Arabian Journal of Chemistry (2021) **14**, 102956

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Facile one-step green synthesis of gold nanoparticles (AuNp) using licorice root extract: Antimicrobial and anticancer study against HepG2 cell line

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Received 28 September 2020; accepted 13 December 2020
Available online 21 December 2020

KEYWORDS

Licorice root;
Gold nanoparticles;
Sterol compounds;
Anticancer;
Antioxidant

Abstract Nanostructures synthesis via green method has been popular due to agitating assets and ground-breaking applications. Cost-effective novel and environment friendly gold nanoparticles (AuNp) synthesized via licorice root extract. The conditions for the eco-friendly AuNp synthesis was optimized by 6 ml salt concentration and 4 ml extract with pH 5 after 2:30 h at 25 °C temperature. A more detailed characterization of green synthesized AuNp was performed using UV–Vis, Fourier-transform infrared (FTIR) and X Ray diffraction (XRD) spectroscopy EDX analysis, transmission and scanning electron microscopy, DLS (dynamic light scattering) and High-performance liquid chromatography to identify the components within licorice root extract. With the aim of retrieving consistent capacity on antioxidant activity ABTS and DPPH two dissimilar broadly applied antioxidant methods were applied. The cytotoxicity of green synthesized AuNps was assessed applying an MTT method upon MCF-7 (breast cancer), HePG-2 (liver) cell-lines. The antifungal with antibacterial activity of green synthesized AuNp was examined by agar well diffusion technique. The as synthesized AuNp showed restrained antibacterial, antifungal activity towards bacterial and fungal strains used and importantly reflective anticancer activity was observed against used cell lines.

1. Introduction

Glycyrrhzia glabra L., commonly known Liquorice root is Fabaceae family member, conventional plant (Karkanis et al., 2018). It is also known as Western licorice (Li et al., 2019), which is believed to be one of the widely spread oldest plant used in medical field (Komes et al., 2016). It is general herb of the Asian region and is commonly used in the diet

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.arabjc.2020.102956

(Shinde et al., 2016). The triterpenoid glycyrrhizin present in it responsible for its sweet taste (Chakravarthi et al., 2012), comparatively its 50 times more sweet alongside sucrose (Page and Hennessy, 2008). The roots are long, cylindrical, thick and multibranched (Gupta et al., 2008) restrain diversity of secondary metabolites amino acids resins carbohydrates. The roots show range of activities including antioxidant, antimutagenic dropping cortisol level. Licorice contribute in lowering the blood cholesterol, enhancing the memory, act as antidepressant (Bahmani et al., 2014). Till date, Licorice have given roughly 400 different chemical contents, counting about 300 flavonoids including chalcones, isoflavones, flavones flavonols, and beyond 20 triterpenoids which got extensive interest due to their structural variety and important bioactivities (Wang et al., 2019). These metabolic compounds are well known for the pharmacological properties of the Licorice (Bahmani et al., 2014). It also used to reduce throat infections, inflammation and eye and liver diseases in Indian ayurveda system. Commonly Licorice exhibit antiviral, anticancer, antidiabetic, antiallergenic, and expectorant activities (Chidambaram, 2017).

Recent years nanotechnology has grown lone exhilarating vanguard fields amongst other important one (Bhushan, 2017), and received great attention from scientists and researchers. Nanotechnology through its application within every scientific field, pharmacy, engineering(Al-Radadi, 2018), food, agriculture (Satpathy et al., 2019), biomedical including bio-imaging and drug delivery (Ramalingam et al., 2016), in other sectors like electronics, biology, medical treatment (LIU et al., 2016), is flourishing as an essential area of research (Al-Radadi and Al-Youbi, 2018(a)). Now a days it is well known that nanomaterials have unique optical, electrical, thermal and magnetic properties (Liu et al., 2016) but still there is space for further work on anticancer and antimicrobial activities. Two main approaches were used for preparing metal nanoparticles; First bottom up and top down, bottom-up method is used for preparation of nanoparticle because it is most acceptable and effective method, in which nanoparticles are "grown" using reaction precursors which are simpler molecules (Saif et al., 2016). Green synthesis, a bottom up approach (Hussain et al., 2016). The green method of synthesis, also well-known as phytosynthesis (Gopinath et al., 2016). Green synthesis is environment friendly, effortlessly scaled up while large scale production concern (Rafique et al., 2017), nontoxic and low-cost (Al-Radadi and Al-Youbi, 2018(b)). In nanoscience and nanotechnology, green way of noble metal NPs synthesis has emerged interesting field owing to its wide use like cosmetic products tooth paste, detergents, besides medical pharmaceutical diagnostic and drug delivery applications Metallic nanoparticles are having vast scope with superb chemical and physical properties, like high heat transfer (thermal conductivity) and high surface-to-volume ratio. Gold nanoparticles (AuNps) are inert in nature relatively resistant to bacteria have unique optical, physical and chemical properties with a rich history since ancient times and an intense future in the field of biological and chemical sciences. There is increased requirement for building up eco-friendly methods to synthesize nanoparticles devoid of applying toxic. The range of eco-friendly nanoparticle synthesis approaches include using enzyme, microorganisms, plant extracts, medicinal plants (Song et al., 2009; Kshirsagar et al., 2017; Borah, 2020; Ali et al., 2019).

While performing evidences on Licorice mediated nanoparticle synthesis we are not finding any related work. From this standpoint it was to be explored if the gold nanoparticles synthesized using Licorice root will it show straight cytotoxic, antimicrobial effects and anti-coagulative properties relevantly. With the intention of solving this issue an aqueous extract was used to synthesize gold nanoparticles examined for significant anticancer activity against HepG2 and MCF7 cells, anti-coagulative and antimicrobial activity against Gram-negative and Gram-positive microbes for the first time. Also facile one step method for gold nanoparticle production from Licorice root is developed here.

2. Experimental details

2.1. Preparation of licorice root extract

To drain out the impurities licorice root was washed thoroughly using double distilled water. 2 g of licorice root are heated in 70 ml double-ionized water with 20 ml ethanol, filter the aqueous extract. The filtrate is used as reducing agent.

2.2. Gold nanoparticle synthesis

$HAuCl_4{\cdot}3H_2O$ derived from Sigma-Aldrich with high purity 6 ml volume of 1×10^{-3}M concentration mixed with several volumes of licorice root extract (1 ml, 2 ml, 3 ml, 4 ml). Allowed the reaction to take place and monitored using UV vis Spectroscopy time to time. The remarkable color change from yellow to violet to dark violet monitored. Further different pH and temperature, gold salt concentration and extract concentration was optimized.

2.3. Characterization

Gold nanoparticles formation was monitored by UV–Vis spectroscopy using double beam spectrophotometer at wavelengths rang 350–700 nm. The dry powder of gold nanoparticles was used for X-Ray Diffraction (XRD) analysis using Shimadzu XRD-6000. FTIR (Fourier transforms infrared) spectrometry performed on thermo a Nico-let 6700 in range of 400–4000 cm^{-1} in order to analyze the possible function group present. The examination of size, surface morphology carried out on JEOL/JEM 2100 transmission electron microscope operated at 90 KV and FESEM (Field Emission-Scanning electron microscope). The energy dispersive X-ray analysis (EDX) of purified AuNps was performed on JSM-5610LV from JEOL EDX. The bioactive compounds in licorice extract were recognized and enumerated via HPLC. Size distribution and zeta potential performed on Malvern Zeta sizer version 7.13.

2.4. Cytotoxicity study

Cytotoxicity is the toxicity of prepared chemicals immune-mediator cells or natural compounds towards particular cell-lines. An MTT (3–14,5- dimethylthiazol-2-yl)-2,5-diphenylte trazolium bromide) is a compound accurately measures the cytotoxicity. In this assay cell culture ($1x10^4$ cells/ml) plated on 96 well plate with 100 pl/well. The known sample extract which was diluted mixed in to each well with known concen-

trations 0, 1, 2, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250 and 500 ug/ml and incubated. Finishing 24 h of incubation MTT liquid compound dissolved and further 24 h incubated. The 50% concentration causing growth inhibitory action (IC_{50} value) to tumor cells was estimated using following formula

$$Cell\ viability\% = \frac{OD\ value\ of\ experimental\ sample\ (mean)}{OD\ value\ of\ experimental\ control(mean)} \times 100$$

2.5. Antimicrobial activity

Antimicrobial activity of licorice root extract and AuNP against ten different microbial pathogens was examined by well diffusion technique.

Total five bacterial strains *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhi (ATCC 6539)* with five fungal cultures *Aspergillus niger* (RCMB 002007), *Candida albicans* (ATCC 10231), *Fusarium oxysporum* (RCMB 008002), *Aspergillus flavus* (ATCC 16883) and *Penicillium citrinum* (RCMB 001011) were used for testing. Primary, the developed microbial strains were equally seeded onto Petri plates surface containing 10 ml medium. Each well was filled with 1 ml (0.5 mg/mL) sample. A well with 5 mm radius was created in the petri plate and incubated 72 h at RT for fungal culture and 24 h at 37 °C for bacterial cultures. The compounds having antimicrobial property inherited within samples were circulated inside of medium plus interrelated to microbes. An activity was measured in the zone of inhibition by calculating the diameter in mm. The samples were dissolved in DMSO and it showed no inhibitory action against studied microbial cultures validating negative impact on microbial growth.

2.6. Analysis of antioxidant activity of AuNps

DPPH (1,1-diphenyl -2-picryl-hydrazine) is a compound generally used to determine the free radical scavenging activity. AuNps synthesized using Licorice root extract was investigated for antioxidant assay. DPPH reagent was prepared in methanol was mixed in various concentration (100, 80, 60, 40, 20 µg/mL) of AuNp after addition shake well and incubated for 30 min. at RT. Later the incubation, the optical density was observed at 517 nm wavelength. The percent inhibitory (PI) action of free radical DPPH investigated using following formula

$$PI = \frac{AC - AT}{AC} \times 100$$

where AT = Sample absorbance, AC = Control absorbance. Ascorbic acid was used as the reference.

3. Results and discussion

3.1. Phytochemical analysis of licorice (Glycyrrhiza glabra)

HPLC has ability to separate and identify the compounds present in any specific sample that can be dissolved in a liquid in trace concentrations (Gupta et al., 2013). Phytochemical screening: is a broad name for a wide variety of compounds produced by plant (History and Chauhan, 2016). In general extract of plant act such as a possible replacement towards reducing as well as stabilizing agents (Deokar and Ingale, 2016; Deokar and Ingale, 2018) because of multiple important bio-components are present like flavonoids, phenolics, glycosides, organic acid, proteins, amino acids and fatty acid (Ahmad and Kalra, 2020).

3.1.1. Phenolic, flavonoids, glycosides and organic acid

We used the HPLC device to study the neutral antioxidant in licorice root plant and contain phenolic, flavonoids, glycosides and organic acids as shown in the (Fig. 1) and (Table1). Plants are prospective supply of precious antioxidants. Secondary metabolites in plant are phytochemical or Natural source of antioxidants. Phenolics compound provide distinctive flavor, taste and healthiness encouraging assets originated within fruits as well as vegetables (Ghasemzadeh and Ghasemzadeh, 2011). Phenols are very heterogeneous compounds as much by their composition as by their structure (Delgado et al., 2019). The highest concentrations found for phenolic acids were: Sinapic, Ellagic, protocatechulic and ferulic respectively. The flavonoid it is important because of their biological activities (anticarcinogenic, anti-inflammatory effects) (Botsoglou, 2001). Flavonoids are polyphenolic compounds, are antioxidants and may prevent lipid peroxidation and the formation of atherosclerotic plaques (“the_sugar_moiety_is_a_major_determinant_of_the_ab-wageningen_university_and_research_46008.pdf,” n.d.). Among the flavonoids are: luteolin, rutin and kampherol (dos Santos et al., 2017). Glycosides used to lower blood pressure (Investigation and Profile, 2020). Also, the highest amounte of glycosides is linamarin, pinoresinol, lorcinadols. The natural compound organic acid contributes to the acidic nature of the Licorice root (Maduwanthi and Marapana, 2019). Organic acids contain succinic acid, ascorbic acid, gallic acid and fumaric acid. Glycyrrhizin (GL), also known as glycyrrhizic acid, is a natural triterpene glycoside, glycyrrhetinic acid as an active component in licorice is responsible for the sweet-tasting properties of licorice. The glycyrrhizin sweet taste is considered to be 50–200 times higher than sucrose and this important characteristic makes it a powerful natural sweetener. It is used as food additive could provide foods with health benefits in preparing some specified products for persons suffering from low blood pressure, Glycyrrhizin is considered a main ingredient in various medicines such as antimicrobial, anti-ulcer, anti-hepatotoxic and antivirus activities (Abd El-Lahot et al., 2017; Noori et al., 2018; Zhao et al., 2017). It is combination of couple of glucuronic acid molecules plus single glycyrrhizic acid molecule. GL also possesses antipyretic, antimicrobial, antiherpes and anxiolytic activities. GL has hepatoprotective activities inhibits production of pro-inflammatory cytokines. GL is prepared by the glycyrrhizic acid calcium and potassium salts (González-Reyes et al., 2016; Peng et al., 2017; Sakai-Sugino et al., 2017). Licorice root contain high amount of glycyrrhizin as shown in (Table 1 **and** Fig. 1). The differences in constituents may perhaps due to ecological factors (humidity, light, temperature) and genetic factors and cultural practices too (Gundogdu et al., 2011). Sterols are useful biomarker, Sterols are indispensable bio-molecules which contribute towards membrane function along with fluidity of membrane (Crandall et al.,

