ejpmr, 2021,8(11), 466-473

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM THE AQUEOUS LEAF EXTRACT OF *CASSIA ALATA* AND ITS ANTI-INFLAMMATORY, ANTI-DIABETIC & CHARACTERIZATION STUDIES

Sidharth K.[1]* and Dr. K. Narayanasamy[2]

[1]Post Graduate Student, Dpt. of Biochemistry, Sree Narayana Guru College, Coimbatore.
[2]Professor & Head, Dpt. of Biochemistry, Sree Narayana Guru College, Coimbatore.

***Corresponding Author: Sidharth K.**

Post Graduate Student, Dpt. of Biochemistry, Sree Narayana Guru College, Coimbatore.

Article Received on 14/09/2021 **Article Revised on 04/10/2021** **Article Accepted on 24/10/2021**

ABSTRACT

Nano science is an inspiring and influential discipline of science which has numerous novel and cost effective yields and applications. The main challenge in the development of catalytic NPsis to prepare nanomaterial's that are highly active, selective, stable, robust, and inexpensive. There has been a growing need to replace the chemical synthetic procedures with clean, nontoxic, and environmentally acceptable green chemistry methods. Water soluble plant metabolites and co- enzymes present in plant extracts can be used to reduce metal ions to nanoparticles in a single-step green synthesis process. Among the metal nanoparticles, SNPs are considered to be of great importance because of their properties such as antiviral, antibacterial, antifungal, electrical conductivity, chemical stability and catalytic activity. Silver nanoparticles are nanoparticles of silver, i.e. silver particles of between 1 nm and 100 nm in size. *Cassia alata* (also known as *Senna alata)* is a shrub belonging to the fabaceae family, found in tropical areas. In the present scenario deals with the exploration and development of cheaper, effective plant based silver Nano particle with better bio active potential and least side effects. In this study involves the synthesis of AgNPs by an aqueous leaf extract of *Cassia alata*. The biologically synthesized silver nanoparticles showed effective anti- inflammatory, anti- diabetic activities.

KEYWORDS: *Cassia alata*, green synthesis, AgNPs, characterization studies,anti-diabetic activity, anti-inflammatory activity.

INTRODUCTION

Nano science is an inspiring and influential discipline of science which has numerous novel and cost effective yields and applications. There has been a growing need to replace the chemical synthetic procedures with clean, nontoxic, and environmentally acceptable green chemistry methods. Metal based nanoparticles are synthesized for numerous applications from the extracts of different plant parts such as leaves, roots, flower, seeds, etc. Water soluble plant metabolites and co-enzymes present in plant extracts can be used to reduce metal ions to nanoparticles in a single- step green synthesis process. Extracts of a diverse range of plant species have been successfully used to nanoparticles. Among the metal nanoparticles, SNPs are considered to be of great importance because of their properties such as antiviral, antibacterial, antifungal, electrical conductivity, chemical stability and catalytic activity.

Silver nanoparticles are nanoparticles of silver, i.e. silver particles of between 1 nm and 100 nm in size. Green synthesis is effective, eco-friendly and without difficulty scaled up on a large scale. There is no need of high pressure, energy, temperature, and toxic chemicals. The inhibitory effect on silver on microbes, it was detected in the past, is generally used in medical and industrial processes. It is generally recognized that silver nanoparticles may attach to the cell wall, thus disturbing cell-wall permeability and cellular respiration. The nanoparticles may also penetrate inside the cell causing damage by interacting with phosphorus and sulfur containing compounds such as DNA and protein.

Generally, silver does not adversely affect viable cells and does not easily provoke microbial resistance. Hence silver containing materials used in various fields. *Cassia alata* (also known as Senna alata) is a shrub belonging to the fabaceae family, found in tropical areas. It is commonly known as candle bush, with reference to the shape of inflorescence. It is annual or bi annual shrub with an offensive smell, 1-4 m tall, preferring sunny and moist areas. Leaves, flowers and fruits of C. alata is used as anti-diabetic, anti-inflammatory, analgesic, against digestive problems and infectious diseases (as anti-bacterial and anti-fungal agents). Senna alata is native to Mexico, can befound in diverse habitat. Senna alata often

called ring worm bush because of it very effective fungicidal property, for treating ringworm and other fungal infection of the skin. Its effective ingredients include the yellow chrysophanic acid. Its laxative effect, due to its anthraquinone content is also well proved. In the present scenario deals with the exploration and development of cheaper, effective plant based silver Nano particle with better bio active potential and least side effects. In this study involves the synthesis of AgNPs by an aqueous leaf extract of Cassia alata. The biologically synthesized silver nanoparticles showed effective anti- inflammatory, anti- diabeticstudies.

