

Green Synthesis of Silver, Copper and Bimetallic (Ag-Cu) Nanoparticle using Aqueous Extract of *Achyranthes Aspera* for Hepatoprotective activity on HEPG2 Cell lines against CCl₄ induced toxicity

B Sirisha^{1,2*}, D Meera¹ and Ch. Venkatramana Devi¹

¹Department of Biochemistry, Osmania University, Hyderabad-500007, Telangana, India

²Telangana Social Welfare Residential Degree College for Women, Mahendrahills, Hyderabad, Telangana, India

ABSTRACT

One of the prominent rich resources used in the synthesis of medication is medicinal plants. Nanoparticles exhibit considerable potential across various domains and disciplines in Medicine. The synthesis of nanoparticles from plant sources has advantages over traditional chemical methods. This study explores an eco-friendly process by which the synthesized silver nanoparticles along with Copper nanoparticles (AgNPs/ CuNPs) utilize *Achyranthes aspera* and evaluates their hepatoprotective effects using HepG2 cell lines. The nanoparticles were produced and characterized through UV-Vis spectroscopy, SEM, XRD, and FTIR analyses. The present study results indicate that silver-copper nanoparticles generated from *A. aspera* seeds have the capability to serve as protective agents for the liver against damage caused by carbon tetrachloride.

Keywords: Silver Copper Nanoparticles, *Achyranthes aspera*, In vitro hepatoprotective activity

1. Introduction

According to the WHO, 21,000 plants reutilized for medicinal purposes worldwide, and India has a rich tradition of using medicinal herbs and spices. There are 2,500 species in India, while 150 of them are used extensively for economic purposes [1]. Day-by-day usage for plant-based medications is rising globally including in India [2]. Due to the presence of phytochemicals, most of the plants show healing properties to many diseases and showing anti-bacterial, characteristics which include antifungal, anticancer, antidiuretic, anti-inflammatory, along with anti-diabetic [3-7]. Presently many countries are looking for an alternative to allopathic treatment for many diseases like diabetes, cancers, and bacterial and viral infections. Medicinal plants are rich sources of components that are used in the production of medication. There are numerous pharmaceutical companies that specialize on producing medications using plants as raw materials [8]. Due to the extraction of active compounds used in the production of different medications; medicinal plant raw materials are frequently used. Similar to laxatives, blood thinners, antibiotics, or antimalarial medications, all of these medications contain plant-based components [9].

26 September 2024: Received

24 October 2024: Revised

20 November 2024: Accepted

15 December 2024: Available Online

Citation: B Sirisha, D Meera and Ch. Venkatramana Devi (2024). Green Synthesis of Silver, Copper and Bimetallic (Ag-Cu) Nanoparticle using Aqueous Extract of *Achyranthes Aspera* for Hepatoprotective activity on HEPG2 Cell lines against CCl₄ induced toxicity. *Acta Pharma Reports*.

DOI: <https://doi.org/10.51470/APR.2024.03.02.18>

*Corresponding Author: B Sirisha

Email Address: boyinisirisha24@gmail.com

Copyright: © 2024 by the authors. The license of *Acta Pharma Reports*. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

Nano particles synthesis of silver and gold, its usage in the numerous advancements in the area of cancer diagnostics and treatment via nano particles (NPs), and medicinal applications have constantly increased [10]. Due to the demanding usage of nano particles, they are synthesized using hazardous substances with both physical and chemical techniques. To overcome this, many researchers are focused on the synthesis of eco-friendly nano particles from enzyme [11], fungus [12], and algae [13-14] were reported successfully in the production of Au-NPs. Additional to this, synthesizing the nano particles from plants and plant parts and its usage in diseases management are very effective compare to the plants alone [15].

