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RESEARCH ARTICLE

REVISED Green synthesis and characterization of zinc oxide nanoparticles using bush tea (*Athrixia phylicoides* DC) natural extract: assessment of the synthesis process.

[version 3; peer review: 1 approved, 1 approved with reservations, 1 not approved]

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Abstract

Background: Nanoparticles are globally synthesized for their antimicrobial, anti-inflammatory, wound healing, catalytic, magnetic, optical, and electronic properties that have put them at the forefront of a wide variety of studies. Among them, zinc oxide (ZnO) has received much consideration due to its technological and medicinal applications. In this study, we report on the synthesis process of ZnO nanoparticles using *Athrixia phylicoides* DC natural extract as a reducing agent.
Methods: Liquid chromatography–mass spectrometry (LC-MS) was used to identify the compounds responsible for the synthesis of ZnO nanoparticles. Structural, morphological and optical properties of the synthesized nanoparticles have been characterized through X-ray diffraction (XRD), Ultraviolet-visible spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS).
Results: LC-MS results showed that different flavonoids and polyphenols, as well as Coumarin, an aromatic compound, reacted with the precursor to form ZnO nanoparticles. XRD and UV-Vis analysis

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confirmed the synthesis of ZnO nanoparticles, with a spherical shape showed in SEM images. The quasi-spherical ZnO crystals had an average crystallite size of 24 nm. EDS and FTIR analysis confirmed that the powders were pure with no other phase or impurity.
Conclusions: This study successfully demonstrated that the natural plant extract of *A. phylicoides* DC. can be used in the bio-reduction of zinc nitrate hexahydrate to prepare pure ZnO nanoparticles, thus, extending the use of this plant to an industrial level.

Keywords
ZnO nanoparticles, green synthesis, bush tea, reducing agent, natural extract.

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REVISED **Amendments from Version 2**

This version includes a modified introduction as well as Figure 8.

Any further responses from the reviewers can be found at the end of the article

Introduction

Nanomaterials with a size less than 100 nm are globally synthesized owing to their various properties such as, antimicrobial, anti-inflammatory, wound healing, catalytic, magnetic, optical, and electronic properties, that have put them at the forefront of a wide variety of studies (Gunalan *et al.*, 2012; Jamdagni *et al.*, 2018). Compared to their counterpart bulk materials, they present a reduced surface to volume ratio that increases as the size is reduced (Ohno *et al.*, 2010), thus placing them on the borderline between single molecules and bulk materials (Mansoori and Soelaiman, 2005).

The introduction of nanoparticles in the consumer industry, health, food, space, chemical, and cosmetics, has called for a green and environmentally responsible strategy for their production (Rao and Gautam, 2016). Metal oxides and dioxides such as zinc oxide, silver, gold and titanium dioxide have received copious consideration because of their multiple properties and applications (Dobrucka and Długaszewska, 2016). However, their synthesis has been done through numerous physicochemical methods. Laser ablation, microwave irradiation and vapour deposition have been reported to date (Satyanarayana and Reddy, 2018), they involve forces of condensation, dispersion, or fragmentation of bulk particles into nanoparticles, as well as some toxic chemicals, harmful to the environment (Dhandapani *et al.*, 2014; Krupa and Vimala, 2016).

Synthesis of nanomaterials through biological systems assisted by some biotechnological tools is an emerging asset of nanotechnology that provides a safe, cost-effective and eco-friendly synthesis process (Shinwari and Maaza, 2017). Plants, diatoms, fungi, yeast, algae, bacteria, and human cells have been used. Their proteins and other metabolites have been well reported to have a reductive capacity that can transform metal ions into metal nanoparticles (Dobrucka and Długaszewska, 2016; Parveen *et al.*, 2016). The biological synthesis of nanoparticles provides more advantages than chemical and physical ones (Kharissova *et al.*, 2013). Numerous metal oxide nanoparticles, such as TiO_2, CuO, and ZnO have been produced by total green chemistry. Among them, ZnO, an n-type semiconductor, has gained interest owing to its easy production, cost-effectiveness, and safety of synthesis and usage (Agarwal *et al.*, 2017). Several studies have successfully been led to synthesize ZnO nanoparticles using different organisms such as bacteria, fungi, algae, and plants (Agarwal *et al.*, 2017).

Among all biological systems, plant phytosynthesis of nanoparticles using plants has shown great potential. Plant-mediated nanoparticle synthesis is simple, eco-friendly, and provides antibacterial assets (Gunalan *et al.*, 2012; Iravani *et al.*, 2015; Thema *et al.*, 2015). A variety of metabolites such as terpenoids, polyphenols, sugars, alkaloids, phenolic acids and proteins have been reported to have metal ion reduction assets (Parveen *et al.*, 2016). Several studies dedicated to the green synthesis of ZnO nanoparticles using plant extracts as capping or reducing agents have shown the use of different plant aerial parts, such as leaves and fruits of different species such as *Aloe vera*, *Hibiscus sabdariffa*, *Allium sativum*, *Allium cepa*, *Petroselinum crispum, Moringa oleifera* and *Camellia sinensis,* for the synthesis of nanoparticles (Mahendiran *et al.*, 2017; Matinise *et al.*, 2017; Senthilkumar and Sivakumar, 2014; Stan *et al.*, 2015). Bush tea, mostly known as a medicinal tea plant in southern Africa where it originates has high concentrations of phenolic compounds such as tannins and flavonoids (Lerotholi *et al.*, 2017). However, data explaining the synthesis processes of nanoparticles using this plant are lacking. Hence, the objective of this study was to contribute to the explanation of the compounds induced in the synthesis process of ZnO nanoparticles using *Athrixia phylicoides* leaf extract.

