16. Toyang NJ, Ateh EN, Davis H, Tane P, Sondengam LB, Bryant J, and Verpoorte R. In vivo antiprostate tumor potential of Vernonia guineensis Benth. (Asteraceae) tuber extract (VGDE) and the cytotoxicity of its major compound pentaisovaleryl sucrose. J Ethnopharmacol. 2013a; 150(2): 724–728. https://doi.org/10.1016/j.jep.2013.09.028.
17. Toyang NJ, Krause MA, Fairhurst RM, Tane P, Bryant J, and Verpoorte R. Antiplasmodial activity of sesquiterpene lactones and a sucrose ester from Vernonia guineensis Benth. (Asteraceae) J Ethnopharmacol. 2013b; 147(3): 618–621. https://doi.org/10.1016/j.jep.2013.03.051.
18. Toyang NJ, Wabo HK, Ateh EN, Davis H, Tane P, Sondengam LB, Bryant J, and Verpoorte R. Cytotoxic sesquiterpene lactones from the leaves of Vernonia guineensis Benth. (Asteraceae), J Ethnopharmacol. 2013c; 146(2): 552–556. https://doi.org/10.1016/j.jep.2013.01.022
19. Noumi E. Ethno medicines used for treatment of prostatic disease in Foumban, Cameroon. African J. of Pharmacy and Pharmacology, 2010; 4(11): 793–805.
20. Wouamba STN, Happia GM, Lenta BN, Sewald N, Kouam SF. Vernoguinamide: A new ceramide and other compounds from the root of Vernonia guineensis Benth. and their chemophenetic significance Biochemical Systematics and Ecology, 2020b; 88, 103988 https://doi.org/10.1016/j.bse.2019.103988
21. Griffin S, Tittikpina NK, Al-Marby A, Alkhayer R, Denezhkin P, Witek K, Gbogbo KA, Batawila K, Duval RE, Nasim MJ, Awadh-Ali N A, Kirsch G, Chaimbault P, Schäfer K-H, Keck C M, Handzlik J, and Jacob C. Turning waste into value: Nanosized natural plant materials of Solanum incanum L. and Pterocarpus erinaceus poir with promising antimicrobial activities. Pharmaceutics, 2016; 8: 11. https://doi.org/10.3390/pharmaceutics8020011.
22. Griffin S, Alkhayer R, Mirzoyan S, Turabyan A, Zucca P, Sarfraz M, Nasim MJ, Trchounian A, Rescigno A, Keck CM and Jacob C. Nanosizing Cynomorium: Thumbs up for Potential Antifungal Applications Inventions 2017; 2: 24 https://doi.org/10.3390/inventions2030024
23. Abraham AM, Alnemari RM, Jacob C, Keck CM. PlantCrystals-Nanosized Plant Material for Improved Bioefficacy of Medical Plants. Materials. 2020; 13(19): 4368. https://doi.org/10.3390/ma13194368.
24. Jahan N, Aslam S, ur Rahman K, Fazal T, Anwar F & Saher R (2016) Formulation and characterisation of nanosuspension of herbal extracts for enhanced antiradical potential, Journal of Experimental Nanoscience, 2016; 11(1): 72–80, https://doi.org/10.1080/17458080.2015.1025303
25. Banso A, and Adeyemo S (2006) Phytochemical screening and antimalarial assessment of Abutilon mauritianum, Bacopa monnifera and Datura stramonium. Biokemistri, 2006; 18: 39–44. https://doi.org/10.4314/biokem.v18i1.56390
26. Ismail M, Noor-Ul-Haq, Ali S, Ali I, Shaheen R, Murtaza G and Abdurrazaq (2017) Qualitative analysis for phytochemicals of selected medicinal plants from Gilgit-Baltistan, Pakistan Asian Journal of Chemistry; 2017; 29(9): 1929–1932. https://doi.org/10.14233/ajchem.2017.20583.
27. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis, Springer Science et Business Media, 1998.
28. Yeo D, Dinica R, Yapi HF, Furdui B, Praisler M, Djaman AJ, N'Guessan JD. Évaluation de l'activité anti-inflammatoire et screening phytochimique des feuilles de Annona senegalensis. Thérapie, 2011; 66(1): 73–80. https://doi.org/10.2515/therapie/2010076
29. Ayoola GA, Coker HB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria. Trop. J. Pharm. Res. 2008; 7: 1019–1024. https://doi.org/10.4314/tjpr.v7i3.14686

