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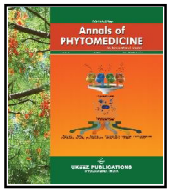

Original Article : Open Access

Green synthesis of silver nanoparticle and characterization of *Woodfordia floribunda* Salisb. aqueous leaf extract and their anti-inflammatory activity

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Abstract

This study focused on the biogenic preparation of silver nanoparticles. The (AgNPs) synthesised by using silver nitrate and water-extracted leaves from *Woodfordia floribunda* Salisb. Biosynthesized crystallites had an average size of roughly 23.18 nm. As a precursor and a capping reducing agent, silver nitrate as well as leaves extract were utilised, respectively. Energy dispersive spectroscopy (EDS), infrared spectroscopy (FTIR), transmission electron microscope, field emission scanning electron microscope, and UV-Vis spectrophotometer were employed to characterise and identify the biogenic AgNPs (FTIR). The *W. floribunda* AgNPs exhibit anti-inflammatory activity, with diclofenac (5 mg/kg), showing the strongest activity. The release of histamine (1 h), serotonin and bradykinin (2 h), and prostaglandins (3 h) was additionally inhibited by the AgNPs, demonstrating additional anti-inflammatory effect. This indicates that *W. floribunda* derived AgNPs have the desired inhibition (76.66%). The AgNPs anti-inflammatory properties are due to the release of prostaglandins (48.21%), histamines (68.34%) and bradykinins (80.48%). Mean and standard error were calculated using one-way ANOVA. Values of $p<0.05$ were considered statistically significant

1. Introduction

The medicinal plant has significant role in medicinal chemistry. The researcher mentioned in the Ayurveda, the pharmacological study and biological activity (Shrilakshmi *et al.*,2022). The human being facing the problem of arthritis after the age of 60 years. For that purpose, the best remedy is the medicinal plant. The *W. floribunda* was found in the mountain of Sahyadri region of Maharashtra. Since it enables the development of known materials with new features, nanotechnology has experienced a recent surge in popularity. The *W. floribunda* is a shrub with a long life. The plant's powerful phytochemical components had been used for centuries as a wound healer and for a variety of other medical conditions, including leprosy, burning sensation, skin conditions, diarrhoea, dysentery, fever, headaches, haemorrhoids, herpes, internal haemorrhage, leucorrhea, liver disorders, and menorrhagia. In addition to having anti-inflammatory and antitumor characteristics, this plant's various components also have hepatoprotective and free radical scavenging abilities (Nautiyal *et al.*, 2017). Although, the entire plant possesses therapeutic qualities, its blooms in particular have become quite popular in domestic and foreign markets that specialise in the production of herbal medicines (Kumar *et al.*,2016). The biomedical applications like drug delivery, targeted therapy and diagnosis of various diseases were performed using AgNPs. In the literature, *W. floribunda* had been reported that the family Lythraceae found alkaloids, triterpenoids flavonoids, phenolic, tannins and other compounds are presents. Plant extracts have been used to create a variety of nanoparticles, including copper oxide, gold and silver. Plant extracts are favoured over other biological materials for the creation of nanoparticles because they do not require the time-consuming procedure of maintaining cell cultures. Silver nanoparticles stand out among other metal nanoparticles due to their excellent conductivity, stability, and antibacterial activity. Silver nanoparticles' biological activity is affected by a number of variables, including dispersion, size, particle composition, shape, surface chemistry, particle morphology, capping, agglomeration, and others (Carlson *et al.*, 2008). Due to its numerous benefits, including being eco-friendly, quick, non-pathogenic, and affordable, the use of diverse plant extracts for the synthesis of silver nanoparticles is becoming increasingly important (Mudasir *et al.*, 2016). The combination of biomolecules found in plant extracts, like proteins, enzymes, alkaloids, saponins, terpenoids, phenolics, tannin, and vitamins; amino acids results, in the reduction and stability of silver ions (Arora *et al.*, 2022). Using a UV-Visible spectrophotometer, it is possible to observe how plant extracts convert $AgNO_3$, which creates Ag3+ ions, to AgO ions (Soni and Raizada, 2021). The aim of this research article to find out the anti-inflammatory activity of AgNPs of the *W. floribunda*.

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2. Materials and Methods

2.1 Sample collection

The leaves of plant were collected at Gardani road, near shivdoh waterfall, Akole, Ahmednagar and Maharashtra, India. The taxonomic identification was made by BSI Pune (No. BSI/WRC/Iden.Cer./2021/1905210003955). The leaves were washed thoroughly in tap water to detach adhere materials and finally washed with sterile distilled water. The plant leaves dried for 2-7 days.

2.2 Preparation of plant leaves extract

The dried leaves grind to make fine powder. Weigh 40 g leaves powder and 250 ml sterilized distilled water to boil the mixture for 10 min before decanting the mixture. The prepared plant leaf broth stored at 4°C in order to use for further studies.

Figure 1: The *W. floribunda* leaves.

2.3 Synthesis of silver nanoparticle

For the synthesis of silver nanoparticles (AgNPs), 85 ml of a 1 mM $AgNO_3$ was added to 15 ml of aqueous leaf extract. The mixture was incubated at dark for 20 h. After incubation, formation of AgNPs were observed by colour change yellow to reddish brown colour. Then, the synthesized silver nanoparticles were collected through centrifugation at 20,000 rpm for 20 min. The pellet was collected and dried at room temperature. (Sudha *et al.*, 2020).

