E-ISSN: 2278-3229

IJGHC, September 2020 –November 2020 Sec.B; Vol.9, No.4, 578-591.
DOI: 10.24214/IJGHC/HC/9/4 /57891.

International Journal of Green and Herbal Chemistry

An International Peer Review E-3 Journal of Sciences

Available online at www.ijghc.com

Section B: Herbal Chemistry

Research Article

CODEN (USA): IJGHAY

Phytofabricated silver nanoparticles using *Vernonia conferta* aqueous leaves extract enhance wound healing in experimental rats

Moïse Henri Julien Nko'o[1], Philippe Belle Ebanda Kedi[2], Simone Veronique Fannang[1], Manfred Dimitri Kevin Yvon Kotto Modi[1], Vasily Gvilava[3], Alex Spieß[3], Agnes Antoinette Ntoumba[2], François Eya'ane Meva[1,3]*, Christoph Janiak[3], Nnanga Nga Emmanuel[1], Emmanuel Albert Mpondo Mpondo[1]

[1]Department of Pharmaceutical Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, PO Box 2701 Douala, Cameroon

[2]Department of Animal Biology and Physiology, Faculty of Science, University of Douala, PO Box 24157 Douala, Cameroon

[3]Department of Chemistry and Structural Chemistry, Heinrich-Heine-University Dusseldorf, Dusseldorf, Germany.

Received: 13 October 2020**; Revised:** 27 October 2020; **Accepted:** 04 November 2020

Abstract: Interest in green nanotechnology is rapidly growing due to simplest, affordable, cost-effective and environmental friendly procedures to generate nanomaterial for biomedical application. Plant-based nanoparticles have been reported to exhibit enhanced biological effects as compare to their medicinal extracts counterpart. The present study highlights the wound healing effect of crude extract (AEVC) and silver nanoparticles (AgNPs-VC) synthesized using *Vernonia conferta* leaves. The crude extraction was carried out using distilled water meanwhile AgNPs-VC were obtained by blending plant extract with silver nitrate solution (10:50 v/v). Synthesized AgNPs-VC

were characterized using UV-Visible spectroscopy (UV-Vis), fourier transform infrared spectroscopy (FTIR), powder X-ray diffraction (PXRD), scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). The AEVC and AgNPs-VC were prepared as 5% (v/v) solution and suspension respectively and evaluated for wound healing activity using excision wound model in rats. The changing colour of the reaction mixture and UV-Vis absorbance spectrum validated the formation of AgNPs-VC, showing a characteristic peak at 465 nm. Fourier Transform Infrared Spectroscopy (FTIR) spectrum showed that particles interface were coated with organics and X-ray diffraction pattern showed nanocrystallite nature of pure silver with mean diameter size of 16.25 nm. Highly aggregated spherical/cuboidal silver grain were depicted with SEM. EDS analysis reveals particles consisting of silver as major element. Both AEVC and AgNPs-VC formulations significantly ($P < 0.01$) increased wound contraction rate and reduced period of epithelialization as compared to control group. The wound healing activity of AgNPs-VC treatment was greater than AEVC and trolamine used as standard. These findings clearly demonstrate that *Vernonia conferta* mediated silver nanoparticles enhanced wound healing property of the plant. Hence, the potential advantages of phytopharmacology and nanopharmacology can be combined to result in a new area of drugs discovery.

Keywords: Green nanotechnology, silver nanoparticles, aqueous extract, *Vernonia conferta*, wound healing effect, rat

INTRODUCTION

Skin constitute the outer barrier between the environment and inner organs providing a fundamental role of protection to the body. It is permanently challenged by several external aggressors such as micro-organisms, radiations, chemicals, pressure, mechanical impacts and variation of temperature causing injuries [1,2]. When the skin is wounded, its ability to work as an effective barrier is impaired and the wound healing process should be triggered. Wound repair is a restoration of structure and function of an injured tissue in order to approximate pre-wound characteristics [3]. It is a complex biological process which depends on the type of injury, the underlying disease, systemic mediators, and local wound factors [4]. The effective management of wounds remains a major healthcare concern. It has been reported that many as 1-2% of worldwide populations will acquire a chronic wound during their life-time [5]. Although traditional medicine approaches depending almost entirely upon natural resources have shown promises and continue to be widely practiced, most of the bioactive compounds responsible of plant therapeutic activities are reported to possess insoluble character leading to lower bioavailability and increased systemic clearance [6]. Therefore, developing new strategies to improve the pharmacological action of medicinal plants becomes mandatory. One such alternative is the use of plant-based nanoparticles since their biological effects have been reported to be superior to their medicinal extracts counterpart [7].

Green nanotechnology is the use of biological systems (plants and micro-organisms) to generate materials at the nanoscale level (1-100 nm). Within this size range all the properties (chemical, physical and biological) changes in fundamental ways of both individual atoms/molecules and their corresponding bulk [8]. Due to simplest, affordable, cost-effective and environmental friendly synthetic procedures, and their exceptional properties the use of metallic nanoparticles mediated bio-extract in biomedical area is exponentially increasing. The strategy has been successfully developed over the years as the combination of inorganic nanoparticles to plant secondary metabolites allows a synergistic promoting effect hence,

gain in efficacy [9]. Physicist, chemists, biologists and material scientists have recognized the limitless scientific opportunities and challenges in the applications of of biomelecules-nanoparticles hybrid systems [10]. Bionanosilver have demonstrated exceptional potential exhibiting antimicrobial, anti-inflammatory and wound healing action [11-14].