Methods

Material

Leaves of bush tea (*A. phylicoides* DC) were used to reduce zinc nitrate hexahydrate. Analytical grade $Zn(NO_3)_2.6H_2O$ of 99% purity was purchased from Sigma-Aldrich, South Africa. Bush tea leaves were harvested from the wild in Thohoyandou (22.8785°S; 30.4818°E) in the Limpopo province, South Africa. Following the harvest, the leaves were washed with deionized water and freeze-dried for 72 hours at -50°C at a pressure of 0.32 Kpa, hereafter they were ground and kept for further usage.

Material preparation

Extract preparation

Ten grams of ground bush tea leaves were weighed and mixed with 300 ml of deionized water. The mixture was heated at 60°C for 30 minutes until the water changed to a dark green colour. After centrifugation using a Hermle Labortecnik GmbH Z 216-M benchmark centrifuge at 4000 rpm for 10 minutes, the mixture was filtered twice using Whatman filter paper number 1, and the extract was kept in an airtight container in a fridge at ≈4°C for analysis and ZnO nanoparticles synthesis.

Synthesis of ZnO nanoparticles

In this study, zinc nitrate hexahydrate [Zn $(NO_3)_2.6H_2O$] was used as the precursor. One gram of the precursor was mixed with 25 ml of *A. phylicoides* extract. The mixture was kept on a magnetic stirrer at 300 rpm at 60°C for 30 minutes and then left to cool down at room temperature for 12 hours, a precipitate was observed. The mixture was centrifuged for 15 minutes at 4000 rpm. The supernatant was collected and transferred to LC-MS vials for analysis and the precipitate was dried at 60°C for one hour and then annealed at 800°C for two hours. The obtained powder was then kept for characterization.

Bush tea compounds profiling and identification

The determination and profiling of different compounds present in the extract before the synthesis as well as the supernatant after synthesis were performed using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS) using a Bruker impact II (Germany). After peak integration and Pareto scaling, the liquid chromatography-mass spectrometry (LC-MS) data were transformed into buckets using the Bruker Compass data analysis programme version 4.3.110 (https://www.bruker.com/en/). Peaks were determined using real mass, MS/MS, and retention time (RT). The accuracy of the mass and MS/MS spectral data was compared to the Kyoto standard Encyclopaedia of Genes and Genomes (KEGG) and ChemSpider databases using the MetFrag 2.2 online software (Tete *et al.*, 2020). Principal component analysis (PCA) and T-tests were performed using MetaboAnalyst 4.0.

ZnO nanoparticle characterization

The characterization of the obtained ZnO nanopowders was done using X-ray diffraction (XRD), Ultraviolet-visible spectroscopy (UV-Vis), and Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS). The crystallite size of ZnO nanoparticles was estimated using the modified Scherrer equation:

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

where λ is the X-ray wavelength, β the peak width at half maximum weight, $K = 0.9$, and the Scherrer constant (Monshi *et al.*, 2012).

Results

Assessment of the synthesis process

Evaluation of extract composition relative to the synthesis of ZnO nanoparticles using LC-Q-TOF-MS

The crude extract from bush tea leaves and the supernatant after the synthesis of ZnO nanoparticles were investigated. The differences between the composition of the crude extract and the supernatant after synthesis are represented in Figure 1. The results from the PCA co-variance of data show that two distinct groups were observed from the three principal components with 85.1%, 8.6% and 2.1% respectively for principal components 1, 2 and 3. The compounds (represented in red) resulting from the supernatant after synthesis of ZnO nanoparticles clustered together following the Y-axis of the PCA while the compounds of crude extract were on the Z-axis. The differences observed are due to the reaction between the plant extract and the precursor to form ZnO nanoparticles. The synthesis of ZnO nanoparticles involves a reaction between the plant extract and the precursor resulting in the reduction of Zn^{+2} ions into ZnO nanoparticles (Hussain *et al.*, 2019).

Figure 2 shows the different compound peaks observed using LC-Q-TOF-MS analysis. The dissection of observed spectra into compounds produced 100 different peaks for the crude extract (Figure 2a) and 84 peaks for the supernatant

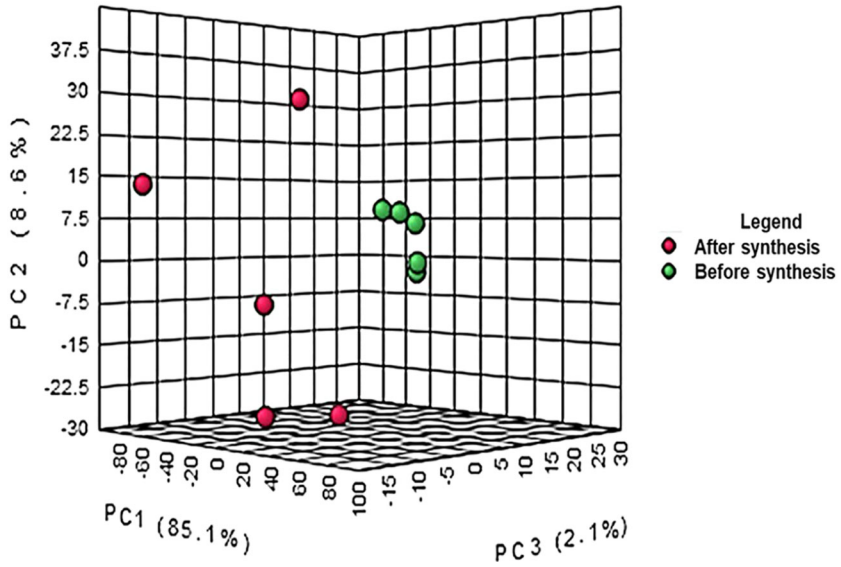


Figure 1. Principal component analysis of liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS) peak intensities of 10 bush tea (*Athrixia phylicoides* DC.) leaf extracts before and after ZnO nanoparticle synthesis.

