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Anti-Helicobacter Pylori, Anti-Diabetic and Cytotoxicity Activity of Biosynthesized Gold Nanoparticles Using Moricandia Nitens Water Extract

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THIS STUDY aims to synthesize gold nanoparticles (AuNPs) using water extract of *Moricandia nitens* (Viv.) E. A. Durand & Barratte aerial parts water extract. The presence of various phytochemicals viz. alkaloids, carbohydrates, glycosides, flavonoids, tannins, cumarines, protein and saponins by following standard biochemical methods was investigated. The effect of different parameters such as extract concentration, pH and temperature on the size and morphology of the gold nanoparticles (AuNPs) were studied. The synthesized gold nanoparticles (AuNPs) were characterized by using UV-Vis spectroscopy, FTIR and XRD. The synthesized nanoparticles were found to be spherical in shape with average size in the range of 5 to 20 nm. The green synthesized GNPs were found to be potent inhibitors of α-glucosidase with an IC_{50} value of 159.3 µg/ml and showed anti-*Helicobacter pylori* activity against multidrug resistant *H. pylori* strains with an MIC 31.25 (µg/ml). Furthermore, biogenic AuNPS possess more significant anticancer activity against HepG2 and HCT-116 withIC_{50} = 44.6 ± 0.8 µg/ml and IC_{50} = 36 ± 0.7 µg/ml respectively.

Keywords: *Moricandia nitens*, Anti-Helicobacter pylori, Anti-diabetic, AuNPs, Anticancer activity, HepG2, HCT-116

Introduction

Natural products such as plant extracts provide unlimited opportunities for new drug discoveries because of unmatched availability of chemical diversity, either as pure compounds or as standardized extracts [1]. These medicinal plants rich with phenolic compounds, alkaloids, di-terpenoid, and other compounds which inhibit the development of various microorganisms [2]. Besides these, phytochemicals in the plant extract it act as reducing and capping agent in the reduction of metal ions to metal nanoparticles [3]. The biosynthesis of metal nanoparticles using natural products as plant extracts has received a great attention as a suitable alternative to existing chemical procedures and physical methods [4,5]. Green approaches with controlled shape and size have been developed and thereby it can be used as an economic and valuable alternative for the large scale production of metal nanoparticles. Many researchers have reported the biosynthesis of metal nanoparticles by using various plant extracts [6,7].

Gold nanoparticles (AuNPs) have been widely investigated for their immense potential in various biomedical applications such as imaging, photo diagnostics and photo thermal therapy. Early studies showed that AuNPs improved blood glucose level, liver enzymes and pro-inflammatory cytokines causing control of hyperglycemia, which therefore induce the possible role of AuNPs as a cost-effective therapeutic medication in the treatment of diabetes and its complications [8]**.** It has been reported that AuNPs functionalized with chitosan and liposomes are highly stable in gastric acid, and capable of fusing with bacteria

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at physiological pH, making them suitable to treat gastric pathogens such as *Helicobacter pylori* infections [9]. Several studies reported that AuNPs cause cellular damage to mammalian cells through unintended mechanisms including induction of necrosis and apoptosis [10], An earlier report suggests that the AuNPs can be used in the destruction of cancer cells and act as potential therapeutic agents [11].

Moricandia nitens (Viv.) E. A. Durand &Barrattebelong`s to family *Brassicaceae*, which is an economically important family for its many food and oil seed crops as well as containing many important ornamental plants and noxious weeds. Crucifers are characterized by the presence of a group of secondary compounds called glucosinolates [12,13].

From the previous studies GC-MS analysis of the aerial parts and roots of *M. nitens* showed the presence of 50 phytochemical constituents form Glucosinolates [13]. Gluclates are a diverse class of S- and N-containing secondary metabolites that are found mainly in members of the Brassicaceae [14]. Glucosinolates play a variety of roles for plant defense responses and cancer prevention. They are relatively nonreactive, hydrophilic, nonvolatile compounds that are stored within plant vacuoles [15,16].

The present study deals with the synthesis and characterization of AuNPs using aqueous extract of *Moricandia nitens* and their anti-diabetic activity, Anti-*Helicobacter pylori* activities and cytotoxic effect.

Experimental

$HAuCl_4.H_2O$ 99.9% was purchased from Aldrich. *Moricandia nitens* (Viv.) E. A. Durand &Barratte was collected at full flowering stage from Ageba area, Mersa-Mattruh at April 2016, then the aerial parts were air dried, then grounded to fine powder and kept to be used for different analysis. Preparation of *Moricandia nitens* aerial parts extract: The plant extract (2% w/v) was prepared by boiling 2.0g of dried, well grinded *Moricandia nitens* arial parts for 20 min, filtrating, and completing to the volume100 ml deionized water. The extract was freshly prepared for each experiment.

phytochemical screening

Freshly collected plant of *Moricandia nitens* aerial parts were dried and then coarsely powdered. One hundred gm of the coarse powder were extracted using deionized water until clearness. The extract was filtered and subjected to qualitative tests for the identification of various phytochemical constituents [17-19]. Presence of alkaloids were assessed using Dragendorff's test [20], while carbohydrates and proteins using Molisch and Biuret tests, respectively [21]. In addition, cardiac glycosides were assessed using Concentrated H_2SO_4 test [22], coumarin using alcoholic sodium hydroxide, flavonoids by Pew's tests [23], saponin using Foam [20], tannins by Ferric chloride test and terpenes using Salkowski's test [22]. Moreover, volatile oils were assessed using oil distillation method. All these procedures are underlined by Allen S.E. [24].