30. Singleton VL, Orthofer R, Lamuela-Raventós RM. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent Methods in Enzymology, 1999; 299: 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1
31. Hegazy AE and Ibrahium MI. Antioxidant Activities of Orange Peel Extracts World Applied Sciences Journal, 2012; 18(5): 684–688. https://doi.org/10.5829/idosi.wasj.2012.18.05.64179
32. Darkal A K, Zuraik Mhd M, Ney Y, Nasim M J, Jacob C, Unleashing the biological potential of *Fomes fomentarius* via dry and wet Milling. Antioxidants, 2021; 10(2): 303. https://doi.org/10.3390/antiox10020303
33. Vaso M, Ali W, Masood MI, Muhammad Jawad Nasim MJ, Lilischkis R, Schäfer K-H, Schneider M, Papajani VT, Jacob C. Nanosizing Nigella: A cool alternative to liberate biological activity, Current Nutraceuticals, 2021; 2: 37–46. https://doi.org/10.2174/2665978601999200930143010
34. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity LWT - Food Science and Technology, 1995; 28(1): 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5
35. Mukherjee PK. Phyto-Pharmaceuticals, Nutraceuticals and Their Evaluation, in Quality Control and Evaluation of Herbal Drugs, Evaluating Natural Products and Traditional Medicine Pages, 2019: 707–722
36. García-Pérez ME, Kasangana PB, Stevanovic T. Bioactive Polyphenols for Diabetes and Inflammation in Psoriasis Disease, in Studies in Natural Products Chemistry, 2017; 52: 231–268. https://doi.org/10.1016/B978-0-444-63931-8.00006-0
37. Hannah C, Simone P, John S, Martha T, Josiemer M. The Role of Polyphenols in Human Health and Food Systems: A Mini-Review. Frontiers in Nutrition, 2018; 5: 1–9. https://doi.org/10.3389/fnut.2018.00087
38. Jideani AIO, Silungwe H, Takalani T, Omolola AO, Udeh HO & Anyasi TA. Antioxidant-rich natural fruit and vegetable products and human health, International Journal of Food Properties, 2021; 24(1): 41–67, https://doi.org/10.1080/10942912.2020.1866597
39. MbossoTeinkela JE, Yoyo Ngando LP, Bamal HD, Ekounè Kamè E, Mpondo Mpondo EA, Assob Nguedia JC, Fannang SV. Evaluation of in vitro antiplasmodial, antiinflammatory activities and in vivo oral acute toxicity of *Trichoscypha acuminata* Engl (Anacardiaceae) stem bark. National Journal of Pharmaceutical Sciences 2023; 3(1): 107–114
40. Feduraev P, Chupakhina G, Maslennikov P, Tacenko N, Skrypnik L. Variation in Phenolic Compounds Content and Antioxidant Activity of Different Plant Organs from *Rumex crispus* L. and *Rumex obtusifolius* L. at Different Growth Stages. Antioxidants (Basel). 2019; 8(7): 237. https://doi.org/10.3390/antiox8070237.
41. Sarfaraz D, Rahimmalek M, Saeidi G. Polyphenolic and molecular variation in Thymus species using HPLC and SRAP analyses. Sci Rep. 2021; 11(1): 5019. https://doi.org/10.1038/s41598-021-84449-6.
42. Kabtni S S, Dorra D, Ines BB, Mattew S, Trifi-Farah N, Fauconnier M-L and Marghali S. Influence of climate variation on phenolic composition and antioxidant capacity of Medicago minima populations. Sci Rep. 2020; 10: 8293. https://doi.org/10.1038/s41598-020-65160-4
43. Miyazawa T, Itaya M, Burdeos GC, Nakagawa K, Miyazawa T. A Critical Review of the Use of Surfactant-Coated Nanoparticles in Nanomedicine and Food Nanotechnology. Int J Nanomedicine. 2021; 16: 3937–3999. https://doi.org/10.2147/IJN.S298606.
44. Hartmann NB, Jensen KA, Baun A, Rasmussen K, Rauscher H, Tantra R, Cupi D, Gilliland D, Pianella F, Riego Sintes JM. Techniques and protocols for dispersing nanoparticle powders in aqueous media-is there a rationale for harmonization? J Toxicol Environ Health Part B, 2015; 18: 1–28. https://doi.org/10.1080/10937404.2015.1074969
45. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, Khorasani S, Mozafari MR. Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems. Pharmaceutics. 2018; 10(2): 57. https://doi.org/10.3390/pharmaceutics10020057.
46. Sadat SM, Jahan ST, Haddadi A Effects of size and surface charge of polymeric nanoparticles on in vitro and in vivo applications. J. Biomater. Nanobiotechnol. 2016; 7: 91. https://doi.org/10.4236/jbnb.2016.72011
47. Clogston JD and Patri AK (2011) Zeta Potential Measurement Scott E. McNeil (ed.), Characterization of Nanoparticles Intended for Drug Delivery, Methods in Molecular Biology, 2011; 697, https://doi.org/10.1007/978-1-60327-198-1_6 63–70 2011