Achyranthes aspera Linn is a plant species. belongs to the Amaranthaceae family, which is also known as Apamarga. It is commonly found as a weed in India. Furthermore, it is used as traditional medicine in tropical Asian and African countries [16]. With fixed branches, thick, elliptic leaves, along with greenish-white blooms, it can reach a height from 0.3 - 1 m. Antibacterial, antifungal, thyroid-stimulating, antiperoxidative, anti-inflammatory, antiarthritic, immunomodulatory, wound-healing, anti-obesity, anticonvulsant, anticancer, along with hepatoprotective qualities had been among the various biological qualities demonstrated by *A. aspera*. Numerous phytochemical classes, including alkaloids, steroids, flavonoids, phenolic compounds, saponins, or terpenoids have been reported to occur in this plant [17].

2. Material and Methods

2.1 Collection of the plant material

Achyranthes aspera plants were procured locally, with Dr. Shashikanth, a taxonomist of the Department of Botany at Osmania University in Hyderabad, verifying it. Following plant harvest, the surface was carefully cleaned with running tap water to remove if any remaining dust particles of the plant separating the leaves, root and stems from the plant. Separated plant parts leaves, root and stems were shade dried and ground separately into powder for further experimental analysis.

2.2 Preparation of Extracts

25g of dry coarsely powdered plant material (Root, Leaf, and Stem) separately was submerged in a 250ml of double distilled. The mixture was heated for 20 minutes at 60°C while stirring occasionally. The mixture is cooled to room temperature and filtered using Whatman no.1 filter paper. The filtered extracts (Root, Leaf, and Stem) separately were stored at 4°C for further experimental analysis [26].

2.3 Synthesis of Silver nanoparticles (AgNPs)

A. aspera leaf, stem, and root extract (10 mL) were added to 90 mL containing 1 mM aqueous silver nitrate solution, which was then heated to 80 °C for three hours while being constantly stirred in order to create silver nanoparticles. The shift to yellow to a rich brown coloration indicated the production of AgNPs. Labeling was done on the final green-synthesized silver nanoparticles, which then remained at 4°C until additional experimental examination [27].

2.4 Synthesis of Copper nanoparticles (CuNPs)

A. aspera copper nanoparticles have been made by mixing 50 mL (5 mM) copper sulfate solution with 5 mL of aqueous plant extracts of the leaf, stem, along with root. NaOH (1 N) solution had been added to lower the mixture's pH to 7.0. The resultant green mixture was additionally stored for further experimental analysis at room temperature [28].

2.5 Synthesis of bimetallic Silver-Copper nanoparticles (Ag-CuNPs)

A. aspera bimetallic silver-copper nanoparticles have been established by mixing equal volumes of nanoparticles of silver and copper (50:50) and holding them at 4°C until additional experimental investigation.

2.6 In Vitro - Hepatoprotective Activity

HepG2 cells were used to examine the test extract's in-vitro hepatoprotective properties. The toxicological assay involved incubating cells with DMEM in DMSO (0.05% v/v) for 12 hours, followed by 1.5 hours of DMEM treatment with 40 mM CCl₄. Normal control cells were cultured with DMEM in DMSO (0.05% v/v) for 12 hours. Cells were treated in 40 mM CCl₄ for 1.5 hours after being cultured with DMEM containing various extracts at concentrations of 10, 25, 50, 100, and 150 µg/mL for 12 hours. By reducing the MTT salt to chromophore formazan crystals, the cells with metabolically active mitochondria produce precipitates at the end of the incubation time. For solubilized crystals with DMSO, the optical density is evaluated at 570 nm using a microplate reader. The following formula has been employed to determine the growth inhibition percentage.

$$\% \text{ Inhibition} = \frac{100 (\text{Control} - \text{Treatment})}{\text{Control}}$$

