Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2019.0502.33**

PHARMACOLOGICAL ACTIVITY OF SILVER NANOPARTICLES, ETHANOLIC EXTRACT FROM *JUSTICIA GENDARUSSA* (BURM) F PLANT LEAVES

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ABSTRACT: *Justicia gendarussa* (Burm) f. is one of the common, well known and important medicinal shrub. *Justicia gendarussa* (Burm) f. plant leaves medicinal properties such as, anti arthritic, anti-inflammatory and immunosuppressive activities and also this plant to cure many diseases. Ethanol and aqueous extract of the dry plant leaves were collected and qualitatively analyse the phytochemical compounds. Silver nano particles (AgNPs) were synthesised biologically from the aqueous extract of the *Justicia gendarussa* (Burm) f plant leaves. Then the AgNPs were characterised and confirmed by the analysis of UV, HR-TEM, and FE-SEM. In this study to explore the Antioxidant and anti-microbial activities using ethanolic extract and Silver nanoparticles of *Justicia gendarussa* (Burm) f plant leaves. The antioxidant assays are performed by ABTS, DPPH, NO and reducing power scavenging activity. The antimicrobial activity of the ethanolic extract and AGNPs were tested against 5 bacterial species. This study confirms the significant activities of antioxidant and antimicrobial against the ethanol extract and silvernano particles of *J. gendarussa* plant leaves.

KEYWORDS: Silver nanoparticles, FE-SEM, HR-TEM, Anti-oxidant activity and Antimicrobial activity.

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1. INTRODUCTION

Worldwide medicines play an important role in the wellbeing of the population. Medicines are obtained from various sources like plants, animals and minerals. Indian medicinal systems are well

known traditional medicine systems of the world [1]. Medicinal plants are one of the cheapest and good health cares for the poor and marginalized peoples. There are many medicinal plants still unexplored in the field of medicine and science. The therapeutic active compounds present in the medicinal plants identified and used to treat against the disease [2]. Acanthaceae is the family name for *Justicia gendarussa* (Burm) f plant. This plant is a branched, smooth, undershrub and 0.8 to 1.5 m height with long leaves, small flowers with the terminal spikes of purple spots. It is found all over Asian countries such as India, Malaysia, Indonesia and Srilanka. Use of this plant based remedies are spread in so many countries and numerous pharmaceuticals are using this plant compounds. Indian traditional medicine system used the plant *Justicia gendarussa* (Burm) f. leaves to treat fever, hemiplegia, rheumatism, arthritis, headache, earache, muscle pain, respiratory disorders and digestive trouble [3]. Studies prove that the phytochemicals are responsible for medicinal activity of plants. Plant have two types of phytochemicals, one is primary and another is secondary metabolites. These metabolites are responsible for the pharmacological activity against various diseases. This plant phyto constituents of this plant is reported to have an antioxidant and antimicrobial activity significantly [4, 5]. In this modern world nanoparticles are most effectively studied and are made from noble metals such as Ag, Zn, Au, Pt and Palladium. Among them silver Nano- particles were proven to be the most efficient as they possess good antimicrobial and antioxidant activities. Silver Nano particles are synthesised by chemical and biological methods. The chemical synthesis of AgNPs leads to the presence of some toxic chemicals that may have harmful or adverse effect in medical application. But the biological synthesis is reported to be more effective than the chemical synthesis and are inexpensive, eco-friendly and size-controlled nanoparticles [6]. The aim of the present study is to analyse phytochemical compounds and also to analyse the antioxidant and antimicrobial activity using ethanolic extract and synthesised silvernano particles of *J. gendarussa* plant leaves. This is the first study done on the antioxidant activity and antimicrobial activity comparatively along with AgNPs and ethanolic extract of *J. gendarussa* (Burm) f. plant leaves.

2. MATERIALS AND METHODS

Collection and preparation of plant extract

The *Justicia gendarussa* (Burm) f (Figure 1) plant leaves were collected (Ichadi, Pudukkottai District, Tamilnadu, India) in the natural environment. This plant was authenticated by (specimen - SJCBOT2183) by Dr. S. Soosairaj, Assistant Professor, Department of Botany, St. Joseph's College, Tiruchirappalli District, Tamilnadu, India. Fresh *J. gendarussa* plant leaves were washed, shade dried and ground into fine powder. This powdered material was soaked in 500 ml ethanol and was kept in a shaker at room temperature for 48 hrs. This material was filtered through muslin cloth and Whatman no.1 filter paper. Following which the powder is dried in a rotary evaporator at 37 ºC and was stored in a refrigerator [7].