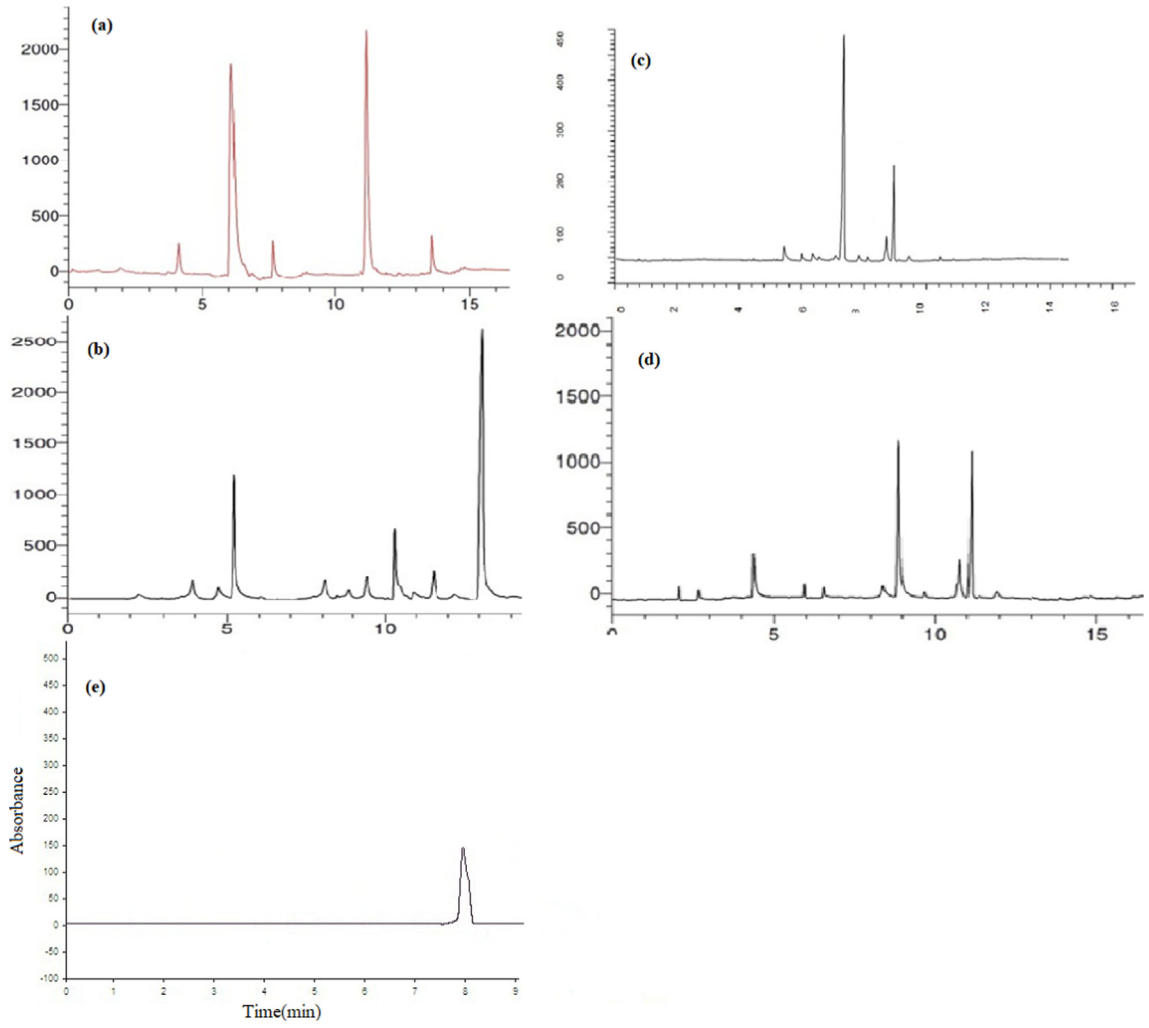

Fig. 1 HPLC chromatograms of phenolic (a), flavonoids (b), glycosides (c), organic acid (d), Glycyrrhizin (e) present within Licorice root extract respectively.

2016). Sterols are biomolecules with water hating nature plus it have a tendency to connect towards organic matter. They also omnipresent multipart imperative component of cells (Biache and Philp, 2013). Fig. 2 shows the sterols constituents of *Glycyrrhiza glabra* tincture and mass fragmentation is representing in Fig. 3. The tincture contains three analyzed sterols: Beta-sitosterol (29.3Ug/mg), Dihydrostigmasterol (14.6Ug/mg), ergosterol (1.4Ug/mg). Out of these the Beta-sitosterol is comparativelylargest (Khalaf et al., 2012).

3.1.2. Antioxidant activity

The total constituent of licorice root phenolic and flavonoid was quantified by HPLC. The results obtained in (Tables 2) (Diaz et al., 2012). The antioxidant activity of the Licorice root extracts both ABTS and DPPH assay was performed as shown in Table 2 (Komes et al., 2016), The differentiation of outcomes of ABTS method (Eyupoglu, 2019) with DPPH method (Yeo and Shahidi, 2019), expressed in IC50 values (Rahman et al., 2019) is suggestion of diverse reaction of antioxidants within extracts. Believing the information which DPPH act in response with only lipophilic antioxidants, whereas ABTS counter amid together lipophilic antioxidants and hydrophilic, these fact are now turning very noticeable (Komes et al., 2016). The aqueous phase antioxidants in the study were Glutathione (GSH) and Ascorbic acid (ASC). The GSH value of licorice root was 18.26 ± 0.84 μmol/g FW and amount of ASC was 10.51 ± 1.37 μmol/g FW in licorice root (Hamad et al., 2015). The necessary nutrients amino acids play a key role in the human health. Several amino acids like histidine, arginine and lysine react with carbohydrates to produce melanoidins an antioxidant (Zhang et al., 2019). Little amino acid deficiency in our body could result to sluggish metabolism, trouble losing weight, trouble building muscles mass, low energy levels and poor concentration, mood swing, muscle, bone and joint pain (Ogagaoghene et al., 2019). 17 Amino acids were identified in licorice root plant Out of 23 amino acids (Ibrahim et al., 2019). showed significant difference in their amino acid contents were studied in (Table3) and (Fig. 4), like L -Methionine, Glutamic acid, L –Valine, L -Threonine, Aspartic acid, Glycine, Cysteine, L -Lysine, Serine, proline, L -Isoleucine, L -Phenyl alanine, Alanine, Arginine, Histidine, L -Leucine, Tyrosine respectively. Licorice root are rich in antioxidant as we showed above.

Table 1 Concentration of Phenolic, Flavonoids, Glycosides and Organic acids (mg/g) in licorice root plant.

Phenolic				
Concentration in licorice root	Sinapic	Ellagic	Protocatechulic	Ferulic
	25.14	18.09	9.12	7.04
Retention time	11.02	6.2	13.5	7.5
flavonoids				
Concentration in licorice root	Luteolin	Rutin	Kampherol	–
	50.15	27.1	12.5	–
Retention time	13.1	5.4	10.3	–
glycosides				
Concentration in licorice root	linamarin	pinoresinol	laricinesol	–
	50.12	18.30	4.22	–
Retention time	6.8	9.5	6.8	–
Organic acids				
Concentration in licorice root	Succinic acid	Ascorbic acid	Fumaric acid	Gallic acid
	13.41	10.87	2.17	4.56
Retention time	9.1	11.0	10.7	4.21
Triterpenes				
Concentration in licorice root	Glycyrrhizin			
	81.737			
Retention time	8.112			

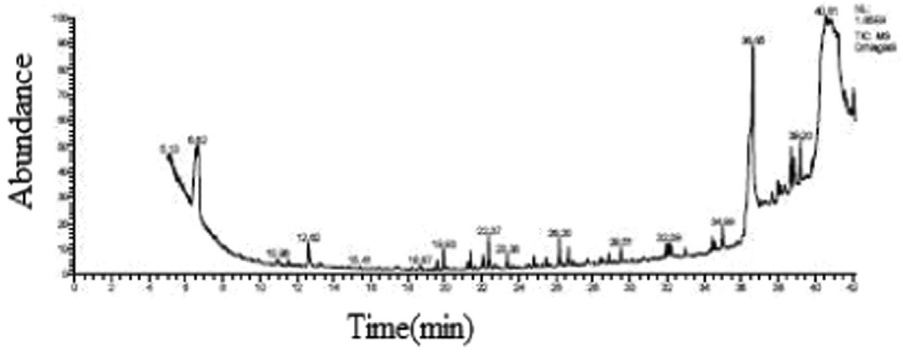

Fig. 2 The chromatogram from the GC–MS analysis of the licorice root of setorel copmpund.

3.1.3. Dietary fiber content

Dietary fiber is an integral part of plants and also plays vital role in human nutrition. Generally, it is categorized into soluble and insoluble dietary fiber. Pectic substances and hydrocolloids are soluble dietary fiber whereas, cellulose, hemicelluloses and lignin arre insoluble in water (Ahmed et al., 2016). Dietary fiber support favorable physiological property counting laxation (Bunzel et al., 2002) and cleanse the bowel (Awotedu et al., 2020) help in reducing the blood lipids level, thus dropping the cardiovascular diseases risk (Aremu et al., 2019). Dietary Fiber is called Antioxidant (Herrera-Balandrano et al., 2020). Analysis of licorice root it is contain fiber soluble (14.2%) and contain fiber insoluble (5.36%).

3.1.4. Vitamins

The use of HPLC for the study of water-soluble vitamins in licorice root has been demonstrated to be a fast, simple, and reliable method (Sami et al., 2014). Vitamins are organic compounds having little molecular weight necessary in tiny amount for different physiological and chemical functions to usual growth and development. Out of the 13 vitamins (Abibu et al., 2019) none vitamin given the peak in chromatograph as shown in (Fig. 5).

3.1.5. Minerals

The mineral concentration of licorice root are varieties as shown in (Table 4) (Butkute et al., 2018), seven minerals were quantified in licorice root. The major mineral present in licorice root is Calcium (387.1 mg/100 g) followed by Potassium (104.5 mg/100 g), Silicone (39.6 mg/100 g), Phosphorus (14.9 mg/100 g) and contain few amount of Iron (3.8 mg/100 g), Sulfur (1.23 mg/100 g) and Magnesium (0.5 mg/100 g) (Matsuo et al., 2019). The mineral constituents are conveyed like mg/100 g plant matter (Datta et al., 2019). Calcium plays vital task of cell–cell signaling within immune reaction against unrelieved infection consequential of *M. tuberculosis* resulting tuberculosis. Calcium contributes human body in many ways like reinforcing teeth and bones, regulating muscle, relaxation and contraction, regulation of enzyme function, blood clotting, and heart function, communication of messages within nervous system. It also acts a major role in mineralization of skeletal. Calcium deficiency results in variety of diseases such as Alzheimer's, gout, arthritis, heart and res-