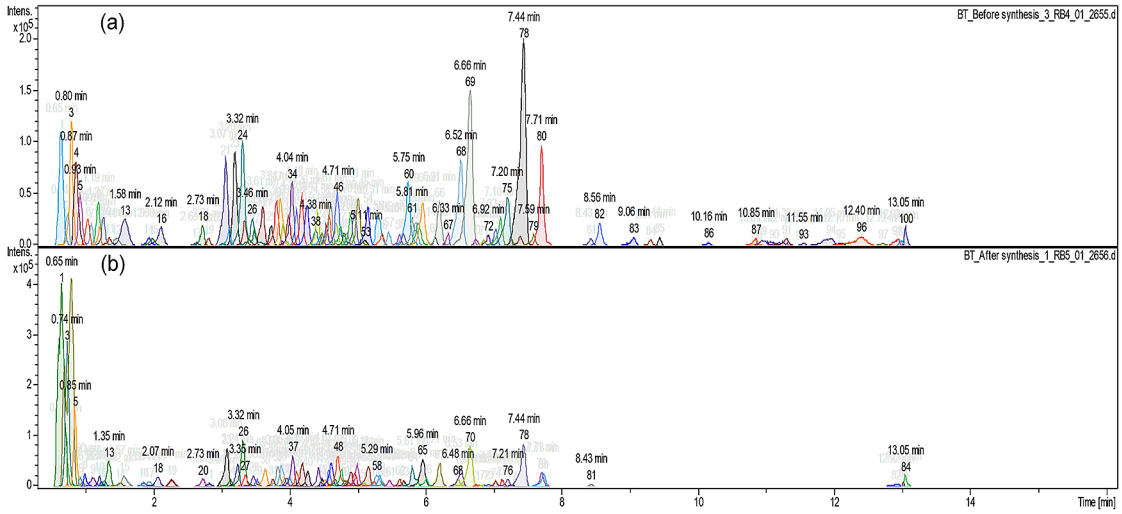


Figure 2. Compound dissection (a) before the synthesis process (100 peaks) (b) after the synthesis process (84 peaks).

after synthesis (Figure 2b). The reduction in the number of compounds confirms that the synthesis took place and secondary metabolites from *A. phylicoides* DC. extract has reacted with the precursor reducing Zn^{2+} ions into ZnO nanoparticles.

Bush tea extract compounds identification before and after synthesis

Different peaks identified, after chromatogram dissection (Figure 2), from LC-Q-TOF-MS revealed the presence of several compounds in both the crude extract and the supernatant after ZnO nanoparticle synthesis, with a reduction of the compound's amount in the supernatant collected after synthesis. Thus, revealing the presence of an interaction between the precursor and the extract mainly by the oxidation, reduction or degradation of the phytochemical compounds that occur during nanoparticle formation (Jeevanandam *et al.*, 2016). Table 1 presents the secondary metabolites investigated for both the crude bush tea extract and the supernatant solution after synthesis, respectively.

Table 1. Liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS) bush tea extract compounds identified before ZnO nanoparticles synthesis using MetFrag software (KEGG and ChemSpider databases, 50 ppm).

	Compound name	Formula	RT [sec]
1	(+)-7-Isojasmonic acid	$C_{12}H_{18}O_3$	294
2	(6Z,9Z,12Z)-Octadecatrienoic acid	$C_{18}H_{30}O_2$	543.6
3	10-Oxo-11,15-phytodienoic acid	$C_{18}H_{28}O_3$	357.6
4	13-hydroxy-9Z,11E-octadecadienoic acid	$C_{18}H_{32}O_3$	652.2
5	17-Hydroxylinolenic acid	$C_{18}H_{30}O_3$	340.8
6	1-O,6-O-Digalloyl-beta-D-glucose (tannin)	$C_{20}H_{20}O_{14}$	351
7	3,6-Anhydroglucose	$C_6H_{10}O_5$	228.6
8	3-hydroperoxy-4-phenyl-pentan-1-ol/Loliolide	$C_{11}H_{16}O_3$	309
9	3-tert-Butyl-5-methylcatechol	$C_{11}H_{16}O_2$	426
10	4-Heptyloxyphenol	$C_{13}H_{20}O_2$	282.6
11	4”-Hydroxyacetophenone	$C_8H_8O_2$	71.4
12	4-Hydroxyestradiol-17beta	$C_{18}H_{24}O_3$	443.4
13	5,7,3'-Trimethoxy-6,4',5'-trimethoxyisoflavone	$C_{18}H_{16}O_8$	690
14	7-Hydroxy-2”,4”,5”-trimethoxyisoflavone	$C_{18}H_{16}O_6$	726
15	Naringenin 7-O-beta-D-glucoside	$C_{21}H_{22}O_{10}$	435.6
16	17-Hydroxylinolenic acid	$C_{18}H_{30}O_3$	372.6
17	Adenine	$C_5H_5N_5$	783
18	alpha-Curcumene	$C_{15}H_{22}$	609.6
19	5S-Hydroperoxy-18R-HEPE	$C_{20}H_{30}O_5$	274.8
20	Atropaldehyde	C_9H_8O	345
21	Scullcapflavone II	$C_{19}H_{18}O_8$	462.6
22	Cinnamaldehyde	C_9H_8O	354
23	Cisapride	$C_{29}H_{27}N_3O_3$	115.8
24	Coumaric acid/Caffeic Aldehyde	$C_9H_8O_3$	285
25	Coumarin	$C_9H_6O_2$	76.8
26	D-Norvaline	$C_5H_{11}NO_2$	48
27	Homovanillate/Dihydrocaffeic acid	$C_9H_{10}O_4$	288.6
28	Lancerin	$C_{19}H_{18}O_{10}$	348.6
29	Lophophorine/Stovaine	$C_{13}H_{17}NO_3$	55.8
30	Mallotophenone	$C_{21}H_{24}O_8$	432
31	Malonyldaidzin	$C_{24}H_{22}O_{12}$	207.6
32	Melampodin A	$C_{21}H_{24}O_9$	399.6
33	Montanol	$C_{21}H_{36}O_4$	513.6
34	Myrcene/(E)-beta-Ocimene	$C_{10}H_{16}$	321.6
35	Nafenopin glucuronide	$C_{26}H_{30}O_9$	291
36	Neocnidilide/4-Hexyloxyphenol	$C_{12}H_{18}O_2$	421.8
37	Pentalen-13-ol/Nonylphenol	$C_{15}H_{24}O$	411
38	Petasin/Cafestol	$C_{20}H_{28}O_3$	558.6
39	Pinosylvin	$C_{14}H_{12}O_2$	276.6
40	Quinestrol	$C_{25}H_{32}O_2$	232.2
41	Traumatic acid	$C_{12}H_{20}O_4$	403.8