Figure 1: *Justicia gendarussa* (Burm) f plant

Phytochemical analysis

The extract was qualitatively screened for various constituents (alkaloids, saponins, tannins, sterol, flavonoids, terpenoids, simple sugars) using standard protocol [8].

Biosynthesis of AgNPs

1g of the plant leaf powder was mixed with 20ml of distilled water at 60º C for 10 min. then 5 ml of the prepared aqueous leaf *Justica gendarusa* (Burm) f. extract and the reductant used for this study was added to 95 mL of 1 mM aqueous silver nitrate (Qualigens – 99.8%) (1mM silver nitrate in 100ml aqueous). The substrate is kept at room temperature in dark place for 24 h to attain the reduction of Ag+ ions. The AgNPs were purified by centrifugation at 10,000 rpm for 10 min at 4ºC. Then the pellet was suspended in Millipore water and stored at 4ºC for further analysis [9, 10].

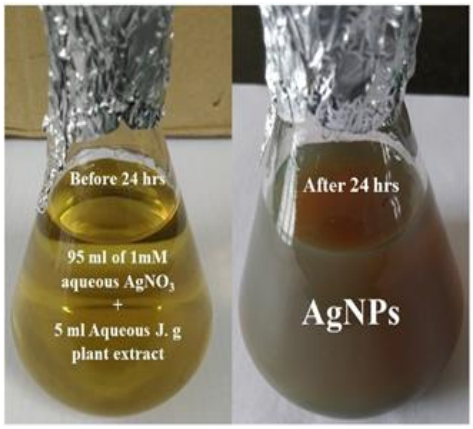


Figure 2: Color change of AgNPs using aqueous extract of *J. gendarussa* (Burm)f.

Characterization of AgNPs

The colour change to brown is a preliminary confirmation of AgNPs synthesis. 200–600 nm range was chosen in the Hitachi double beam equipment (Model Lambda 35) to perform UV–Vis spectrometric measurements. Compound analysis were done by FTIR instrument. Fe-SEM analysis was performed on a JSM-7610F Scanning Electron Microscope instrument to analyse the shape and size of the AgNPs. HR-TEM measurements were performed on a Jeol/JEM 2100 instrument operated at an accelerating voltage of 200 kV.

Antioxidant activity

The *in vitro* antioxidant activity of the crude ethanolic extract of the *Justicia gendarussa* (Burm) f plant leaves were analysed by basic methods such as ABTS, DPPH, Nitric oxide, reducing power free radicals were expressed as % of inhibition and they were compared with standard antioxidant BHT. Butyl hydroxytoluene BHT was taken as reference standard. The percentage of inhibition versus concentration was plotted and the concentration required for 50% inhibition radical was expressed as IC_{50} value.

ABTS assay

In-vitro antioxidant activity was analysed by ABTS assay method. As per the previous study [11] the stock solution contained 7.4 mM 2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) solution and 2.6 mM potassium persulfate solution [12]. The working solution prepared by mixing of these two stock solution in equal volume and were allowed to react at room temperature for 12 h in dark. This solution was diluted by mixing ABTS solution with methanol to obtain an absorbance 0.736 ± 0.01 at 734 nm. Fresh solution was prepared for each ABTS assay. Then, 1 ml of sample (crude ethanolic extract and AgNPs of the *Justicia gendarussa* (Burm) f plant leaves) was mixed with 1 ml of ABTS solution, and the absorbance at 734 nm was measured after 10 min incubation at room temperature. Finally, percentage of inhibition was calculated by following formula,

$$\% \text{ ABTS-scavenging activity} = [1 - (A_{sample}/A_{control})] \times 100$$

