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Formulation and Characterization of Silver Nanoparticles Loaded with Aqueous Extract of *Lantana Camara* Linn Leaves

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ABSTRACT

In the present study, the *In vitro* antibacterial activity and *In vivo* anti-bacterial, anti-inflammatory, and wound healing activity of the leaf extract in solvents ethanol and aqueous extracts of the selected plant *Lantana camara*. The synthesis of silver nanoparticles using aqueous extract of fresh leaves of *Lantana camara* as bio reducing agents. This method allowed the synthesis of nanoparticles which was confirmed by UV-Visible spectroscopy, FTIR, particle size, and zeta potential. Anti-bacterial activity of ethanol and aqueous leaf extract of *Lantana camara* was separately tested for gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli*. Again anti-bacterial activity of *Lantana camara* mediated synthesis silver nanoparticles was tested by disc diffusion assay against standard organisms like *Escherichia coli* and *staphylococcus aureus* bacteria and *in-vivo* studies designed to evaluate the carrageenan-induced paw edema and Excision wound model activities of ethanol and aqueous extract of leaf of *Lantana camara* Linn. The ethanol and aqueous extracts of leaves give positive for all the phytochemical constituents etc. The particle size of silver particles prepared in all conditions was in the range of 2657.2nm. The Zeta potential of silver nanoparticles was found to be 0.3mv so it indicates the dispersion and stability.

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INTRODUCTION

Nanoparticles represent a particle with a nanometer size of 1–100 nm. The most widely studied nanoparticle materials are metal nanoparticles because they are easier to synthesize [1]. In the present work, *Lantana camara* (also known as red sage) leaf extract was used to synthesize silver nanoparticles for antimicrobial studies. Some of the most studied metallic nanoparticles include silver, gold, platinum, and palladium. Ag nanoparticle is an interesting metal to be studied, especially in the field of health and medicine. Silver is a strong antibacterial and also toxic to cells [2]. Silver has the ability to damage bacterial cell walls, inhibits bacterial cell growth, and disrupts cell metabolism because of the

interaction between Ag ions with macromolecules in cells, such as proteins and deoxyribonucleic acid. The Ag nanoparticles are chemically more reactive than silver in their bulk. The plant *Lantana camara* Linn. (Verbenaceae) is an ornamental herb. It is found in South India, America, and Africa, mostly native to subtropical and tropical countries. The number of species is available with genus *Lantana*. Some species of *Lantana* are *Lantana trifolia*, *Lantana salvifolia* Jacq. and *Lantana indica* Roxb. These leaves are rich in various chemical constituents such as triterpenes, glycosides, steroids, flavonoids, and essential oils [3]. The plant contains various medicinal properties and used in the treatment of skin diseases such as dermatitis, itching, scabies, leprosy, and chickenpox.

MATERIALS

Fresh plant of *Lantana camara* was collected from S.V. University, Thirupathi. Silver Nitrate purchased from Wise scientific, India. Gentamicin purchased from Aristo Pharmaceuticals, Mumbai, India. Cepftrione purchased from Ranbaxy Pharmaceuticals, Mohali, India. Diclofenac purchased from Cipla pharmaceuticals. India. All analytical grade and purchased from Hi Media (Mumbai).

Methods

Cold Extraction

Lantana Camara was separately shade dried, crushed to crude powder was macerated with ethanol and aqueous based on their polarity following cold extraction method, and filtrates were concentrated by rotary evaporator to yield was about 4% dense residues [4]. Based on the phytochemical analysis and 99% ethanol and aqueous extract were selected for further experiments. Ethanol and aqueous extract of *Lantana camara* were prepared their anti microbial activity was done using microbial maximum inhibitory concentration.

Phytochemical Screening

Test for flavonoids: Alkaline reagent´s, Shinoda test

Test for alkaloids: Dragendroffs test, Mayers test, Wagner's test.

Test for carbohydrates: Benedict's test, Molischs test, Fehling's test

Test for proteins: Biuret test, Xanthoproteic test, Millon's test

Test for glycosides: Liebermann's test, Salkowskis test, Killer Killian test

Test for phenols: Ferric chloride test, Test for tannins, Gelatin solution

Test for free amino acids: Ninhydrin test, Test for quinines, HCL test; Test for the steroids: Liebermann Burchard test; Test for saponins: Foam test, Froth test [5].