Anti-diabetic potential Evolution

Determination of α-glucosidase inhibitory activity: The α-glucosidase inhibitory activity was measured according to the method described by You, Q. et al. [25]. Briefly 0.1 mg of each sample or acarbose at different concentration (1000- 1.95 µg/ml) was incubated with 500 µl of 1.0 U/ml α-glucosidase solution in100 mM phosphate buffer (100 mM, pH 6.8) at 37 ^{0}C for 15 minutes. Thereafter, 250 µl of p- nitrophenyl glucopyranoside (pNPG) (5 mM) in the same buffer was added and the reaction mixture was further incubated at 37 ^{0}C for 20 min. The absorbance of the released p-nitrophenol was measured at 405 nm using a microplate reader **(**BioTeK Instruments, Inc., Winooski, VT**)**. The inhibition percentage was calculated using as follow:

$$\% \text{ Inhibition} = \frac{Abs_{control} - Abs_{Sample}}{Abs_{Control}} \text{ x } 100$$

Where, abs control is the absorbance of the control reaction (containing all reagents except the test sample) and abs sample is the absorbance of the test sample. The IC_{50} value was defined as the concentration of a-glucoside inhibitor to inhibit 50% of its activity under the assay condition.

Helicobacter pylori activity assay

Determination of the minimal inhibitory concentration (MIC)

Antibacterial activity of tested compound against helicobacter pylori was determined by a micro-well dilution method described by Bonacorsi, C. et al. [26].

The inoculum of helicobacter pylori was prepared and the suspension was adjusted to 10^6

CFU/ ml. The compounds under investigation and standard drug (Clarithromycin) were prepared in dimethyl sulfoxide (DMSO) and subsequent twofold dilutions (1000-0.03 µg g) were prepared in a 96 - well plate. Each well of the microplate included 40 µl of the growth medium (Brain Heart (BHI) plus 10% fetal bovine serum (FBS), 10 µl of inoculum and 50 µl of the diluted compounds. The Clarithromycin and DMSO are used as positive controls, respectively. The plates were incubated at 37 ^{0}C for 3 days, in 5% O_2,10%CO_2, and 85% N_2 atmosphere. After that ,40 µl of 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide(MTT) at a final concentration 0.5 mg/ml freshly prepared in water was added to each well and incubated for 30 min. The change to purple color indicated that the bacteria were biologically active. The inhibition percentage was calculated using the formula:

$$\% \text{ Inhibition} = \frac{Abs_{control} - Abs_{Sample}}{Abs_{Control}} \text{ x } 100$$

The concentration of samples (Inhibitor) required for 90%of inhibition (MIC 90) was determined to form corresponding dose-response curves. The MIC was taken to the lowest concentration, where no change of color of MTT was determined using an automatic reader at 620 nm. The MITT were done in triplicate.

The cytotoxicity assay

The cytotoxicity assay performed according to the micro culture MTT method with slight modifications [27]. The cells were harvested (1.5×10^4 cells/well) and inoculated in 96-well microtiter plates. They were washed with phosphate-buffered saline (PBS) and the cultured cells were then inoculated with and without the sample (1 mg/ml). After 72 h of incubation, the medium was aspirated. Ten micro-liters of MTT solution (5 mg/ml in PBS, pH 7.2) was added to each well and the plates were incubated for 4 h at 37 °C. After incubation, 100 µl of dimethyl sulfoxide (DMSO) was added to the wells followed by gentle shaking to solubilize the formazan dye for 15 min. Absorbance was read at 540 nm and the surviving cell fraction was calculated. Suramin (100 µM) was used as the reference standard for anticancer activity, and H_2O_2 (1 mM) was used as the cytotoxic agent against normal liver cell lines. The inhibition of cell viability and COX was calculated using the formula: % viability = A_t/A_c x100, where A_t = Absorbance of the test sample and A_c = Absorbance of the control sample.

Synthesis of AuNPs Moricandia nitens aqueous extract:

To synthesize nanoparticles using *Moricandia nitens,* a certain volume of *Moricandia nitens* extract was added to 0.05ml of $HAuCl_4$ at room temperature and the mixture was shacked well then completed to 10 ml with deionized water. The final concentration of Au was 1.4 x 10^{-4} M. The reduction process of Au^{3+} to AuNPs was followed by the change in the color of the solution and UV–visible spectroscopy. The nanoparticles prepared at different pH values, the pH of the solutions (1.4x10^{-4} M $AuCl^{4-}$and 2.6ml extract in 10 ml flask) were adjusted using 0.1 N HCl or 0.1 N NaOH solutions.

