

CYTOTOXIC EFFECT OF ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF CASSIA AURICULATA

Running title: cassia Auriculata ,Antibacterial Activity,

Abstract

Introduction

Cassia auriculata, also known as tanner's cassia, belongs to the family Caesalpiniaceae is a drug used by Ayurvedic practitioners. The root, bark and leaves of this plant is traditionally used for the treatment of diarrhoea, leprosy, uterine and liver disorders. This study aimed to find the Antibacterial activity of Cassia auriculata ethanolic extract preparation.

Materials and Methods

Cassia auriculata in 5g of 50ml of ethanol then it kept in the orbital shaker for 72 hours. After 72 hrs it was boiled using heating Mantle at 60-70 °C for 10 mins. Then it was filtered using Whatman - No. / filter paper. The filter extract was again concentrated using heating Mantle.

Results :

The extract shows very good Antibacterial activity of Cassia auriculata ethanolic extract preparation was effective.

Conclusion

The Antibacterial activity of *Cassia auriculata* ethanolic extract preparation was effective.

Keywords

Cassia auriculata, ethanolic extract, pharmaceutical preparations, traditional medicine.

Introduction

Herbal medications have been used for centuries because of their safety, efficacy, cultural acceptability, and lack of negative effects. Plants and plant products have been used to cure and prevent diseases with varied degrees of effectiveness throughout history. Plant sources that are employed in traditional medical systems can be used to produce therapeutically intriguing and important medications. More than 5000 plants are used by different ethnic groupings in India, and the Indian traditional system of medicine is built on empirical knowledge of observations and experience spanning millennia. Cassia is a medicinal plant genus native to India, with Cassia auriculata having a great biodiversity in south India and

having a large biodiversity in north India. The current communication is a review of the medicinal qualities and pharmacological effects of the Indian traditional medicines *Cassia auriculata*. Diabetes mellitus is a serious metabolic illness that affects people all over the world. Several plants are being studied for diabetic control.

Cassia auriculata is a herb that is well-known for its nutritional content as well as its health advantages. However, there is just minimal scientific information on the explanation of its root bioactives and biological activity. Plants have long been utilised to cure a variety of health issues, not only as a dietary addition to live organisms. Many plants' medicinal properties are yet unknown due to the numerous activities of chemicals responsible for later. There is a pressing need to discover novel antimicrobial chemicals, given the increased prevalence of bacteria that are resistant to antibiotics. Plants have been utilised to treat infectious ailments from the beginning of time. Indian traditional medicines such as Siddha, Ayurveda, and Unani have used the therapeutic potential of various plants. Because medicinal herbs are nontoxic and inexpensive, there has been an increase in their use and demand. *Cassia auriculata* L., also known as tanners cassia and “avaram” in Tamil, is a shrub belonging to the Caesalpiniaceae family. The shrub is particularly well-known for its lovely yellow blossoms, which are used to heal skin conditions and body odour. Rheumatism, conjunctivitis, and diabetes are all treated with it in traditional medicine. It offers a wide range of therapeutic qualities. Its bark is astringent, the leaves and fruits are anthelmintic, the seeds are used to treat eye problems, and the root is used to treat skin ailments.

Plant materials, on the other hand, remain valuable resources in the fight against global diseases. Our team has extensive knowledge and research experience that has translated into high quality publications (Rajeshkumar *et al.*, 2018; Nandhini, Rajeshkumar and Mythili, 2019; M. Gomathi *et al.*, 2020; Rajasekaran *et al.*, 2020; Vairavel, Devaraj and Shanmugam, 2020), (Santhosh Kumar *et al.*, 2019), (Raj R, D and S, 2020), (Saravanan *et al.*, 2018), (Gheena and Ezhilarasan, 2019), (Ezhilarasan, Sokal and Najimi, 2018), (Ezhilarasan, 2018), ((Dua *et al.*, 2019; A. C. Gomathi *et al.*, 2020; Vairavel, Devaraj and Shanmugam, 2020), (Ramesh *et al.*, 2018; Duraisamy *et al.*, 2019; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Arumugam, George and Jayaseelan, 2021; Joseph and Prasanth, 2021) ., (Gnanavel, Roopan and Rajeshkumar, 2019), (Markov *et al.*, 2021)