DPPH Assay

The stock solution of this assay was prepared by dissolving 10 mg of DPPH (2, 2-diphenyl1-picrylhydrazyl) radical solution in 1ml of methanol [13]. Working solution is prepared by this stock solution diluted with methanol and the absorbance was measured at 515 nm against (methanol) reference samples such as crude ethanolic extract and AgNPs of the *Justicia gendarussa* (Burm) f plant leaves. The percentage of DPPH scavenging activity of the sample was calculated by following equation,

$$\% \text{ scavenging capacity} = [1 - (A_{sample}/A_{control})] \times 100$$

Nitric oxide Assay

Sodium nitroprusside reagent produces nitric oxide (NO) physiological pH interacts with oxygen to produce nitric ions, which were measured by using the Griess reaction reagent at 546 nm [14]. 3ml of reaction mixture containing sodium nitroprusside (100 mM in PBS) and different concentration of plant extract and AgNPs were incubated at room temperature (25° C for 150 min). BHT used as a standard and NO radicals are used as positive control. Nitric oxide Assay was calculated by following equation,

$$\% \text{ NO scavenging activity} = [1 - (A_{sample}/A_{control})] \times 100$$

Ferric Reducing power assay

The reducing power was determined by [15] with slight modification. Different concentrations (50,100 150,200, 250 g/mL) of crude ethanolic extract and AgNPs of the *Justicia gendarussa* (Burm) f plant solution were mixed with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of 1 % potassium ferricyanide. Then the mixture was allowed to incubate at 50^{o} C for 20 min and then was cooled rapidly. Then 2.5 ml of TCA (10%w/v) was added. This mixture was centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was collected in separate tubes containing 2.5 ml distilled water. Finally, 0.5 ml 1% ferric chloride and the absorbance of the samples were measured using spectrophotometer at 700 nm against blank. Increased absorbance of the reaction mixture indicates increased reducing power. The obtained results were compared with BHT which was used as a positive control. The percentage of reducing power was calculated by the following equation.

$$\% \text{ Ferric Reducing power scavenging activity} = [1 - (A_{sample}/A_{control})] \times 100$$

Antibacterial activity

Antibacterial activity of the crude ethanolic extract and synthesised AgNPs of the *Justicia gendarussa* (Burm) f plant were determined mined by disc diffusion method. In this study five bacterial cultures (Source K.A.P. Viswanathan Govt. Medical College Tiruchirappalli) were used. Overnight inoculated cultures were spread over the freshly prepared agar plates. The sterile 6 mM disc (Himedia) were kept on plate. Further the plant extract and AgNPs (40 µl) was added to the disc. The Ciproflaxin disc (antibiotics disc- 20 µl) was also kept on the plate and was incubated at 37^{o} C for24 h. The antimicrobial property of ethanolic extract and AgNPs was determined by measuring the zone of inhibition around the discs after incubation.

3. RESULTS AND DISCUSSION

Phytoconstituents of the ethanolic and aqueous extract of the *Justicia gendarussa* (Burm) plant leaves were shown in Table 1.

The ethanol and aqueous extracts of the plant leaves contain Carbohydrates, Alkaloids, Flavonoids, Tannins, phenols, Saponins and Steroids. These phyto compounds shows Pharmacological activity, such as antimicrobial, antioxidant properties as per the previous study. Flavonoids are reported to inhibit the bacterial growth [16] and the tannins, flavonoids, phenolic compounds may be responsible for antioxidant properties. As per the previous reports, phyto compounds of the plant leaf possess biological properties such as anthelminthic, anti-hypertensive, diuretic, anti-malarial, anti-diabetic, antirheumatic, anti-cancerous, anti-inflammatory, antiviral etc., [17].

Table 1: Phytochemical compounds of the *Justicia gendarussa* (Burm) plant leaves

S.No	Phytoconstituents	Ethanol	Aqueous
1	Carbohydrates	+	+
2	Alkaloids	+	+
3	Flavonoids	+	+
4	Tannins	+	+
5	Phenols	+	+
6	Saponins	+	-
7	Steroids	+	+
8	Terpenoids	+	+

Characterization of AgNPs

In this study biologically synthesised AgNPs were analysed and confirmed by UV, Fe-SEM, & TEM. AgNPs have proven to possess antioxidant activity [18]. As the plant was mixed in the aqueous.

