Annals of Biology **39** (2) : 164-168, 2023

Impact Score : 0.32 (Scopus)

Antioxidant Potential of Lantadene A-nano Silver Purified from Leaves of *Lantana camara*

ZAHRAA R. SHAMSEE*, FATIMAH A. SABRY[1] AND AHMED F. ALSHANON[2]

Al-Turath University College, Medical Laboratory Techniques Department, Baghdad, Iraq
**(e-mail: phd.zahraa89@gmail.com; Mobile: +96 91452 56324)*

(Received: January 2, 2023; Accepted: February 11, 2023)

ABSTRACT

The present study examined the potential antioxidant activity of purified Lantadene A bind with nano silver extracted from *Lantana camara* after purification of LA using thin-layer chromatography, silica gel chromatography and high-performance liquid chromatography. UV visible spectra result showed high absorption at 422 wavelengths by lantadene silver nanoparticles. The characterization of nano particles were measured by field scanning electron microscope, UV and atomic forces microscope. Lantadene-Ag increased significantly scavenging activity and it ranged between 70-80% in DPPH and ABTS assay comparing with ascorbic acid. At the same time 200-100 µg/ml of lantadene-Ag showed antioxidant properties and increased significantly reactive oxygen species in hepG2 cell line.

Key words: AgNps, *Lantana camara*, lantadene A, hepG2 cell line

INTRODUCTION

Cancer is a significant threat to human health and is the second factor of mortality after cardiovascular disease (WHO, 2018). Since there is no one therapy that works for all types of cancer, cancer treatment poses a considerable difficulty, in addition to traditional chemotherapy which utilize cytotoxic medicines with harmful side effects (Zugazagoitia *et al.*, 2016). Hence, natural active compounds of medical plants were crucial in the treatment and prevention of human diseases. It is commonly considered that herbal drugs are safer and cheaper as compared to synthetic drugs and contain high amounts of bioactive secondary metabolites used as anti-cancer and anti-proliferate (Tasneem *et al.,* 2019).

Many active medicinal chemicals, as pentacyclic triterpenoids, may be isolated easily from various portions of *L. camara* that have anti-proliferative properties. Following thorough *in vivo, in vitro* and clinical testing, such benefits could give a good alternative for tumor treatment in humans (Shamsee *et al.*, 2019). *L. camara* is a flowering aromatic plant which is part of Verbenaceae family that contains a number of phytoconstituents such as: terpenoids, flavonoids, alkaloids that have therapeutic properties for a variety of ailments, including ulcers, asthma and cancer (Kumar *et al.*, 2020). Lantadenes are pentacyclic triterpenoids found as a natural form in several parts of *L. camara* including lantadene A, B, C and D (Shamsee *et al.*, 2019). Lantadene A (LA) considered as the most hepatotoxic compounds present in the leaves of lantana plants (Kumar et al., 2018).

One of the most significant technologies is nanotechnology, which is used in a variety of field including technology, biology, medicine and pharmaceuticals (Nakamura *et al.*, 2016). Silver nanoparticles (AgNPs) appeared many advantages that coordinate in drug delivery, biochemical application and other gene therapy in respect of their high surface to volume ratio, tiny size, biocompatibility, constancy and special heat characteristics (Khandanlou *et al.*, 2018).

Plant extracts have shown to be a simple and effective stabilizer in the green production of nanoparticles, affordable and environmentally benign technique. This method creates nanoparticles with some degree of control over their size, shape and absorbency by adjusting the temperature, pH and concentration of plant extract. Lantadene A showed potent chemo preventive capabilities against a variety of cancer forms when tested on

[1]Al-Rafidian University College, Pharmacy Department, Baghdad, Iraq.
[2]Center of Biotechnology Research, Al-Nahrain University, Baghdad, Iraq.

animals showing remarkable survival rates (Shabestarian *et al.*, 2016). The goals of the current investigation were creating AgNPs from LA and evaluating their *in vitro* anticancer activities against hepG2 cells line through studying reactive oxygen species and DPPH scavenging activity.

MATERIALS AND METHODS

A purified LA, already isolated from *L. camara,* was supplied from College of Biotechnology, Al-Nahrain University. The LA underwent purification and identification procedures using thin-layer chromatography, silica gel chromatography and high-performance liquid chromatography.