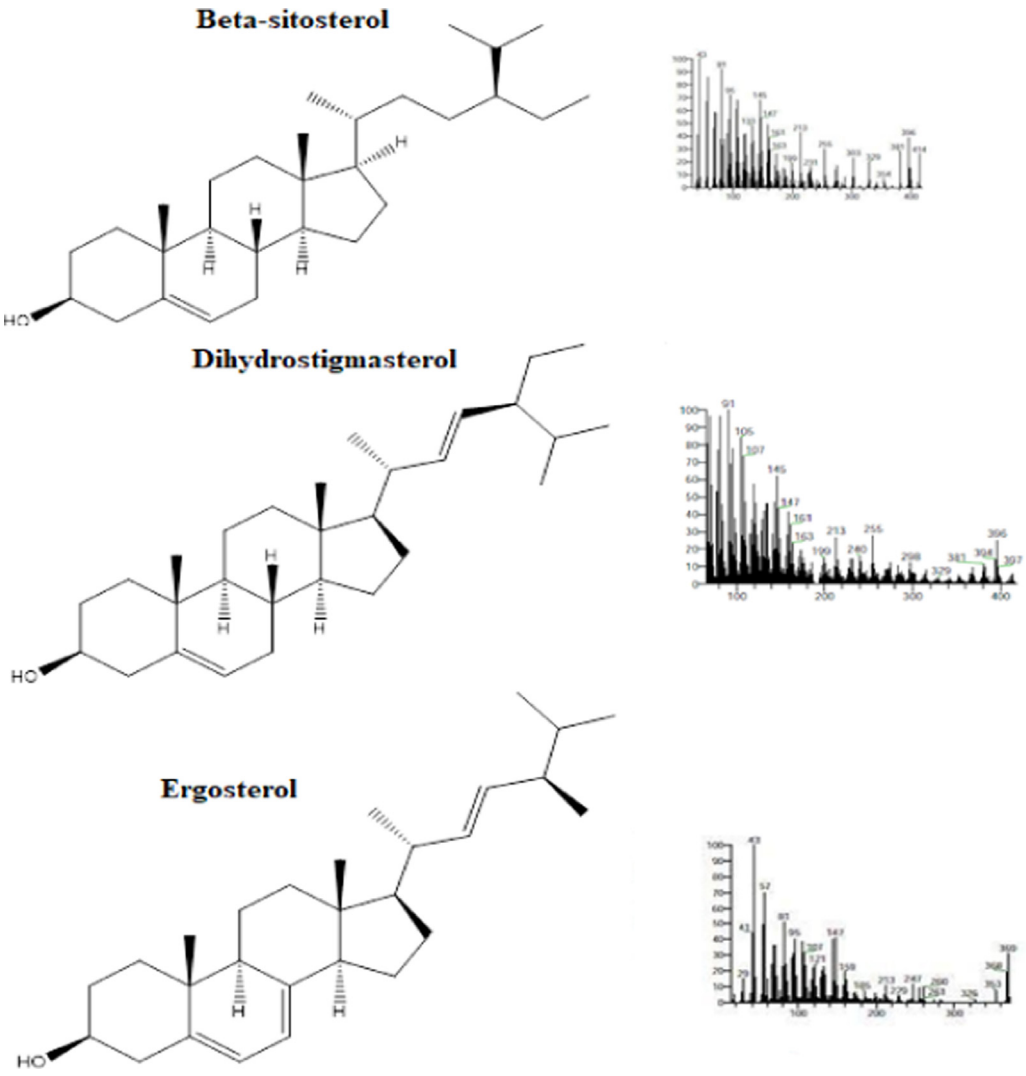

Fig. 3 Mass Spectrum of compounds of sterol in licorice root.

Table 2 Total phenolic and flavonoids content, and antioxidant activity of licorice root.

Licorice root content (mg/g)		Antioxidant (μg/ml)	
Total Phenolic	Total Flavonoids	DPPH	ABTS
15.26 ± 0.72	25.14 ± 1.98	86.90 ± 23.7	83.81 ± 9.3

piratory track related disorders (Devi et al., 2016) and calcium deficiency may be overcome by dairy products intake regularly (Cormick and Belizán, 2019). Calcium is a source of food for women, especially because they are at risk of pregnancy and must be eaten in a high quantity and useful to reduce blood pressure, although it must not be consumed within huge amount because it leads to water retention in the body. Potassium is also an important plant nutrient and performs various vital functions (Bakker, 2018).

3.1.6. Fatty acids

The fatty acid (FA) content is an indispensable pointer for nutritional assessment (Ben Mansour et al., 2018). Table 5 represents the fatty acid content of licorice root with mass fragmentation data (Fig. 6 **and** Fig. 7), Chiefly within unsaturated fatty acid content was Oleic acid, along with Palmitic acid and linoleic acid observed (Popa et al., 2012). The existence of these essential fatty acids within considerable value be able to create the oils advantageous to human health and may be utilize in the pharmaceuticals (Rezaei et al., 2017).

3.1.7. Proximate compositions

Licorice root is considered a good source of Ash, Moisture, total proteins, total carbohydrates and total lipids. The high amount of ash content indicated a high amount of minerals present in licorice root (Zafirah et al., 2019). Moisture composition is indirectly related to the shelf life span of fruits, high moisture content less nutritive affluent while low levels considered long shelf life (Oyeyinka and Afolayan, 2019). Proteins are complex macromolecules contains diverse amino acids. Proteins perform significant task of regulation of cellular structure and functions, including additional metabolic actions within every living system. Therefore Proteins show initial significance within consumers every day diets (Natesh, 2017). Carbohydrates are used as an energy source, have a plastic

Table 3 Concentrations of amino acids (mg/100 g) in licorice root plant.

Amino acid	Glutamic acid	L -Valine	Alanine	Proline	L -Phenyl alanine	Serine
Content (mg/100 g)	10.45	10.25	5.24	6.21	5.26	7.19
Retention time (min)	28.9	24.8	42.2	47.6	42.3	52.4
Amino acid	L -Threonine	Glycine	L-Isoleucine	L -Methionine	Tyrosine	L-Leucine
Content (mg/100 g)	9.66	8.82	5.69	12.78	0.0	0.0
Retention time (min)	19.2	40.1	27.7	31.9	58.5	51.3
Amino acid	Arginine	L-Lysine	Aspartic acid	Histidine	Cysteine	
Content (mg/100 g)	4.98	7.23	9.24	3.76	8.07	
Retention time (min)	20.6	58.7	12.8	50.2	59.7	

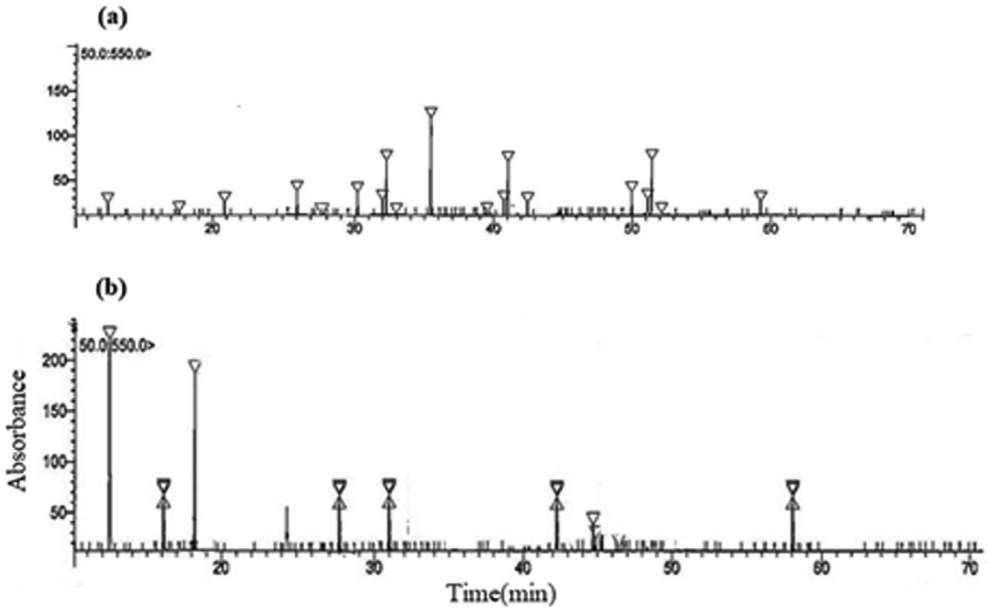

Fig. 4 HPLC charts for separation of amino acids (a) non-essential and (b) essential present within Licorice root extract.

role, they increase the body's resistance to toxic substances (Krishi Vidyapeeth et al., 2019). Proximate analysis of licorice root indicated the presence of ash, moisture, total proteins, total carbohydrates and total Lipids. The proximate composition of licorice root as assessed is contained on (Table6) (Vol and June 2017). Licorice root does not Content of total Cholesterol, Triglyceride, HDL and LDL is (0.0 mg/g) in licorice root.

3.1.8. Analysis of aromatic compounds

Licorice root was subject to GC–MS study so that the aromatic compounds either volatile or semi-volatile can be detected. These compounds are responsible for unique taste in the licorice root (Das et al., 2014) There were 28 compounds identified (Hong et al., 2013) in Table 7 and Fig. 8, which shows dissimilarity content in licorice root. The three most abundant compounds were E, E, Z-1,3,12-Nonadecatriene-5, 14-diol, 1-hexanol and 1-pentanol (Noguera-Artiaga et al., 2020).

3.1.9. Sugars

Carbohydrates in the plant root acting as a energy source and functionally, structurally it's a key role player in plant growth and enzyme regulating movement in gene expression for plant growth (Ma et al., 2014). In accordance to (Table 8), glucose, sucrose and fructose found the plentiful amount within licorice root (Akšić et al., 2019; Kelebek et al., 2009).

3.1.10. Hormones

Hormones of plant are secondary-metabolites taking place naturally with the intention of vital part within plants entire life cycle (Fu et al., 2011). They not only help in plant development but also incorporate extracellular signals to for regulation and optimization of performance and growth of plant within tiny concentration.

The hormones studied in Licorice root were Abscisic (29.4 mg/ml), auxin (11.30 mg/ml), Salicylic acid (10.22 mg/ml), gibberellins (8.04 mg/ml) we use GC–MS analysis of hormones as shown in Fig. 9.

3.2. UV–VIS spectroscopy

It is a fundamental technique for obtaining SPR (surface plasmon resonance) of prepared metal nanoparticles via UV Vis spectroscopy (Kumar et al., 2011; Das et al., 2010). During the reduction reaction $HAuCl_4$ ions converted to Au ions using licorice root aqueous extract was easily observed under UV Vis spectroscopy using different conditions (Ankamwar, 2010).

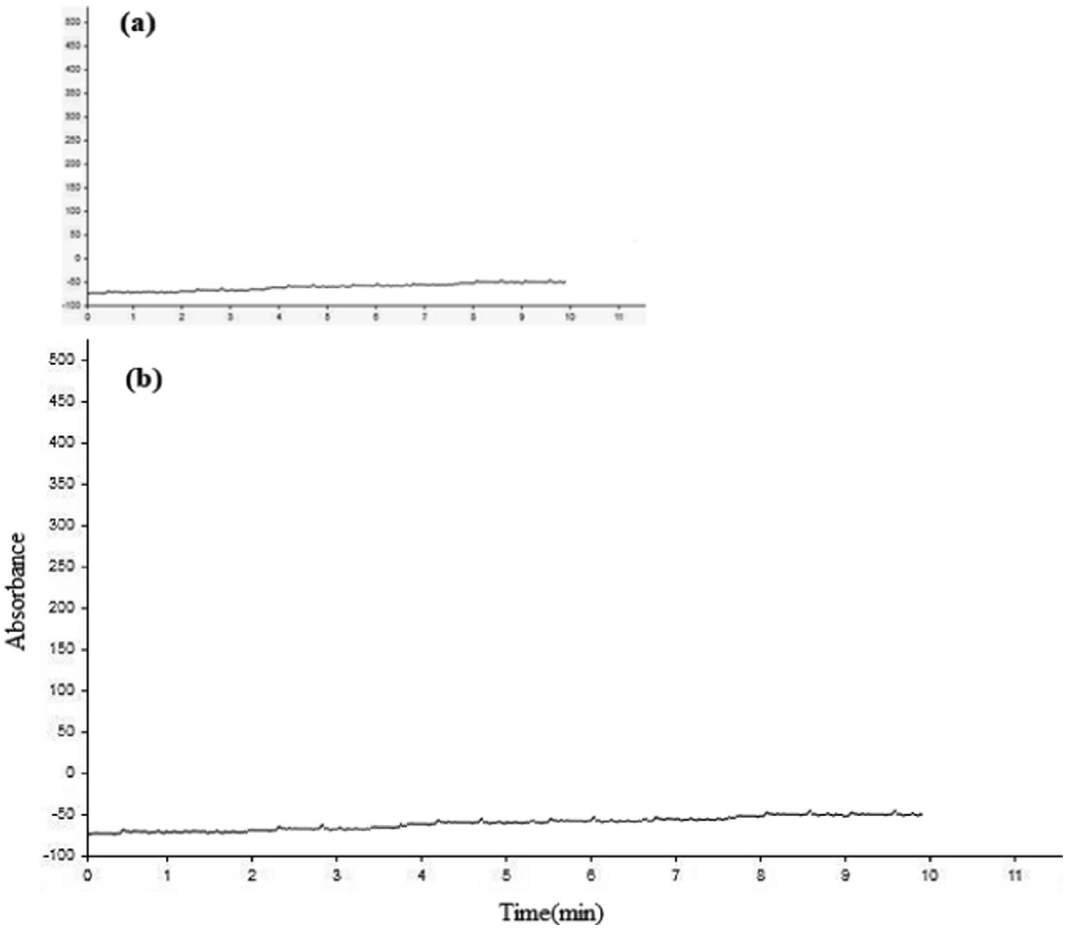

Fig. 5 chromatograph of FSV (a), WSV (b).

Table 4 Minerals content (mg/100 g dry weight) of analyzed licorice.