Table 1. *Continued*

	Compound name	Formula	RT [sec]
42	Tricin	$C_{17}H_{14}O_7$	379.8
43	Umbelliferone/4-Hydroxycoumarin	$C_9H_6O_3$	209.4
44	4″-Hydroxyacetophenone	$C_8H_8O_2$	1.19

Table 2 present the compounds identified from the supernatant after synthesis of ZnO nanoparticles. The secondary metabolites investigated present a reduced number compared to the ones from the crude extract, thus revealing that a reaction has taken place between bush tea natural extract metabolites and the precursor resulting in the formation of ZnO nanoparticles.

Table 2. Liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS) bush tea extract compounds identified after ZnO nanoparticles synthesis using MetFrag software (KEGG and ChemSpider, 50 ppm).

	Compound name	Formula	RT [sec]
1	Indanone	C_9H_8O	348.6
2	Mallotophenone	$C_{21}H_{24}O_8$	432.6
3	Melampodin A	$C_{21}H_{24}O_9$	399.6
4	Sterigmatocystin	$C_{18}H_{12}O_6$	268.8
5	Umbelliferone	$C_9H_6O_3$	211.8
6	Salicylate	$C_7H_6O_3$	182.4
7	Resolvin E2	$C_{20}H_{30}O_4$	265.8
8	Scullcapflavone II	$C_{19}H_{18}O_8$	463.8
9	Myrtenol	$C_{10}H_{16}O$	306
10	3-tert-Butyl-5-methylcatechol	$C_{11}H_{16}O_2$	427.8
11	(+)-7-Isojasmonic acid	$C_{12}H_{18}O_3$	404.4
12	Traumatic acid	$C_{12}H_{20}O_4$	91.2
13	4-Heptyloxyphenol	$C_{13}H_{20}O_2$	282.6
14	4,4″-Dihydroxystilbene	$C_{14}H_{12}O_2$	276.6
15	1,3-Diphenylpropane	$C_{15}H_{16}$	309.6
16	Geranyl hydroquinone	$C_{16}H_{22}O_2$	781.2
17	Syringin	$C_{17}H_{24}O_9$	232.8
18	3-Hydroxybenzaldehyde	$C_7H_6O_2$	280.2
19	6-Hydroxyluteolin 7-glucoside	$C_{21}H_{20}O_{12}$	256.2
20	6-Methoxyaromadendrin 3-O-acetate	$C_{18}H_{16}O_8$	388.8
21	Adenine	$C_5H_5N_5$	72.6
22	9S-hydroxy-10E,12Z,15Z-octadecatrienoic acid	$C_{18}H3_0O_3$	372.6
23	9E-Heptadecenoic acid	$C_{17}H_{32}O_2$	337.2
24	Carboxymethyloxysuccinate	$C_6H_8O_7$	81
25	Coumarin	$C_9H_6O_2$	219
26	Pent-7alpha-Hydroxykaur-16-en-19-oic acid	$C_{20}H_{30}O_3$	319.8
27	Etherolenic acid	$C_{18}H_{28}O_3$	357.6
28	Icariin	$C_{33}H_{40}O_{15}$	240

Assessment of the implication of bush tea compounds in ZnO nanoparticles synthesis

In this study, compound identification was carried out using Bruker data analysis and data profiling tools. The KEGG and ChemSpider databases were consulted to find the name and the chemical formula of each identified compound. The different compounds with mass to ratio (m/z) values as well as their retention time (in seconds) were shown with a variable importance in progression (VIP) score plot (Figure 3). The concentration of eight compounds were found to be high in the crude extract compared to the supernatant after synthesis of ZnO nanoparticles where their concentrations were low.

Table 3 present the various compounds that were involved in the synthesis process of ZnO nanoparticles including five flavonoids and two polyphenol compounds, as well as one aromatic compound, which highly reacted with the precursor to form ZnO nanoparticles. Studies have shown that the synthesis of nanoparticles using plant extracts involves terpenoids, flavonoids, alkaloids and phenolic acid, which act as reducing, capping, and stabilizing agents (Kuppusamy *et al.*, 2016).