MATERIALS AND METHODS:

Chemicals and Reagents:

The chemicals DPPH(1,1-diphenyl-2-picrylhydrazyl), and butylated hydroxyanisole (BHA) were obtained. *Cassia auriculata* in 5g of 50ml of ethanol then it kept in the orbital shaker for 72 hours After 72 hrs it was bold using heating Mantle at 60-70 C for 10mins .Then it was filtered using whatman filter paper,the filter extract was again concentrated using heating mantle.

Plant collection and Extraction:

Leaves of *Cassia auriculata* were collected from Saveetha dental college during July-september 2021. The species was identified and authenticated by a Taxonomist and voucher specimens were deposited. Shade dried and coarsely powdered leaves of *Cassia auriculata* (1g) were sequentially extracted with ethanol at room temperature for 48 hrs. The extracts were filtered and concentrated under reduced pressure using a rotary evaporator to get completely dried extracts (PSE Ext). The yield of the leaf crude extract was about 80 g.

Cytotoxic activity – BSLA

BRINE SHRIMP LETHALITY ASSAY:

Salt water preparation :

2g of iodine free salt was weighed and dissolved in 200ml of distilled water. 6 well ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (20µL, 40 µL, 60 µL, 80µL, 100 µL). Then the nanoparticles were added according to the concentration level. The plates were incubated for 24 hours. After 24 hours, the ELISA plates were observed and noted for number of live nauplii present and calculated by using following formula, $\text{number of dead nauplii} / (\text{number of dead nauplii} + \text{number of live nauplii}) \times 100$

ALBUMIN DENATURATION ASSAY:

The anti-inflammatory activity for *Solanum tarvum* gel was tested by the following convention proposed by Muzushima and Kabayashi with specific alterations (Pratik Das et al., 2019). 0.05 mL of *Solanum tarvum* gel of various fixation (10µL, 20µL, 30µL, 40µL, 50µL) was added to 0.45 mL bovine serum albumin (1% aqueous solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid. These samples were incubated at room