Vernonia conferta Benth. (Asteraceae) is a medicinal plant mostly distributed in tropical regions including Cameroon. It is used in folk medicine against numerous ailments including stomachache, whooping-cough, convulsive-cough, bronchitis, asthma, skin infection, wounds, jaundice, poisoning, diarrhoea, constipation and worms [15]. Despite the wide spread uses of this plant, there is no scientific evidence to support the claims made by traditional healers. Furthermore, the need for and effective wound-healing agents prompted the present study. Hence, *Vernonia conferta* aqueous extract was screened for phytochemical composition and its silver nanoparticles where generated. The nanoparticles where characterized by ultraviolet visible spectroscopy, infrared spectroscopy, powder X-ray diffraction, scanning electron microscopy and energy dispersive x-ray spectroscopy. The wound healing activity of both aqueous extract and silver nanoparticles was evaluated using excision wound model in rats.

2. MATERIAL AND METHODS

2.1. Chemicals and reagents: All chemicals used in this study were of analytical grade. Silver nitrate ($AgNO_3$) was purchased from Sigma Aldrich Co Ltd Germany. Ketamine, acepromazine and trolamine were obtained commercially and used as received without any further purification. Distilled water was used throughout the reactions.

2.2. Collection, authentication and preparation of extract: *Vernonia conferta* leaves **(Figure 1)** were harvested at Dibombari (N04°11.585; E009° 39.585'), Littoral region, Cameroon, in March 2019 and authenticated at the Cameroon National Herbarium in comparison with a voucher specimen previously deposited (N°29458/HNC). The freshly collected material was thoroughly washed with running tap water followed by distilled water to remove all surface contaminants and finely cut. 10 g of the plant was introduced into a conical flask containing 100 ml preheated distilled water (80°C) and stirred for 5 minutes using hot plate equipped with magnetic stirrer. After cooling at room temperature, the solution was filtered using Whatman paper n°1, introduced into a Petri dish and left overnight in an oven at 45 °C resulting in the complete evaporation of the solvent. The dried extract was weighed and the amount of plant extract present in the initial solution was calculated.

Figure 1:*Vernonia conferta* leaves

2.3. Qualitative phytochemical investigation: The prepared aqueous extract of *Vernonia conferta* (AEVC) was qualitatively screened for its phytochemical composition according to the common phytochemical methods described by Malahubban and collaborators (2013) [16]. The tests were based on the visual observation of colour change or formation of a precipitate after addition of a specific reagents.

2.4. Biological synthesis of silver nanoparticles: For the synthesis of silver nanoparticles the procedures described by Eya'ane Meva and co-workers were followed [12]. 10 mL of AEVC prepared as described in section **2.2** before drying was added to 50 mL silver nitrate aqueous solution (1 mM) for bioreduction process. The mixture was incubated at room temperature in the dark to minimize the photoactivation of silver nitrate under static conditions until changing color appearance. The color change involved the formation of silver nanoparticles. The mixture was then centrifuged (Hettich D-7200 Tuttlingen, Germany) at 6000 rpm for 20 min and washed twice with distilled water and once with ethanol 95°. Purified pellets were kept into petri dish, dried in the oven at 60°C for 24 h and used for characterization studies.

2.5 Characterization of silver nanoparticles

2.5.1 UV-Visible spectroscopic measurement: The formation of silver nanoparticles was monitored by recording UV-Visible spectrum of the reaction mixture (plant extract and silver nitrate solution) on day 14, 18, 22 and 24 after incubation using an UV–visible spectrophotometer (Uv-line 9100 single beam, halogen light source, 1 nm resolution). Distilled water was used as a blank.

2.5.2 Fourier transform infrared spectroscopy*:* The Fourier transform infrared spectrum was recorded at room temperature trough the potassium bromide pellet method. Samples were ground with KBr, pressed into pellets placed in the infrared path and the spectrum was obtained using a Nicolet IS5 model of ThermoScientific operating at a resolution of 0.4 cm^{-1}.

2.5.3 Powder X-ray diffraction: Powder X-ray diffraction measurements of purified silver nanoparticles were carried out at ambient temperature using a BRUKER D2 Phaser diffractometer (Cu Kα, λ = 1.54182 Å, 30 kV) by preparing a film of the silver-organic nanopowder on a flat, low-background silicon sample holder.

2.5.4 Scanning electron microscopy and energy dispersive X-ray spectroscopy: The biosynthesized silver nanoparticles were subjected to scanning electron microscopy (SEM) for morphology determination. A small amount of sample powder was deposited on a carbon coated carbon grid and coated with carbon using a coating sputter coater (Quorum Q 150 TES) to increase the conductivity of the sample. SEM images were taken using Carl Zeiss Auriga Field Emission Scanning Electron microscope (FEG SEM) imagine at 5 keV. Energy dispersive X-ray spectroscopy (EDS) spectrum for elemental analyses was collected with an Oxford Instruments X-Max solid-state silicon drift detector operating at 20 keV coupled to a TECNAI G2 HRSEM.