3. Results

Silver, copper and silver-copper combined Nanoparticles synthesized from the *Acyranthus aspera* root, leaf and stem extracts and have been studied for their *in-vitro* hepatoprotective activity using the HepG₂ cells. HepG₂ cells treated with CCl₄ (40mM) alone and CCl₄ along with Root extract and Root silver, copper and Silver-Copper nanoparticles for the concentrations of 10-150 µg/ml. CCl₄ alone showed the inhibition of 52.55±0.526 at the concentration of 40 mM CCl₄. A combination of CCl₄ (40mM) and Root extract treated with the 150 µg/ml showed an inhibition of 33.1±0.256 whereas the Root Silver-Copper nanoparticles showed an inhibition with 24.57±0.327 followed by Root Copper nanoparticles with the inhibition 25.25±0.268 and Root Silver nanoparticles with 27.3±0.325 of inhibition, (Table 1 & Figure 1).

Table 1: In-vitro hepatoprotective activity Root extract and synthesized Nanoparticle

Concentration (µg)	Root Extract	Root Silver NPs	Root Copper NPs	Root Silver-Copper NPs
CCl ₄ (40mM)	52.55±0.526	52.55±0.526	52.55±0.526	52.55±0.526
CCl ₄ (40mM)+10	50.68±0.471	50.17±0.507	49.48±0.468	47.61±0.483
CCl ₄ (40mM)+25	46.75±0.419	45.22±0.466	44.02±0.423	43.85±0.445
CCl ₄ (40mM)+50	43±0.384	40.44±0.423	37.88±0.387	36.68±0.416
CCl ₄ (40mM)+100	38.39±0.349	35.49±0.416	33.1±0.354	29.86±0.348
CCl ₄ (40mM)+150	33.1±0.256	27.3±0.325	25.25±0.268	24.57±0.327

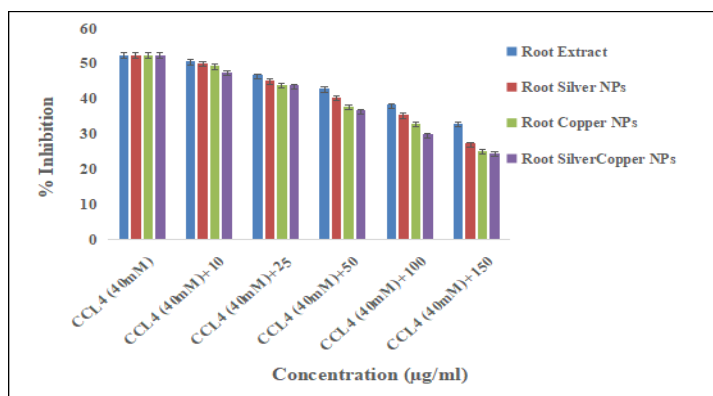


Figure 1: In-vitro hepatoprotective activity Root extract and synthesized Nanoparticles.

Combination of CCl₄ (40mM) and Leaf extract treated with the 150 µg/ml showed the inhibition of 38.73±0.433 where as the Leaf Silver-Copper nanoparticles showed the inhibition with 34.3±0.216 followed by Leaf Copper nanoparticles with the inhibition 35.32±0.382 and Leaf Silver nanoparticles with 37.37±0.294 of inhibition (Table 2 and Figure 2).

Table 2: In-vitro hepatoprotective activity Leaf extract and synthesized Nanoparticles

Concentration (µg)	Leaf Extract	Leaf Silver NPs	Leaf Copper NPs	Leaf Silver-Copper NPs
CCl ₄ (40mM)	52.55±0.526	52.55±0.526	52.55±0.526	52.55±0.526
CCl ₄ (40mM)+10	51.87±0.583	50.68±0.472	49.82±0.653	48.8±0.753
CCl ₄ (40mM)+25	49.14±0.562	48.29±0.434	47.09±0.641	44.88±0.724
CCl ₄ (40mM)+50	46.07±0.513	45.22±0.407	43±0.629	41.29±0.328
CCl ₄ (40mM)+100	42.49±0.471	40.95±0.368	38.39±0.437	37.03±0.238
CCl ₄ (40mM)+150	38.73±0.433	37.37±0.294	35.32±0.382	34.3±0.216