RESULT



Figure 1: *Cassia auriculata* ethanolic extract

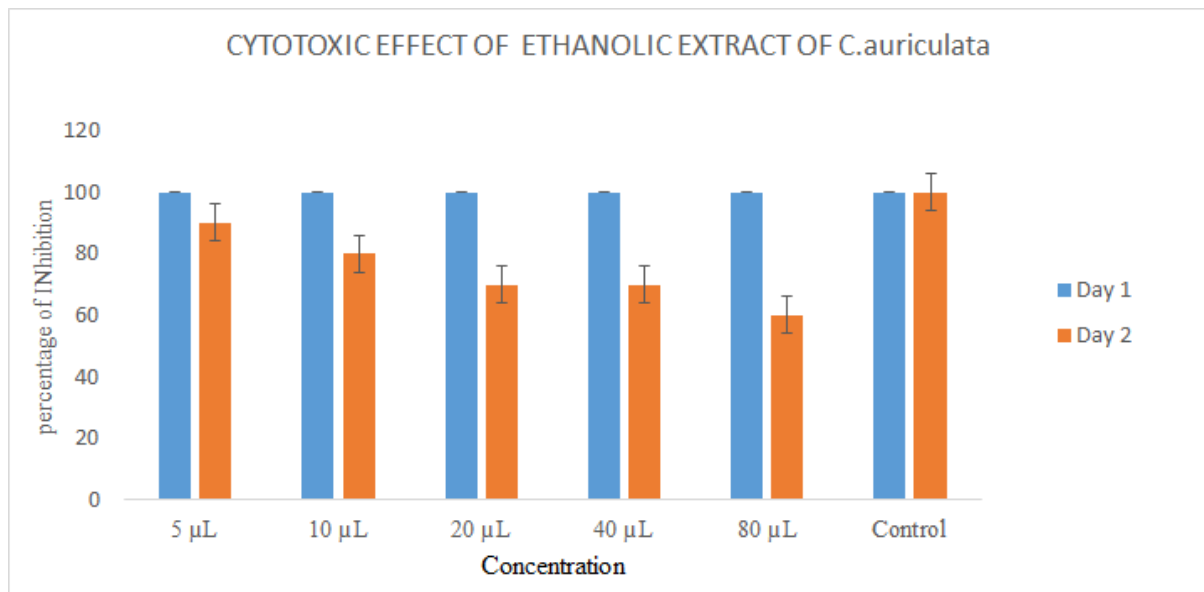


Figure 2 : The above graph depicts the antioxidant activity with an increased percentage of inhibition with a concentration in microlitres. X axis denotes concentration and Y axis denotes the percentage of inhibition of *Cassia auriculata*.

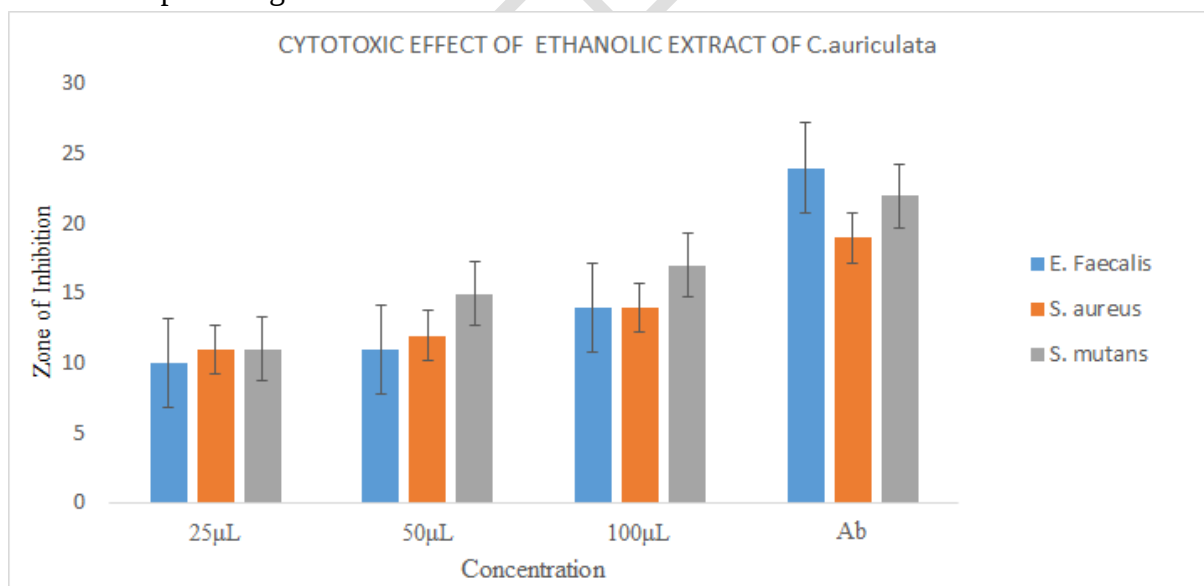


Figure 3 : The above graph depicts the Antibacterial activity of *Cassia auriculata* ethanolic extract on Hydrogen peroxide increased percentage of inhibition with a concentration in microlitres. X axis denotes concentration and the Y axis denotes the percentage of inhibition of *Cassia auriculata*.