MATERIALS AND METHODS

Collection of leaf samples from *C. alata*

Leaves that appeared healthy were collected from different branches of *C. alata* from Ottapalam, Palakkad, Kerala (fig.1). The plant authentication were done in Botanical Survey of India, Southern Regional Centre, Coimbatore with reference no: BSI/SRC/01/18/2021/Tech/93.

Preparation of plant extract

The plant leaf sample were washed thoroughly with running tap water followed by double distilled water twice, to remove the adhering dust particles and the leaves were dried under the shades for two weeks. It is then grinded into fine powder form and stored in an airlift container. The plant extract of *C. alata* was prepared by, boiled the 20g of leaf powder with 250ml distilled water .After 15 minutes the aqueous extract was cooled and filtered through What man No.1 filter paper and get clear plant extract.. The aqueous leaf extract was used as reducing agent for the synthesis of silver nanoparticles.(fig.2).

Phytochemical analysis (Qualitative)

The qualitative phytochemical study was performed on the extracts by using standard tests.

Preparation of 1mM silver nitrate solution

Dissolve 0.169 AgNO3 (silver nitrate) in 1000ml of distilled water and used for the green synthesis of silver nanoparticles (AgNPs).

Green synthesis of silver nanoparticles

10 ml of filtered aqueous extract of *cassia alata* leaves was added to 90 ml of 1mM AgNO3in a 250 ml Erlenmeyer flask . then kept in room temperature for 48 hours at dark. The process was continued till the change of colour occurred from yellow to dark brown indicating the completion of silver nanoparticle synthesis (fig.2). After 24 hour centrifuge the reaction mixture and discard the supernatant. Added 1 ml of distilled water to the pellet and washed by centrifugation. The pellet was collected and the nanoparticles were stored for further characterization.

Characterization of silver nanoparticles

UV-Visible spectral analysis

UV-Visible spectroscopy is an important technique for analyzing the formation of SNPs in aqueous solution. The bio reduction of pure silver ions to silver nanoparticles was observed by UV-Visible spectroscopy. Taking 4ml of the sample, compared with 4ml of 1mM silver nitrate used as blank. The absorption maxima were measured by using UV spectrophotometer between 300-800nm wavelength.

Fourier transform infra-red spectroscopy (FTIR)

The fourier transform infrared spectroscopy (FTIR) analysis was carried out to know the different functional groups that act as bioreductors to reduce Ag+ ions to Ag0. 2mg of the dried sample (AgNPs) were mixed with 200mg of KBr and pressed into a pellet. it was placed into the sample holder for FTIR analysis, in which the samples were irradiated by a broad spectrum of infra-red light and the level of absorbance at a particular frequency was plotted after fourier transformation of the data. Compounds contained in the dried sample were identified according to standard infra-red chart.

Scanning Electron Microscopy (SEM Analysis)

SEM analysis was carried out to determine the particle morphology. The biologically synthesized silver nanoparticle sample was centrifuged. The pellet was collected and dried. The fine sample was used for SEM analysis. SEM analysis was made using FEI QUANTA 200 SEM machine.

Energy dispersive X-ray spectroscopy (EDX)

The composition of the synthesized silver nanoparticles was analyzed by Energy Dispersive X-ray Microanalysis Spectroscopy. EDX analysis was made by using EDAX Genesis XM4 machine. (fig.6)

Thermo gravimetric analysis (TGA analysis)

Thermo Gravimetric Analysis or Thermal Gravimetric Analysis (TGA) is a method of thermal analysis in which the mass of a sample is measured over time as the temperature changes. TGA can be used to evaluate the thermal stability of a material. A TGA analysis is performed by gradually raising the temperature of a sample in a furnace as its weight is measured on an analytical balance that remains outside of the furnace. In TGA, mass loss is observed if a thermal event involves loss of a volatile component.

Anti-Inflammatory Activity Inhibition of protein denaturation

Inhibition of protein denaturation was evaluated by the method of Mizushima and Kobayashi 1968 and Sakat et al. 2010 with slight modification. 500 µl of 1% bovine serum albumin was added to 100 to 500 µL of plant extract. This mixture was kept at room temperature for 10 minutes, followed by heating at 51°C for 20 minutes. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm.