48. Borthagaray G, Mondelli M, Facchin G, Torre MH, Chap. 8 - Silver-containing nanoparticles in the research of new antimicrobial agents against ESKAPE pathogens, Editor(s): Alexandru Mihai Grumezescu, Inorganic Frameworks as Smart Nanomedicines, William Andrew Publishing. 2018, 317–386. https://riquim.fq.edu.uy/items/show/5734.
49. Rajpoot K, and Tekade RK. Chapter 10 - Microemulsion as drug and gene delivery vehicle: an inside story. Rakesh K. Tekade Ed., In Advances in Pharmaceutical Product Development and Research, Drug Delivery Systems, Academic Press, 2019: 455–520, https://doi.org/10.1016/B978-0-12-814487-9.00010-7.

Figures

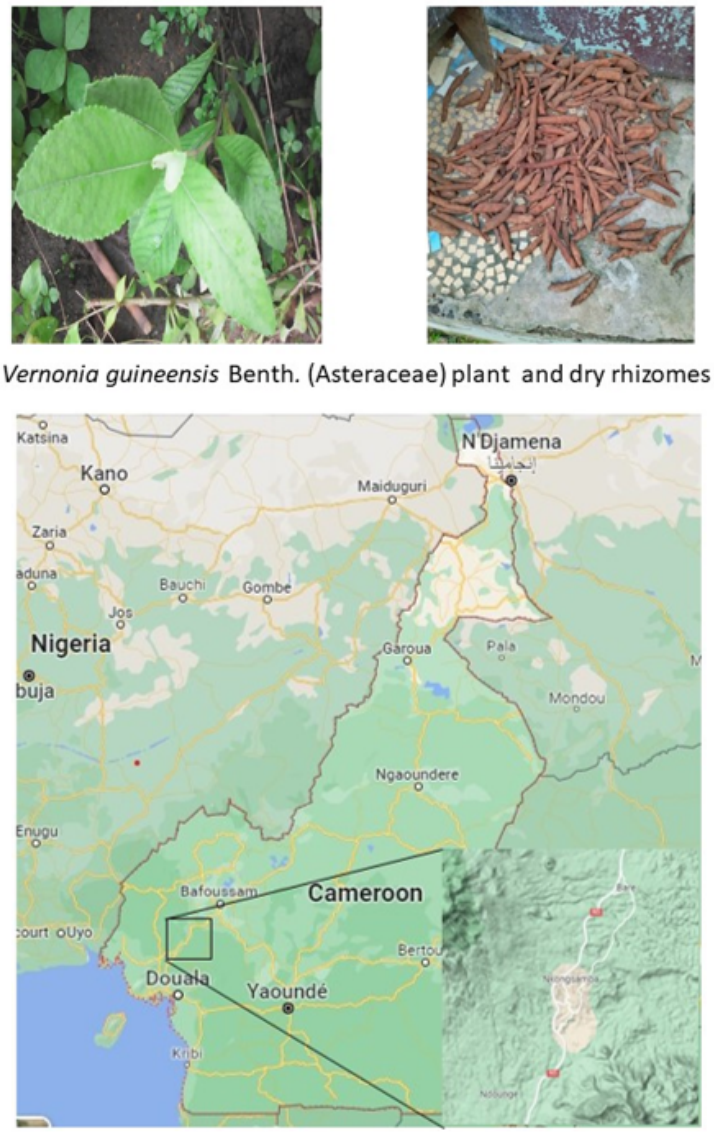

Figure 1

Vernonia guineensis Benth. (Asteraceae) and geographic location (The maps have been reproduced employing Google Maps).