Discussion

As we compare with the previous study Silver NPs are derived biogenically during a controlled environment of *C. fistula* and Ag⁺ rich media. The evaluation of toxic properties of synthesis AgNPs, using *C. fistula* is crucial when considering public health protection because exposure to Ag may result in undesirable effects on consumers. Few reports suggest that oral administration of *C. fistula* extract didn't produce any significant toxicity concerning several biochemical analysis and histopathological examination in mice model. Within the present paper, AgNPs were characterised using physical techniques: namely, UV-vis spectroscopy, DLS, FTIR, FE-SEM and therefore the potential antioxidant, antimicrobial and cytotoxic activities proposed to use for pharmaceutical formulations. The DLS data revealed that synthesis AgNPs also can be utilized in conjunction with the quality drugs to reinforce their efficacy. Herbal medicine is gaining growing interest due to its cost effective and eco friendliness. There is a reduction of the metal ions through plant extracts resulting in the formation of silver nanoparticles has been explained before but the potential of parts of the plant like fruits as a capping and reducer has not been explained in previous studies. Within the present study, we found that fruits were an honest source for the synthesis of silver nanoparticles. it's many advantages like , ease with which the methods are often scaled up, economic viability and to get smaller particle size. This study demonstrated the antimicrobial activity of using biologically synthesized silver nanoparticles against various oral pathogens. The preparation of silver nanoparticle by using *Solanum xanthocarpum* extracts has excellent antimicrobial activity against *Enterococcus faecalis*. Hence this technology are often applied in therapeutics

As we compare with the previous study H. Padalia et al. Green synthesis of ZnO NPs using avaram leaf extract is low cost, eco friendly, and straightforward . The reduction of zinc ions by avaram leaf extract resulted within the formation of stable nanoparticles. The optical absorbance peak was recorded at 370 nm confirms the formation of ZnO NPs. SEM analysis demonstrated that ZnO NPs are spherical and irregular in shape and average particle size is 68.64 nm. ZnO NPs showed a broad spectrum of antimicrobial activity and also exhibited better synergistic antimicrobial activity as compared to plain antibiotics. Synthesized ZnO NPs showed potent cytotoxic effects against HeLa neoplastic cell lines. Hence, this green-synthesized ZnO NPs are often effectively used as potential antimicrobial agents to inhibit various micro- bial pathogens and features a great potential within the preparation of medicine used as a completely unique medicine in therapeutic application in disease like cancer.

In the present study, green synthesis of gold nanoparticles from aqueous extract of avaram was administered . The outstanding prerequisite for applying gold nanoparticles in cancer therapy is that the nanoparticles should be produced in biologically providential media. Usually, the plant leaves are an important source for antioxidant molecules like polyphenols and flavonoids which have the ability to scale back metal ions. These antioxidant molecules produce gold nanoparticles by reduction of gold ions (Au³⁺) (Roy et al., 2011).

Conclusion

Based on our observations, it was confirmed that *Cassia Auriculata* (Leaf) showed strong in vitro Antibacterial effect in the cell free system. Phytochemical research is needed to identify

the active principles responsible for this biological activity of this medicinal plant. Further studies are aimed at the isolation and identification of bio-active molecules from the ethanolic extract of *Cassia auriculata*.

The increased demand has placed a great strain on the natural populations of *Cassia auriculata*. Collectors of medicinal plants are resorting to unsustainable exploitation causing serious threat to the survival of the species. Thus, the species is reported to show a poor regeneration capacity. Cultivation on a substantially high scale is yet to be started. Therefore, there is a need to conserve the species for the benefit of mankind. More importantly, critical elements of effective conservation strategies need to be discussed.

NOTE:

The study highlights the efficacy of "Ayurved" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCE

Arumugam, P., George, R. and Jayaseelan, V. P. (2021) 'Aberrations of m6A regulators are associated with tumorigenesis and metastasis in head and neck squamous cell carcinoma', *Archives of oral biology*, 122, p. 105030.

Dua, K. *et al.* (2019) 'The potential of siRNA based drug delivery in respiratory disorders:

Recent advances and progress', *Drug development research*, 80(6), pp. 714–730.

Duraisamy, R. *et al.* (2019) 'Compatibility of Non Original Abutments With Implants: Evaluation of Microgap at the Implant-Abutment Interface, With Original and Unoriginal Abutments', *Implant dentistry*, 28(3), pp. 289–295.