Acetyl salicylic acid was taken as a positive control. The experiment was carried out in triplicates and percent inhibition for protein denaturation was calculated using:

$$\text{Percentage inhibition} = \frac{100 - (\text{O.D. of test} - \text{O.D. of product control}) \times 100}{\text{O.D. of Control}}$$

Anti- Diabetic Activity

Alpha Amylase Inhibition Assay

α-amylase activity was carried out by starch-iodine method. 10 µl of α-amylase solution (0.025 mg/ml) was mixed with 390 µl of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) containing different concentration of extracts. After incubation at 37° C for 10 min, 100 µl of starch solution (1%) was added, and the mixture was re-incubated for 1 h. Next, 0.1 ml of 1% iodine solution was added, and after adding 5 mL distilled water, the absorbance was taken at 565 nm. Sample, substrate and α-amylase blank determinations were carried out under the same reaction conditions (Sheikh et al., 2008).Inhibition of enzyme activity was calculated as.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}}$$

Alpha Glucosidase Inhibition Assay

α-glucosidase enzyme inhibition assay were carried out by adding 225 ml of 80mM Phosphate buffer pH 7.0/ Positive control/ Different concentration of test samples +75 ml of α-glucosidase . Pre-incubated at 37 C for 30 minutes. Kept in boiling water bath for 2 minutes, cooled and added 250 ml of glucose reagent Incubated at room temperature for 10 mins.Measured OD at 510 nm.

Table1: Anti-Inflammatory Activity (Protein Denaturation Inhibition Assay).

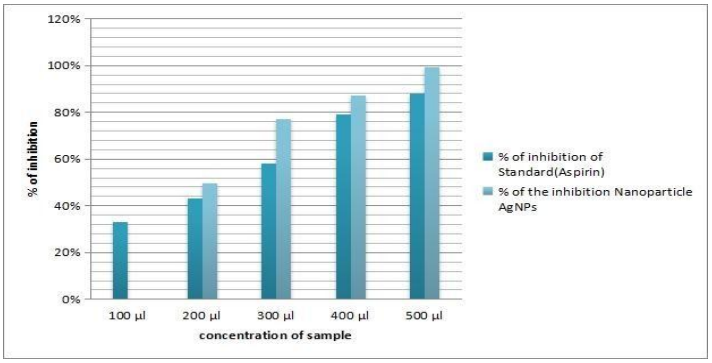
Concentration	% of inhibition ofStandard (Aspirin)	% of the inhibition Nanoparticle AgNPs
100 µl	33 %	39. 00 %
200 µl	43 %	49.50 %
300 µl	58 %	77.09 %
400 µl	79 %	87.00 %
500 µl	88 %	99.08 %

Table 2: Anti Diabetic ActivityAlpha Amylase Inhibition Assay.

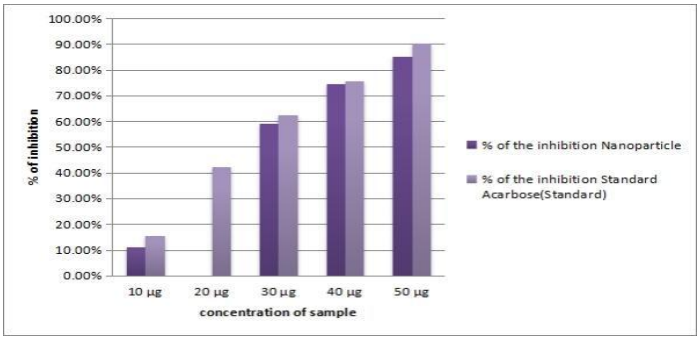
Concentrationof the sample	% of the inhibition Nanoparticle	% of the inhibition Standard Acarbose (Standard)	IC50 Value of AgNPs	IC50 Valueof Standard
10 µg	11.22 %	15.60 %	24.3 µg	25.6 µg
20 µg	33.44 0%	42.25 %		
30 µg	59.20 %	62.39%		
40 µg	74.53 %	75.65 %		
50 µg	85.17 %	90.26 %		

Table 3: Alpha Glucosidase Inhibition Assay.