Characterization of AuNPs

UV–visible spectral analysis

The bio-reduction of $AuCl_4^-$ ions in solution was monitored by measuring the UV–vis spectra of the reacted mixtures at wavelengths between 300-1000nm on a λ-Helios SP Pye- Unicam UV-Vis spectrophotometer using 10 mm optical path length quartz cuvettes.

Transmission electron microscopy (TEM)

The size and morphology of the nanoparticles were examined and the TEM images were obtained on a JEOL-1200JEM. Transmission electron microscopy samples of AuNPs were conducted by placing a drop of the bio-synthesized AuNPs suspension onto carbon coated copper grids and allowing the solvent to evaporate in air.

X-Ray Diffraction

XRD measurement of the AuNPs was done on a Shimadzu XRD-6000 diffractometer operating at a voltage of 40 KV and current of 20 mA with Cu Kα radiation. (λ= 1.54Å). Fourier transform infrared (FTIR) spectroscopy: Nicolet 6700 FTIR spectrometer was used to obtain FTIR spectra at room temperature. The bio-reduced chloroauric acid solution was centrifuged at 10,000 rpm for 15 min and the sample was dried and grinded with KBr in the form of round disk and it was analyzed to get FTIR of capped nanogold. FTIR of *Moricandia nitens aerial* parts was obtained by grinding dried with KBr.

Results and Discussion

The phytochemical screening

The results for preliminary phytochemical screening carried out on the *Moricandia nitens*, were summarized in Table 1. It showed the presence of flavonoids, carbohydrates, glycosides,

tannins, coumarins, proteins, fatty acids, phenolic compounds, alkaloids, and saponins while terpenes and volatile oils are absent.

UV–visible spectroscopy and TEM Studies

Effect of *Moricandia nitens aerial parts* extract concentration: The formation of AuNPs was confirmed by the visual color change from yellow into mauve, purple (or brilliant red color) and green according to size and shape of formed AuNPs as shown in Fig. 1. Synthesis of AuNPs using a constant HAuCl4 concentration ($1.4x10^{-4}$ M) (5x10-3 w/v) and different concentrations of *Moricandia nitens aerial parts* extract is followed by Uv-visible spectroscopy measurements. Using low *Moricandia nitens* extract concentrations (0.2% w/v) a broad band centered at 560 nm is obtained indicating the formation of AuNPs [28,29]. Increasing the *Moricandia nitens* extract concentration from 0.2 ml to 2.6 ml, the SPR band intensity is found to increase with a blue shift in the band from 560 nm to 543 nm, this blue shift indicates the formation of uniformly polydispersed spherical shaped nanoparticles, with particle size ranging from 5 to 30nm.

As shown in TEM images taken for AuNPs synthesized using 0.4ml of Moricandia *nitens* aerial parts extract*, different* nano-shapes (nano-triangles, nano-spheres and nano-hexagons) with different sizes ranging from 10 to 30 nm, while using 2.6ml of extract the size of the nanoparticles decrease in range 5 - 20 nm with spherical shape (Fig. 2). Further increase of the extract concentration the SPR band starts to decrease again indicating the agglomeration of nanoparticles by secondary reduction phenomenon between capping phytochemicals on the surface of the preformed nuclei [30-32]

Effect of contact time

The rate of the *Moricandia nitens* aerial parts extract mediated biosynthesis of AuNPs is studied by monitoring the absorption intensity of the SPR band at 537 nm at time intervals of 5 min for 40 min. As shown in Fig.3, the bio-reduction started within 2 min. and increased dramatically in the first 20 min. indicating a high initial reaction rate after that only slight increase in the absorption intensity was observed, after 35 min there was nearly no change in the absorption intensity indicating that the reaction was completed within 40 min.

Effect of extract pH

The pH of the extract used in the biosynthesis of AuNPs is a critical factor affecting the size, shape and composition of the nanoparticles. With the help of Uv-vis spectroscopy and TEM analysis the impact of *Moricandia nitens* aerial parts extract pH on biosynthesis of AuNPs is studied. At pH 3 abroad SPR band with undefined peak is absents increasing pH from 4 to 8 the SPR absorption band increase accompanied with a blue shift in the wavelength from 545 nm to 536 nm suggesting the formation of smaller nanoparticles [33]. Increasing pH of extract solution, the SPR decrease with change in SPR maximum indicating that at pH higher than 8 affect the reducing ability of the extract.

These results were consistent with several studies which reported that at low pH, the gold nanoparticles prefer to aggregate to form larger nanoparticles rather than to nucleate and form new nanoparticles (aggregation of nanoparticles is favored over the nucleation). However, higher pH facilitates the nucleation and subsequent formation of large number of nanoparticles

TABLE 1. The preliminary screening of water aerial parts extract of Moricandia nitens.