2.6. Formulation of drug: Administrated medications were prepared as solution and suspension respectively for AEVC and AgNPs-VC following the procedure described by the Encyclopedia of Pharmaceutics Excipient with slight modifications [14]. Briefly, 2.5 mg of oven-dried AEVC was introduced into a Becker containing 2 mL distilled water and grinded. Then, 2 mL water was added and the mixture was grinded. The addition of water and grinding step were repeated until obtaining homogenous mixture. Finally, the volume of the mixture was adjusted to 50 mL. Similar steps were repeated for the preparation of AgNPs-VC suspension.

2.7. Evaluation of wound healing activity:

2.7.1 Animal and ethical considerations: Healthy adult Wistar albino rats of either sex weighing between 150 - 200 g were obtained from the Laboratory Animal Centre of the Department of Pharmaceutical Science, Faculty of Medicine and Pharmaceutical Science, University of Douala, Cameroon. The animals were housed in standard polypropylene cage and maintained under standard conditions of temperature (24 ± 2°C) and light (approximately 12 h/12 h light/dark cycle). They had free access to standard

laboratory diet and tap water *ad libitum*. All experimental procedures were in strict compliance with the approved protocol by the Institutional Ethical Committee of the University of Douala (Protocol approval number CEI-UDo/1399/04/2018/T).

2.7.2 Animals grouping and treatment: After one week of acclimatization in laboratory conditions, the experimental animals were randomly divided into four groups of six rats per group. Animals in group I received no treatment and were considered as control. Group II was treated with trolamine (0.67 %) ointment as a standard drug. Group III and group IV were treated with 5 % AEVC solution and AgNPs-VC suspension respectively. Treatment was gently applied topically by blotting the wound with cotton swab soaked in each formulation to cover the wounded area until reaching complete healing. Experiments were performed during the light phase of the nyctemeral cycle and animals were used only once. All the treatments were given once per day.

2.7.3 Wounds excision: Animals were anesthetized prior to and during creation of the wounds using intra-peritoneal injection of ketamine (50 mg/kg) and acepromazine (10 mg/kg). The rats were inflicted with excision wounds as described by Morton and Malone[17]. After wound area preparation with 70% alcohol, the dorsal fur of the animals was shaved with an electric clipper and the anticipated area of the wound to be created was outlined on the back of the animals on the dorsal thoracic region (1 cm away from vertebral column and 5 cm away from the ear) using a stainless steel stencil. Full thickness excision wounds sized about 12 mm^2 and 2 mm depth (extending down to adipose tissue) were created along the markings using toothed forceps, scalpel and pointed scissors. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. The entire wound was left open to the environment. The animals were then placed in separate cages to avoid any disturbance and the bedding was changed daily. All treatments were given 24 h after wound creation as described above. Wound area, wound contraction and epithelialization period were monitored during the whole healing process.

2.7.4 Determination of wound healing rate: The wound closure rate was assessed as described by Shetty *et al.*, 2012 [18]. Briefly, the wound area of each animal was measured on days 0, 4, 8, 12, 16, 18, and 20 post-wounding using semi-transparent tracing paper and a permanent marker. The tracing paper was placed on a 1 mm^2 graph sheet and traced out. The recorded wound areas were used to calculate the percent wound closure using the equation [1] below. The epithelialization time was noted as the number of days required after wound infliction for the scab to fall off leaving no raw wounds behind.

$$\% \text{ of wound closure} = [(D_0 - D_t)/D_0] \times 100 \qquad [1].$$

Where D_0 is the wound size on day 0, and D_t is the wound size on day t.

2.8 Statistical analysis: Results are express as the mean ± SEM. The difference between treated groups and control group was compared using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. The analyses were carried out using GraphPad Prism Software version 5.03 (Inc., San Diego, CA). P values less than 0.05 were considered significant.

3. RESULTS

3.1. Extraction yield and phytochemical analysis: The extract yield obtained from *Vernonia conferta* leaves using distilled water as solvent was found to be 17% (w/v). Table 1 summarize the metabolite content of AEVC. The qualitative phytochemical analysis reveals the presence of coumarins, saponins, phenols, sterols, and terpenoids which are known to possess several biological activities.

Table 1: secondary metabolites present in AEVC

Metabolites	AEVC
Saponins	+
Coumarins	+
Flavonoids	-
Anthraquinones	-
Sterols	+
Terpenoids	+
Phenols	+

(+) Present (-) Absent

3.2 Visual observation of synthesized nanoparticles: When the AEVC was mixed with silver nitrate solution, the colour of the reaction mixture change slowly from clear yellow to brownish indicating the formation of nanoparticles as depicted on **Figure 2**. The observed changing colour due to formation of Plasmon at the colloid surface occurs 18 days following incubation. No further change was observed indicating the stabilization of the synthesized nanoparticles.

Figure 2: Silver nitrate solution (left), AEVC (middle) AgNPs-VC solution (right)

3.3 UV-Visible spectral analysis: UV-Visible spectra of the reaction mixture recorded against distilled water as a function of time of reaction is shown on **figure 3**. The characteristic Plasmon resonance band confirming the presence of nanoparticles in the sample was observed at 465 nm. The peak appears 18 days following incubation and increased with increasing contact time indicating the bio-reduction of silver ions in aqueous solution.