Minerals	Calcium	Potassium	Silicone	Magnesium	Phosphorus	Iron	Zinc
Licorice root content	387.1	104.5	39.6	0.5	14.9	3.8	0.0
Minerals	Manganese	Sulfur	Copper	Boron	Selenium	Sodium	
Licorice root content	0.0	1.23	0.0	0.0	0.0	0.0	

Table 5 Composition in fatty acids (saturated and unsaturated) for licorice root.

Fatty acid	(C8)-Caprylic acid	(C10)-Capric acid	(C12)-Lauric acid	(C14)-Myristic acid
Licorice root content (%)	0.0	0.0	0.21	4.08
Retention time(min)	22.3	24.9	26.1	26.8
Fatty acid	(C18)-Stearic acid	(C18:1)-Oleic acid	(C18:2)-Linoleic acid	(C18:3)-Linolenic acid
Licorice root content (%)	7.63	35.72	10.83	3.24
Retention time(min)	33.8	35.7	37.2	40.04
Fatty acid	(C24)-Lignoceric acid	(C22)-Behenic acid	(16:1)-Palmitoleic acid	(C17)-Heptadecanoic acid
Licorice root content (%)	0.0	0.0	0.27	2.26
Retention time (min)	43.1	42.3	30.9	31.7
Fatty acid	(C20)-Arachidic acid	(C16)-Palmitic	(C15)-Pentadecanoic acid	(C20:1)-Arachidonic acid
Licorice root content (%)	8.16	25.36	1.89	0.35
Retention time (min)	39.4	30.2	27.9	39.8

Experimentally we found the alteration in color to dark violet from yellow when treated with different volumes of Licorice root extract. It was successfully noted that 1 ml licorice root extract is capable of giving strong SPR peak at 549 nm wavelength indicating synthesis of nanoparticles of gold successfully worked out after 150 min. (Fig. 10) (Khoshnamvand et al.,

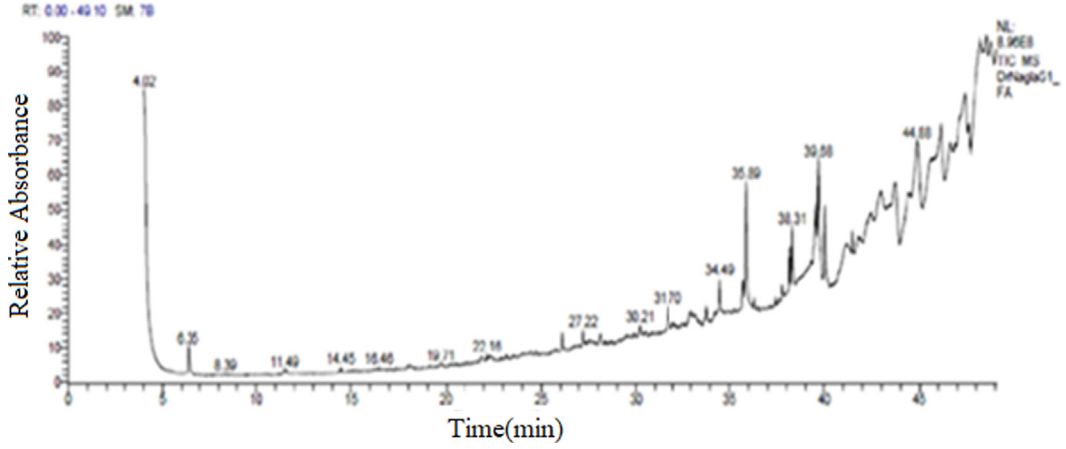

Fig. 6 The chromatogram from the GC–MS analysis of fatty acid in licorice root.

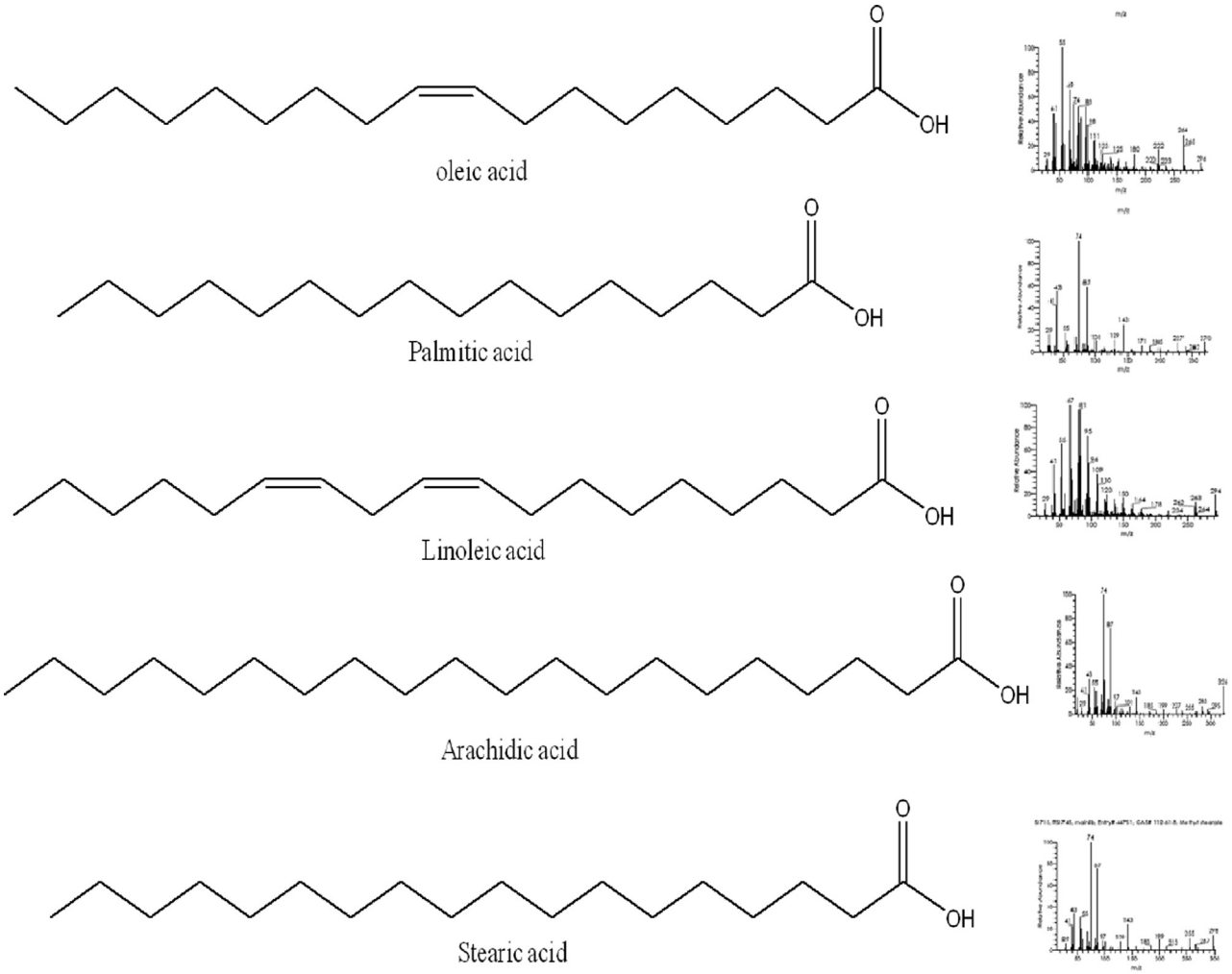

Fig. 7 Mass spectrum of compounds of fatty acid in licorice root.

2019a). While increased the volume of Licorise extract results in the rise of sharp and broad intensity band at 540 nm which relates to the gold nanoparticles (Mapala and Pattabi, 2017; Balasubramanian et al., 2019). The electron microscopy analysis showed shape and size related outcomes, mentioned in Fig. 19.

Another study with the effect of $HAuCl_4$ volume (1 ml, 2 ml, 3 ml, 4 ml, 6 ml) Fig. 11 represented the UV–vis spectra of AuNp synthesized via constant (4 ml) Licorice root extract (Alle et al., 2020; Latha et al., 2019). The large amount of volume of $HAuCl_4$ produced more gold nanoparticle, which could be indicated by the higher absorbance value (Aji et al.,

Table 6 Proximate composition (g/100 gm dry weight) of licorice root.

Proximate composition	Ash	Moisture	Total Proteins	Total Lipids	Total Carbohydrates
	(g/100 mg dry weight)	(g/100 mg dry weight)	(g/mg dry weight)	(mg/g)	(g/mg dry weight)
Licorice root content	17.22	16.4	21.42 ± 0.94	87.1 ± 9.5 mg/g	24.51 ± 31.3

Table 7 Aromatic compound contents (%) in licorice root detected by GC–MS.

Volatile components				
Licorice root content (%)	2-pentanal	1-hexanol	1-pentanol	2-propenoic acid, 2-methyl, 2-methyl-2-propen-1-yl ester
	0.71	41.3	17.49	0.31
Retention Time (min)	5.98	6.87	6.92	9.06
Licorice root content (%)	B-Cymene	Cineole	D-Limonene	2-Heptenal
	0.09	0.24	0.42	0.38
Retention Time (min)	19.57	22.62	24.86	25.72
Licorice root content (%)	Hexadecanoic acid, ethyl ester	9,12-Octadecadienoic acid	Linoleic acid	Linoleic acid ester
	0.39	1.47	0.61	1.25
Retention Time (min)	27.17	27.39	29.65	29.88
Licorice root content (%)	Geraniol	Linalool	2-pentylfuran	Eicosenic acid
	0.46	0.93	1.21	0.27
Retention Time (min)	35.53	36.6	37.97	38.28
Licorice root content (%)	decanal	Z-(13,14-Epoxy) tetradic-11-en-1-ol acetate	Nonanal	E, E, Z-1,3,12-Nonadecatriene-5, 14-diol
	1.59	1.63	4.19	14.55
Retention Time (min)	40.96	41.01	42.11	43.87
Licorice root content (%)	α-terpineol	3,4-dihydro-2H-1,5-(3″-T-bityl)-benzodioxepine	g-Terpinene	Hexa Decadienal
	0.57	1.33	0.26	5.66
Retention Time (min)	34.99	26.60	16.9	39.38
Licorice root content (%)	Tetramethyl pyrazine	Terpinen-4-ol	1-hydroxy-4-methyl-2,6-di-Tert-butylbenzene	Nonane
	1.39	0.73	0.5	0.07
Retention Time (min)	38.88	32.26	26.18	12.03

2019). When the $HAuCl_4$ increased from (1 ml to 2 ml), there was no intensity observed of the plasmon absorbance. With increase in plasmon intensity observed at 540 nm when volume of $HAuCl_4$ (3–6 ml) increased. So that reduced plasmon intensity may associated with nanoparticles aglomerization (Mortazavi-Derazkola et al., 2020). TEM image of synthesized AuNPs at 6 ml of $HAuCl_4$ and licorice root extract at 4 ml are mainly spherical in shape, and did not form agglomerates, small size and homogeneous as shown in Fig. 19 (Biao et al., 2018).

pH is one more important parameter which influence the shape and size of nanoparticle. The absorption spectra of AuNPs at different pH of (1, 2, 3, 4, 5, 6) were observed under UV–VIS spectrophotometer (Fig. 12) (Khan et al., 2018). At pH 5 observed a narrow band at 540 nm. By contrast, the solution at pH 4 showed a weak SPR intensity of AuNPs formations because reducing species under acidic pH are generated very low that lead to obtaining low yield of AuNPs. Furthermore, the solution at pH 6 became broad band was observed, which may be due to the aggregations of AuNPs (Parab et al., 2016). According to our previous study, the SPR of synthesized Au nanoparticle increased by increasing pH of the solution until to 5 and decreased at higher ph. At lower pH (lower than 4) the synthesis of Au nanoparticles was not done. Hence, pH 5 was considered as optimum one. The results showed that at pH = 5 the synthesized AuNPs are the most uniform particles (Paquin et al., 2015). TEM images also evidenced that the pH = 5 directed the synthesis of non aggregated, homogeneous circular AuNps.