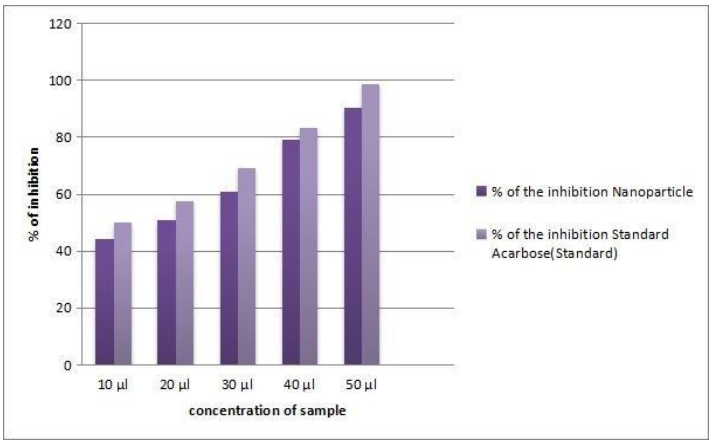
Sl. No	Concentration ofthe sample	% of the inhibition Nanoparticle	% of the inhibition Standard Acarbose(Standard)
1	10 µl	44.2	50.00
2	20 µl	50.8	57.73
3	30 µl	60.8	69.34
4	40 µl	79.1	83.48
5	50 µl	90.5	98.56
IC-50		10 µg	20 µg



Graph 1: Anti Inflammatory Activity.



Graph 2: Alpha Amylase Inhibition Assay.



Graph 3: Alpha Glucosidase Inhibition Assay.

Fig. 1: *Cassia alata* Plant Showing Leaves And Inflorescence.

Fig. 2: Aqueous leaf extract of *Cassia alata* plant Before and After the Formation of AgNPs.

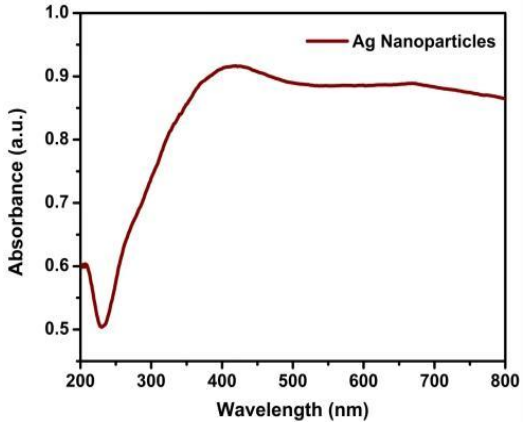


Fig 3: UV-Visible spectrum of silver nanoparticles synthesized from the aqueous leaf extractof *Cassia alata.*

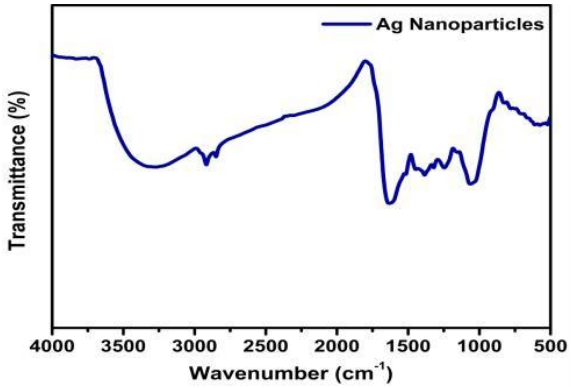


Fig 4: FTIR spectra of AgNPs synthesized from the aqueous leaf extract of *C.alata.*