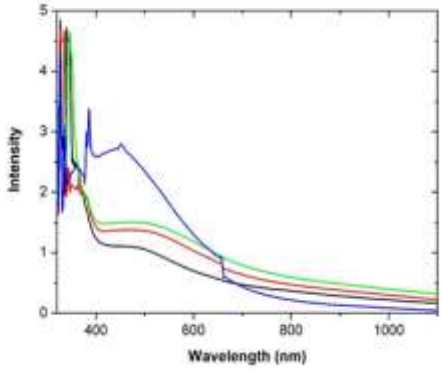

Figure 3: UV-Vis spectra recorded after 18 (black), 22 (red), 24 (green) and 60 (blue) days of incubation.

3.4 Fourier transform infrared spectroscopy results: The infrared spectrum of silver nanoparticles mediated *Vernonia conferta* recorded over the spectral range from 500 cm^{-1} to 4000 cm^{-1} is depicted in Figure 4 and functional groups which acted as capping agents are listed in Table 2. Characteristics IR peak centred at 3435 cm^{-1} is associated with a strong sharp O-H stretch. The peak centred at 2919 cm^{-1} is attributed to stretching mode of C-H bonds. Absorption band at 2846 cm^{-1} is due to stretching vibration of C-H group. The absorption occurring at 1631 cm^{-1} can be attributed to C=C diene compounds. The band at 1029 cm^{-1} is attributed to the stretching vibration mode of C-O bond of alcohol. The absorption band at 551 cm^{-1} is due to the presence of C-Br bonds.

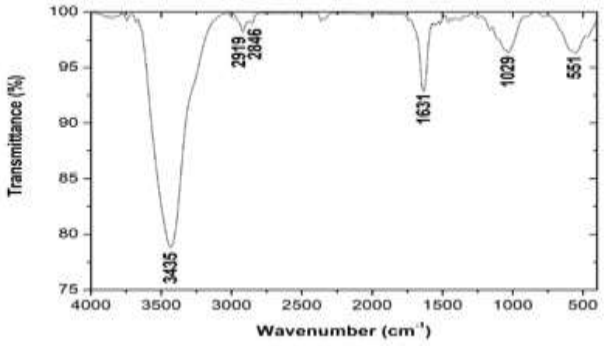

Figure 4: Fourier Transformed Infrared spectrum of silver nanoparticles from *Vernonia conferta*

Table 2: Functional groups at a given wavenumber for the spectra of *Vernonia conferta*

Absorption (cm^{-1})	Appearance	Functional groups	Compound class
3435	Strong sharp	O-H	Alcohol, intramolecular bounded
2919	Sharp	C-H stretch	Alkyl, methylene
2846	Medium	C-H stretch	Alkane
1631	Strong sharp	C=C stretch	Diene
1029	Medium	C-O stretch	Alcohol, carboxylic acid, ester
551	Medium	C-Br strech	Alkyl halides

3.5 Powder X-ray diffraction results: **Figure 5** shows the powder X-ray diffraction pattern of biosynthesized silver nanoparticles from *Vernonia conferta* and their principal characteristics are summarized on table 3. The pattern is compatible with the cubic phase of silver with reflections at 2θ values of 38.07°, 44.16°, 64.33°, and 77.40° that can be indexed to the (111), (200), (220), and (311) planes, respectively of the face-centred cubic structure (JCPDS file: 65-2871). No other characteristic peaks were indicated by the XRD analysis suggesting the high purity of the as-prepared AgNPs-VC. The average crystalline size of synthesized particles was calculated using the Debye-Scherrer formula as follows:

$$Dv = \frac{K\lambda}{\beta \cos\theta}$$

Where *Dv* is the average crystallite size; *K* is a dimensionless shape factor, with a value close to unity (0.99); λ is the wavelength of Cu Kα; β is the full width at half-maximum of the diffraction peaks, and θ is the Bragg angle.

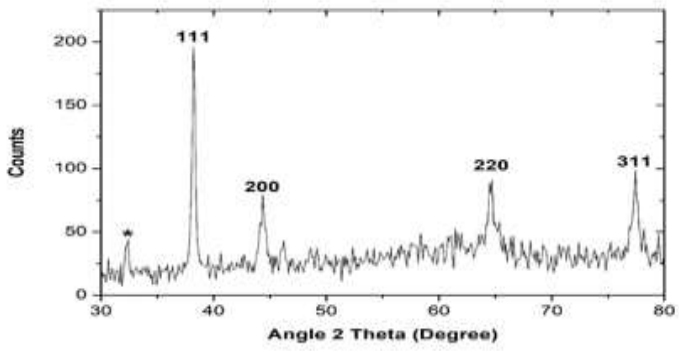

Figure 5: X-ray diffractogram pattern of silver nanoparticles from *Vernonia conferta*

Table 3: PXRD characteristic of silver nanoparticles from *Vernonia conferta*

N°	2θ	HKL	FWHM (degree)	I/I_0	Crystallite size-D (nm)
1	37.95	111	0.4380	1000	20.04
2	44.06	200	328.96	0.6571	13.63
3	63.94	220	369.73	1.5331	6.38
4	81.13	311	163.73	0.4380	24.95

3.6 Scanning electron microscopy and energy dispersive X-ray spectroscopy results: The SEM image and EDS spectrum of AgNPs-VC are depicted on **Figure 6A and 6B** respectively. It can be observed that synthesized nanoparticles are highly crystalline aggregates, spherical in shape with varied size. The EDS spectrum shows an intense optical absorption band peak at **3 keV** corresponding to silver. The elemental composition of synthesized nanoparticles is resumed on **Table 4**.