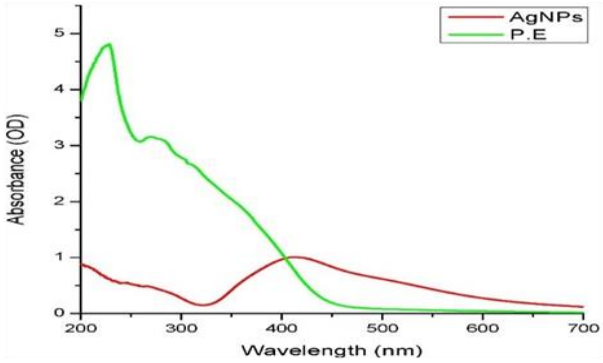


Figure 3: UV-Vis absorption spectra of silver nanoparticles present at 430nm, which is comparable to aqueous extract of the *J. gendarussa* (Burm) f. plant.

Mixed in the aqueous solution of the silver ion complex, a colour change was observed which may be due to reduction of silver ion, which may be the indication of silver nanoparticles formation [19]. The plant mediated silver nano solutions after incubation time, showed the color change from light to dark brown color (Figure 2). UV-Vis spectra recorded after 24h incubation time from aqueous solution of silver nitrate with *J. gendarussa* (Burm) f. *extrac*t. The samples display an optical absorption band peak at about 430 nm, typical of absorption for Ag particles, due to the Surface Plasmon Resonance (SPR). The absorption peaks around 380–440 nm could be attributed to AgNPs in size range of 25–50 nm [20]. Previous reports have predicted that the AgNPs in the region of around 410–450 nm can be attributed to spherical nanoparticles [21].

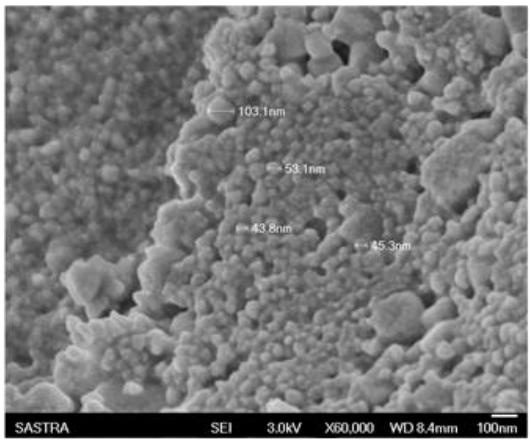


Figure 4: Fe-SEM image of synthesized AgNPs at room temperature using aqueous extract of *J. gendarussa* (Burm) f. plant.

The morphology and size of particles was determined by FE-SEM. Figure 4 shows that the particles are irregular spherical in shape with range of 20 to 100nm. HR-TEM images of AgNPs derived from the extract of *J. gendarussa* (Burm) f., are shown in Figure 5 (a). HR-TEM image of the AgNPs that are irregular spherical in shape and slightly agglomerated with the size ranging from 12 to 30 nm with the scale bar of 20 nm.

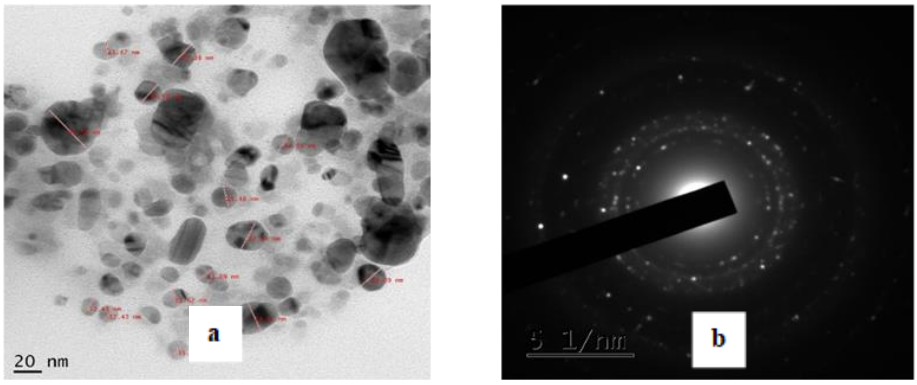


Figure 5: (a) HR-TEM image of the synthesized nanoparticles. (b) Selected area electron diffraction showing the characteristic crystal planes of elemental silver

HR-TEM analysis showed that most particles had a size of about 12 nm. Figure 5 (b) shows selected area electron diffraction pattern (SAED) of the silver nanoparticles. The silver particles are crystalline, as can be seen from the selected area diffraction pattern recorded from one of the nanoparticles [22].