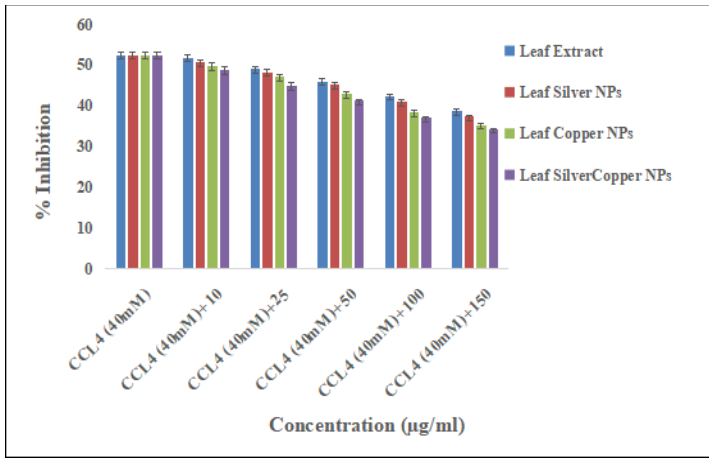


Figure 2: In-vitro hepatoprotective activity Leaf extract and synthesized Nanoparticles

A combination of CCl₄ (40mM) and Stem extract treated with 150 µg/ml showed the inhibition of 31.22±0.411 where as the Stem Silver-Copper nanoparticles showed the inhibition of 22.01±0.145 followed by Stem Copper nanoparticles with the inhibition 25.42±0.166 and Stem Silver nanoparticles with 29.01±0.207 of inhibition (Table 3 and Figure 3).

Table 3: In-vitro hepatoprotective activity Stem extract and synthesized Nanoparticles

Concentration (µg)	Stem Extract	Stem Silver NPs	Stem Copper NPs	Stem SilverCopper NPs
CCl ₄ (40mM)	52.55±0.526	52.55±0.526	52.55±0.526	52.55±0.526
CCl ₄ (40mM)+10	44.53±0.467	43.51±0.362	41.46±0.591	39.07±0.293
CCl ₄ (40mM)+25	42.15±0.415	40.1±0.328	38.73±0.365	37.03±0.223
CCl ₄ (40mM)+50	38.22±0.724	36.68±0.295	33.78±0.472	32.59±0.255
CCl ₄ (40mM)+100	35.15±0.617	32.08±0.262	28.83±0.243	26.45±0.177
CCl ₄ (40mM)+150	31.22±0.411	29.01±0.207	25.42±0.166	22.01±0.145

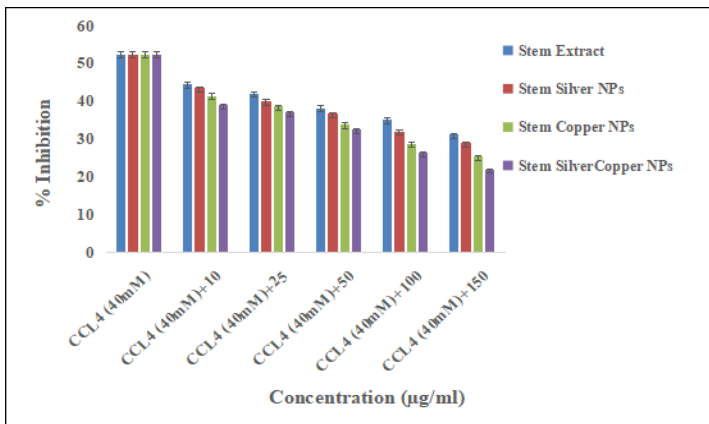


Figure 3: In-vitro hepatoprotective activity Stem extract and synthesized Nanoparticles