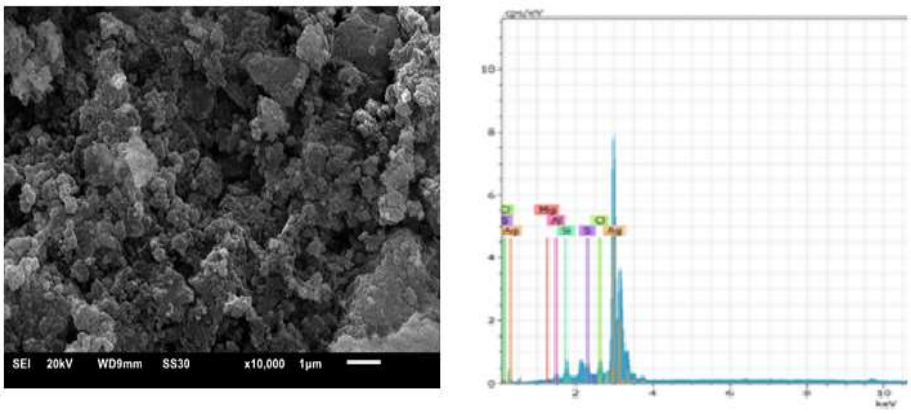

Figure 6: SEM image (left) and ED's spectrum (right) of AgNPs-VC

Table 4: Elemental composition of silver nanoparticles from *Vernonia conferta*

Element	Atomic mass	Serie	Unnormalized weight (%)	Normalized weight (%)	Atomic %
Ag	47	L	87.48	94.07	81.62
Si	14	K	2.11	2.26	7.55
S	16	K	1.95	2.09	6.11
Al	13	K	0.64	0.69	2.40
Cl	17	K	0.82	0.89	2.32
Total	-	-	93.00	100	100

3.7 Wound healing results: Progressive wound contraction was observed in all treated animals during the experimental period as compared to control **(Table 5).** The wound closure started significantly ($P<0.05$) increase from day 4 in AgNPs-VC treated group, exhibiting better contraction than trolamine used as standard. Less contraction activity was recorded with AEVC treatment as compared to control. Epithelialization period were found to be 17.67±0.33 days, 22.67±0.21 days and 16.33±0.33 days for trolamine, AEVC and AgNPs-VC respectively. The untreated group took longer time (23.17±0.30 days) to achieve epithelialization.

Table 5: Wound healing activity and epithelialization period of AEVC and AgNPs-VC

Post-wounding period (day)	Wound area (mm^2) and percentage of wound contraction			
	Control	Trolamine	AEVC	AgNPs-VC
0	12.42±0.20	12.08±0.08	12.33±0.21	12.25±0.17
4	12.33±0.16 (0.67)	11.92±1.37 (1.37)	12.17±0.16 (1.35)	11.75±0.17* (4.08)
8	11.50±0.22 (7.38)	8.00±0.37*** (33.80)	11.30±0.11 (8.78)	7.42±0.20*** (39.50)
12	9.75±0.17 (21.48)	6.00±0.36*** (50.34)	9.08±0.27 (26.35)	4.66±0.21*** (61.90)
16	6.75±0.11 (45.60)	3.25±0.11*** (73.10)	5.75±0.17*** (53.40)	2.42±0.15*** (80.30)
20	3.08±0.08 (75.17)	0.00±0.00*** (100)	2.33±0.21*** (81.08)	0.00±0.00*** (100)
Epithelialization Period (day)	23.17±0.30	17.67±0.33***	22.67±0.21	16.33±0.33***

Values are expressed as means ± SEM in each group (n=6); $^{*}P<0.05$ and $^{***}P<0.001$ as compared with control. Values between parentheses represent the percentage of wound closure. AgNPs-VC, silver nanoparticles; AEVC, aqueous extract of *Vernonia conferta*

DISCUSSION

Interest in green nanotechnology is rapidly growing due to effortlessness, rapid, low cost and eco-friendly procedures for nanomaterial production. Particular attention is being focus on the use of medicinal plants

to generate metallic nanoparticles which exhibit exceptional biological activities. For the first time, this study reports the enhanced wound healing effect of silver nanoparticles mediated *Vernonia conferta*.

Water extraction among other extraction methods has been retained for the preparation of crude extract using *Vernonia conferta* leaves. Being the mostly used in traditional medicine, aqueous extracts have shown many benefits in health systems including facile preparation and administration, low toxicity and rapid physiologic absorption [19]. The oven-drying aqueous extract gave a darkish solid mass of 1.7 g having an extract yield inferior to methanol, hexane or dichloromethane extracts [15]. The preliminary phytochemical screening of *Vernonia conferta* aqueous extract indicated the presence of coumarins, phenols, sterols, saponins and terpenes. These compounds are reported to possess anti-inflammatory and anti-microbial activities indicating the extract capability of promoting wound healing [20].