In an Erlenmeyer flask, 100 ml of silver nitrate solution at various concentrations (1-5 Mm) were created. Then maintaining the solution's concentration in 10 ml of silver nitrate solution 1 Mm 1, 2, 3, 4 and 5 ml of plant extract was added. Fresh 2 g *L. camara* fruits were collected and heated at 55-60°C in 20 ml of 95% methanol for 10 min after being cleaned with Milli-Q water. After cooling, Whatman No. 1 paper was used to filter the pale greenish yellow 2 ml extract. Pink colour was obtained after 6 h as a result of reduction.

Samples of AFM were made by placing on the glass slides, droplets of LA-AgNPs solution by drying them at room temperature. A NTEGRA (NT-MDT, Russia) instrument was used to evaluate these slides. This evaluation was carried out in the Materials Research Department, Ministry of Science and Technology.

Scanning Electron Microscope was applied to investigate the morphology of LA and LA-AgNPs. SEM images were captured by a VEGA 3 (TESCAN, Czech Republic) apparatus containing an energy distributing X-ray (EDX) spectrometer which determine the element that make up molecules. The SEM and EDX analysis was carried out in the Materials Research Department, Ministry of Science and Technology.

Antioxidant activity of *L. camara* extract, synthesized LA, AgNPs and LA were determined by two *in vitro* antioxidant analysis. The antioxidant assay estimates were 2,2-diphenyl-1- picrylhydrazyl free radical scavenging assay (DPPH) and 2,20-Azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid radical cation scavenging assay (ABTS) as described by Irondi *et al.* (2017).

HepG2 cell line of human liver cancer (Passage No. 23) was supplied by Al-Nahrain Biotechnology Center of Al-Nahrain University. The RPMI-1640 medium containing 10% fetal bovine serum with 103 IU/100 ml penicillin G increment was used to subculture the cells. Streptomycin (Ajanta Pharm) at 0.001 g/100 ml prevented microbial contamination. The cells were humidified by incubation at 37°C with 5% CO_2. HepG2 cells were placed into T 25-cm^2 flasks tissue culture (Thermo Scientific) for growth initiation. Cells were extracted after a brief trypsinization (50 mg/ml of trypsin) when they had entered the exponential growth phase (36-48 h) to be implanted at the correct concentration.

The evaluation of intracellular ROS levels indicated the anticarcinogenic impact caused by Lantadene A silver nanoparticles. Reactive oxygen species played a crucial role in both apoptosis and cancer cell growth. HepG2 cells (1×10^4) were planted in 96-well plates for 24 h before being exposed to various concentrations of Lantadene A extract. Fifty microliter of staining solution (DMEM), with 500 nM Hoechst 33342 and 2.5 mg dihydroethidium (DHE) were inserted into each well. The plates were incubated at 37°C for 30 min. PBS containing 3.5% formaldehyde was used to fix the cells for 15 min at room temperature. Cells were rinsed with phosphate-buffer saline and the plate's performance was assessed using an ArraySacn HCS analyzer (Thermo Scientific, USA).

RESULTS AND DISCUSSION

Visible-ultraviolet spectroscopy is an essential method for getting spectrum and stabilizing metal nanoparticles. Change in colour is used as an indicator for the formation of AgNPs due to light reflection or adsorption in creating silver nanoparticles (Lee *et al.*, 2016).

High absorption at 422 wave length was observed by formation of lantadene silver nanoparticles (Fig. 1).

Scanning electron microscopy (SEM) micrographs of AgNPs revealed that they were sphere-shape, well dispersed AgNP. The morphological examination showed nanoparticle crystal size ranging from 134.9-50.28 nm (Fig. 2).

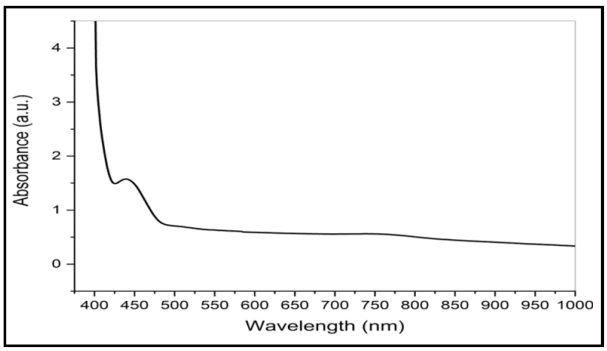

Fig. 1. UV-visible spectra of nano methanol.