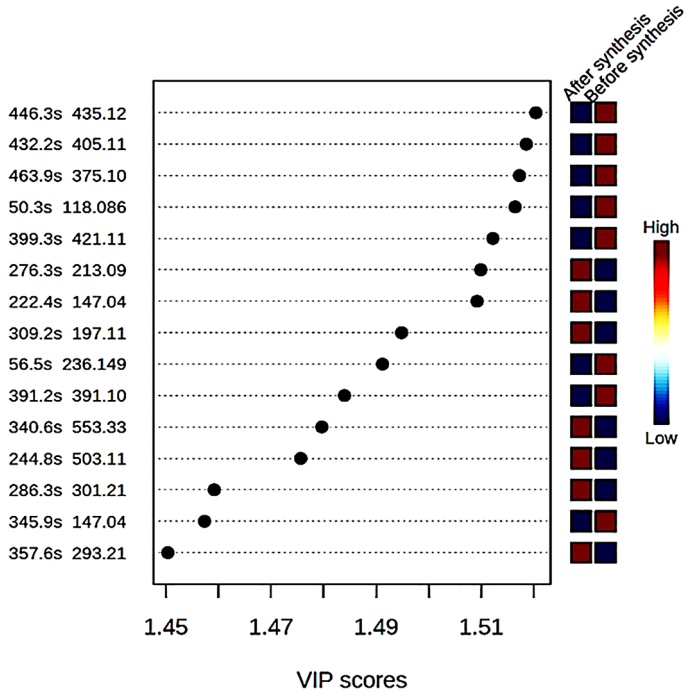


Figure 3. Variable importance in progression (VP) score plot of different compounds found in the bush tea crude extract before synthesis and the supernatant after synthesis of ZnO nanoparticles.

Table 3. Identified compounds reported having mostly interacted with the precursor to form ZnO nanoparticles.

Compound name	Formula	Type
Naringenin 7-O-beta-D-glucoside	$C_{21}H_{22}O_{10}$	Flavonoid
Scullcapflavone II	$C_{19}H_{18}O_8$	Flavonoid
Mallotophenone	$C_{21}H_{24}O_8$	Polyphenol
6-Methoxyaromadendrin 3-O-acetate	$C_{18}H_{16}O_8$	Flavonoid
2-Phenylacetamide	C_8H_9NO	Polyphenol group
7-Hydroxy-2",4",5"-trimethoxyisoflavone	$C_{18}H_{16}O_6$	Flavonoid
Coumarin	$C_9H_6O_2$	Aromatic
Malonyldaidzin	$C_{24}H_{22}O_{12}$	Flavonoid

ZnO nanoparticles characterization

XRD analysis

The XRD analysis was done to confirm the crystallinity of the synthesized ZnO nanoparticles using a Bruker AXS (Germany) D8 advance X-ray diffractometer. Figure 4 presents the XRD pattern of the ZnO nanoparticles. The crystallinity of the powder resulting from the synthesis using *A. phylicoides* DC extract. The peaks (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202) are lattice planes. The diffraction peaks reveal that the synthesized ZnO nanoparticles are essentially crystalline, in accord with the ICDD #897102 in the wurtzite structure (Noman *et al.*, 2020). The same results have been observed by the green synthesis of ZnO nanoparticles using *Ocimum basilicum* (Salam *et al*., 2014) and *Agathosma betulina* (Thema *et al*., 2015). The average crystallite size of obtained ZnO nanoparticles calculated using the modified Scherrer equation was approximately 24.53 nm.

Fourier-transform infrared spectroscopy

The PerkinElmer Frontier FTIR spectrometer was used to perform FTIR analyses using Potassium bromide (KBr) (Potassium bromide) optics. The presence of ZnO nanoparticles was confirmed by the peak at 479 cm^{-1} as shown in Figure 5. The other

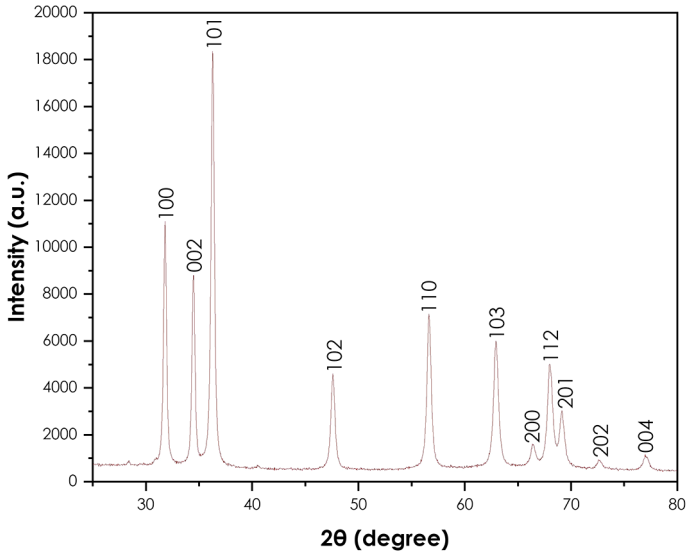


Figure 4. X-ray diffraction pattern of ZnO nanoparticles.