In-vitro antibacterial activity

Disc diffusion method

Prepare the inoculums

10^8 colonies forming unit per ml bacterial inoculums in a nutrient broth which is prepared by picking 3-5 isolated colonies from the plate [6]. The winning streak the swab on the surface going from nutrient agar go away for 5-10 minutes to dry the surface going from agar that permitting the bacteria to establish for themselves and on the media. Invert the plate and incubate them at 37^{o}C for 18-24 hours (Table 1).

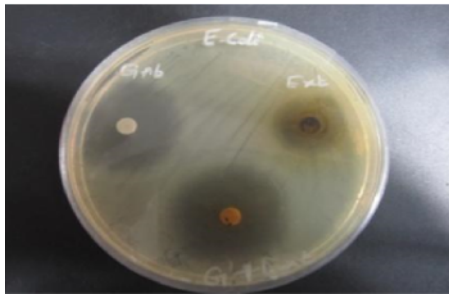

Figure 1: Zone of inhibition of gentamicin, ethanol extract of *Lantana camara*, gentamicin with LELC in *E.coli* culture plate

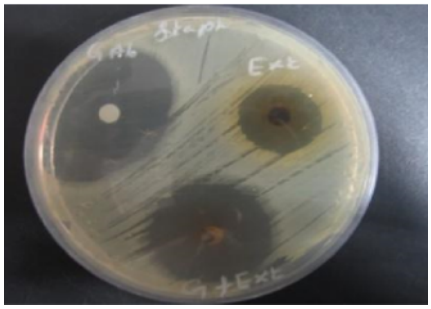

Figure 2: Zone of inhibition of gentamicin, ethanol extract of *Lantana camara*, gentamicin with ethanol extract of *Lantana camara* in *staphylococcus aureus* culture plate

In-vivo Anti bacterial activity

The mice were randomly divided into five groups (n=2), for the preparation of inoculating the bacteria on to nutrient agar and incubated at 37^{o}C overnight. Before inoculation, the mouse models of bacterial skin infection were sedated with either [7]. The flanks of the sedated mice were shaved with clippers when necessary and cleansed with an ethanol solution and then apply the bacterial solution on the skin. The treatment with antimicrobial used in this

Table 1: Treatment schedule for *In-vitro* antibacterial activity

Groups	Treatment				
	Media	Inoculums	Antibiotic	Standard Dose	Aqueous Extract of *Lantana camara* Leaves AgNPs
Normal	Nutrient agar media	*Staphylococcus aureus*, *Escherichia coli.*	-	-	-
Standard	Nutrient agar media	*Staphylococcus aureus, Escherichia coli.*	Gentamicin	10mg/kg	-
Control	Nutrient agar media	*Staphylococcus aureus, Escherichia coli*	-	-	-
Extract	Nutrient agar media	*Staphylococcus aureus, Escherichia coli*	-	-	10 mg/kg

Table 2: Treatment schedule for *In-vivo* studies of anti-bacterial activity

Groups	Treatment
Group 1	Animal served as the positive control (normal animals) were administered 0.9% normal saline solution
Group 2	Animal served as the negative control (disease animal) were administered 0.9% normal saline solution
Group 3	Animal treated with standard drug Gentamicin
Group 4	Animals were administered graded doses of 200mg/kg bodyweight of the ethanol extract of *Lantana camara* leaves respectively twice daily.
Group 5	Animals were administered graded doses of 200mg/kg bodyweight of the Aqueous Extract of *Lantana camara* leaves respectively twice daily.

Table 3: Treatment schedule for *In-vivo* studies of anti-inflammatory activity

Groups	Treatment
Group 1	Animal served as the positive control (normal animals) were administered 0.9% normal saline solution
Group 2	Animal served as the negative control (disease animal) were administered 0.9% normal saline solution
Group 3	Animal treated with standard drugs Diclofenac.
Group 4	Animals were administered graded doses of 200mg/kg bodyweight of the ethanol extract of *Lantana camara* leaves respectively twice daily.
Group 5	Animals were administered graded doses of 200mg/kg bodyweight of the Aqueous Extract of *Lantana camara* leaves respectively twice daily.