The AuNp preparation was supervised by various reaction timings (1, 1:30, 2, 2:30, 3, 3:30). The AuNp prepared using licorice root extract were located at 540 nm wavelength (Dudhane et al., 2019). We observed at (1, 1:30) there was no reduction of gold ion into AuNPs as there was no SPR observed (Fig. 13). However, as time progressed, we found that at (2 h) the formation of AuNPs was started as there

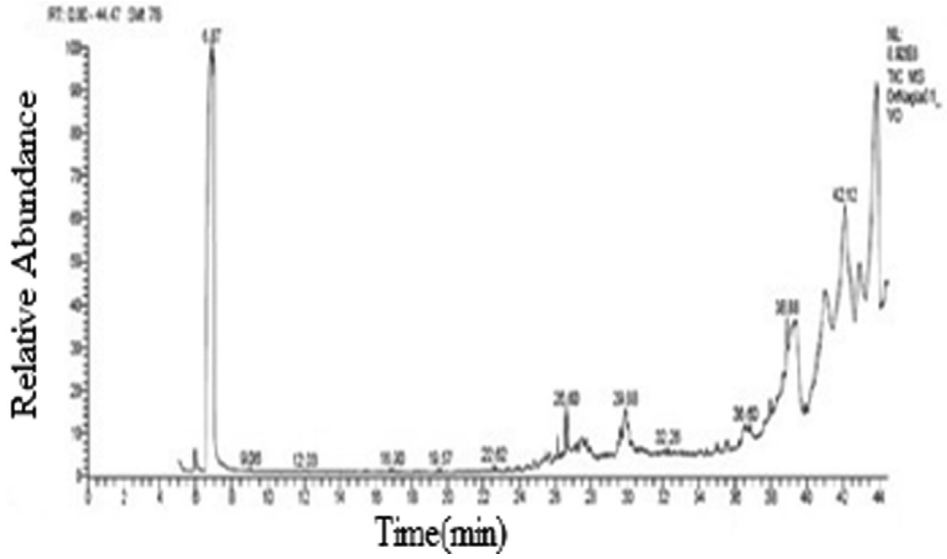

Fig. 8 Aromatic compound in licorice root detected by GC–MS analysis.

Table 8 Sugars composition on sucrose, glucose, xylose, arabinose, mannose, galactose, lactose, rhamnose and fructose of analyzed licorice root (mg/100 g).

Sugars	Sucrose	glucose	Xylose	Arabinose	mannose
Licorice root content	149.6	57.2	0.0	0.0	0.0
Retention time (min)	8.8	7.9	3.5	5.3	6.2
Sugars	**galactose**	**Lactose**	**Rhamnose**	**fructose**	
Licorice root content	1.3	0.0	0.0	4.1	
Retention time (min)	7	9.5	10.5	4.7	

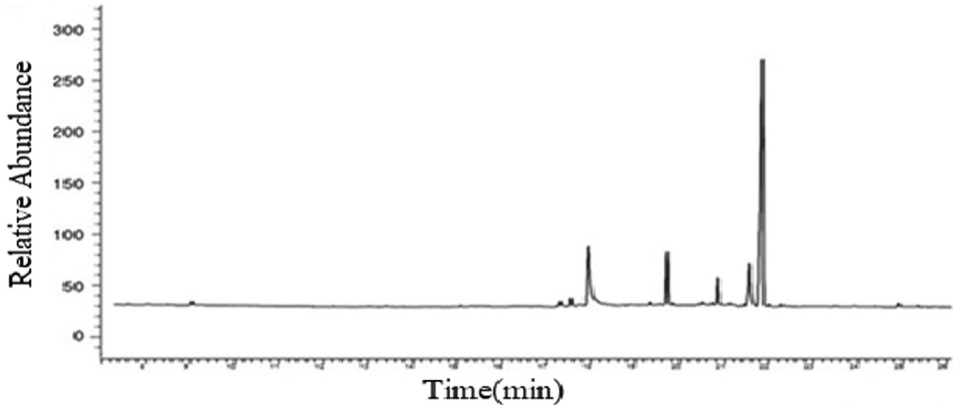

Fig. 9 The hormones chromatogram from the GC–MS analysis of the licorice root.

was some signal of SPR. After (2:30 h) of reaction time, we observed a complete SPR formation from the mixture of gold ion and licorice root extract at $\lambda_{max} = 540$nm. The blue shift appeared at 3 and 3:30 h, that means the absorbance peak moved towards the lesser wavelength (Izadiyan et al., 2018). We need 2:30 h (150 min) to synthesis of AuNPs with licorice root extract (Fig. 14) (Najmeh Aboutorabi et al., 2019). Fig. 14 revealed absorbance maxima by reaction time. The λ max turn into stable following 2:30 h (150 min) reaction time because of full reduction of present gold salt within the medium [105–109]. This analytically proved by TEM (Transmission Electron Microscopy) experiments in Fig. 19. The nanoparticles predominately adopt a spherical morphology and are not agglomerated at 2:30 h and 4 ml of licorice root extract, 6 ml of $HAuCl_4$ at pH 5 [110–12].

3.3. Fourier-Transform infrared spectroscopy (FT-IR)

The FTIR technique was executed for reorganization of functional compounds occur resting upon AuNp surface (Guo

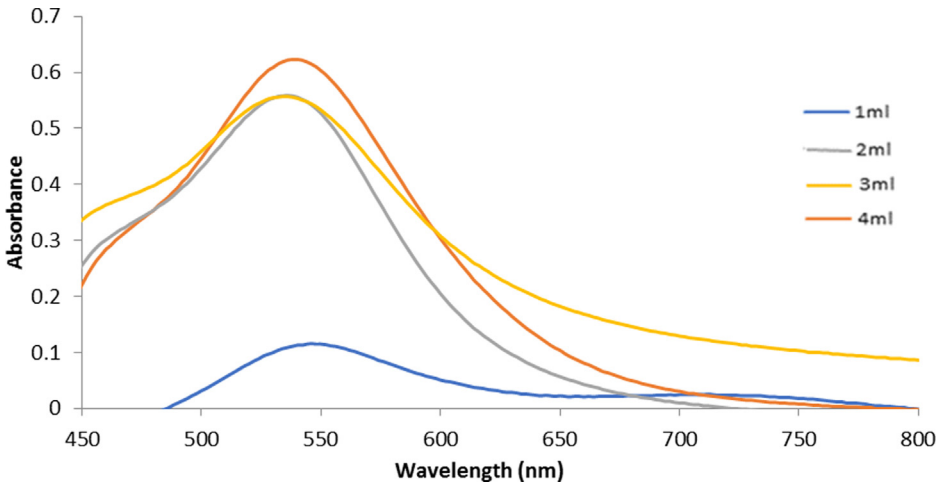

Fig. 10 UV–Visible spectrum of AuNp synthesized using various volumes **Licorise** (Glycyrrhiza glabra) extract (1 ml, 2 ml, 3 ml, 4 ml) with 6 ml)1 × 10^{-3}) M $HAuCl_4$ solution after 150 min of addition at 25 °C.

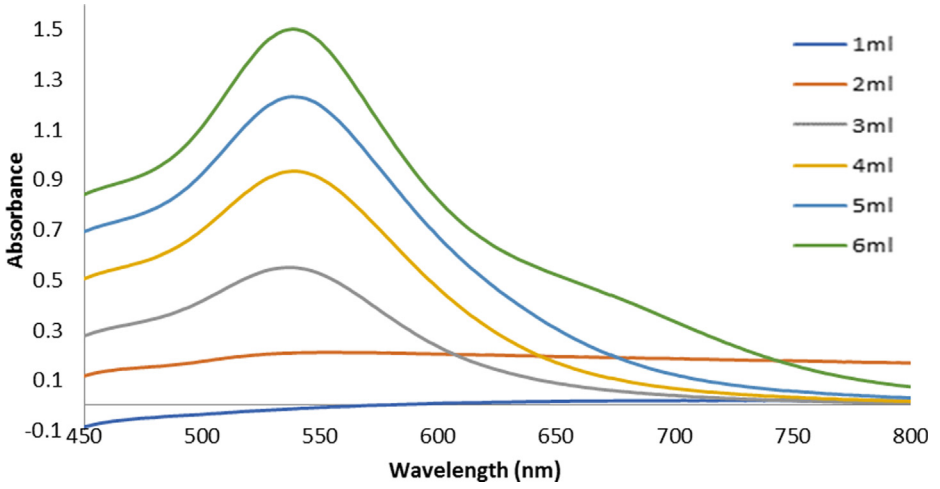

Fig. 11 UV–visible spectrum of AuNp produced via various volumes (1–6) ml 1 × 10^{-3} M $HAuCl_4$ solution with 4 ml Licorice (Glycyrrhiza glabra) extract after 150 min of addition at 25 °C.

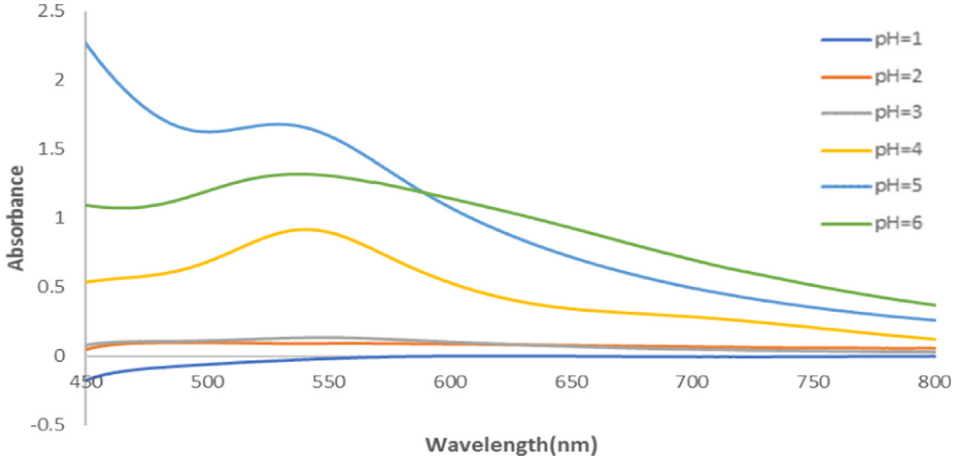

Fig. 12 AuNp UV–Vis spectra as a purpose of effect of different (1–6) pH of 6 ml 1 × 10^{-3} M $HAuCl_4$ stock solution and 4 ml of glycyrrhiza glabra extract after 150 min of addition at 25 °C.

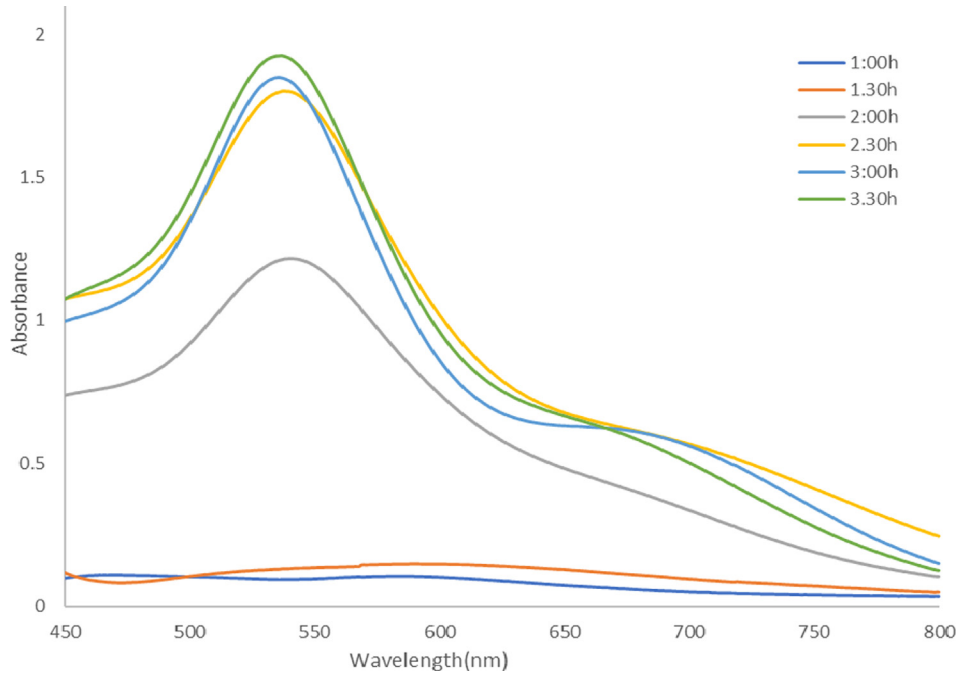

Fig. 13 UV–visible spectrum of AuNp as a task of 6 ml 1 × 10^{-3} M $HAuCl_4$ solution and 4 ml of Licorice (Glycyrrhiza glabra) root extract after 150 min of addition at 25 °C.