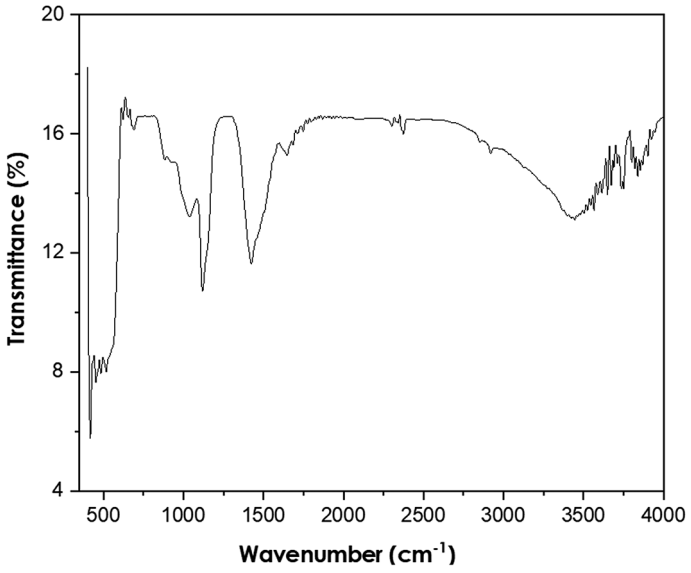


Figure 5. Fourier-transform infrared spectra of ZnO powder annealed at 600°C.

observed peaks are attributed to the phytochemical components present in the extract solution. The peak at 1113 cm^{-1} is attributed to the C-O stretching of primary alcohols. The peak at 1427 cm^{-1} corresponds to the O-H bending of the carboxylic acid. The peak observed at 2351 cm^{-1} is attributed to the O=C=O stretching of carbon dioxide. The FTIR spectra of bush tea extract, presented in Figure 6, show the presence of carboxylic acid bonding, primary alcohol stretching as well as the intramolecular hydrogen bond.

UV-Vis analysis

UV-Vis analyses were performed at a resolution of 1 nm at 250–800 nm wavelength range using a PerkinElmer Lambda 650S UV-Vis spectrometer. The absorption of ZnO nanoparticles is observed in the wavelength range of 250–400 nm (Kolekar *et al.*, 2011). The measured peak at 380 nm (as shown in Figure 7) reveals the presence of ZnO nanoparticles with a band gap energy of 3.11 eV, smaller than the bulk ZnO of 3.37 eV. Thus, the presence of hexagonal wurtzite structures in the analysed samples is indicated, in accordance with the XRD results.

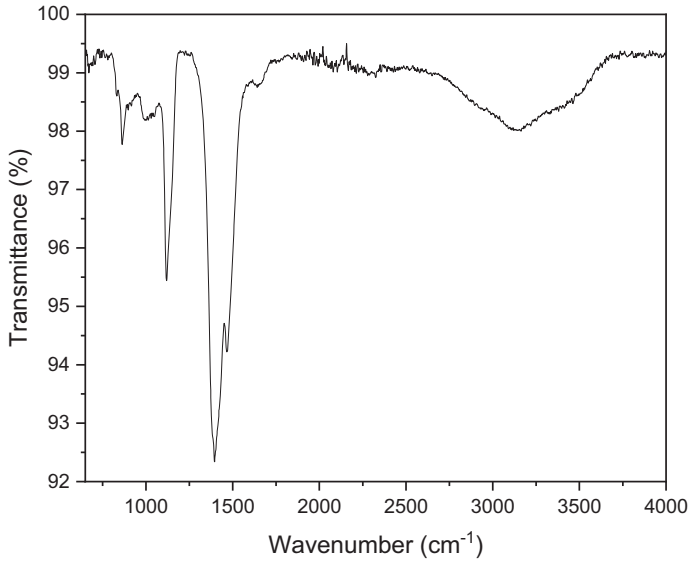


Figure 6. Fourier-transform infrared spectra of Bush tea leaf extract.

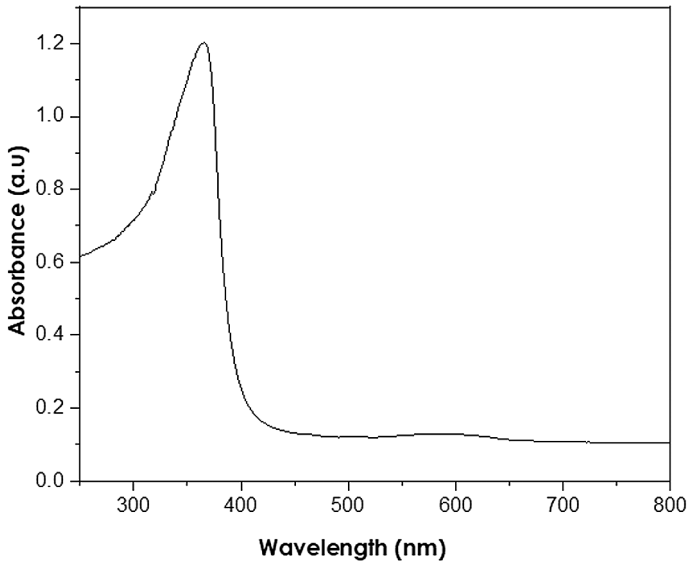


Figure 7. Ultraviolet-visible spectra of as-synthesized ZnO nanoparticles.