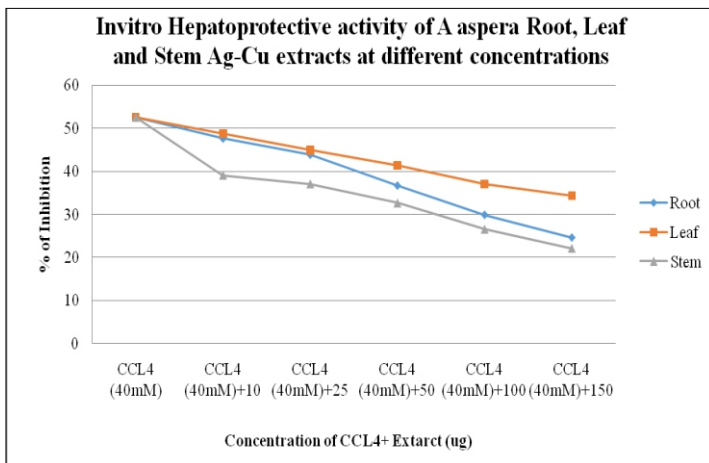


Figure 4: Comparative Analysis of Hepatoprotective Activity Root, Leaf and Stem Ag-Cu A.aspera Extracts

A comparison of the Hepatoprotective activity of Root, Leaf, and Stem Ag-Cu A.aspera Extracts has shown that the highest concentration of Stem Ag-Cu extract has shown a significant increase in the percentage of Inhibition as compared to Root and Leaf.

4. Discussion

Hepatotoxic chemicals impact liver cells by inducing oxidative damage, or the liver is crucial for the metabolism along with detoxification of substances that into the body which have the potential to induce hepatic injury. Both synthetic as well as natural medications are available to treat liver disease. Liver disorders have traditionally been treated with natural therapies, and in recent years, plant-based herbal medications have become increasingly important in the fight against drug-induced toxicity [18].

The *in-vitro* hepatoprotective efficacy of gold nanoparticles made from *Moringa oleifera* pods was investigated; Likewise, ethanolic, chloroform, and aqueous extracts revealed that *M. oleifera* might have hepatoprotective properties [19]. Aqueous leaf extract from *Azima tetraacantha* has been employed to generate silver nanoparticles, which demonstrated potent hepatoprotective efficacy against CCl₄-induced hepatocyte damage [20]. In our present study, a combination of Silver-Copper nanoparticles synthesized from the *Acyranthus aspera* leaf extract showed highest hepato-protective activity compared to the silver. A study on the hepatoprotective and antioxidant effects of cortex dictamni aqueous extract in rat liver damage caused by carbon tetrachloride (CCl₄) revealed that rats of both sexes with CCl₄-induced liver damage had noticeably low levels of aspartate aminotransferase, alkaline phosphatase, glutamate pyruvate transaminase, and total bilirubin [21]. Clerodendrum paniculatum Flower Extract showed better hepatoprotective activity in CCl₄-induced hepatotoxicity due to the isolated fraction containing the rich flavanoids in female Wistar Albino rats [21].

Green silver nanoparticles from *Herpetospermum darjeelingense* were produced, and it was found that these particles improved hepatoprotective function in addition to antimicrobial and antioxidant activity [23]. Nanoparticle (AgNPs) synthesized from the ethanol extract showed *in vitro* hepatoprotective efficacy along with resistance to paracetamol-induced hepatotoxicity in rats evaluated for *Broussonetia papyrifera* L., *Alangium salvifolium*, while *Abutilon indicum* L. AgNPs produced by *Broussonetia papyrifera* L., *Alangium salvifolium*, along with *Abutilon indicum* L.

shown notable hepatoprotective effect by lowering the metabolic parameters altered by CCL₄ along with paracetamol [24].