Phytochemical compound	Results
Alkaloids	+
Carbohydrates	+
Glycosides	+
Flavonoids	+
Tannins	+
Cumarines	+
Protein	+
Saponins	+
Terpenes	-
Fatty acids	+
Volatile oil	-

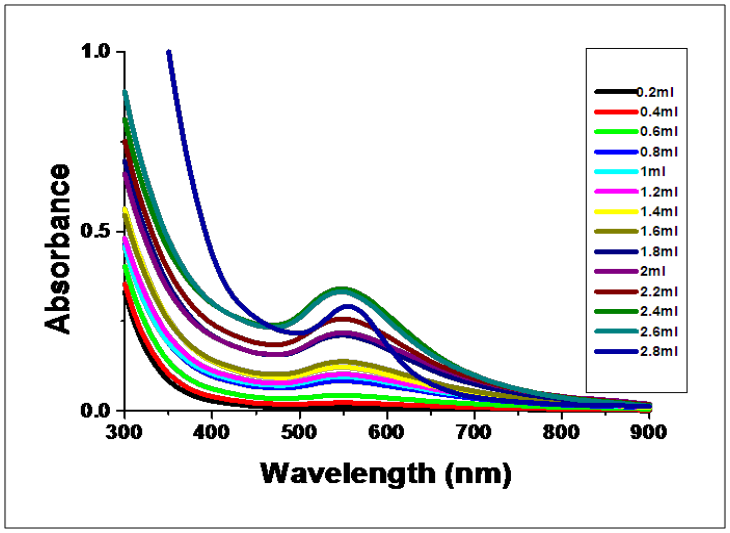

Fig. 1. Uv-vis spectra of gold nanoparticles using constant HAuCl4 concentration ($1.4x10^{-4}$M) and different Moricandia nitens aerial parts extract concentrations.

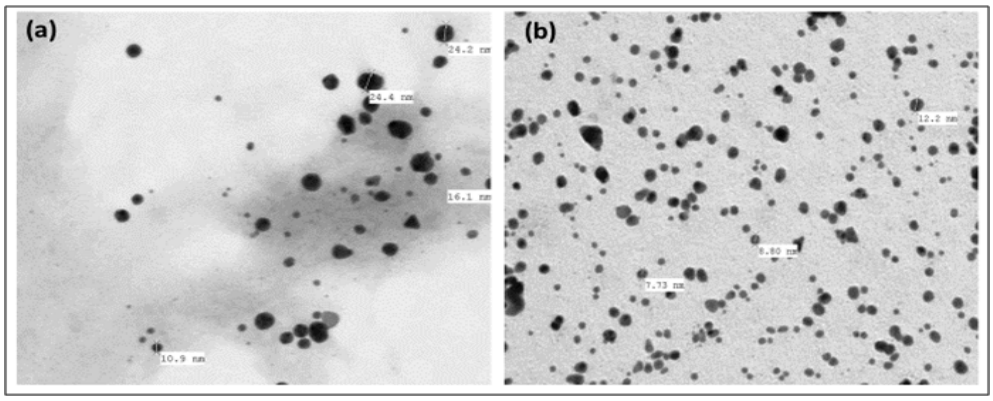

Fig. 2. TEM images of AuNPs synthesized using HAuCl4 ($1.4x10^{-4}$M) and (a) 0.4ml and (b) 2.6 ml of Moricandia nitens aerial parts extract.

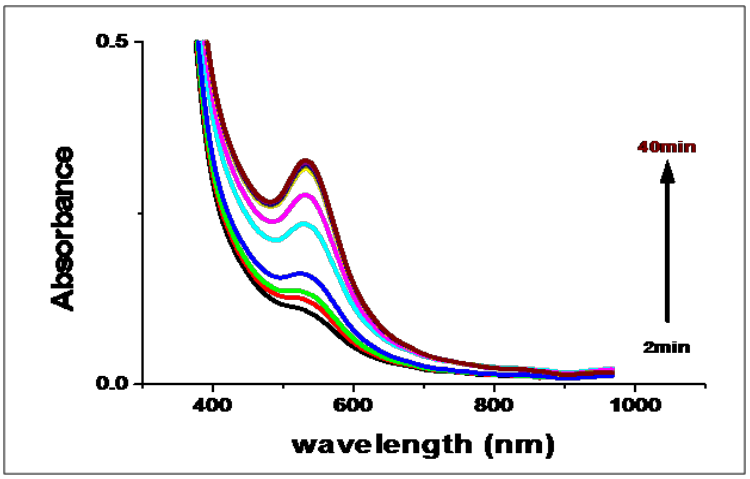

Fig. 3. UV–Vis spectra recorded from reduction of $1.4x10^{-4}$ M $HAuCl_4$ using 2.6 ml of Moricandia nitens aerial parts extract at various time intervals of 5 minutes for 40 minutes.