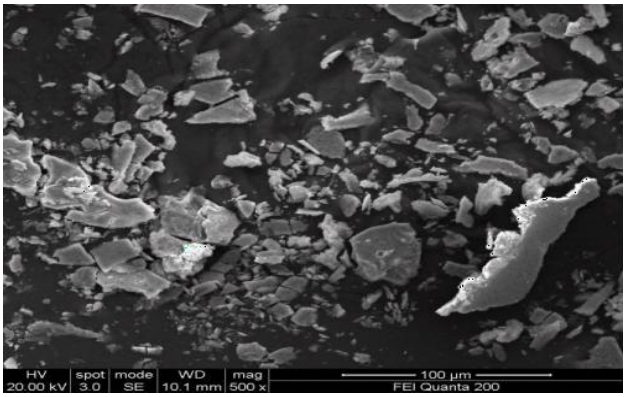


Fig. 5: SEM images of AgNPs synthesized from aqueous leaf extract of *C.alata.*

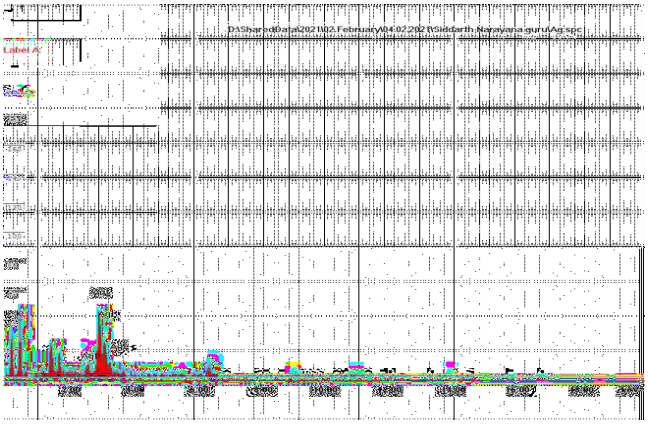


Fig. 6: EDX Spectra of AgNPs synthesized from aqueous leaf extract of *C.alata.*

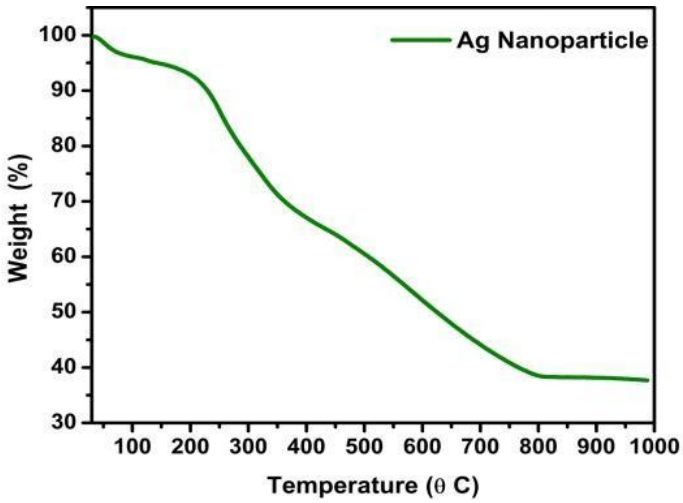


Fig. 7: TGA of AgNPs synthesized from the aqueous leaf extract of *C.alata.*

RESULTS

Phytochemical analysis

The plant extract showed the positive result for phytochemical analysis. From the qualitative phytochemical test it was revealed that the Cassia alata leaf extract shows the maximum presence of phytochemicals.

UV-Visible spectral analysis

The absorption spectrum showed maximum peak at 430nm for the nanoparticle synthesized using aqueous extract of Cassia alata leaves.(fig.3)

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR analysis of nanoparticle sample (aqueous leaf extract of Cassia alata) shows peaks at 3564.45, 3525.88 indicate the presence of OH group. The peak at 2927.94 indicates the presence of OH stretch. The peaks at 2364.73 indicates NH stretch .The peak value of 1516.05 indicates the presence of C=C groups (preferably belonging to benzene ring). The peak in between 1000-1500 indicates strong C-F stretching (fluro compound).(fig.4)

SEM Analysis

SEM images shows similar appearance for the presence of silver nanoparticles synthesized from C. alata. The SEM image shows cluster of nanoparticles in different size. The particles are spherical in shape .The SEM analysis confirmed the presence of nanoparticle. (fig.5)

Energy dispersive X-ray spectroscopy (EDX)

The energy dispersive spectroscopy (EDX) data show very strong silver signals. Which indicate that the reduction of silver ions to elemental silver possibly originated from the molecules attached to the surface of the AgNPs (fig.6)

Anti-Inflammatory Activity

Inhibition of protein denaturation

Green synthesized AgNPs from aqueous leaf extract of C.alata were able to inhibit protein denaturation in a concentration –dependent manner, and the inhibitory effect of AgNPs and drug aspirin at different concentrations (10-50 µg/ml) on protein denaturation is shown in table.1. Inhibition % of protein denaturation of the AgNPs was within the range from 39.00% to 99.08% at the concentration range of 10-50 µg/ml. Green synthesized AgNPs from aqueous leaf extract of C.alata exhibited a significantly higher level of inhibition compared to the standard drug aspirin. (graph.1)