Antioxidant Activity

Anti-Oxidants significantly play an important role in many disorders like inflammation, rheumatoid arthritis, asthma, psoriasis, and contact dermatitis leading to oxidative stress. As per the previous report the phenol, flavonoids, terpenoids etc. plant compound act as an antioxidant in the health promotion [23]. In this present study, the *Justicia gendarussa* (Burm) f plant leaves significantly scavenged the ABTS, DPPH, Nitric Oxide, reducing power results were represented in the Figure 6.

Our plant results were compared with BHT standard.

ABTS

The graph of the ABTS (figure 6) radical scavenging shows the potential of the biosynthesised silver nano particles and Ethanolic extract of the plant at the concentration of 50-250 µg/ml. ABTS scavenging is widely used for the antioxidant which is commonly present in the plant components. The free radicals of ethanol extract and AgNPs were higher in its concentration. The plant extract high range of activity present 75% and the AgNPs were 68% at 250 µg/ml. The scavenging activity of plant extract and AgNPs significantly lower than the BHT standard at 89% in 250 µg/ml. As per the previous reports of the plant extract, AgNPs scavenging activity results are supports our study [24, 25, 26]

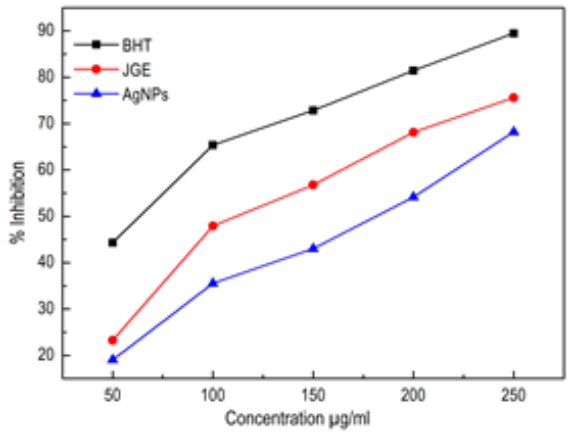


Figure 6: ABTS activity of the ethanolic extract and synthesised AgNPs of the *J. gendarussa* (Burm) f. plant in comparison of BHT.

DPPH assay

The DPPH activity were commonly used antioxidant assay for the free radical scavenging active compounds of the plant extract. The graph (figure 7) shows the DPPH activity of ethanolic extract and synthesised AgNPs of the *J. gendarussa* (Burm) f. plant. they reveal that with an increase in concentration DPPH scavenging activity got increase which is compared to the BHT standard in *in vitro* study. Both ethanol extract, AgNPs of the plant were significantly dose dependent inhibition of DPPH activity. The plant extract resulted in high range of activity at 56% and the AgNPs were 49% at 250 µg/ml (IC_{50}). [27] Earlier study reports the DPPH was a stable compound and accept hydrogen or electrons from silver nanoparticles. Our results of the DPPH assay show that the free radical scavenging activity was significantly inhibited by both ethanolic extract, AgNPs comparably with BHT standard relatively for the previous studies [28, 29, 30].

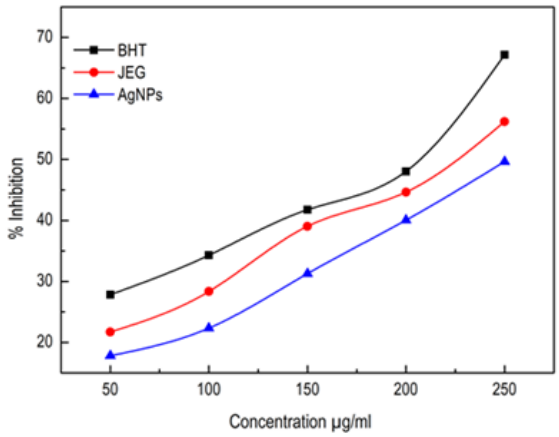


Figure 7: DPPH activity of the ethanolic extract and synthesised AgNPs of the *J. gendarussa* (Burm) f. plant in comparison of BHT.