Methanolic leaf extract of *Acalypha indica*, silymarin and quercetin reduced the toxicity CCL₄ induction by the antioxidant and anti-inflammatory properties since the extract contains flavonoids [25]. In our present study, we have synthesized the silver, copper and combination of silver-copper NPs from the *Acyranthus asper* leaf, root and stem extracts and evaluated the *in vitro* hepatoprotective activity against the CCL₄ induced toxicity in HepG2 cells. The combination of silver-copper nanoparticles of stem showed the better hepatoprotective activity as compared to silver-copper nanoparticles from leaf and root.

5. Conclusion

The combination of silver-copper nanoparticles from leaf, root and stem showed the hepatoprotective activity compared to the individual nanoparticles of silver and copper nanoparticles from *Acyranthus aspera* leaf, root and stem extracted nanoparticles. Combination of silver-copper nanoparticles from the stem showed a prominent hepatoprotective activity than the combination of leaf and root-derived silver-copper nanoparticles. According to our research, silver-copper nanoparticles generated from *A. aspera* stem have the capability to serve as protective agents of liver against damage caused by carbon tetrachloride. Further investigations are warranted to confirm our studies.

Credit authorship contribution statement

B Sirisha Writing – original draft, Validation, Methodology, Investigation, Conceptualization. Ch Venkataramana Devi: Supervising – original draft, Project administration, Conceptualization.

Declaration of competing interest

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

Acknowledgements

The authors are thankful to the Department of Biochemistry and Botany, University College of Science, Osmania University, Hyderabad for providing infrastructure and necessary support.

6. References

- Seth SD, Sharma B. Medicinal plants in India. *Indian J Med Res.* 2004 Jul;120(1):9-11. PMID: 15299226.
- Bodeker, G., K.K.S. Bhat, J. Burley & P. Vantomme, Eds. 1997. *Medicinal plants for forest conservation and health care.* - Rome, FAO (Non-wood Forest Products 11).
- Sule Wf, Okonko Io, Joseph Ta, Ojezele Mo, Nwanze Jc, Et Al. In-Vitro Antifungal Activity Of *Senna Alata L.* Crude Leaf Extract. *Res J Biol Sci.* 2010, 5(3): 275-284. DOI: [10.3923/rjbsci.2010.275.284](https://doi.org/10.3923/rjbsci.2010.275.284)
- Timothy Sy, Lamu Fw, Rhoda As. Acute Toxicity, Phytochemistry And Antibacterial Activity Of Aqueous And Ethanolic Leaf Extracts Of *Cassia Alata L.* *Int Res J Pharma.* 2012, 3(6): 73-76.