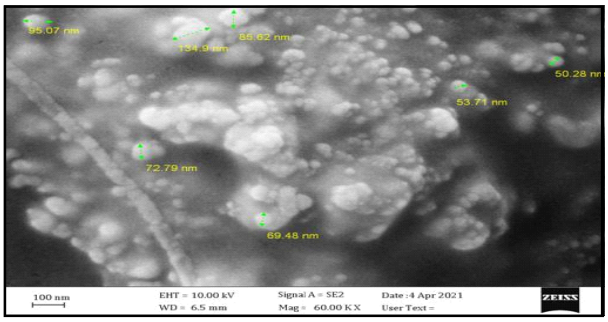

Fig. 2. SEM images of methanol lantana extract.

The three-dimensional data in the AFM images allowed for the quantitative measurement of the nanoparticles' height. SEM only measured two-dimensional images, though AFM speeds were slower than SEM (Hummel *et al.*, 2021) (Fig. 3).

Two hundred µg/ml concentration extract inhibited 80% of free radicals, while LA and ascorbic acid revealed 75.5 and 86% inhibition, respectively. Since IC_{50} rate of LA-AgNPs was 84.56 µg/ml, whereas LA and ascorbic acid were 16.18 and 36.09 µg/ml, respectively. (Fig. 4). These results were compatible with the result of Mittal *et al.* (2016) who produced AgNPs from *S. cumimi* fruit extract. In contrast to the standard of 22.19 µg/ml, synthesized AgNPs had an IC_{50} value of 31 µg/ml.

Inhibition was 16-67% of LA and 25-70% for LA-AgNPs at the concentration range of 12.5-200 µg/ml. With an increase in LA-AgNPs concentration, the reaction mixture absorbance showed a stable increase (Fig. 5). This result is in consistence with the result

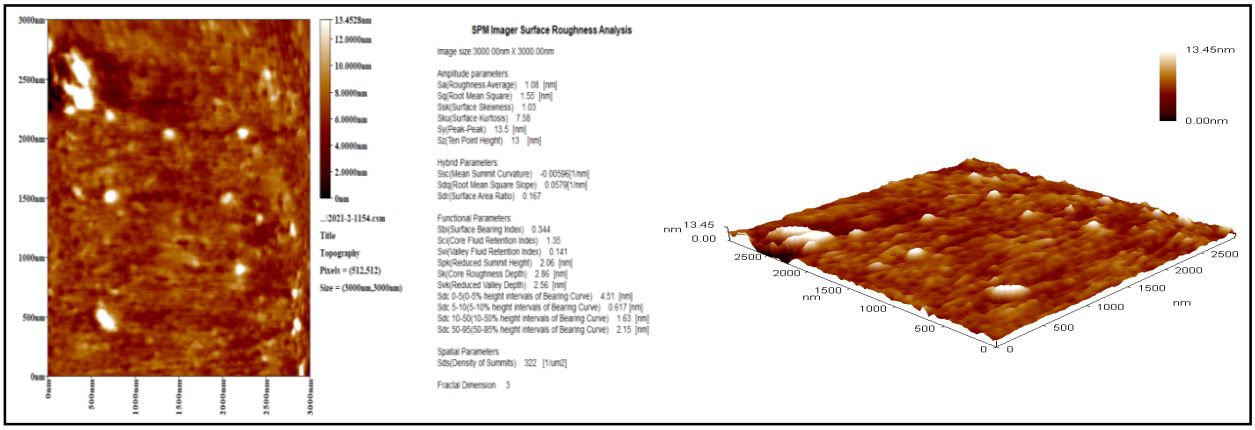

Fig. 3. AFM images of synthesized of AgNPs of methanol lantadene extract.