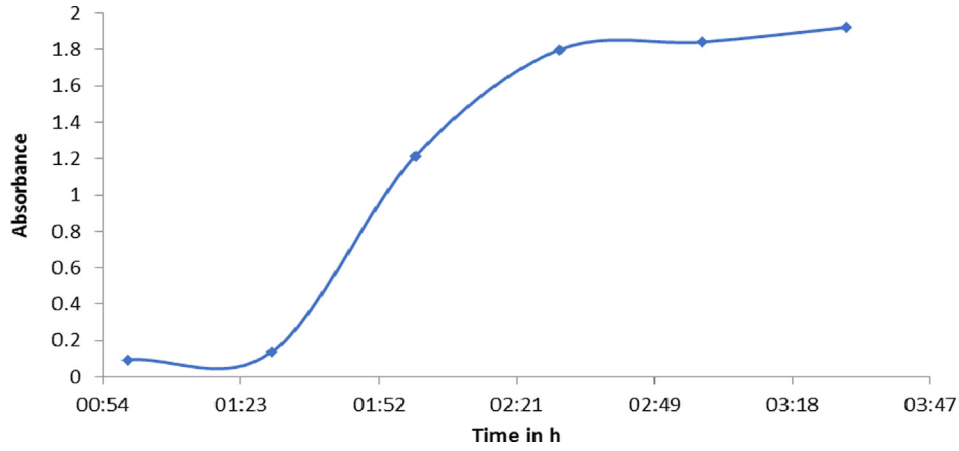

Fig. 14 Intensity variant within UV–Visible spectra of AuNp as a function of 6 ml 1 × 10^{-3} M $HAuCl_4$ stock solution and 4 ml of Licorice (Glycyrrhiza glabra) root extract after 150 min of addition at 25 °C.

et al., 2020). The results illustrate significant absorption spectra ranging from 400 to 4000 cm − 1 (Fig. 15) (Abbasi et al., 2019). The analysis results of licorice root observed many peaks at 3421, 2937, 1643, 1053, 1080, 1375, 623, 893 and 609 cm^{-1} (Fig. 15 a). The powerful wide band at 3421 cm^{-1} was caused by O—H str vibrations from phenolic, carboxylic and alcoholic group. The wide peak present almost at 2937 cm^{-1} reveals C—H stretching from alkanes. The band at 1643 cm^{-1} reflects the C═O stretch(Al-Radadi, 2018). At 1058 cm^{-1} reflects to C—O stretching. Aromacity may be mentioned between 893 and 609 cm^{-1}.1080 and 1375 cm^{-1} due to C—N stretching vibrations coming by the aromatic plus aliphatic amines. The band 623 cm^{-1} is hydroxyl compound that was out of plane bend. Although, moving of bands were noted due to synthesis of AuNPs shown in Fig. 15-**b** (Ravichandran et al., 2019; Yilmaz Öztürk, 2019). There are significant shifts in the OH and peaks, at 3419 for (NH_2 / OH) stretching, 2924 for C—H stretching, 1631 for C═O and amide and 1053 for C—O stretching as shown in Fig. 15-**a**. these spectral changes may be related to AuNPs coating by compounds from licorice root extract (Banasiuk et al., 2020; Stalin Dhas et al., 2019).

3.4. X-Ray diffraction (XRD) analysis

The crystalline nature of AuNp was studied with XRD analysis. The powder XRD pattern of AuNp revealed the four distinct peaks may be assigned to (111), (200), (220) and (311) crystalline planes to the 2Θ = 38.1°, 44.5°, 64.6° and 76.8° respectively of gold (Fig. 16-**a**) (David and Moldovan, 2020; Veena et al., 2019). These indices specify the face centered cubic structure of AuNp (Abdelghany et al., 2019). In

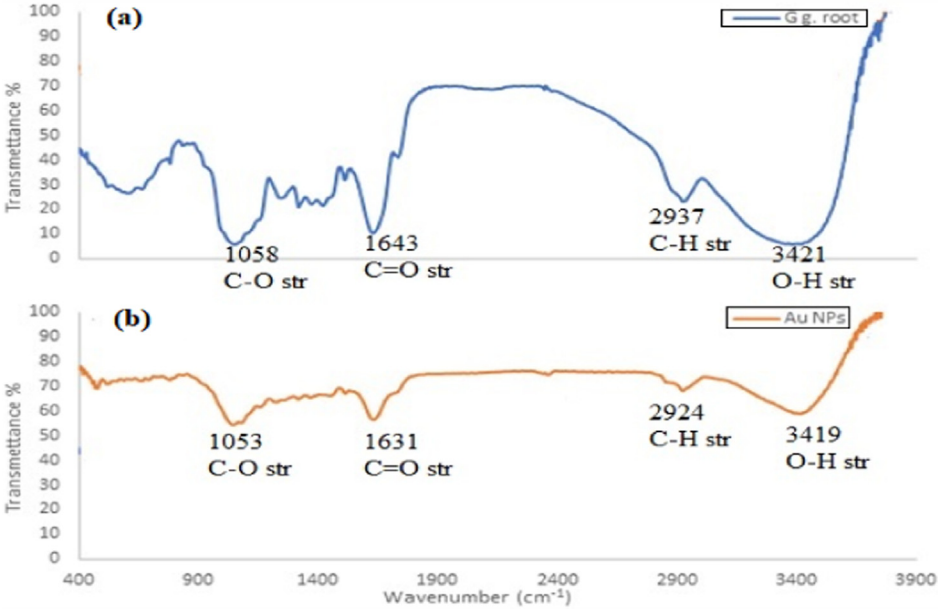

Fig. 15 FTIR spectra of AuNPs and licorice root extract.

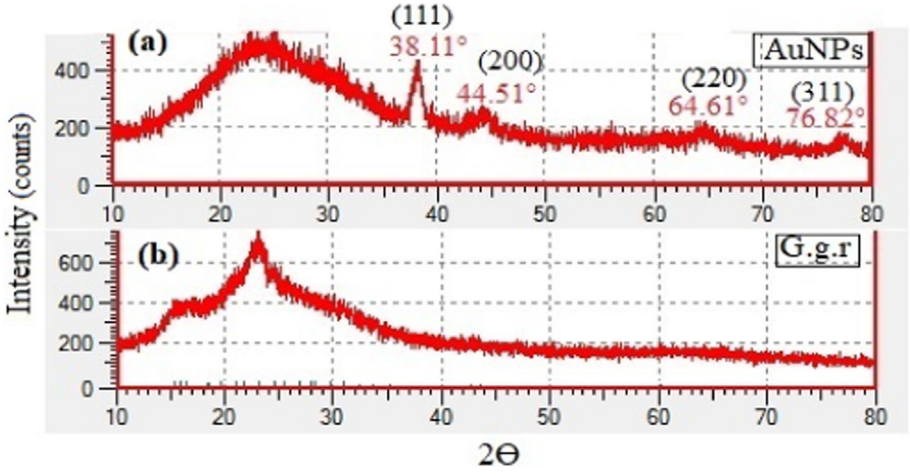

Fig. 16 XRD spectrum of synthesized gold nanoparticles with licorice root extract.

Fig. 16-**b** the extra peak of 25° is related to the amorphous nature of the extract (Khatami et al., 2018). Average size of the AuNp calculated from the Debye–Sheerer equation $D = k\lambda/\beta \cos \theta$ (Nabi et al., 2019). The band resultant to (1 1 1) was strong enough compared to rest of the planes signifying that synthesized AuNp has crystalline nature and (1 1 1) was principal orientation (Suganthy et al., 2018).

3.5. Energy dispersive X-ray spectroscopy analysis

The occurrence of Au atoms within AuNp synthesized using licorice root extract was validated using EDX analysis. The EDX graph exhibiting presence of strong optical signals at 3, 8 and 8.9 KeV indicating the presence of Au (Yadav et al., 2019) (Fig. 17). The other phytochemicals were also additionally present clearly indicating the capping of Licorice root chemicals like oxygen, potassium and calcium acting capping role (Elbagory et al., 2016; Vijaya Kumar et al., 2018).

3.6. Dynamic light scattering (DLS)

Dynamic light scattering interprets the average particle size considering the hydrodynamic diameters. The AuNp synthesized using Licorice root extract showed the average particle size 53.7 nm through size distribution histogram (Fig. 18-**a**) (Nag et al., 2018). The stability of AuNp is more important when these items are utilized in biomedical applications (Mohammed Siddiq et al., 2019). The average zeta potential of triplicate analysis was observed −26.5, which clearly indicated the AuNps were stable at 25 °C (Fig. 18-**b**) while the PDI value 0.399 observed. The discrepancy of particle size in the DLS and TEM are due to the information that DLS analysis contain thick layer of bio-compounds surrounded on the

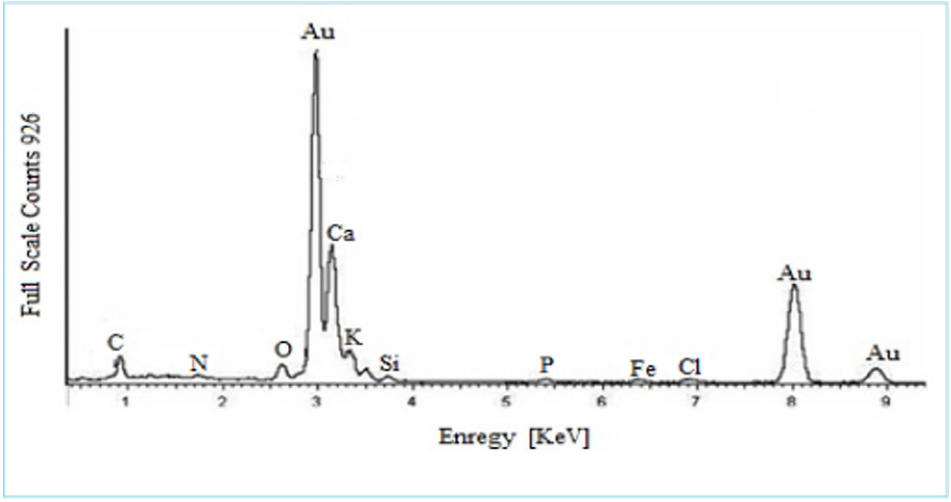

Fig. 17 EDX spectrum of AuNPs with licorice root extract.

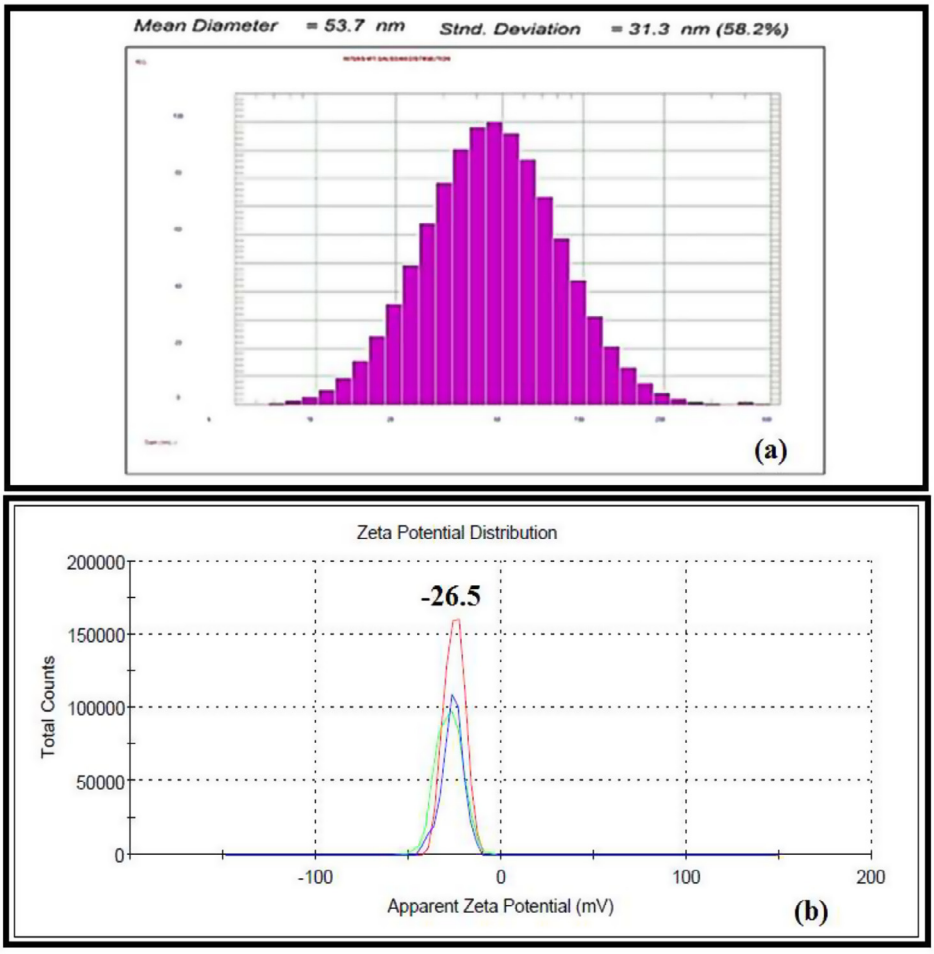

Fig. 18 DLS Size Distribution image (a) and Zeta potential (b) of AuNps synthesized by Licorice root extract.

Licorice root extract mediated synthesized AUNps (Ahmad et al., 2016).