Anti- Diabetic Activity

In vitro Alpha amylase inhibition assay

In this study the in-vitro α- amylase inhibitory activity of the AgNPs was investigated. The result of experiment showed that, there was a dose dependent increase in percentage inhibitory activity against α- amylase enzyme. The inhibitory effect of AgNPs and the standard drug acarbose at different concentrations (10-50 µg/ml) on α- amylase has been depicted in Table2, The green synthesized AgNPs (10-50 µg/ml) exhibited mild potent α- amylase inhibitory activity in a dose dependent manner. The AgNPs showed inhibitory activity from 11.22% to 85.17% with an IC50 value of 24.3µg extract. Acarbose is a standard drug for α- amylase inhibitor. Acarbose at concentration of (10-50µg/ml) showed α-amylase inhibitory activity from 15.60% to 90.26% with an IC50 value of 25.6 µg.(graph.2)

In Vitro Alpha Glucosidase Inhibition Assay

The AgNPs revealed a mild significant inhibitory action of α- glucosidase enzyme. The result of experiment showed that, there was a mild dose dependent increase in percentage inhibitory activity against α- glucosidase enzyme. The inhibitory effect of AgNPs and the standard drug acarbose at different concentrations (10-50 µg/ml) on α- glucosidase The AgNPs showed inhibitory activity from 44.2% to 90.5% with an IC50 value of 10 µg extract.(table.3) Acarbose is a standard drug for α-glucosidase inhibitor. Acarbose at concentration of (10-50µg/ml) showed α-glucosidase inhibitory activity from 50.00% to 98.56% with an IC50 value of 20 µg.(graph.3).