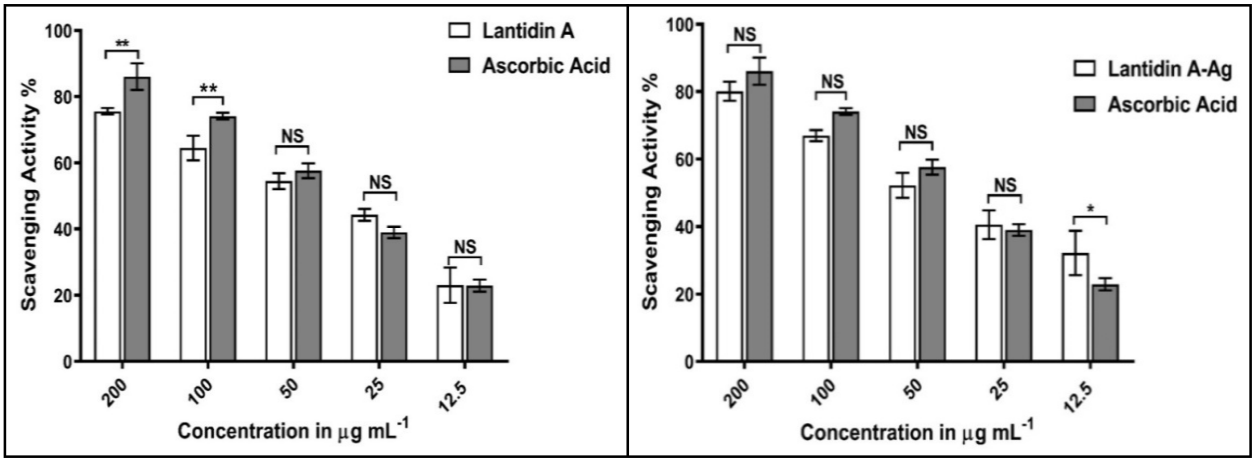

Fig. 4. DPPH scavenging action. A: Scavenging activity of lantadene A. and B: Scavenging activity of lantadene A-Ag separated from *L. camara* leaf extracts.

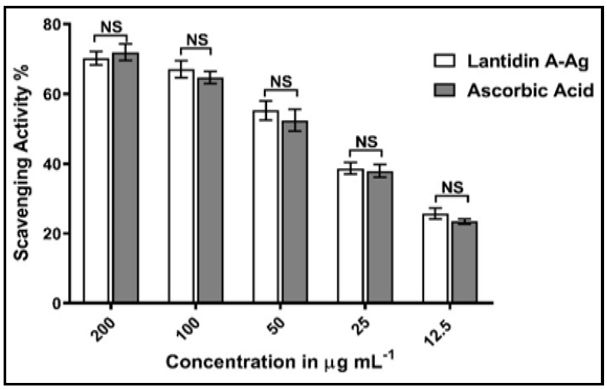

Fig. 5. Radical scavenging action (ABTS): ABTS scavenging action of LA-AgNPs.

of Muniyappan and Nagarajan (2014), using *Elephantopus scaber* leaf extract. Kharat and Mendhulkar (2016) also created AgNPs, which significantly increased the antioxidant action of Es-AgNPs after DPPH assay (Bhakya *et al.*, 2016).

The higher concentrations of LA-AgNPs extract showed substantial increases in the level of ROS at 100 and 200 µg/ml in contrast to untreated cells (Fig. 6). Test was improved with the Thermo Scientific Arrayscan HCS Reader.

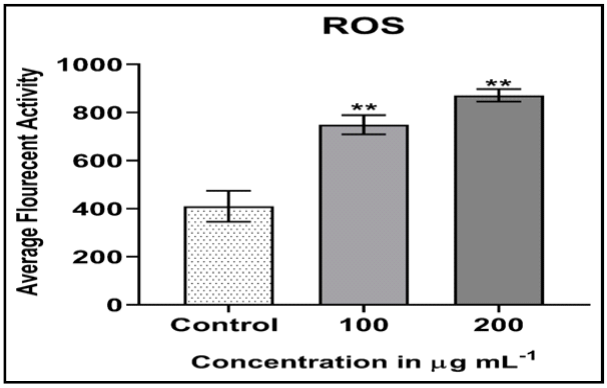

Fig. 6. Impact of various concentrations of *L. camara* on ROS production in hepG2 cells.

ROS was strongly induced in hepG2 cells by LA-AgNPs extract, at 200 µg/ml concentration as compared with untreated cells using High Content Screening system (Fig. 7). Increased ROS production was linked to the regulation of several interconnected molecules, such as: antioxidant enzymes, when cell damage or chemical exposure substantially altered the intracellular balance of antioxidant enzymes, resulting in ROS overproduction (Czarnocka and Karpinski (2018).

Plant leaf extract using AgNPs determinates anti-cancer activity against human breast cancer cells (MCF-7; Poor *et al.*, 2017).