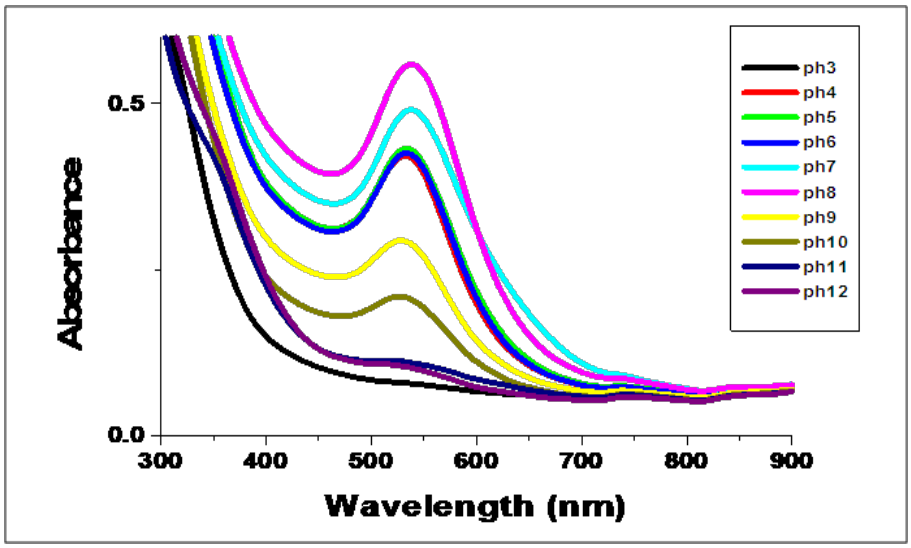

Fig. 4. Uv-Vis spectra of AuNP synthesized using 2.6 ml of Moricandia nitens aerial parts extract using $HAuCl_4$ at different pH of the extract (pH 3-12).

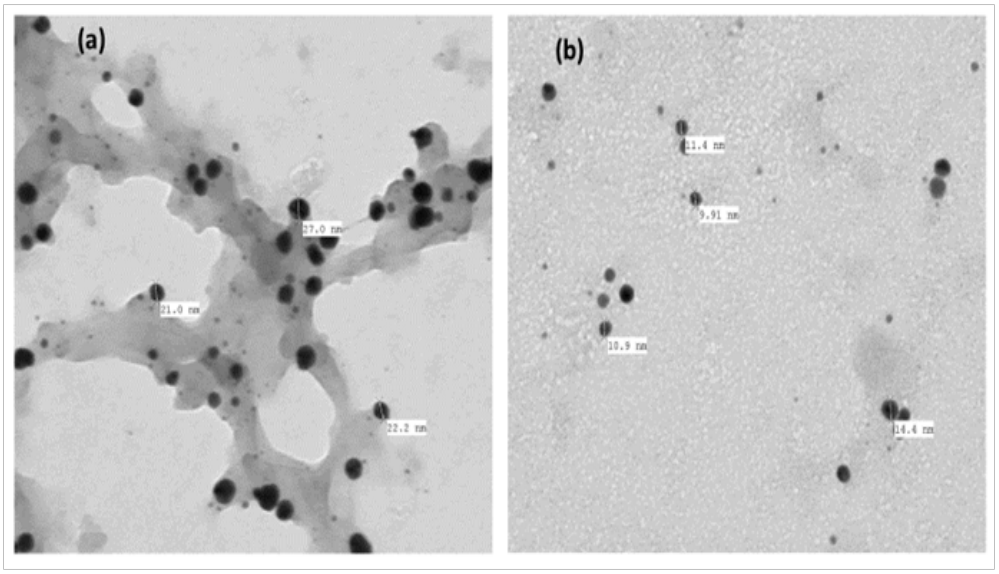

Fig. 5. TEM images of AuNPs (a) at pH4 and (b) at pH8 of plant extract.

with smaller diameter, also more functional groups (carbonyl and hydroxyl) are available for gold binding thus, a higher number of Au (III) complexes would bind to the biomolecules at the same time [34-36]. Increasing alkalinity of the surrounding media above pH 8 induced a change in the electron density on the surface of AuNPs so it affects surface plasmon band and band intensity decreased.

Effect of reaction temperature on AuNPs synthesis

Figure 6 shows the effect of temperature in the nanoparticles synthesis. The SPR of nanoparticles increases with increasing the reaction temperature using Moricandia *nitens* extract with blue shifted form 556 nm to 530 nm indicating formation of small size of AuNPs with increasing reaction temperature. The higher rate of reduction was occurred at higher temperature due to the consumption of gold ions in the formation of nuclei whereas the secondary reduction was stopped on the surface preformed nuclei [30].

X-Ray diffraction study

The green-synthesized nanoparticles were clearly analyzed using XRD measurements.

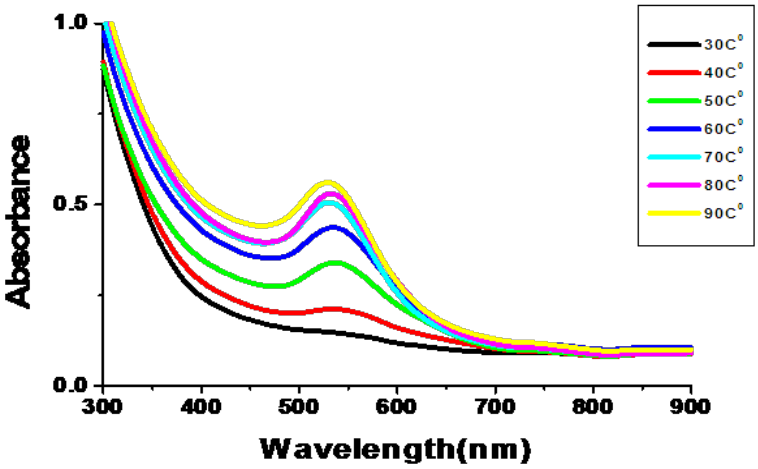

Fig. 6. Uv–vis spectra of AuNPs as a function of time.