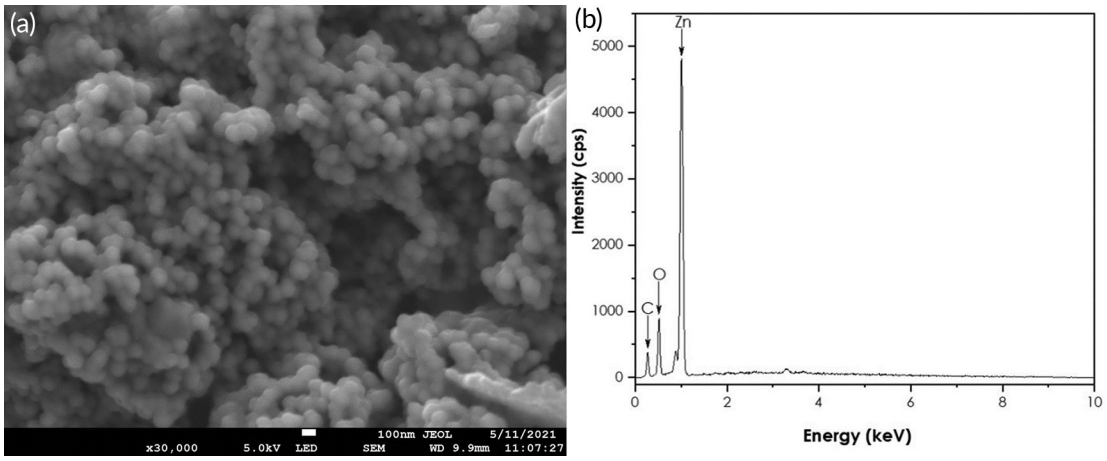


Figure 8. (a) Scanning electron microscopy image and (b) Energy-dispersive X-ray spectra of ZnO nanoparticles.

SEM and EDS analyses

A JEOL JSM-7500F field-emission scanning electron microscope (FE-SEM) coupled with a JXA-8230/SXEDS/EDS/WDS energy-dispersive X-ray spectrometer (EDS) was used to get the morphology and the purity of the ZnO nanoparticles. SEM results are represented in Figure 8. The image shows quasi-spherical shaped ZnO nanoparticles agglomerated together. The EDS confirmed the presence of Zn and O. These findings are supported by Nethavhanani (2017) using natural extracts of *Aspalathus linearis* as a reducing agent (Nethavhanani, 2017).

Discussion

Understanding the process of nanoparticles synthesis using the green route is key to the efficiency of the process and the outcome. Following the lack of data on chemical interactions of plant extracts with different metals to form nanoparticles, this study aimed to investigate the interaction of compounds with zinc nitrate to form ZnO nanoparticles. The identification of plant metabolites was performed using LC-MS tools by means of different databases such as KEGG, ChemSpider or Metfrag (Cecilia *et al.*, 2020). Henceforth, the differences in the extracts resulting from the synthesis of ZnO nanoparticles were shown by means of PCA and the VIP score plot. Bush tea leaves contain a high percentage of flavonoids and tannins, apart from non-structural carbohydrates, proteins, fatty acids, and minerals, such as calcium, magnesium, phosphorus, potassium, sodium, iron, manganese, zinc, copper, aluminium, sulphur and fluoride (Lerotholi *et al.*, 2017). Hence, the synthesis process resulted in the complete use of some metabolites as shown in Figure 2. The supernatant recorded low quantities of 8-C-Glucosylnaringenin/Naringenin 7-O-beta-D-glucoside, Scullcapflavone II, Mallotophenone, 6-Methoxyaromadendrin 3-O-acetate, 2-Phenylacetamide, 7-Hydroxy-2″,4″,5″-trimethoxyisoflavone, Coumarin, Malonyldaidzin (Figure 3). A variety of metabolites, such as terpenoids, polyphenols, sugars, alkaloids, phenolic acids, and proteins can reduce metal ions into nanoparticles (Marslin *et al.*, 2018). Flavonoids, polyphenols as well as an aromatic compound interacted most with the precursor to form ZnO nanoparticles (Table 2). UV-Vis is a wonderful tool for the examination of the size and the shape of nanoparticles (Raut Rajesh *et al.*, 2009). The analysed samples show the presence of a wurtzite structure at 380 nm. These findings are supported by (Krupa and Vimala, 2016) who reported the synthesis of ZnO nanoparticles absorbing light at 368 nm. The wavelength of 380 nm corresponds to the bulk band-edge of 3.2 eV for ZnO (Kolekar *et al.*, 2011).

Conclusion

In this study, bush tea metabolites were screened to understand their interaction with metal ions to form nanoparticles. The LC-MMS peaks in both the crude extract before ZnO nanoparticles synthesis and the supernatant after synthesis revealed a significant difference, shown by the PCAs. Different flavonoids, polyphenols and an aromatic compound were found to react with zinc nitrate to form zinc nanoparticles. The FTIR as well as the XRD and UV-Vis analyses confirmed the formation of ZnO nanoparticles with a hexagonal wurtzite structure.

Data availability

All data underlying the results are available as part of the article and no additional source data are required.

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Open Peer Review

Current Peer Review Status: ✗ ✓ ?

Version 3

Reviewer Report 12 August 2022

https://doi.org/10.5256/f1000research.125894.r145453

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Karina Chávez
Instituto de Investigación en Metalurgia y Materiales, Universidad Michoacana de San Nicolás de Hidalgo, UMSNH, Morelia, Mexico

The manuscript discusses about studying the zinc oxide nanoparticles by a green method using the bush tea. But there are some important major notes about this manuscript:

1. Check for grammatical error, typo error, etc. (especially in the introduction section).

2. If possible, the authors should present a scheme for the method using the Bush tea as a reducing agent, which has advantages or differences from other techniques. Otherwise, it is not easy for us to get the novelty.

3. For the XRD results, I strong suggest that the standard pattern of ZnO should be added in Figure 4, then the figure will more readable to readers.

4. The details of the FTIR results of figures 5 and 6 should be described in the figure, such as the compounds or the wavenumber.