Table 4: Treatment schedule for *In-vivo* studies of Wound-healing activity

Groups	Treatment
Group 1	Animal served as the positive control (normal animals) were administered 0.9% normal saline solution
Group 2	Animal served as the negative control (disease animal) were administered 0.9% normal saline solution
Group 3	Animal treated with standard drugs Neosporin.
Group 4	Animals were administered graded doses of 200mg/kg body weight of the ethanol extract of *Lantana camara* leaves respectively twice daily.
Group 5	Animals were administered graded doses of 200mg/kg body weight of the Aqueous extract of *Lantana camara* leaves respectively twice daily.

Table 5: Phytochemical test of *Lantana camara* Leaves

Phytochemical Constituents	Ethanol	Aqueous
Flavonoids		
Alkaline reagent	+	+
Shinoda test	+	+
Alkaloids		
Wagner's test	+	-
Mayers test	+	-
Carbohydrates		
Benedicts test	+	+
Molischs test	+	+
Fehling's test	+	+
Proteins		
Biuret test	+	+
Xanthoproteic test	+	+
Millon's test	+	+
Glycosides		
Liebermann's test	+	-
Salkowskis test	-	+
Keller Killian's test	+	-
Phenols		
Ferric chloride test	+	+
Tannins		
Gelatin test	-	+
Braymer's test	-	+
Amino acids		
Ninhydrin test	+	+
Steroids		
Liebermann Burchard test	+	-
Quinines		
HCL test	-	-
Saponins		
Foam test	-	+
Froth test	-	+

Note: (+) Present, (-) Absent

Table 6: Zone of inhibition of Ethanol Squeeze of *Lantana camara* leaf

Organisms	Gentamicin (2mg)	Ethanol Squeeze of leaf (2mg)
Escherichia coli	5.5	3.5
Staphylococcus aureus	6	4.5

Table 7: FTIR on *Lantana camara* leaf extract of silver nanoparticles

S.No	Type of Vibration	Wave Number		Possible Serious For Shift Alteration
		Plant Extract	Nano Silver	
1	N-H stretching vibration of secondary amines	3334	3329	Chelation of N-H groups with silver
2	O-H stretching vibration of carboxylic acids	2117	2131	Interaction of silver with OH group of flavonoids
3	Stracting vibration of C=O aldehydes	1637	1637	Chelation of C=O groups with silver
4	C-H grouping of phenyl ring substitution	621	581	Interaction with phenyl ring or alkynes with silver
5	C-H group of phenyl ring substitution or alkynes	524	525	C-H group of phenyl ring

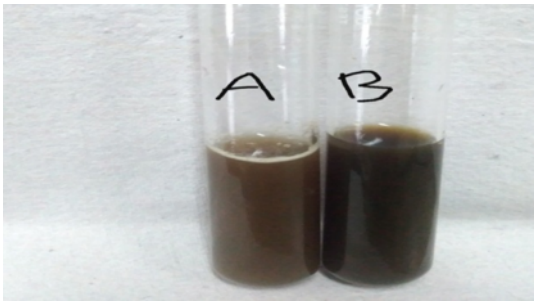

Figure 3: Synthesized AgNPs solution several response time (A) 20 min (B) 70 min

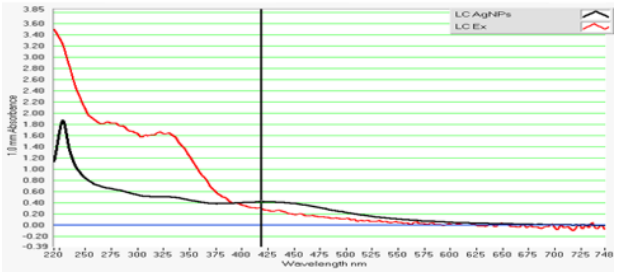

Figure 4: UV-Visible spectroscopy of *Lantana camara* leaf extract of silver

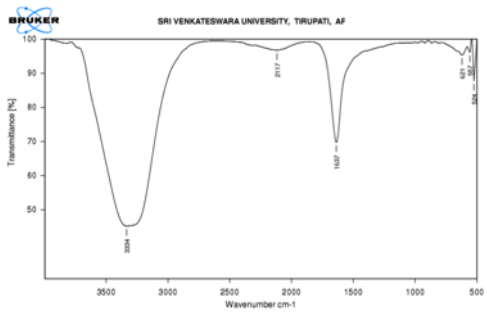

Figure 5: FTIR of *Lantana camara* leaf extract

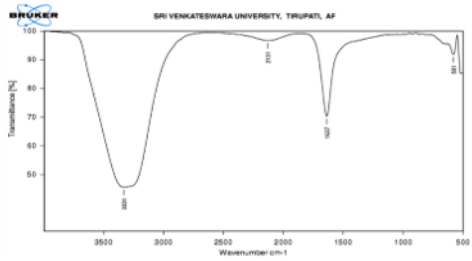

Figure 6: FTIR of *Lantana camara* Silver Nanoparticles

study begins after 4 hours of bacterial inoculation and continued at the regimens of 7 days (Table 2).