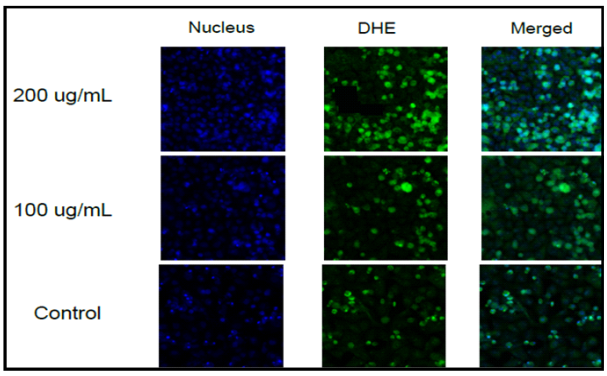

Fig. 7. HCS analysis with several parameters of hepG2 cells treated with *L. camara* extract after incubation for 24 h at 37°C. Hoechst 33342/ blue (Ex 330 nm/Em 420 nm) dye used for cells staining, which allowed for surveillance of nuclear morphology alters and DNA content, Permeability dye (Green) (Ex 491 nm/Em 509 nm) for membrane permeability monitoring, MMP Dye (Red) (Ex552 nm /Em 576 nm) for mitochondrial membrane potential changes. Goat anti-mouse secondary antibody conjugated with DyLight™ for Cytochrome C dismissing.

Additionally, silver nanoparticles were produced by Kathiravan *et al.* (2015) from *Melia dubai* for antitumor effects on human breast cancer cells.

L. camara leaves extract using AgNPs revealed cytotoxic activity against hepG2. AgNPs resulted in released Ag+ interferes with mitochondrial enzymes then interacted with proteins that had sulfhydryl groups which depleted glutathione (GSH). It made GSH's less effective in scavenging ROS and caused oxidative stress (Ratan *et al.*, 2020). As a result, NPs rose intercellular ROS generation and stimulated caspase-3 protein, that caused cell to enter G2/ M phase and initiated cell death. ROS produced by NPs also caused mitochondrial damage. Consequently, the cell cycle stopped and necrotic death took place (Rai *et al.*, 2016).

CONCLUSION

The straightforward and environmentally favourable greener AgNPs was synthesised from *L. camara* extract. HPLC analysis, UV visible spectroscopy, SEM and AFM approved the synthesis of silver nanoparticles. The scavenging effect on DPPH free radicals was shown using nano methanol lantana extract. According to the HCS examination nano methanol extract showed harmful effectiveness on hepG2 cells in a dose-depended manner at 200 µg/ml.

REFERENCES

Bhakya, S., Muthukrishnan, S., Sukumaran, M. and Muthukumar, M. (2016). Biogenic synthesis of silver nanoparticles and their antioxidant and antibacterial activity. *Appl. Nanosci.* **6**: 755-766.

Czarnocka, W. and Karpinski, S. (2018). Friend or foe? Reactive oxygen species production, scavenging and signalling in plant response to environmental stresses. *Free Rad. Biol. Med.* **122**: 04-20.

Hummel, S., Elibol, K., Zhang, D., Sampathkumar, K., Frank, O., Eder, D., Schwalb, C., KotakoskI, J., Meyer, J. C. and Bayer, B. C. (2021). Direct visualization of local deformations in suspended few-layer graphene membranes by coupled *in situ* atomic force and scanning electron microscopy. *Appl. Phys. Letters* **118**: 103104. *https://doi.org/10.1063/5.0040522.*

Irondi, E. A., Akintunde, J. K., Agboola, S. O., Boligon, A. A. and Athayde, M. L. (2017). Blanching influences the phenolics composition, antioxidant activity and inhibitory effect of *Adansonia digitata* leaves extract on α-amylase, α-glucosidase and aldose reductase. *Food Sci. Nutr.* **5**: 233-242.

Jaafar, N. D., Al-Saffar, A. Z. and Yousif, E. A. (2020). Genotoxic and cytotoxic activities of Lantadene A-Loaded gold nanoparticles (LA-AuNPS) in MCF-7 Cell line: An *in vitro* assessment. *Int. J. Toxicol.* **39**: 422-432.

Kathiravan, V., Ravi, S., Ashokkumar, S., Velmurugan, S., Elumalai, K. and Khatiwada, C. P. (2015). Green synthesis of silver nanoparticles using *Croton sparsiflorus* Morong leaf extract and their antibacterial and antifungal activities. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **139**: 200-205.