Since applying nano-improvement to plant extracts has proven to be simple, eco-friendly, cost-effective and obtained nanoparticles are less toxic with enhanced pharmacological effect, the general procedures developed in previous studies consisting in blending plant extract with silver nitrate aqueous solution has been retained. The observed changing colour of the reaction mixture from clear yellow to brownish, occurring 18 days post-incubation indicated the formation of AgNPs-VC. The nanoparticles formation and stability in aqueous colloidal solution were further confirmed using UV-Vis spectral analysis. The absorption spectra showed a characteristic peak at 465 nm due to the formation of plasmon at the colloid surface, due to coherent oscillation of conduction electrons on the surface as previously reported [7]. No further colour change was observed after 18 days, indicating the end of nanoparticles synthesis reaction. Marslin and co-workers [21], have shown that the synthesis of nanoparticles from plant extracts uses a wide range of molecules. The participation of terpenoids and polyphenols in the reduction of metal ions into nanoparticles and in supporting their subsequent stability has also been postulated [22]. The long reaction time for the generation of silver nanoparticles mediated *Vernonia conferta* can be explained by the relative low content or low reactivity of reducing agent in the plant extract.

A typical FTIR spectrum of generated nanoparticles to study their surface functionalization was obtained. Functional groups of molecules responsible for the reduction of silver ions and coating of the bioreduced silver nanoparticles were associated with absorption bands of various stretching modes including O-H, C-H, C=C, C-O and C-Br. A β-sitosterol named stigmast-5-ene-3β-ol isolated from the dichloromethane fraction of the ethanol extract of *Vernonia conferta* leaves showed approximately similar peaks in infrared spectrum suggesting that AgNPs-VC are capped with β-sitosterol among other phytochemicals giving characteristic peaks in the spectrum [15,23]. Moreover, β-sitosterol have been reported to possess wound healing effect [24].

The X-ray diffractogram analyses of synthesized nanoparticles indicated that synthesized nanoparticles were face-centered cubic (JCPDS file: 65–2871). The obtained peaks at 2θ value of 38.07°, 44.16°, 64.33° and 77.40° suggest crystalline and amorphous organic phases. The mean size of synthesized silver nanoparticles determined using the Scherrer equation was found to be 16.25 nm. The week signal observed in the powder X-ray diffractogram at 32° (2θ value) can be related to another crystalline silver phase. Such findings have been previously reported while using aqueous extract of *Psidium guajava* [25].

The morphology and elemental composition of synthesized silver nanoparticles were investigated using SEM and EDS respectively. The particles were found to be highly aggregated in the solid state. Spherical and cuboidal grains coalesce given the whole material was observed. The elemental mapping gives a material composed of silver as major constituent suggesting that slow reduction process can favour the formation of pure silver. Silver nanoparticles have the tendency to agglomerate due to their high surface tension of ultrafine nanoparticles [26]. The fine particle size results in a large surface area that, in turn, enhances the nanoparticle activities. Nanocrystallites of AgCl are generally formed together with pure

Ag. The relative low content of chlorine (0.89 normalized weight %) in the nanograins could be responsible of the absence of AgCl when chorine high reactivity is considered. The slow rate of formation is a second factor that can favour pure silver nanograins [27].

Pharmaceutical products comprise many formulation for topical application including ointments, creams, lotions and gels among others. It is well known that the type of formulation is important to the efficacy of the active component and anatomy of body parts [11]. Thus, choosing the appropriate formulation that will give optimum drug delivery is imperative. In this study, aqueous extract and silver nanoparticles mediated *Vernonia conferta* leaves were formulated as solution and suspension respectively and used for the treatment of wounds in rats. Generally, topical applications of drugs are effective in increasing wound healing rate because of its greater availability at the infected wound site [28]. Skin wound healing starts immediately after injury and consists of three phases: inflammation, proliferation, and maturation [29]. Though wounds can heal naturally, it is important to accelerate the healing process as several complications may arise [30]. The process involves migration and proliferation of various cells such as fibroblasts, endothelial and epithelial cells [31]. Furthermore, deposition of connective tissue and type I collagen which confers strength and integrity to the tissue matrix playing an important role in epithelialization at the later phase of healing has also been demonstrated [32]. In this study, all treated groups showed significant ($P < 0.05$) wound healing effect as compared to control group. The observed changes in AEVC-treated group might be attributed to β-sitosterol and other bioactive present in the extract [24]. It was suggested that trolamine improves wound healing via enhancing macrophage infiltration into the wound bed [33] meanwhile AgNPs promote wound healing process through the proliferation and migration of keratinocytes [34]. Wounds started significantly close from day 4 in AgNPs-VC treated rats and their period of epithelialization was more reduced than AEVC or trolamine. These findings indicating that apart from the intrinsic potency of the extract in relation to its bioactive components, plant-mediated silver nanoparticles might enhance cells proliferation and migration resulting in a better wound healing activity as previously reported.

CONCLUSION

The present study provides scientific evidence for the use of *Vernonia conferta* nano-hybrids to treat wounds. The nanoformulation strategy developed in this research sustains and credit the plant ethnopharmacological relevance and modernize the application for wound recovery as drugs. The biocompatibility of these new agents is a considerable benefit for future pharmaceutical applications providing novel directions for the wound treatment in clinical practice. Nonetheless, further studies into the stability of the prepared drugs to ensure an efficacious product for wound healing is required.