***In vivo* Method – Anti inflammatory activity**

Carrageen and induced paw edema in rats

Ten healthy, domestic male albino rats, weighing 230-250 gm were used in this study. These mice were kept in separate cages the room temperature was maintained at 20-25°C. Carrageenan-induced paw edema model was used for ethanol and aqueous extract of the *Lantana camara* leaves on inflammation as shown in Table 3. Acute inflammation was produced by the administration of 0.9% mL of car-

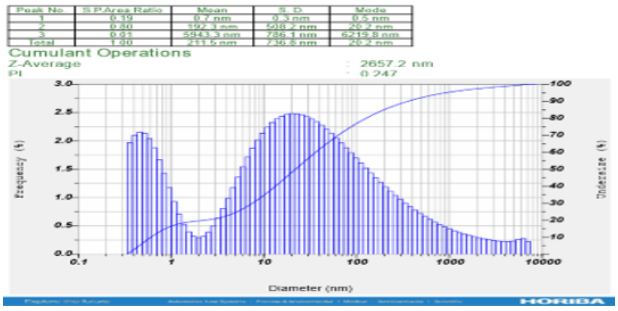

Figure 7: Particle size of *Lantana camara* leaf extract of AgNPs

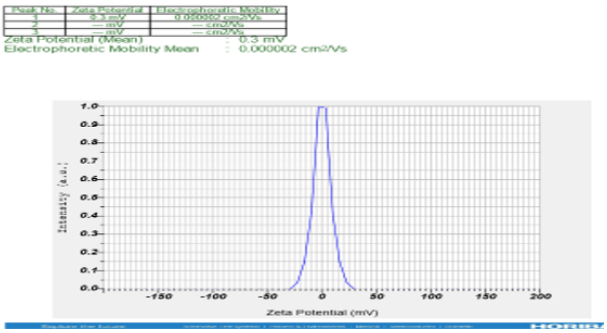

Figure 8: Zeta potential of *Lantana camara* leaf extract of silver Nanoparticles

rageenan in the sub plantar region of the left hind paw of a rat. The standard drug (diclofenac) and extract (50mg) were administered 30 min before the carrageenan injection. Inject to the control group and was treated with vehicle alone in the sub plantar region of the left hind paw of rats [8]. The standard drug was treated with diclofenac (50mg) used for the evaluation of the anti-inflammatory activity. The volume of the paw was measured immediately and also at the end of 2 hours and 4 hours after the administration of carrageenan using plethysmometer. The percentage inhibition of inflammation was calculated by the following formula:

$$\% \ Inhibition = \frac{Increase\ in\ paw\ volume\{(control)-(test)\}}{Increase\ in\ paw\ volume\ (Control)} \times 10$$

In-vivo Method (Wound-healing activity)

The animals were randomly divided into two groups with a sample size of 5 rats/ group. Animals were ketamine (80mg) anesthetized before and during the creation of the wounds [9]. A full-thickness excision wound with a circular area of 200 mm and 2 mm depth was created along the markings using toothed forceps, a surgical blade, and pointed scissors. Animals were topical with the simple ointment (Neosporin) base as a placebo control Animals were treated topically with the 10% ointment of the ethanol (200mg/kg daily) and aqueous(200mg/kg daily) extract of *Lantana Camara* leaves till complete epithelisation as shown in Table 4.

Synthesis Consisting of Silver Nanoparticles on *Lantana camara* Leaves Squeeze

Ethanolic extract of *Lantana camara* leaf 10 ml was added to the 90 ml of 1Mm $AgNO_3$ a ratio of 9:1. This solution was kept at ambient room temperature and stirred continuously for 10 min using a magnetic stirrer [10]. After 24 hours changed green colour to dark brown colour which signifies the production of silver nanoparticles.