- Oladeji So. Thin-Layer Chromatographic Analysis Of Flavonoids And Total Phenolics In Methanolic And Ethanolic Extracts Of *Senna Alata* (L.) Roxb. (Fabales: Fabaceae). *Brazil J Biol Sci.* 2016, 3: 221-225. ISSN 2358-2731 <http://dx.doi.org/10.21472/bjbs.030520>
- Adelowo Fe, Oladeji So. Spectrophotometric Analysis Of Phenolic Compounds In *Senna Alata*. *Am J Adv Sci Res.* 2016, 3(2): 246-253.
- Midawa Sm. Cutaneous Wound Healing Activity Of The Ethanolic Extracts Of The Leaf Of *Senna Alata L.* (Fabaceae). Volume 2, December 2010, *J Biol Sci.* 2010, 2: 63-68.
- Kumar, B., Misra, A., Singh, S. P., Dhar, Y. V., Rawat, P., Chattopadhyay, D., & Srivastava, S. (2021). In-silico efficacy of potential phytochemicals from Ayurvedic herbs as an adjuvant therapy in management of COVID-19. *Journal of Food and Drug Analysis*, 29(4), 559.
- Gangola S, Khati P, Bhatt P, Parul , Anita Sharma. India As The Heritage Of Medicinal Plant And Their Use. *Curr Trends Biomedical Eng & Biosci* 4(4): Ctbeb.Ms.Id.5555641 (2017). DOI: 10.19080/CTBEB.2017.04.555641
- Eman H. Ismail, Aliyah M. A. Saqer, Eman Assirey, Arshi Naqvi And Rawda M. Okasha (2018). Successful Green Synthesis Of Gold Nanoparticles Using A *Corchorus Olitorius* Extract And Their Antiproliferative Effect In Cancer Cells. *Int. J. Mol. Sci.*, 19, 2612 DOI: [10.3390/ijms19092612](https://doi.org/10.3390/ijms19092612)
- K. B. Narayanan And N. Sakthivel, (2011): Facile Green Synthesis Of Gold Nanostructures By NADPH-Dependent Enzyme From The Extract Of *Sclerotium Rolfsii*, *Colloids And Surfaces A: Physicochemical And Engineering Aspects*, Vol. 380, No. 1-3, Pp. 156-161, DOI: [10.1016/j.colsurfa.2011.02.042](https://doi.org/10.1016/j.colsurfa.2011.02.042)
- N. N. Dhanasekar, G. R. Rahul, K. B. Narayanan, G. Raman, And N. Sakthivel (2015): Green Chemistry Approach For The Synthesis Of Gold Nanoparticles Using The Fungus *Alternaria Sp*, *Journal Of Microbiology And Biotechnology*, Vol. 25(7), Pp. 1129-1135 DOI: [10.4014/jmb.1410.10036](https://doi.org/10.4014/jmb.1410.10036)
- Rimsha Chaudhary , Khadija Nawaz , Amna Komal Khan , Christophe Hano , Bilal Haider Abbasi, Sumaira Anjum. An Overview of the Algae-Mediated Biosynthesis of Nanoparticles and Their Biomedical Applications PMID: 33143289 DOI: 10.3390/biom10111498
- H. Khanehzaei, M. B. Ahmad, K. Shameli, Z. Ajdari, M. A. Ghani, And K. Kalantari (2015): Effect Of Seaweed *Kappaphycus Alvarezii* In The Synthesis Of Cu@Cu₂O Core-Shell Nanoparticles Prepared By Chemical Reduction Method, *Research On Chemical Intermediates*, Vol. 41, No. 10, Pp. 7363-7376. DOI: [10.1007/s11164-014-1817-0](https://doi.org/10.1007/s11164-014-1817-0)