BIBLIOGRAPHY

1. Abboud, Y., Eddahbi, A., EI Bouari, A., Aitenneite, H., Brouzi, K., Mouslim, J.: Microwave- assisted approach for rapid and green phytosynthesis of silver nanoparticles using aqueous onion.
2. Abilash G, Ramakrishna P, Ramakrishna M, Lohith K, Chelli J, Apparao MR Catalytic reduction of 4-Nitro phenol using biogenic gold and silver nanoparticles derived from breynia rhamnoides. Langmuir, 2011; 27: 15268-15274.
3. Amin, M., Anwar, F., Janjua, M.R.S.A., Iqbal, M.A., Rashid, U.: Green synthesis of silver nanoparticles through reduction with Solanum xanthocarpum L. Berry extract: characterization, antimicrobial and urease inhibitory activities against Helicobacter pylori. Int. J. Mol. Sci., 2012; 13: 9923–9941.
4. Anbu, M. Anita, R. Sathiya et al., "*In Vitro* Anthelmintic activity of leaf ethanolic extract of *Cassia alata* and *Typha angustifolia*," *MSRUAS-SASTech Journal*, 2013; 14(2): 41–44.
5. B. K. Saha, M. N. Bhuiyan, K. Mazumder et al., "Hypoglycemic activity of *Lagerstroemia speciose.*
6. L. extract on streptozotocin-induced diabetic rat: underlying mechanism of action," *Bangladesh Journal of Pharmacology*, 2009; 4(2): 79–83.
7. B. Sagnia, D. Fedeli, R. Casetti et al., "Antioxidant and antiinflflammatory activities of extracts from *C. alata, E. indica, E.*
8. B. Sarkar, S. Khodre, P. Patel et al., "HPLC analysis and antioxidant potential of plant extract of *Cassia alata*," *Asian Journal of Pharmaceutical Science and Technology*, 2014; 4(1): 4–7.
9. B. Sarkar, S. Khodre, P. Patel et al., "HPLC analysis and antioxidant potential of plant extract of *Cassia alata*," *Asian Journal of Pharmaceutical Science and Technology*, 2014; 4(1): 4–7.
10. B. Singh, J. R. Nadkarni, R. A. Vishwakarma, S. B. Bharate, M. Nivsarkar, and S. Anandjiwala, " hydroalcoholic extract of *Cassia alata* (Linn.) leaves and its major compound rhein exhibits antiallergic activity via mast cell stabilization and lipoxygenase inhibition," *Journal of Ethnopharmacology*, 2012; 141(1): 469–473.
11. Begum, N.A., Mondal, S., Basu, S., Laskar, R.A., Mandal, D.: Biogenic synthesis of Au and Ag nanoparticles using aqueous solutions of Black Tea leaf extracts. Colloids Surf., 2009; 71(1): 113–118.
12. Ben Aissa MA, Tremblay B, Andrieux-Ledier A, Maisonhaute E, Raouafi N, Courty A Copper nanoparticles of well-controlled size and shape: A new advance in synthesis and self- organization. Nano scale, 2015; 7: 3189-3195.
13. Bhainsa, K.C., D'Souza, S.F.: Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillus fumigatus. Colloids Surf B Biointerfaces, 2006; 47: 160–164.
14. C. P. Khare, *Indian Medicinal Plants: An Illustrated dictionary*, Springer Press, London, UK, 2007.
15. C. V. Chang, A. C. Felıcio, J. E. D. P. Reis, M. D. O. Guerra, and V. M. Peters, "Fetal toxicity of *Solanum lycocarpum* (Solanaceae) in rats," *Journal of Ethnopharmacology*, 2002; 81(2): 265– 269.
16. C. Veeresham, "Natural products derived from plants as a source of drugs," *Journal of Advanced Pharmaceutical Technological Research*, 2012; 3(4): 200-201.
17. Chem, 2014; 73: 135–140.
18. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, p. 195, Uppsala University, Uppsala, Sweden, 2015.
19. E. O. Ogunti and A. A. Elujobi, "Laxative activity of *Cassia alata*," *Fitoterapia*, 1993; 64(5): 437–439.
20. E. Ramstad, *Modern Pharmacognosy*, McGraw-Hill, New York, NY, USA, 1959.
21. Esumi, K., Tano, T., Torigoe, K., Meguro, K.: Preparation and characterization of bio metallic Pd–Cu colloids by thermal decomposition of their acetate compounds in organic solvents. Chem. Mater, 1990; 2: 564–587.
22. *Ethnopharmacology*, 1988; 22: 81–90.
23. F. Adelowo and O. Oladeji, "An overview of the phytochemical analysis of bioactive compounds in *S. alata*," *American Chemical and Biochemical Engineering*, 2017; 2(1): 7–14.
24. G. Aviello, I. Rowland, C. I. Gill et al., "Anti-proliferative effffect of rhein, an anthraquinone isolated from Cassia species, on Caco-2 human adenocarcinoma cells," *Journal of Cellular and Molecular Medicine*, 2006–2014; 14.
25. G. K. Varghese, L. V. Bose, and S. Habtemariam, "Antidiabetic components of Cassia alata leaves: identifification through α-glucosidase inhibition studies," *Pharmaceutical*, 2013; 51(3): 345– 349.
26. Goia, D.V., Matijevic, E.: N: Preparation of monodispersed metal particles. J Chem, 1998; 22: 1203–2015.
27. Hayelom DB, Adhena AW, Haile Mariam KB, Tekilt GA Synthesis paradigm and applications of Silver nanoparticles (AgNPs), a Review. SM&T, 2017; 13: 18-23.
28. Henglein, A.: Reduction of Ag (CN) 200 on silver and platinum colloidal nanoparticles. Langmuir , 2001; 17: 2329–2333.
29. A. Ogunwande, G. Flamini, P. L. Cioni et al., "Aromatic plants growing in Nigeria: essential oil constituents of *Cassia alata* (Linn.) roxb. And *Helianthus annuus* L.," *Records of Natural Products*, 2010; 4(4): 211–217.
30. A. Ross, *Medicinal Plants of the World*, Humana Press, Totowa, NJ, USA, 1999.
31. M. Villaseñor, A. P. Canlas, M. Pascua et al., "Bioactivity studies on *Cassia alata* Linn. leaf extracts," *Phytotherapy Research*, 2002; 16(1): 93–96.
32. J. Naowaboot, N. Somparn, S. Saentaweesuk et al., "Umbelliferone improves an impaired glucose and lipid metabolism in high-fat diet/streptozotocin-induced type 2 diabetic rats," *Phytotherapy Research*, 2015; 29: 1388–1395.

33. J. O. Igoli, O. G. Ogaji, T. A. Tor-Anyiin et al., "Traditional medicinal practices among the Igede people of Nigeria (partII),"*African Journal of Traditional Complementary and Alternative Medicine*,2005; 2(2): 134–152.
34. J. S. Kayembe, K. M. Taba, K. Ntumba et al., "*In vitro* antimalarial activity of 20 quinones isolated from four plants.