3.7. Electron microscopy (TEM, SEM) studies

The electron microscopy was applied to determine morphological data and size of synthesized nanoparticles. TEM image (Fig. 19) showed that AuNPs having spherical shape (Tagad et al., 2014; Deokar and Ingale, 2016). All the nanoparticles were "homogeneous" bounded via a layer of faint and slim organic molecules acting as a capping agent (Khan et al., 2019). The Transmission electron microscopic analysis explained the circular AuNp has 43.17 diameter with the 2.647 nm to 16.25 nm size range. (Fig. 19 **inset**) (Moradi Alvand et al., 2019) which was showing 55 nm in DLS. The frequency of spherical AuNp was observed in more number comparatively to different shapes. These circular AuNp point out the thermodynamic stability and minimum surface energy of it (Eskandari-nojehdehi et al., 2016). Size and surface morphology of the prepared AuNp were also imaged through SEM analysis. Fig. 20 displays SEM images of AuNps.

In this analysis Circular and aggregated AuNp were clearly seen.

4. Biological activity

4.1. Antimicrobial activity

4.1.1. Antibacterial activity

The synthesized AuNp were analyzed for antibacterial activity towards Gram-positive and Gram negative bacteria by traditional nutrient agar well diffusion assay. The green synthesized AuNps exhibited showed better antibacterial activity for five bactial strains, which makes contact with a greater amount of bacteria and destruct the bacteria (Yaku et al., 2019). The results in Fig. 21 and Table 9 depicted that the inhibition zone for AuNp increased for *E coli* comparative to other bacterium

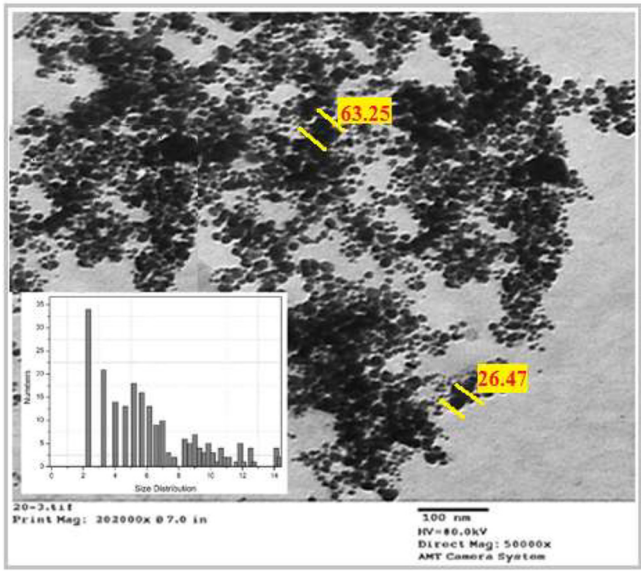

Fig. 19 TEM image of AuNps produced by Licorice root extract, inset showing size distribution.

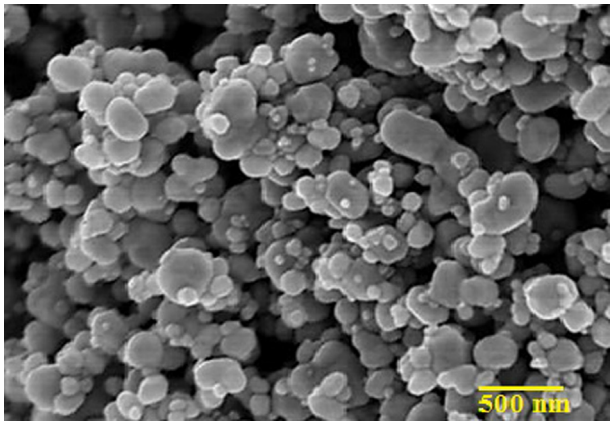

Fig. 20 SEM image of AuNPs synthesized by Licorice root extract.

which means AuNp synthesized using Licorice root extract exhibit good antibacterial activity to Gram negative bacteria (Rajendran, 2017).

4.1.2. Antifungal activity

The antifungal activity of Licorice root extract mediated synthesis of AuNp showed notable findings against said fungal strains (Table 10). The licorice root extract was extremely deadliest towards *A. flavus* (18 ± 0.37) and slightest towards *C. albicans* (12 ± 0.25) while AuNps showed extreme activity against *P. citrinum i.e.* 21 ± 0.21 and least active to *C. albicans i.e.* 15 ± 0.21 (Fig. 22). Every fungal strain used was established sensitivity against experimental licorice root extract and AuNps and by and large as good as nystatin standard antifungal agent

4.2. Anticancer activity

Cytotoxicity action of the AuNPs was studied against the HepG-2 and MCF-7 cell line by MTT assay (Table 11). Cytotoxicity effect on cancerous cell was studied at different concentrations (500, 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, 2, 1, 0 μg/mL) and compared with normal control. The specific values of IC50 were IC_{50} = 23 μg/ml for HepG-2, IC_{50} = 50 μg/ml for MCF-7. The AuNp synthesized by Licorice root extract stopped the increase in the cell line growth within time and dose a dose-dependent way. The bar diagram of efficacy of biosynthesized gold nanoparticles against HepG-2 & MCF7 cells at different concentration (Fig. 24) (Awad et al., 2019; Menon et al., 2018). Half maximal inhibitory concentration (IC_{50}) of licorice root extract mediated synthesized AuNPs observed at concentration of 23 μg/ml towards HepG-2 cell-line while that of 50 μg/ml towards MCF-7 cell line. This result showed that the minimum dose of AuNPs showed marked cytotoxic activity. The bar diagram represents the efficacy of biofabricated AuNPs against HepG-2 and MCF-7 cells at different concentrations (Fig. 24). There was direct relationship observed between concentration of nanoparticles and percent inhibition of cell growth. The Licorice root mediated AuNp presented better antiproliferative activity at high concentration towards MCF-7. The proliferation observed decreasing following 24 h of treatment. Fig. 23 presents the examined cell viabil-

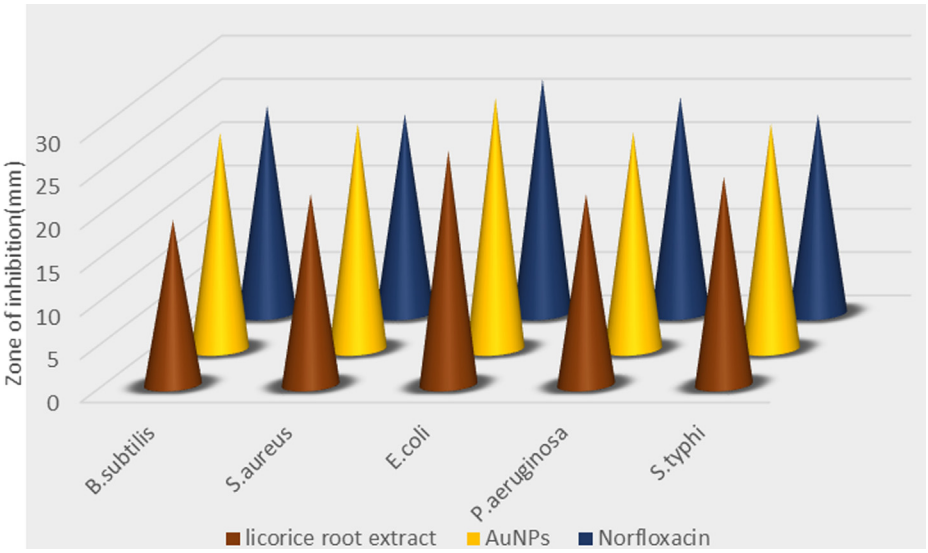

Fig. 21 Bar diagram showing zone of inhibition of antibacterial analysis of AuNp synthesized by Licorice root extract.

Table 9 Antibacterial activity of AuNPs synthesized using licorice root extract.

Bacterial test strains	Average diameter of zone of inhibition (mm)/MIC (minimum inhibitory concentration) (μg/ml).					
	Licorice		AuNPs		Norfloxacin (standard)	
	Inhibition zone	MIC	Inhibition zone	MIC	Inhibition zone	MIC
Bacillus subtilis (ATCC 6633)	19 ± 0. 4	10.41 ± 0.05	25 ± 0. 15	7.51 ± 0.12	24 ± 0.56	3.9 ± 0.12
Staphylococcus aureus (ATCC 29213)	22 ± 0.35	8.67 ± 0.45	26 ± 0.29	3.9 ± 0.5	23 ± 0.5	2.5 ± 0. 35
Escherichia coli (ATCC 25922)	27 ± 0.41	3.9 ± 0.77	29 ± 0.35	1.95 ± 0.4	27 ± 0.98	1.57 ± 0.69
Pseudomonas aeruginosa (ATCC 27853)	22 ± 0.63	7.51 ± 0.62	25 ± 0.17	2.5 ± 0.27	25 ± 0.87	3.9 ± 0.47
Salmonella typhi (ATCC 6539)	24 ± 0.42	6.5 ± 0.08	26 ± 0.15	1.3 ± 0.33	23 ± 0.16	1.57 ± 0.25

Table 10 Antifungal activity of AuNPs synthesized using licorice root extract.

Fungal test strains	Average diameter of zone of inhibition (mm)/MIC (minimum inhibitory concentration) (μg/ml).					
	Licorice		AuNPs		Nystatin (standard)	
	Inhibition zone	MIC	Inhibition zone	MIC	Inhibition zone	MIC
***Candida albicans* (ATCC 10231)**	10 ± 0.25	31.25 ± 0.11	14 ± 0.21	8.67 ± 0.21	15 ± 0.2	7.81 ± 0.16
***Aspergillus niger* (RCMB 002007)**	12 ± 0.19	62.5 ± 0.32	17 ± 0.29	24.25 ± 0.46	18 ± 0.2	20.5 ± 0.52
***Aspergillus flavus* (ATCC 16883)**	15 ± 0.37	41.6 ± 0.2	16 ± 0.15	33.5 ± 0.79	17 ± 0.12	31.25 ± 0.45
***Fusarium oxysporum* (RCMB 008002)**	14 ± 0.14	62.5 ± 0. 5	18 ± 0.33	31.25 ± 0.74	20 ± 0.32	26.4 ± 0.2
***Penicillium citrinum* (RCMB 001011)**	13 ± 0.23	41.6 ± 0.15	19 ± 0.21	31.25 ± 0.45	20 ± 0.20	31.25 ± 0.15

ity against both the cell lines at various concentrations. The morphological observation of the HepG-2 & MCF-7 cell lines showed noteworthy changes in morphology and decreased cell density, which is indication of apoptotic cells (Figs. 25 and 26) (Almatroudi et al., 2020; Pathak et al., 2019). The inhibitory concentration (IC_{50}) value of licorice root extract-AuNPs was 50 μg/ml. In this case higher concentration also showed greater impact. The cell viability decreased upto 83.89% at 500 μg/ml. Additionally, morphological changes in nucleus like failure in stability of membrane and cell clumping was noted within MCF-7 cell line treated by licorice root extract-AuNPs at 500 μg/ml for 24 h (Fig. 26) (Vijayakumar et al., 2019; Al-Radadi and Adam, 2020; Al-Radadi, 2019).

4.3. Antioxidant activity by DPPH and ATBS mathod

The antioxidant capacity of Licorice root extract and the AuNps studied via ATBS and DPPH methods (Francis et al., 2017). The declined absorption spectra supposed as a determined scavenged radicals (SP et al., 2019). The IC50 of licorice root extract, DPPH and AuNPs were 86.90, 78.89 and 74.50 μg/mL, respectively (Fig. 27), The IC50 of licorice root extract, ATBS and AuNPs were 83.81, 79.00 and 77.65 μg/mL, respectively (Fig. 28) (Zangeneh, 2019). The lowest IC50 value of AuNPs indicates their higher antioxidant activity (Mehmood et al., 2019). The flavonoids and phenols were well known for their free radical scavenging activity by

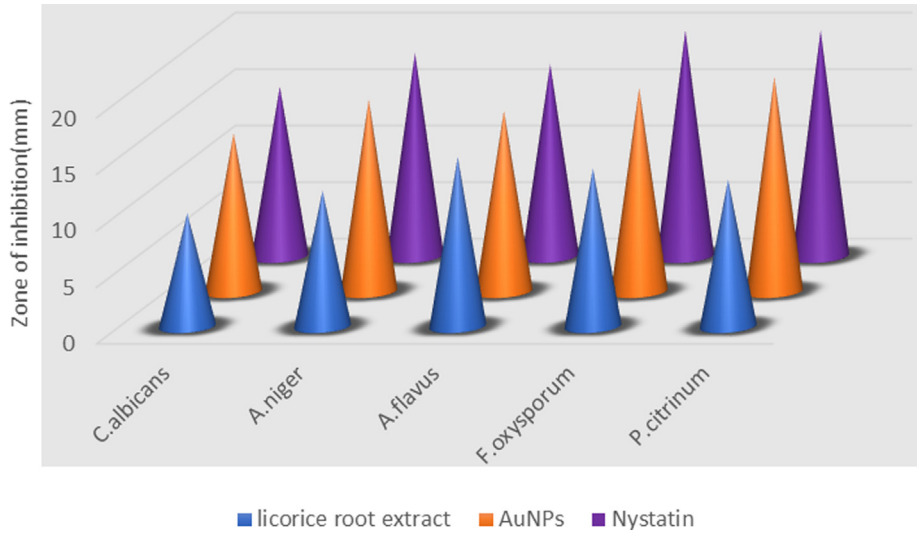

Fig. 22 Bar graph showing zone of inhibition of antifungal analysis of Licorice root extract and AuNp synthesized using it.