Khandanlou, R., Murthy, V., Saranath, D. and Damani, H. (2018). Synthesis and characterization of gold-conjugated *Backhousia citriodora* nanoparticles and their anticancer activity against MCF-7 breast and HepG2 liver cancer cell lines. *J. Mat. Sci.* **53**: 3106-3118.

Kharat, S. N., and Mendhulkar, V. D. (2016). Synthesis, characterization and studies on antioxidant activity of silver nanoparticles using *Elephantopus scaber* leaf extract. *Mat. Sci. Engineer. C.* **62**: 719-724.

Kumar, R., Sharma, R., Patil, R. D., Mal, G., Kumar, A., Patial, V. and Singh, B. (2018). Sub-chronic toxicopathological study of lantadenes of *Lantana camara* weed in Guinea pigs. *BMC Vet. Res.* **14**: 01-13.

Kumar, S., Singh, B. and Yadav, A. (2020). Ethanobotany and phytochemistry of *Lantana camara* L. (Verbenaceae). *Bot. Leads Drug Disc.* **26**: 389-404.

Lee, S. Y., Krishnamurthy, S., Cho, C. W. and Yun, Y. S. (2016). Biosynthesis of gold nanoparticles using *Ocimum sanctum* extracts by solvents with different polarity. *ACS Sustain. Chem. Engineer.* **4**: 2651-2659.

Mittal, A. K., Thanki, K., Jain, S. and Banerjee, U. C. (2016). Comparative studies of anticancer and antimicrobial potential of bioinspired silver and silver-selenium nanoparticles. *J. Mat. NanoSci.* **3** : 22-27.

Muniyappan, N. and Nagarajan, N. S. (2014). Green synthesis of silver nanoparticles with *Dalbergia spinosa* leaves and their applications in biological and catalytic activities. *Process Biochem.* **49**: 1054-1061.

Nakamura, Y., Mochida, A., Choyke, P. L. and Kobayashi, H. (2016). Nanodrug delivery: Is the enhanced permeability and retention effect sufficient for curing cancer? *Bioconjugate Chem.* **27**: 2225-2238.

Organization W. H. Global Health Estimates (2016) Disease burden by cause, age, sex by country and by region, 2000-16. Geneva, 2018.

Poor, M. H. S., Khatami, M., Azizi, H. and Abazari, Y. (2017). Cytotoxic activity of biosynthesized Ag nanoparticles by *Plantago major* towards a human breast cancer cell line. *Rendiconti Lincei* **28**: 693-699.

Rai, M., Ingle, A. P., Birla, S., Yadav, A. and Santos, C. A. D. (2016). Strategic role of selected noble metal nanoparticles in medicine. *Crit. Rev. Microbiol.* **42**: 696-719.

Ratan, Z. A., Haidere, M. F., Nurunnabi, M. D., Shahriar, S. M., Ahammad, A. S., Shim, Y. Y., Reaney, M. J. T. and Cho, J. Y. (2020). Green chemistry synthesis of silver nanoparticles and their potential anticancer effects. *Cancers* **12**: 855. *https://doi.org/10.3390/cancers12040855.*

Shabestarian, H., Homayouni-Tabrizi, M., Soltani, M., Namvar, F., Azizi, S., Mohamad, R. and Shabestarian, H. (2016). Green synthesis of gold nanoparticles using sumac aqueous extract and their antioxidant activity. *Mat. Res.* **20**: 264-270.

Shamsee, Z. R., Al-Saffar, A. Z., Al-Shanon, A. F. and Al-Obaidi, J. R. (2019). Cytotoxic and cell cycle arrest induction of pentacyclic triterpenoides separated from *Lantana camara* leaves against MCF-7 cell line *in vitro*. *Mol. Biol. Rep.* **46**: 381-390.

Tasneem, S., Liu, B., Li, B., Choudhary, M. I. and Wang, W. (2019). Molecular pharmacology of inflammation: Medicinal plants as anti-inflammatory agents. *Pharm. Res.* **139**: 126-140.

Zugazagoitia, J., Guedes, C., Ponce, S., Ferrer, I., Molina-Pinelo, S. and Paz-Ares, L. (2016). Current challenges in cancer treatment. *Clinical Therapeutics* **38**: 1551-1566.