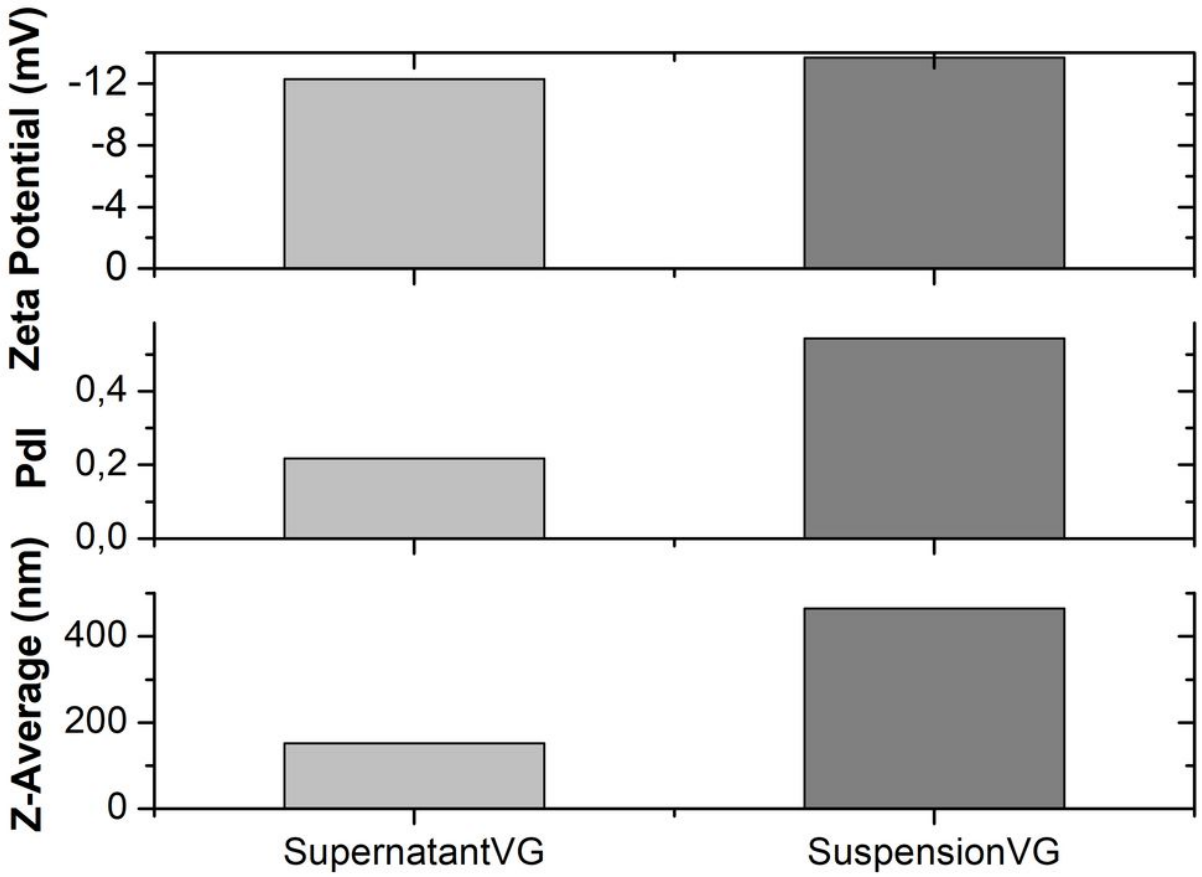

Figure 2

Z-Average, PdI and Zeta Potential for suspension and supernatant of *V. guineensis*