Table 11 Cell viability (%) and inhibitory activity (%) at different concentration of Licorice.

Sample conc. (µg/ml)	HepG-2 Cell viability (%) Inhibitory activity (%)		IC_{50}	MCF-7 Cell viability (%) Inhibitory activity (%)		IC_{50}
	stand	**sample**	23 µg/ml	stand	**sample**	50 µg/ml
500	0.44	**5.8**		1.95	**16.61**	
		94.2			**83.89**	
250	0.67	**16.5**		2.76	**25.7**	
		83.6			**74.3**	
125	0.99	**25**		2.85	**35.8**	
		75			**64.2**	
62.5	1.48	**38**		3.63	**50.3**	
		62			**49.7**	
31.25	0.71	**48**		2.80	**67**	
		52			**33**	
15.6	1.43	**60.3**		0.60	**80**	
		39.7			**20**	
7.8	0.69	**67.9**		0.90	**89**	
		32.1			**11**	
3.9	1.99	**74.7**		0	**94**	
		25.3			**6**	
2	1.30	**79.3**		0	**100**	
		20.7			**0**	
1	2.55	**90**		0	**100**	
		10			**0**	
0	0	**98**		0	**100**	
		2			**0**	

donating the electron. The eleven different concentrations were tested for antioxidant activity of Licorice root mediated AuNp synthesized against ABTS and DPPH radicals (Fig. 27 and Fig. 28). The finding of the antioxidant activity was based on the concentration dependent, as concentration increases antioxidant potential increases in both the cases. The outcomes indicated that the AuNp synthesized using Licorice root has enormous amount of biomolecules which were contributing to antioxidant potential. Licorice root contain sufficient amount of phenolics, ascorbic acid which were taking active part by providing electron and hydrogen atom in the scavenging of ABTS and DPPH radicals (Khoshnamvand et al., 2019b).

4.4. Fibrinolytic activity (anticoagulation of AuNPs)

The fibrinolytic study of Licorice root extract mediated AuNps showed excellent activity since it confirmed the anticoagulative property for human blood (Fig. 29). In this assay, EDTA was taken as a standard anticoagulant exhibited the comparative findings. The microscopic observation exhibited the RBC were well-dispersed while positive control showed lot of blood clots.

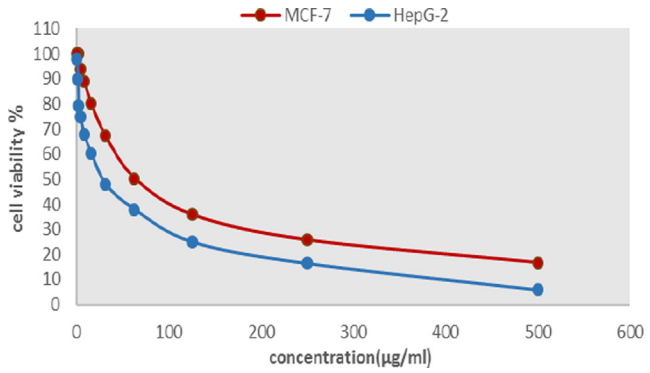

Fig. 23 cell viability (%) at different concentration (ug/mL) of Licorice root mediated AuNps synthesis.

The assay ascertained the AuNPs were non-toxic to platelets of human blood. So that the AuNp synthesized using Licorice root can be used to treat as a anticoagulant in the biomedical application. Here in heparin was helped by AuNp in the enhancement of anticoagulation activity (Lateef et al., 2018, 2016; Raja et al., 2015).

5. Conclusions

The result which showed and proved the green synthesis of AuNPs using licorice root extract provides the most effective simple and eco-friendly method for the true environment. The work provides information on green synthesis of AuNps using licorice root extract. The biosynthesis of AuNps was confirmed using UV–Vis, FT-IR, SEM, TEM, EDX, XRD,

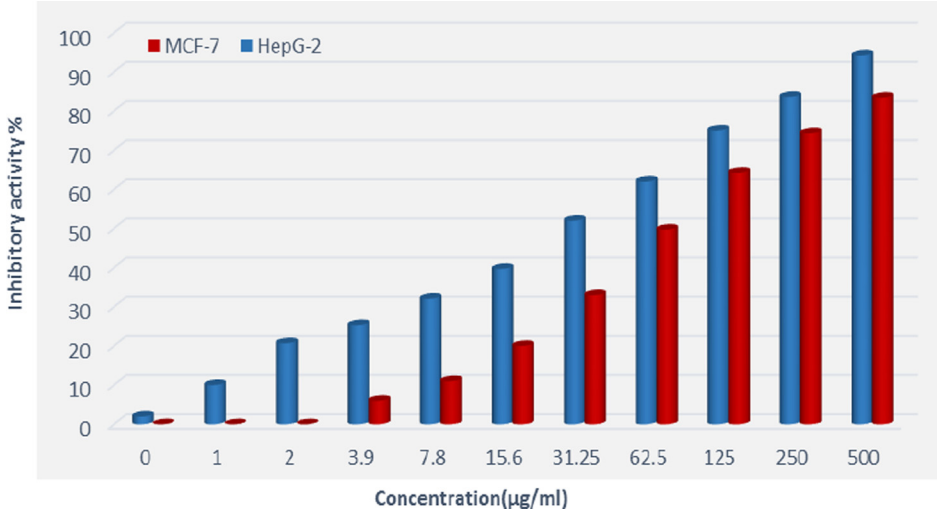

Fig. 24 Inhibitory activity (%) at different concentration of Licorice root extract mediated AuNps synthesis.

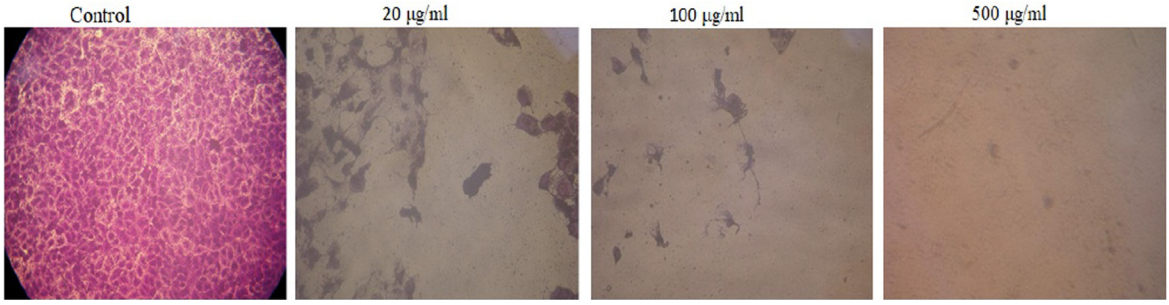

Fig. 25 Morphological changes induced by AuNPs on the liver cancer cell line (HepG-2).

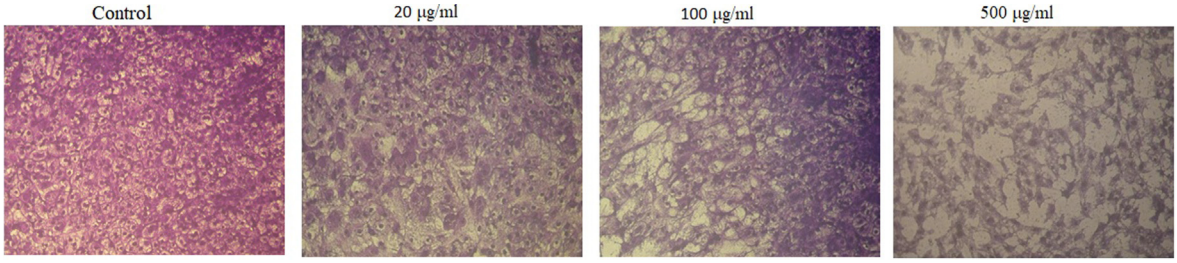

Fig. 26 Morphology image of breast cancer (MCF-7) treatment by AuNPs at 20, 100, 500 µg/ml respectively.

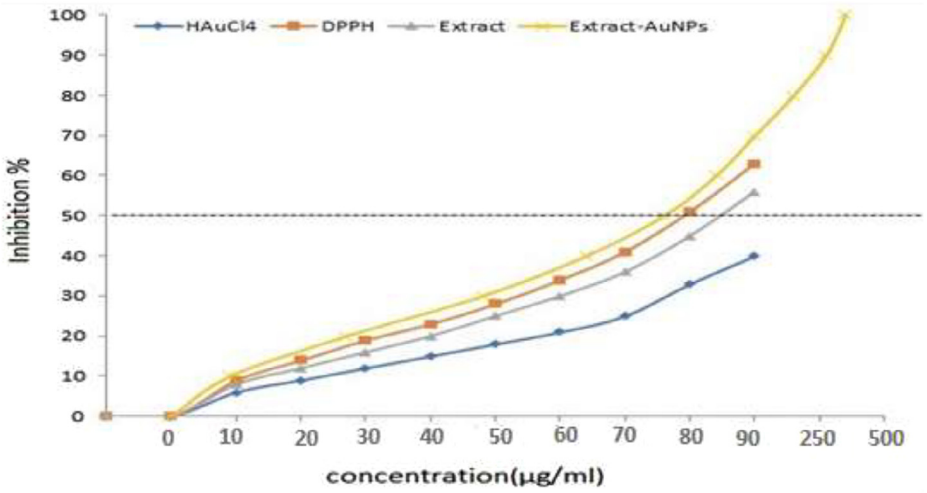

Fig. 27 DPPH free radicals scavenging assays of biosynthesized AuNPs.

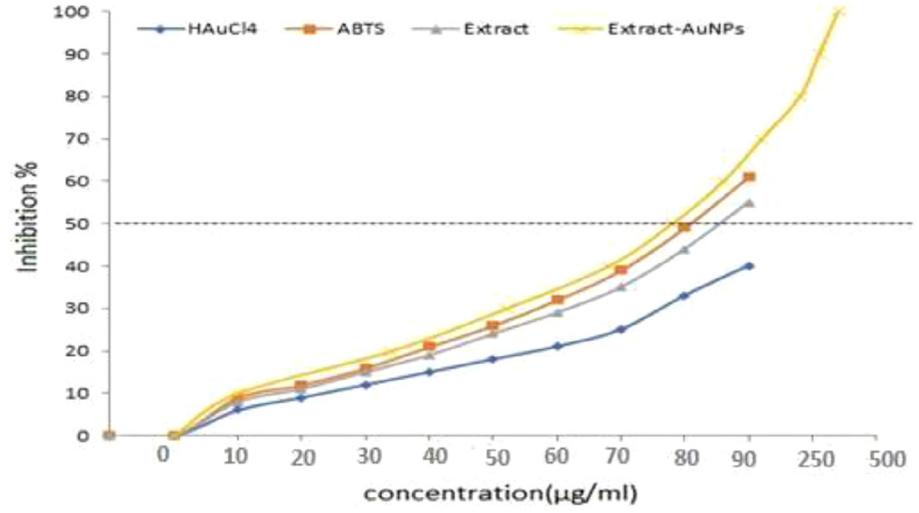

Fig. 28 ABTS free radicals scavenging assays of biosynthesized AuNPs.

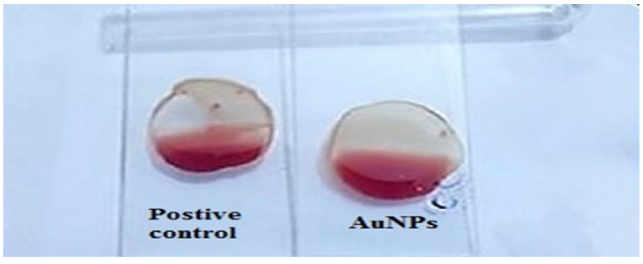

Fig. 29 Anticoagulant activity of biosynthesized AuNPs (Licorice).

DLS. The electron microscopic observation revealed circular shape having 26.47–63.25 nm size range. The AuNp exhibited excellent antimicrobial antioxidant and anticoagulative potential which will allow these AuNp used in biomedical field. The improved cytotoxic effect of licorice root extract can be endorsed towards existence of bioactive compounds on the surface and potential to intrude the cells. In future the AuNp synthesized using this method may directly be applied in the biomedical field without doubt.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The author is grateful to the Department of Chemistry at Taibah University.

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