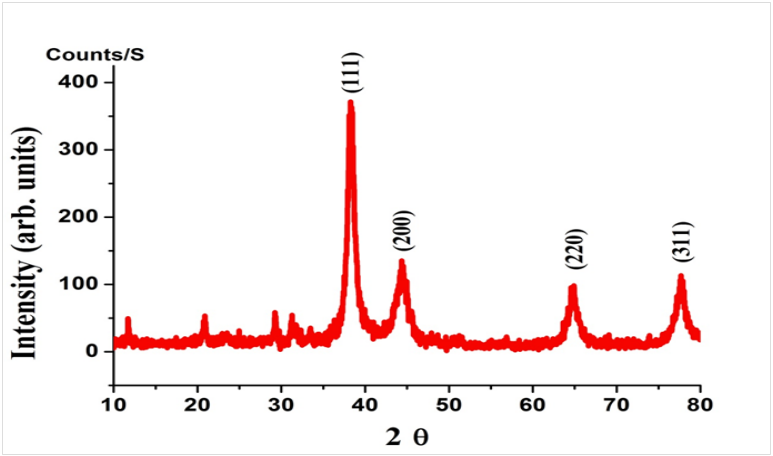

Fig. 7. The X-ray diffraction (XRD) patterns of AuNPs synthesized using Moricandia nitens aerial parts extract.

Figure 7 shows the XRD patterns of the AuNPs prepared using *Moricandia nitens* extract. The XRD patterns revealed that AuNPs corresponded to the crystalline gold face centered cubic phase. The four prominent Bragg reflections obtained at 2θ = 38.33° (1 1 1), 44.35° (2 0 0), 64.83° (2 2 0), and 77.73° (3 11) are identical with those reported for the standard gold metal (Au^0) (Joint Committee on Powder Diffraction Standards-JCPDS, USA). The data revealed crystalline nature of AuNPs.

Fourier transform infrared spectroscopy (FTIR)

FTIR measurements were carried out to identify the possible biomolecules in the *Moricandia nitens* aerial parts extract responsible for the reduction and the stabilization of the synthesized AuNPs. The FTIR spectrum of the aerial part extract of Moricandia *nitens* is shown in Fig.8. The absorption bands at 3406.4 $cm^{-1}$is associated with OH (alcohol) stretching. The peaks at 2922.3, 2852.0 cm^{-1} are associated with anti-symmetric and symmetric stretching of CH_2, respectively. The peak for the carbonyl group was found at 1738.5 cm^{-1}. The synthesized AuNPs revealed a shift in the peaks to 3404.5, 2918.3, 2849.1, and 1730.0 cm^{-1}. The highest absorption peak 3404.5 cm^{-1} reflects that the OH group might be responsible for the reducing property of the extract.

In vitro α-glucosidase enzyme inhibition assay

The in *vitro* α-glucosidase inhibitory studies demonstrated that gold nanoparticles synthesized *using Moricandia nitens* had α-glucosidase inhibitory activity. Gold nanoparticles showed a strong inhibitory potential with an IC_{50} = 159.3µg/ml, Extract IC50 >1000 µg/ml .The Acarbose, the positive control used in this study, inhibited the activity of α-glucosidase with an IC_{50} value estimated at 30.54µg/ml (Fig.9). It should be mentioned here that the calculated IC_{50}values in the current studies is correlated with earlier studies

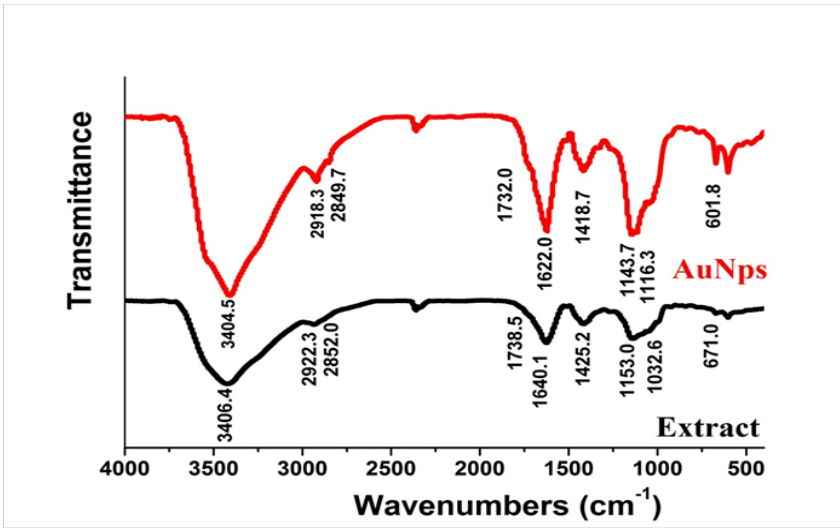

Fig. 8. FTIR of Moricandia nitens extract and capped AuNPs.