Nitric Oxide

Scavenging activity of the *J. gendarussa* (Burm) f plant leaves ethanolic extract and AgNPs against free radicals of Nitric oxide were shown in the figure 8. The plant extract showed significantly high range of activity at 52% and the AgNPs were 44% which was comparable of BHT standard 69% at 250 µg/ml (IC_{50}). The nitric oxide are free radicals in carcinomas and inflammatory process mention in the previous report [31]. Physiological role of nitric oxide is reported to involve in the muscle relaxation process, neural signaling and inhibition of platelet aggregation and regulation of cell mediated toxicity [32]. Several previous reports show the similar observation of the nitric oxide scavenging activity of the plant studied [33, 34].

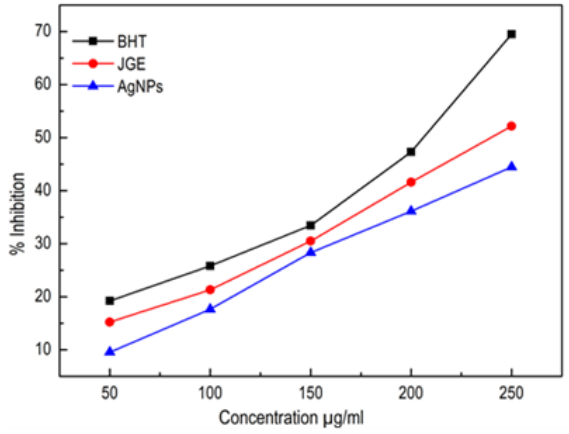


Figure 8: Nitric Oxide scavenging activity of the ethanolic extract and synthesised AgNPs of the *J. gendarussa* (Burm) f. plant in comparison of BHT.

Reducing power

Figure 9 shows the enhanced reducing power of the *J. gendarussa* (Burm) f. ethanolic extract and AgNPs relatively to the BHT standard. 250 µg/ml (IC_{50}) concentration of the reducing power of ethanol extract at 0.549 µg/ml and AgNPs were 0.503 and BHT standard at 0.684 respectively. The reducing activity of AgNPs and ethanol were comparatively low than that of BHT. This reducing

power was used widely in the evaluation of antioxidant components of the phenol [35]. This interpretation was found to be similar to the observations reported [36].

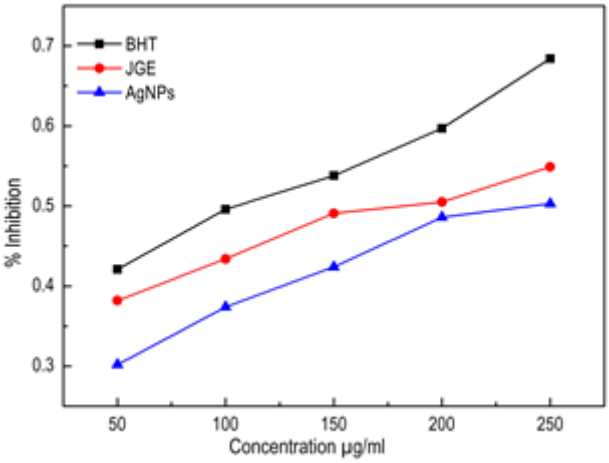
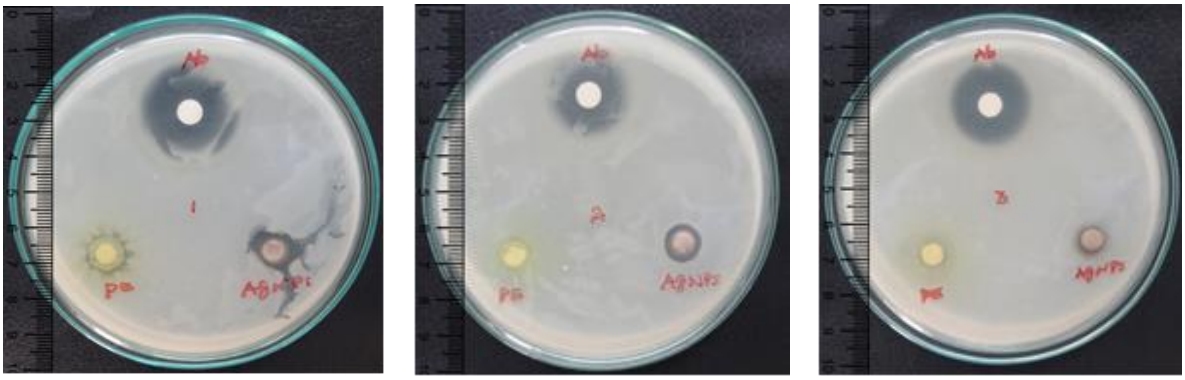