5. The scale of the SEM image (μm) is not comparable for nanoparticles. Add images with higher magnifications or add a TEM analysis. Also, the scale marker is small and not appropriate, I suggest editing it.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
No

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Green synthesis and materials characterization

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 11 May 2022

https://doi.org/10.5256/f1000research.125894.r134572

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Saeid Taghavi Fardood
Department of Chemistry, Faculty of Science, Ilam University, Ilam, Iran

The manuscript is appropriate for indexing.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nanoparticles; green synthesis, photocatalysis, dye degradation; metals oxide.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 17 February 2022

https://doi.org/10.5256/f1000research.80148.r119826

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Saeid Taghavi Fardood
[1] Department of Chemistry, Faculty of Science, Ilam University, Ilam, Iran
[2] Department of Chemistry, Faculty of Science, Ilam University, Ilam, Iran

The manuscript needs a careful major revision, before indexing**.** The comments are given below.

1. Synthesis of zinc oxide nanoparticles has been synthesized using other plant extracts. It is better for the authors to compare the present nanoparticles with previous works.

2. Introduction section should be improved and updated, such as by following these references of mine:

- Green synthesis of zinc oxide nanoparticles using arabic gum and photocatalytic degradation of direct blue 129 dye under visible light[1].
- Sol-gel Synthesis and Characterization of Zinc Oxide Nanoparticles Using Black Tea Extract[2].
- Green Synthesis of ZnO Nanoparticles via Sol-gel Method and Investigation of Its Application in Solvent-free Synthesis of 12-Aryl-tetrahydrobenzo[α]xanthene-11-one Derivatives Under Microwave Irradiation[3].
- Facile green synthesis and characterization of zinc oxide nanoparticles using tragacanth gel: investigation of their photocatalytic performance for dye degradation under visible light irradiation[4].
- Magnetic $Mg_{0.5}Zn_{0.5}FeMnO_4$ nanoparticles: Green sol-gel synthesis, characterization, and photocatalytic applications[5].
- Facile green synthesis, characterization and visible light photocatalytic activity of $MgFe_2O_4$ @$CoCr_2O_4$ magnetic nanocomposite[6].

In the proposed references, in all cases, nanoparticles have been synthesized using green synthesis. In the first 4 references, the green synthesis of ZnO nanoparticles has been studied. The authors should express their work with this method of synthesis in terms of size and quality and express their superiority. In the next two references, the green synthesized nanoparticles have been studied using advanced methods and their application as a suitable photocatalyst has been studied, which can help the authors and readers of the article in how to study their analysis and interpretation.

3. For better comparison of synthesized sample, determination of specific surface area, pore diameter and total pore volume of samples, BET analysis is advised.

4. SEM image scale (μm) is not suitable for nanoparticles. It is better to add image scale around (500 and 200 nm) and high quality. If possible, TEM analysis should be performed.

5. It would have been better if the authors had studied the catalytic, photocatalytic, or biological applications of synthesized nanoparticles. What is the authors' goal of synthesizing nanoparticles without examining their application?

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Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nanoparticles; green synthesis, photocatalysis, dye degradation; metals oxide.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 07 Apr 2022

Gabriel Amani Kaningini

Dear Dr Fardood,

Thank you very much for the comments that have helped us improve our article.
Please see below the response to the comments:

1. The work has been compared to other research in the discussion section.

2. Introduction section amended.

3. BET analysis not done due to the kind of study that was led (ref point 5).

4. Image added at 100 nm scale.

5. The work is mainly to explain the dynamics of compounds involved in the synthesis process of ZnO nanoparticles. We have reported the end product to show that ZnO NPs were synthesized.

Please let us know of any concern.

Kindly
AG Kaningini

Competing Interests: No competing interests were disclosed.

Version 1

Reviewer Report 24 November 2021

https://doi.org/10.5256/f1000research.76914.r100333

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The authors reported on the "Green synthesis and characterization of zinc oxide nanoparticles using bush tea (*Athrixia phylicoides* DC) natural extract: assessment of the synthesis process". This work is of interest and of certain significance for chemical processing. But some major modifications are needed before being accepted for indexing:

1. Importantly, there are no experiments discussing the practical utilization of the so-produced ZnO NPs. For example, photocatalytic activities under UV-vis-light irradiation or biological activities. Please include one application of the synthesized ZnO NPs.

2. TEM image of ZnO NPs must be included in the manuscript.

3. Band gap energy of ZnO NPs must be calculated using Tauc Plot.

4. FT-IR spectra of the leaves of bush tea (*A. phylicoides* DC) should be be included in the manuscript.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Green synthesis of Nanomaterials, Solar cells, Photocatalyst

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 26 Nov 2021

Gabriel Amani Kaningini

Dear Aminuzzaman,
Thank you for the comments on our manuscript. I would like to highlight some particulars regarding the work we have done according to the comments:

1. The study was to confirm the ability of bush tea to synthesize ZnO nanoparticles as the title indicates “assessment of the synthesis process”. This is the reason why we explain and highlight the different compounds involved in the synthesis process, the ZnO nanoparticles being the end product of our work we haven’t tested it for any practical use. However, further work is undergoing for their use as fertilizers or growth-promoting agents.

2. We would really like to add the TEM images, however, we have found ourselves limited to the characterization techniques that we have cited in the work. I do believe that the SEM images of the nanoparticles are enough.

3. We have worked on the bandgap energy (UV-Vis results).

4. We have added the FTIR results of the bush tea leaves extract as recommended.

Competing Interests: No competing interests were disclosed.

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