Characterization of Silver nanoparticles

FT-IR analysis

It turned into performed to spot the functional groups in *Lantana Camara* that have been liable for the reduction of the silver nitrate and spectracular stabilization of silver nanoparticles [11].

Particle size Analyzer

The basic study that offers information about the share out of the numerous smallest sizes of the particles spread in the stratified sample [12].

Zeta potential

The surface attainable of silver nanoparticles this is fact the depiction of the stability of nanoparticles [13].

RESULTS AND DISCUSSION

Phytochemical Screening

Ethanolic extract and aqueous extract of *Lantana camara* Leaf extract were screened and the data was given in Table 5.

In-vitro anti-Bacterial Activity

Disc diffusion method

Ethanol extract of *Lantana camara*, when given for *E.coli* bacteria, shows a zone of inhibition of 4.5mm, 3.5mm for gentamicin (2mg), ethanol extract of *Lantana Camara* (2mg) respectively (Figure 1).

Ethanol extract of *Lantana camara*, when given for *Staphylococcus aureus* bacteria, shows a zone of inhibition of 6mm, 4.5mm for gentamicin (2mg), ethanol extract of *Lantana camara* (2mg) respectively (Figure 2)

Zone of inhibition for gentamicin, was slightly more compound to ethanol extract of *Lantana camara* leaf which is given in Table 6.

From the data of phytochemical screening and *in-vitro* antibacterial utilisation, it is revealed that the liquid extract of *Lantana camara* leaves shows more constituents and good anti-bacterial activity compared to ethanol extract.

So it turned into considered so the aqueous extract of leaves as promising extract which was formulated

as silver nanoparticles to enhance elegance, acceptability and bio-availability.

Characterization of Silver nanoparticles

UV-Vis spectroscopy

A visible colour change delight in see through to brownness inside of 20 minutes signifies the more formation of AgNPs, which was confirmed by UV-Visible spectrometer (Figure 4). After 70 minutes there has been a vital colour to dark brown due to increased reaction time which reinforces the growth of silver nanoparticles (Figure 3).

The intensity of the absorption peak at 418 nm increased with an increasing period of the aqueous component.

Nanoparticles

FT-IR analysis

The infrared Spectrum used to be analyzing the functional grouping present in *Lantana Camara* leaf extract (Figures 5 and 6). The FTIR spectrums of *Lantana camara* leaf extract and silver nanoparticles are represented (Table 7).

Particle size Analyzer

Partial size determination of *Lantana camara* leaf the synthesized AgNPs were shown by the intensity and laser diffraction revealed that particles obtained are a polydisperse mixture with the size ranging from 10 to 30nm. The average diameter of the particles was found to be leaf 2657.2nm (Figure 7).

Zeta potential

The *Lantana camara* leaf sequenced silver nanoparticles are decided in water as a dispersant. it encounter to leaf 0.3mV (Figure 8).

Plants contain many bioactive chemical constituents with various pharmacological activities. Many potent and effective medicinal properties used for treating fatal diseases have been isolated from various medicinal plants. Hence the demand for medicinal herbal drugs is increasing day by day. The study of the medicinal plants makes miracles in the medical field. *Lantana camara* was selected for the present study.

The anti-microbial properties of the plant have been examined by numerous researchers worldwide because of the therapeutic potential accredited to *Lantana Camara*. Collection of *Lantana camara* leaves and preparation of aqueous and ethanol on the cold extraction method. Further Preliminary phytochemical screening of *Lantana camara* extracts to identify phytoconstituents. This study can be used in the traditional medicinal system to cure diseases and therapeutic use. The interaction going from aqueous slot with the silver nitrate solution and showed modernized deepening of color into change yellow color to dark brown color.

CONCLUSION

The present research demonstrates that phytochemical constituents of ethanol and aqueous extract of *Lantana camara* leaves and phytostabilized of AgNPs can act as a unique of antibacterial activity against of organisms like *staphylococcus aureus* and *Escherichia coli* responsible for infections in burns and *In-vivo* methods of *Lantana camara* leaves extract of antibacterial activity, anti-inflammatory activity and wound healing activity. The personalities of water-soluble flavonoids in plant organ extract were responsible for spectracular reduction process of silver nanoparticles. Anti-bacterial activities of ethanol extract of *Lantana camara* were carried out and it shows that the antibacterial activity of ethanol extract of *Lantana Camara* is slightly less compared to gentamicin and ceftriaxone. Zone of inhibition for gentamicin, ceftriaxone was slightly more compound to ethanol extract of *Lantana camara* leaf.