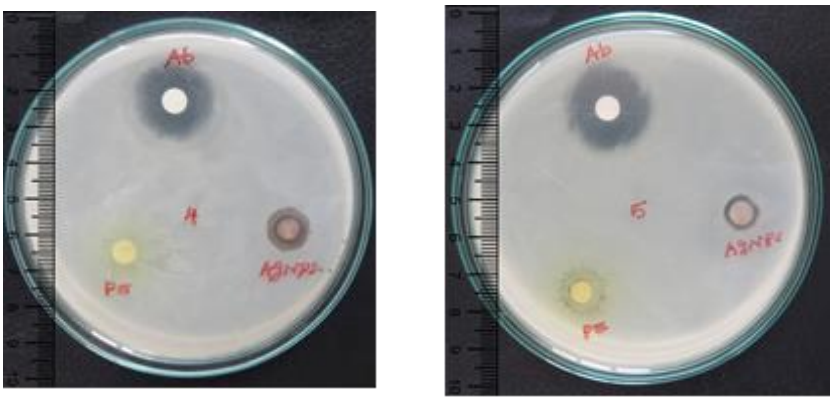
Figure 9: Reducing Power scavenging activity of the ethanolic extract and synthesised AgNPs of the *J. gendarussa* (Burm) f. plant in comparison of BHT.

Antimicrobial activity

AgNPs and ethanolic extract of *J. gendarussa* plant were examined against 5 bacterial pathogens such as, K. pneumonia (gram negative), S. aureus (gram positive), E. coli (gram negative), P. aeruginosa (gram negative), P. vulguris (gram negative) by zone of inhibition method. Figure 10 and table 2 shows the plant extract and AgNPs inhibition zones for all 5 pathogens. The maximum zone of inhibition observed for the silvernano particles against the K. pneumonia (MTCC 3384) pathogens was 15 mm and the minimum inhibition 9 mm was observed for ethanolic extract against S. aureus, P. aeruginosa. These results prove that plant leaves have good antibacterial activity due to the phytochemical compounds. In small concentrations, silver is safe for human cells, but lethal for microorganisms. This results shows the most importantly to prevent the infections [23, 22] study showed that the AgNPs are easy to adsorb on to bandages that could be used to prevent or cure infection and to enhance the healing process of wounds.



K. pneumonia ***S. aureus*** ***E. coli***



P.aeruginosa ***P. vulguris***

Figure 10: Antibacterial activity of the *Justicia gendarussa* (Burm) plant ethanolic extract and AgNPs. Ab - Antibiotics (Ciproflaxin), PE - Plant Extract (ethanol), AgNPs – Silvernano particles.

Table 2: Zone of inhibition (mm) of *Justicia gendarussa* (Burm) plant leaves ethanolic extract and AgNPs against the 5 microbes

S/no	Species name	PE (mm)	AgNPs (mm)	Ab (mm)
1	*K. pneumonia (MTCC 3384)*	12	15	22
2	*S. aureus (MTCC 2940)*	09	12	20
3	*E. coli (MTCC 433)*	10	12	22
4	*P. aeruginosa (MTCC 1034)*	09	12	22
5	*P. vulguris (MTCC 426)*	10	10	20

4. CONCLUSION

This study reveals that the silver nano particles synthesised biologically from the aqueous extract of the *Justicia gendarussa* (Burm) plant leaves. And they ethanolic extract of the plant were proved for their pharmacological action due to their anti-oxidant and antimicrobial activity in *in vitro* procedures. This study suggests that the *Justicia gendarussa* (Burm) plant may be a possible protection against the treatment of disorders associated with oxidative stress and pathogenic infections. However, further research is needed to elucidate its mechanism.

ACKNOWLEDGEMENT

We are grateful to Dr. Mani Jeyaraj, Assistant Professor and Head, Department of Biochemistry, Government Arts College (Autonomous), Kumbakonam and Department of Environmental Biotechnology, Bharathidasan University, Tiruchirappalli for providing all the required facilities to carry out this research.

CONFLICT OF INTEREST

The authors have declared there is no conflict of interest.

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