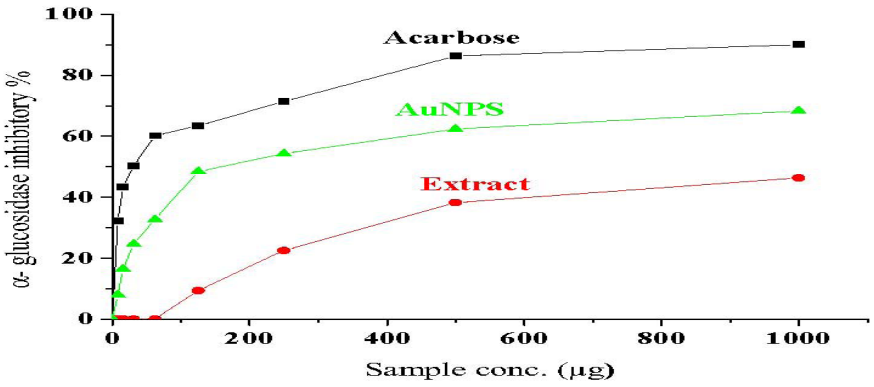

Fig. 9. Anti-diabetic activity of Acarbose, Moricandia nitens extract and AuNPs.

TABLE 2. Anti-diabetic activity (IC_{50}) of Acarbose, extract and AuNPs.

Sample code:	IC_{50}
Acarbose	30.57
Extract	>1000
AuNPs	159.3

TABLE 3. MIC of Clarithromycin, extract and AuNPs.

Sample code:	MIC
Clarithromycin	**1.95**
Extract	**>1000**
AuNPs	**31.25**

[37]. The IC_{50} values of AuNPs of exhibited lower value compared to *Moricandia nitens* extract which suggest that nanoparticles are potent than plant extract in inhibiting α-glucosidase. The strong enzymatic α-glucosidase inhibitory activity shown by green synthesized AuNPs is much better than that of plant extract inhibition. It can be proposed that AuNPs synthesized from *Moricandia nitens* might be a potential resource of α-glucosidase inhibitors formulation that may benefit diabetes treatment.

3-9 In Vitro Anti-Helicobacter Pylori Activity.

MIC90 of AuNPs synthesized using *Moricandia nitens* for their in vitro anti-H. pylori activity revealed inhibition of the bacterial growth relatively at low concentration than extract at 31.25 and >1000 for AuNPS and extract respectively for H. pylori. The AuNPs are effective against H. pylori reflecting that AuNPs could be used as potent anti-H. pylori agents. current studies is correlated with earlier studies [38].

In vitro cytotoxicity of Au NPs

The *in vitro* anticancer efficacy of, AuNP's and aqueous extract of aerial parts confirmed by MTT assay exhibited *with* IC_{50} = 36 ± 0.7 *μg/ml* and IC_{50} = 99.8 ±1.4μg/ml for colon *carcinoma* (HCT-116), IC_{50} = 44.6 ± *0.8* μg/ml and IC_{50} =114 ± 1.4 μg/ml. for *Hepatocellular carcinoma (HepG-2)* respectively. The results clearly reveal that the Au-NPs exhibited selective cytotoxicity against HCL-116 and HepG2 cancer cells. Form thesis results we can say that nano synthesis gives great support and improvement to plant extract in cytotoxicity activities.

Conclusion

Our study is the first report on the green synthesis of gold nanoparticles from *Moricandia nitens* and suggests their strong inhibition of digestive

TABLE 4. Cytotoxicity (IC_{50}) of aqueous extract of Moricandia nitens and the nanoparticles.

Sample code:	IC_{50}= μg/ml ((HCT-116)	IC_{50} = μg/ml (HepG-2)
Extract	99.8 ±1.4	114± 1.4
AuNPs	36 ± 0.7	44.6 ± 0.8

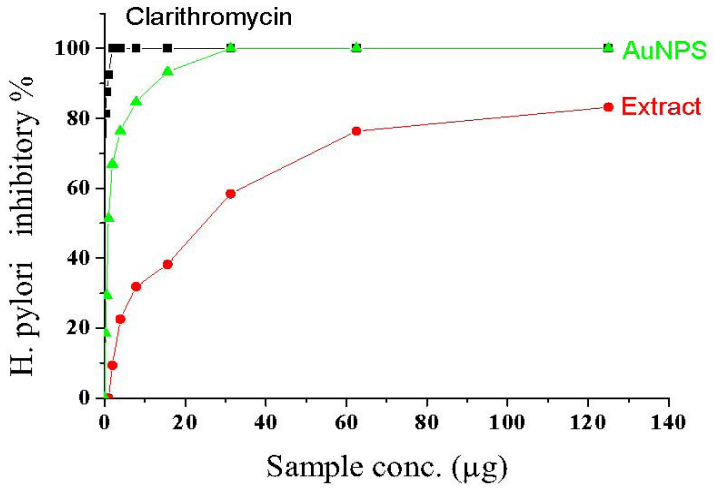

Fig. 10.Anti-Helicobacter pylori Activity of Clarithromycin, Moricandia nitens extract and AuNPs.