15. Kar Xin Lee, Kamyar Shameli, Mikio Miyake, Noriyuki Kuwano, Nurul Bahiyah Bt Ahmad Khairudin, Shaza Eva Bt Mohamad, And Yen Pin Yew (2016). Green Synthesis Of Gold Nanoparticles Using Aqueous Extract Of Garcinia Mangostana Fruit Peels. *Journal Of Nanomaterials* Volume.2016: 7 Pages <https://doi.org/10.1155/2016/8489094>
16. Yadav R, Rai R, Yadav A, Pahuja M, Solanki S, Yadav H. Evaluation Of antibacterial Activity Of *Achyranthes Aspera* Extract Against *Streptococcus Mutans*: An *In Vitro* study. *J Adv Pharm Technol Res* 2016;7:149-52. DOI: [10.4103/2231-4040.191426](https://doi.org/10.4103/2231-4040.191426)
17. Kouadio Ibrahime Sinan, Gokhan Zengin, Dimitrina Zheleva-Dimitrova Ouattara Katinan Etienne, Mohamad Fawzi Mahomoodally, Abdelhakim Bouyahya , Devina Lobine, Annalisa Chiavaroli, Claudio Ferrante , Luigi Menghini, Lucia Recinella, Luigi Brunetti, Sheila Leone And Giustino Orlando. Qualitative Phytochemical Fingerprint and Network Pharmacology Investigation of *Achyranthes aspera* Linn. Extracts. *Molecules* 2020, 25, 1973. doi: 10.3390/molecules25081973.
18. Hongru Zhang , Joe Antony Jacob , Ziyu Jiang , Senlei Xu , Ke Sun , Zehao Zhong , Nithya Varadharaju , Achiraman Shanmugam .Hepatoprotective Effect Of Silver Nanoparticles Synthesized Using Aqueous Leaf Extract Of *Rhizophora Apiculata*. *Int J Nanomedicine*. 2019 May 20;14:3517-3524 DOI: 10.2147/IJN.S198895
19. T. S. Belliraj, Anima Nanda@ And R. Ragonathan, *In-Vitro* Hepatoprotective Activity Of *Moringa Oleifera* Mediated Synthesis Of Gold Nanoparticles , *Journal Of Chemical And Pharmaceutical Research*, 2015, 7(2):781-788
20. E. Prakash, T. Jeyadoss, S. Velavan, *In Vitro* Hepatoprotective Activity Of *Azima Tetracantha* Leaf Extract And Silver Nanoparticle In Hepatocytes, *Der Pharma Chemica*, 2015, 7(10):381-390.
21. Lin Li, Yun-Feng Zhou, Yan-Lin Li, Li-Li Wang, Hiderori Arai , Yang Xu, *In Vitro* And *In Vivo* Antioxidative And Hepatoprotective Activity Of Aqueous Extract Of *Cortex Dictamn*, *World J Gastroenterol*. 2017 Apr 28;23(16):2912-2927. DOI: 10.3748/wjg.v23.i16.2912
22. Remya Kopilakkal, Kaushik Chanda, Musuvathi Motilal Balamurali, Hepatoprotective And Antioxidant Capacity Of *Clerodendrum Paniculatum* Flower Extracts Against Carbon Tetrachloride-Induced Hepatotoxicity In Rats, *ACS Omega* 2021, 6, 26489-26498. DOI: [10.1021/acsomega.1c03722](https://doi.org/10.1021/acsomega.1c03722)
23. Pal, R., Mukherjee, S., Khan, A., Nathani, M., Maji, S., Tandey, R., ... & Mandal, V. (2024). A critical appraisal on the involvement of plant-based extracts as neuroprotective agents (2012-2022): an effort to ease out decision-making process for researchers. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 397(12), 9367-9415.
24. Elegbeleye, J. A., Krishnamoorthy, S., Bamidele, O. P., Adeyanju, A. A., Adebawale, O. J., & Agbemavor, W. S. K. (2022). Health-promoting foods and food crops of West-Africa origin: The bioactive compounds and immunomodulating potential. *Journal of Food Biochemistry*, 46(11), e14331.
25. Mandeth Kodyil Geetha Nambiar, *In Vitro* Hepatoprotective Activity Of Methanolic Leaf Extract Of *Acalypha Indica* Against Ccl4 Induced Hepatotoxicity In Goat Liver Slice Culture, *Trends in Sciences* 2023;20 (4):4562. DOI: [10.48048/tis.2023.4562](https://doi.org/10.48048/tis.2023.4562)
26. Basist, P., Parveen, B., Zahiruddin, S., Gautam, G., Parveen, R., Khan, M. A., & Ahmad, S. (2022). Potential nephroprotective phytochemicals: Mechanism and future prospects. *Journal of ethnopharmacology*, 283, 114743.
27. Tong, Z. W., Gul, H., Awais, M., Saddick, S., Khan, F. S., Gulfranz, M., & Khan, M. I. (2021). Determination of *in vivo* biological activities of *Dodonaea viscosa* flowers against CCL4 toxicity in albino mice with bioactive compound detection. *Scientific Reports*, 11(1), 13336.
28. Eswaran, A., Muthukrishnan, S., Mathaiyan, M., Pradeepkumar, S., Mari, K. R., & Manogaran, P. (2023). Green synthesis, characterization and hepatoprotective activity of silver nanoparticles synthesized from pre-formulated Liv-Pro-08 poly-herbal formulation. *Applied Nanoscience*, 13(3), 2315-2327.