Competing interest: None

Authors' contributions:

MHJN, PBEK, YKDMKM, and FEM conceived and designed the study MHJN, YKDMKM, AS, VG, AAN, SVF, and APM carried out the experiment. MHJN, PBEK analysed and interpreted the results with help of FEM. MHJN and PBEK drafted the manuscript and FEM, EAMM, NNE and CJ revised the manuscript. FEM, NNE, CJ and EAMM, supervised the work at all stages. All authors read and approved the final manuscript.

Availability of data: Datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

ACKNOWLEDGMENTS

PBEK thanks the African-German Network of Excellence in Science (AGNES) for granting a Mobility Grant in 2017 and the International Foundation for Sciences (IFS) for awarding a research grant (I-1-F-6137-1). Support of Word University Service under APA 2668 for providing part equipment used is appreciate. FEM thank the Commonwealth Scholarship Commission for a generous academic fellowship (CMCF-2015-3) and the German Academic Exchange Service DAAD for a generous Professor Fellowship (grant no. 768048). Authors are thankful to the Multidisciplinary Laboratory of the Faculty of Medicine and Pharmaceutical Sciences, Department of Pharmaceutical Sciences for technical support.

Disclosure: The authors report no conflicts of interest in this work.

REFERENCES

1. R. F. Diegelmann, M. C. Evans, Wound healing: An overview of acute, fibrotic and delayed healing. Frontier in Biosciences; 2004, 9, 283-289.
2. G. C. Gurtner, S. Werner, Y. Barrandon, M. T. Longaker, Wound repair and regeneration. Nature; 2008, 453, 314-321.
3. P. G. Bowler, B. I. Duerden, D. G. Armstrong, Wound microbiology and associated approaches to wound management. Clinical Microbiology Review; 2001, 14, 244-269.
4. S. Schreml, R. M. Szeimies, L. Prantl, M. Landthaler, P. Babilas, Wound healing in the 21st century. Journal of American Academy of Dermatology; 2010, 63, 866.
5. I. Garcia-Orue, J. L. Pedraz, R. M. Hernandez, M. Igartua, Nanotechnology-based delivery systems to release growth factors and other endogenous molecules for chronic wound healing. Journal of Drug Delivery Science and Technology; 2017, 42, 2-17.
6. B. V. Bonifácio, P. B. Silva, M. A. Ramos, K. M. Negri, T. M. Bauab, M. Chorilli, Nanotechnology-based drug delivery systems and herbal medicines: a review. International Journal of Nanomedicine; 2014, 9, 1-15.
7. P. Belle Ebanda Kedi, B. Etah Ngangue, V. Deli, A. Gbambie Paboudam, *et al*. In vitro and in vivo anti-inflammatory activity of green synthesized silver nanoparticles from aqueous bark extract of *Mangifera indica* Linn. (Anacardiaceae). International Journal of Green and Herbal Chemistry. Section B; 2020, 9, 345-360.
8. A. Shakeel, A. Mudasir, L. S. Babu, I. Saiqa, A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. Journal of Advance Research; 2016, 7, 17-28.
9. F. J. Costa, P. N. Bandeira, M. R. J. R. Albuqueque, O. D. L. Pessoa, P. E. R. Silveira, R. Braz-Filho, Constituintes quimicos de la Vernonia chalybaea. Mart. Quimica Nova; 2008, 31, 1691-1695.
10. M. Zayats and I. Willner, Biomelecule-Nanoparticle hybrid system in Nanotechnology. Volume 1: Principles and fundamentals. Gunter Schmidt (Ed.), Wiley-CH Verlag GmbH and Co. KGaA, Weinheim; 2008, 139-215.

11. P. F. Builders, T. B. Kabele, M. Builders, B. A. Chindo, P. A. Anwunobi, Y. C. Isimi, Wound Healing Potential of Formulated Extract from *Hibiscus Sabdariffa* Calyx. Indian Journal of Pharmaceutical Science; 2013, 75, 45-52.

12. F. Eya'ane Meva, C. Okalla Ebongue, S. V. Fannang, M. L. Seugnou, etal.; Natural substances for the synthesis of Silver nanoparticles against *Escherichia coli*: The case of *Megaphrynium macrostachum* (Marantaceae), *Corchorus olitorus* (Tiliaceae), *Ricinodendron heudelotii* (Euphorbiaceae), *Gnetum bucholzianum* (Gnetaceae), and *Ipomea batatas* (Convolvulaceae). Journal of Nanomaterials; Vol. 2017. (2017) Article ID 6834726, 6pages.

13. M. S. Luo, T. H. Gao, Encyclopedia of pharmaceutics excipient, Sichuan, Sichuan Publishing House of Science and Technology, 2006, 22.

14. P. Belle Ebanda Kedi, F. Eya'ane Meva, E. L. Nguemfo, Z. C. Bogning, A. A. Ntoumba, A. B. Dongmo, M. Maaza, Green synthesis of silver nanoparticles with enhanced anti-inflammatory activity as a sustainable use of resource method. Humboldt Kolleg, Yaoundé, Cameroun, Apr 3-6th (2019) Book of abstracts p37.

15. I. J. Umoh, O. A. Eseyin, A. A. Ahmadu, Phytosterol from the leaves of Vernonia conferta Benth (Asteraceae). Nigerian Journal of Pharmaceutical and Applied Science Research; 2016, 5, 16-17.