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Conflict of interest

The authors attest that they have no conflict of interest in this study.

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REFERENCES

[1] M. Chandrasekhara Reddy, K. Sri Rama Murthy, A. Srilakshmi, Rao K.R.S. Sambasiva, and T. Pullaiah. Phytosynthesis of eco-friendly silver nanoparticles and biological applications A novel concept in nanobiotechnology. *African Journal of Biotechnology*, 14(3):222–247, 2015.

[2] Ganesh N. Sharma, C. H. Praveen Kumar, et al. Optimization and Characterization of Chitosan Based Nanoparticles Containing Methyl prednisolone Using Box-Behnken Design for the Treatment of Crohn's Disease. *International*

Journal of Applied Pharmaceutics, 12(2):12–23, 2019.

[3] Sanjeeb Kalita, Gaurav Kumar, Loga Nathan Karthik, Kokati Venkata Bhaskara, and Rao. A review on medicinal properties of Lantana Camara Linn. *Research Journal of Pharmacy and Technology*, 5(6):711–715, 2012.

[4] Ganesh Narayan Sharma, Praveen Kumar Ch, Birendra Shrivastava, B Kumar, and Arindam Chatterjee. Box-Behnken design for development and characterization of nanostructured chitosan-based nanoparticles containing sulfasalazine for the treatment of ulcerative colitis. *International Journal of Research in Pharmaceutical Sciences*, 11(1):532–545, 2020.

[5] S M Patil and R Saini. Anti-inflammatory and analgesic activity of methanol extract of the root of Lantana Camara Linn. *Journal of pharmacy research*, 5(2):1034–1036, 2012.

[6] Pradeep Kumar Singh, Kirti Bhardwaj, Parul Dubey, and Asmita Prabhune. UV-assisted size sampling and antibacterial screening of Lantana camara leaf extract synthesized silver nanoparticles. *Journal of Royal Society of Chemistry*, 5(31):24513–24520, 2015.

[7] J Ashok Kumar, S Ramkanth, S Lakshmana Prabu, and V Gopal. Enhancement of Saturation Solubility and In-Vitro Dissolution of Carvedilol Nanoparticles by High Pressure Homogenization Technique. *International Journal of Current Pharmaceutical Review and Research*, 6(6):269–273, 2015.

[8] B. Archana, K. Manjunath, G. Nagaraju, K. B. Chandra Sekhar, and Nagaraju Kottam. Enhanced photocatalytic hydrogen generation and photostability of ZnO nanoparticles obtained via green synthesis. *International Journal of Hydrogen Energy*, 42(8):5125–5131, 2017.

[9] Masese Osoro Brian and S Selvi. Cytotoxic effects of Ceiba pentandra L. mediated silver nanoparticles on HCT-116 colon cancer cell lines through ROS generation and cell membrane damage. *International Journal of Research in Pharmaceutical Sciences*, 10(4):3236–3243, 2019.

[10] Yerikala Ramesh and Chandrasekhar B Kothapalli and. Jayachandra Reddy Peddappi Reddigari, Formulation and Development of Tropicamide loaded Solid lipid nanoparticles enriched in chitosan in-situ Gels for ocular drug delivery. *Journal of Drug Delivery and Therapeutics*, 7(6):139–150, 2017.

[11] P Nagaveni, Y Saravana Kumar, Ramesh, and Ramesh Cn. Pharmacognostic properties and analgesic activity studies of Abrus precatorius leaves. *Journal of Indonesian Tourism and Policy studies*, 3(1):18–23, 2012.

[12] Jayachandra Reddy Peddappi Reddigari, Yerikala Ramesh, and Chandrasekhar B. Kothapalli. Formulation and evaluation of in-situ gels enriched with Tropicamide loaded solid lipid nanoparticles. *International Journal of Research in Pharmaceutical Sciences*, 9(1):216–216, 2018.

[13] F. S. Barreto, E. O. Sousa, F. F. G. Rodrigues, J. G. M. Costa, and A. R. Campos. Antibacterial Activity of Lantana camara Linn Lantana montevidensis Brig Extracts from Cariri-Ceara, Brazil. *Journal of Young Pharmacists*, 2(1):42–44, 2010.

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