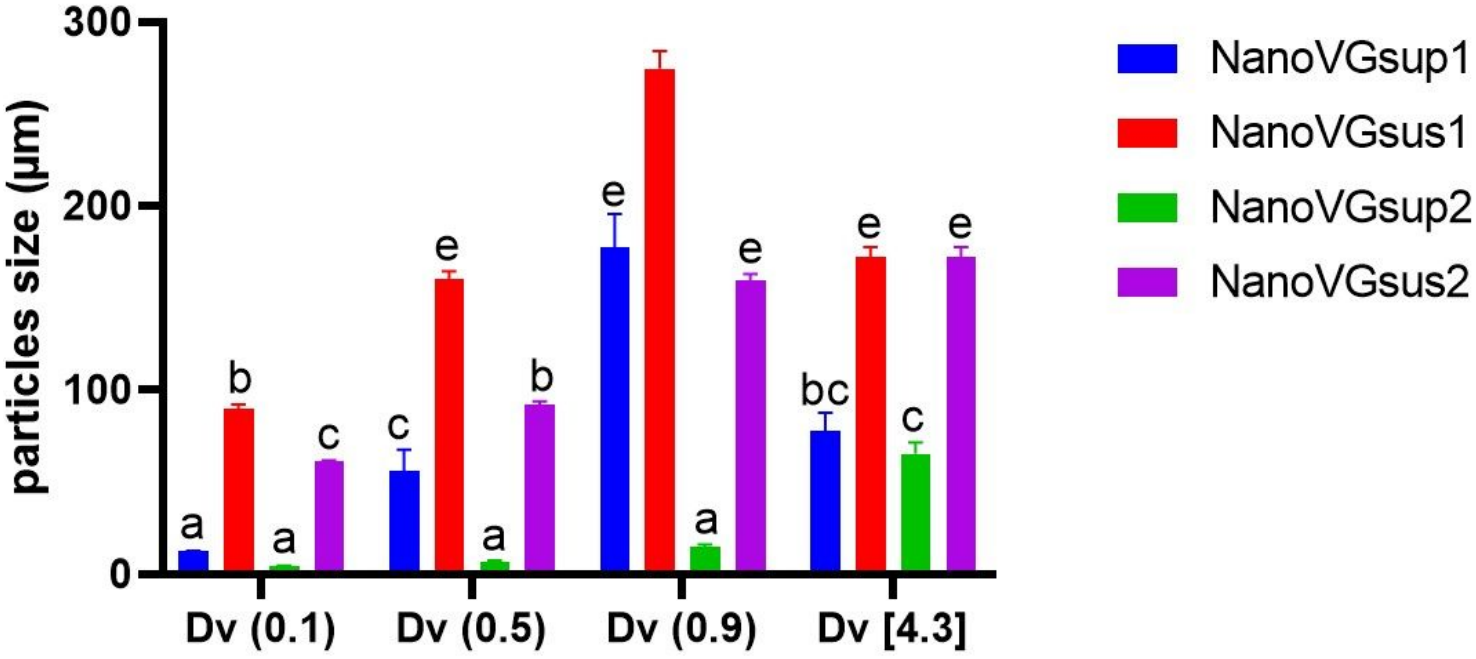

Figure 3

Size distribution by volume for *V. guineensis*, $P < 0.05$ Tukey's multiple comparison test between distributions. Similar letters indicate non-significative comparisons.

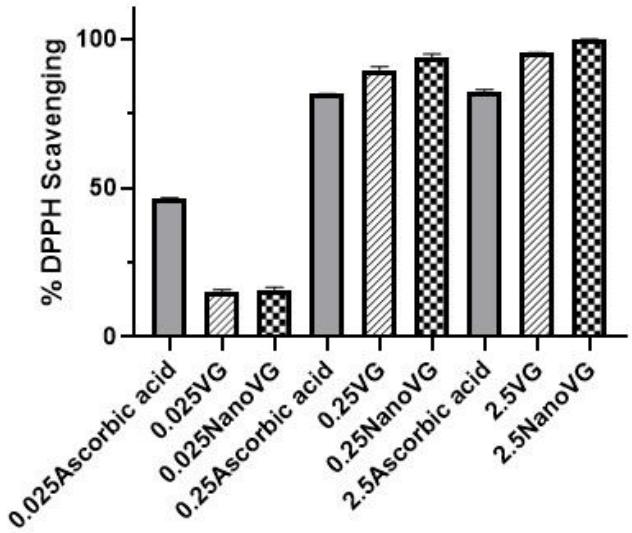

Figure 4

Percentage of DPPH scavenging at different concentrations for *V. guineensis* extracts, their nanoparticles, and vitamin C