16. M. Malahubban, A. R. Alimon, A. Q. Sazili, S. Fakurazi, F. A. Zakry, Phytochemical analysis of *Andrographis paniculata* and *Orthosiphon stamineus* leaf extracts for their antibacterial and antioxidant potential. Tropical Biomedicine; 2013, 30, 467-480.

17. J. J. Morton, M. H. Malone, Evaluation of vulnerary activity by an open wound procedure in rats. Archives Internationales de Pharmacodynamie et de Therapie; 1972, 196, 117-126.

18. R. Shetty, H. Sreekar, S. Lamba, A. K. Gupta, A novel and accurate technique of photographic wound measurement, Indian Journal of Plastic Surgery; 2012, 45(2), 425-429.

19. J. K. Patra, G. Das, L. F. Fraceto, E. V. R. Campos, M. D. P. Rodriguez-Torres, *et al.*; Nano based drug delivery systems: recent developments and future prospects. Journal of Nanobiotechnology; 2018, 16, 71, 1-33.

20. F. Mujeeb, P. Bajpai, N. Pathak, Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from Leaves of Aegle marmelos. BioMed Research International; 2014 (2014) 497606, 1-11.

21. G. Marslin, K. Siram, Q. Maqbool, R. K. Selvakesavan, D. Kruszka, P. Kachlicki, G. Franklin, Secondary Metabolites in the Green Synthesis of Metallic Nanoparticles. Materials; 2018, 11 940. doi:10.3390/ma11060940

22. V. V. Makarov, A. J. Love, O. V. Sinitsyna, S. S. Makarova, I. V. Yaminsky, M. E. Taliansky, N. O. Kalinina, Green nanotechnologies: Synthesis of metal nanoparticles using plants. Acta Naturae; 2014, 6, 35-44.

23. A. Patra, S. Jha, P. N. Murthy, A. Manik, A. Sharone, Isolation and characterization of stigmast5-en-3β-ol (β-sitosterol) from the leaves of *Hygrophila spinosa* T. Anders, International Journal of Pharma Sciences and Research; 2010, 1, 95-100.

24. M. M. Abbas, A. I. N. Rawi, A. M. Abbas, I. A. I. Khateeb, Naringenin potentiated β-sitosterol healing effect on the scratch wound assay. Research in Pharmaceutical Sciences; 2019, 14, 566-573.

25. A. A. Ntoumba, F. Eya'ane Meva, W. Ekoko Eyisap, L. P. Kojom Foko, *et al.;* Anopheles gambiae larvicidal activity of green synthesized Silver nanoparticles from *Psidium guajava* L. aqueous leaves extract. Journal of Biomaterials and Nanobiotechnology; 2020, 11, 49-66.

26. J. Annamalai, T. Nallamuthu, Green synthesis of silver nanoparticles: characterization and determination of antibacterial potency. Applied Nanoscience; 2006, 6, 259-265.

27. F. Eya'ane Meva, J. O. Avom Mbeng, C. Okalla Ebongue, C. Schlüsener, *et al.;* Stachytarpheta cayennensis Aqueous Extract, a New Bioreactor towards Silver Nanoparticles for Biomedical Applications. Journal of Biomaterials and Nanobiotechnology; 2019, 10, 102-119.

28. I. K. R. Agra, L. L. S. Pires, P. S. M. Carvalho, E. A. Silva-Filho, S. Smanniotto, E. Barreto, Evaluation of wound healing and antimicrobial properties of aqueous extract from *Bowdichia virgilioides* stem barks in mice. Annals of the Brazilian Academy of Sciences; 2013, 85, 945-954.

29. T. Kondo, Timing of skin wounds. Legal Medicine; 2007, 9(2), 109-114.

30. S. Y. Gbedema, K. Emelia, A. Francis, A. Kofi, W. Eric, Wound healing properties and kill kinetics of Clerodendronsplendens G. Don, a Ghanaian wound healing plant. Pharmacognosy Research; 2010, 2, 63-68.

31. A. V. Ljubimov and M. Saghizadeh, Progress in corneal wound healing, Progress in Retinal and Eye Research; 2015, 49, 17–45.

32. R. A. F. Clark, Wound repair: overview and general considerations. In: Clark RA and Henson PM. The molecular and cellular biology of wound repair, 2nd ed., New York: Plenum; 1996, 3-50.

33. A. E. Krausz, B. L. Adler, A. Landriscina, J. M. Rosen, T. Musaev, J. D. Nosanchuk, A. J. Friedman, Archives of Dermatological Research; 2005, 307, 583-594.

34. X. Liu X, P. Y. Lee, C. M. Ho, V. C. Lui, Y. Chen, C.-M. Che, P. K. H. Tam, K. K. Y. Wong, Silver nanoparticles mediate differential responses in keratinocytes and fibroblasts during skin wound healing. ChemMedChem; 2010, 5, 468-475.

***Corresponding Author: François Eya'ane Meva[1,3],**

[1]Department of Pharmaceutical Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, PO Box 2701 Douala, Cameroon

[3]Department of Chemistry and Structural Chemistry, Heinrich-Heine-University Dusseldorf, Dusseldorf, Germany. mevae@daad-alumni.de, PO Box 2701 Douala, Cameroon.

Tel: +237698254292

Online publication Date: 04.11.2020