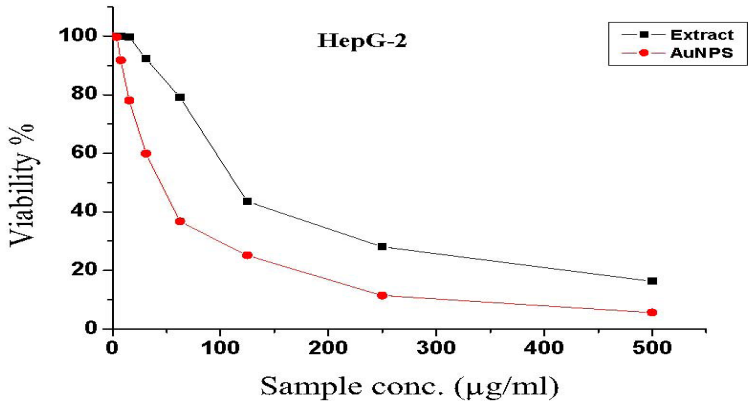

Fig. 11. Cell viability% of AuNPs prepared using Moricandia nitens extract for Hepatocellular carcinoma (HepG-2).

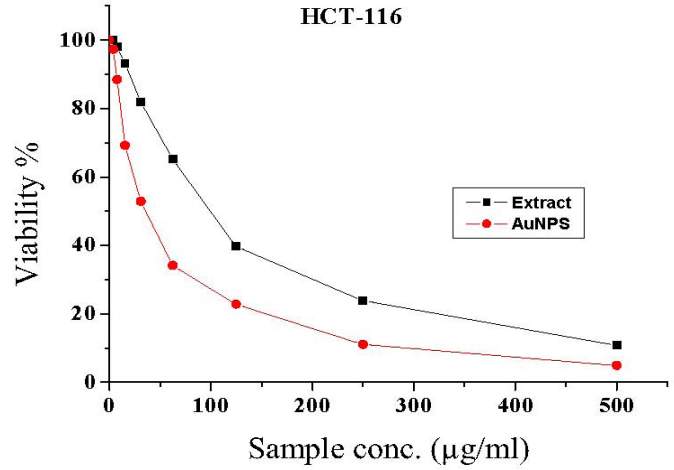

Fig. 12. Cell viability% of AuNPs prepared using Moricandia nitens extract for colon carcinoma (HCT-116).

enzyme α-glucosidase. The efficacy of the gold nanoparticles to inhibit glucosidase and serve as an anti-diabetic agent has been ascertained, exhibited anti-H. pylori activity against multi-drug resistant clinical strains of H. Pylori also synthesized nanoparticles were found to be effective against HepG2 and HCT-116 carcinoma cells.

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مكافحة البكتريا المضادة للهيليكوباكتر، النشاط المضاد للسكري والسمية للخلايا الحيوية بجسيمات الذهب النانوية المحضرة باستخدام المستخلصات المائية لموريكانديا نيتنس

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تهدف هذه الدراسة لتخليق جسيمات الذهب النانونية باستخدام مستخلص المائى لنبات الموركنديا *(snetin aidnaciroM)* *(dnaruD .A .E (.viV)* كما تهدف الى معرفة مايحتويه هذا النبات من العديد من المواد الكيميائية التى تعمل كعامل مختزل لأملاح الذهب وتحويها إلى جسيمات الذهب النانونية. وقد وجد أن المستخلص المائى لهذا النبات يحتوى على العديد من المواد الكيميائية المختلفة مثل الألكالويد ، الكربوهيدرات ، الجليكوسيدات ، الفلافونيدات ، التانينات ، الكومارين، البروتينات والصابونين. وقد تم تحديد العوامل والظروف للتفاعل مثل تركيز المستخلص وتأثير الأس الهيدروجيني و زمن التلامس بين المستخلص وايونات الذهب ودرجات الحرارة للتفاعل . وقد تم توصيف الجسيمات النانونية المحضرة باستخدام التحليل الطيفي للأشعة فوق البنفسجية *RITF ,siV-vU* و*DRX* والميكروسكوب الإلكتروني. حيث وجد ان الجسيمات النانونية جسيمات متناهية الصغر كروية الشكل مع متوسط الحجم في 5-02 نانومتر. وقد تم استخدام هذه الجسيمات في عدة تطبيقات كمثبطات قوية ل *esadisoculg-α* لمرض السكر حيث أظهرت الجسيمات النانوية للذهب قيمة *05CI = (3.951 ميكروغرام / مل)* واستخدمت فى سمية الخلايا السرطانية للكبد والقولون وأظهرت النتائج نصف الجرعة المميتة كالاتى *05CI = 6.44 ± 8.0* ميكروجرام / مل لخلايا الكبد *(2GpeH)* و05CI = 63 ± 7.0 ميكروجرام / مل لخلايا القولون *(611-TCH)*. ايضا استخدمت جسيمات الذهب النانوية الناتجة كمضاد لجرثومة المعدة *(irolyp retcabocileH)* وأعطت نتائج CIM= 52.13 (ميكروغرام / مل).