Ezhilarasan, D. (2018) 'Oxidative stress is bane in chronic liver diseases: Clinical and experimental perspective', *Arab journal of gastroenterology: the official publication of the Pan-Arab Association of Gastroenterology*, 19(2), pp. 56–64.

Ezhilarasan, D., Apoorva, V. S. and Ashok Vardhan, N. (2019) 'Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells', *Journal of oral pathology & medicine: official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*, 48(2), pp. 115–121.

Ezhilarasan, D., Sokal, E. and Najimi, M. (2018) 'Hepatic fibrosis: It is time to go with hepatic stellate cell-specific therapeutic targets', *Hepatobiliary & pancreatic diseases international: HBPD INT*, 17(3), pp. 192–197.

Gheena, S. and Ezhilarasan, D. (2019) 'Syringic acid triggers reactive oxygen species-mediated cytotoxicity in HepG2 cells', *Human & experimental toxicology*, 38(6), pp. 694–702.

Gnanavel, V., Roopan, S. M. and Rajeshkumar, S. (2019) 'Aquaculture: An overview of chemical ecology of seaweeds (food species) in natural products', *Aquaculture*, 507, pp. 1–6.

Gomathi, A. C. *et al.* (2020) 'Anticancer activity of silver nanoparticles synthesized using aqueous fruit shell extract of Tamarindus indica on MCF-7 human breast cancer cell line', *Journal of drug delivery science and technology*, 55, p. 101376.

Gomathi, M. *et al.* (2020) 'Green synthesis of silver nanoparticles using Gymnema sylvestre leaf extract and evaluation of its antibacterial activity', *South African Journal of Chemical Engineering*, pp. 1–4. doi: 10.1016/j.sajce.2019.11.005.

Joseph, B. and Prasanth, C. S. (2021) 'Is photodynamic therapy a viable antiviral weapon against COVID-19 in dentistry?', *Oral surgery, oral medicine, oral pathology and oral radiology*, pp. 118–119.

Markov, A. *et al.* (2021) 'Mesenchymal stem/stromal cells as a valuable source for the

treatment of immune-mediated disorders', *Stem cell research & therapy*, 12(1), p. 192.

Nandhini, N. T., Rajeshkumar, S. and Mythili, S. (2019) 'The possible mechanism of eco-friendly synthesized nanoparticles on hazardous dyes degradation', *Biocatalysis and agricultural biotechnology*, 19, p. 101138.

Rajasekaran, S. *et al.* (2020) 'Collective influence of 1-decanol addition, injection pressure and EGR on diesel engine characteristics fueled with diesel/LDPE oil blends', *Fuel*, 277, p. 118166.

Rajeshkumar, S. *et al.* (2018) 'Biosynthesis of zinc oxide nanoparticles using *Mangifera indica* leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells', *Enzyme and microbial technology*, 117, pp. 91–95.

Raj R, K., D, E. and S, R. (2020) ' β -Sitosterol-assisted silver nanoparticles activates Nrf2 and triggers mitochondrial apoptosis via oxidative stress in human hepatocellular cancer cell line', *Journal of biomedical materials research. Part A*, 108(9), pp. 1899–1908.

Ramesh, A. *et al.* (2018) 'Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study', *Journal of periodontology*, 89(10), pp. 1241–1248.

Santhoshkumar, J. *et al.* (2019) 'Toxicology evaluation and antidermatophytic activity of silver nanoparticles synthesized using leaf extract of *Passiflora caerulea*', *South African Journal of Chemical Engineering*, 29, pp. 17–23.

Saravanan, M. *et al.* (2018) 'Synthesis of silver nanoparticles from *Phanerochaete chrysosporium* (MTCC-787) and their antibacterial activity against human pathogenic bacteria', *Microbial pathogenesis*, 117, pp. 68–72.

Vairavel, M., Devaraj, E. and Shanmugam, R. (2020) 'An eco-friendly synthesis of *Enterococcus* sp.-mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells', *Environmental Science and Pollution Research*, 27(8), pp. 8166–8175.