3. Results

3.1 UV-visible spectrum

The UV-visible spectrum of silver nanoparticles biosynthesized using *W. floribunda* leaf extract. To enable UV-visible spectrophotometric measurements, the AgNPs were sonicated into uniform dispersion in distilled water at a concentration of 0.1 weight per cent (Mohanty, 2018). Ag nanoparticle absorption peak curve between 200 and 450 nm is shown in Figure 2. The LABINDIA analytical UV3092 UV-visible spectrometer was utilised to conduct the UV-visible analysis.

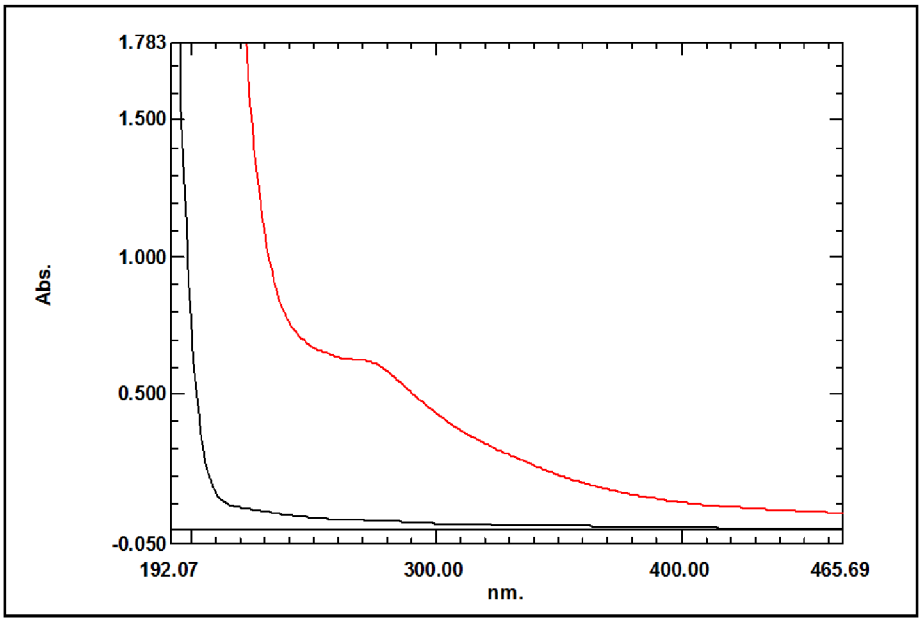

Figure 2: UV–vis spectrum of the synthesized AgNPs in water.

3.2 Fourier transform infrared spectroscopy (FTIR)

Figure 3 shows the Ag nanoparticles' FTIR spectrum, which was recorded between 400 and 4000 cm^{-1}. The FTIR spectra (Figure 2) of AgNPs showed bands around 3617.56 cm^{-1}, which indicates O-H stretching vibration. The absorption range about 3428.98 cm^{-1} reveals the broad stretching of hydroxyl (O-H), which is due to bending vibrations of water. In a solution of AgNPs, well-known absorbance bands were seen at approximately 1,018.89, 1,074, 1,316.89, 1,381, 1,602.15, and 2,263. The detected peaks represent, respectively, C-O-C, C-O, and C = C groups or aromatic rings (Elbagory *et al.*, 2019). These bands represent the stretching vibrational bands that are responsible for compounds like flavonoids and terpenoids and they may be held accountable for the effective stability and capping of produced AgNPs.

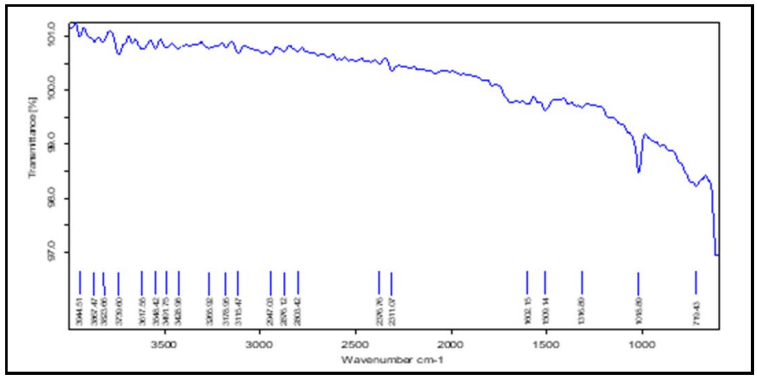

Figure 3: FTIR of the synthesized Ag NPs.

3.3 Field emission scanning electron microscope (FESEM)

The instrument specification for FESEM are Carl Zeiss model Supra 55 Germany was used. The Figure 4 shows FESEM images of silver nanoparticles. It reveals that silver nanoparticles were spherical and particles form cluster (Bhattacharjee *et al.*,2016).The images showed the synthesized nanoparticles in various magnifications, 100 nm, 200 nm and 300 nm (Mag.= 20,50,60,75 and 100 KX, EHT = 5.21 KV and WD = 2.7 and 2.8 mm) which distinctly gives physical morphology, particle size and aspect ratio.

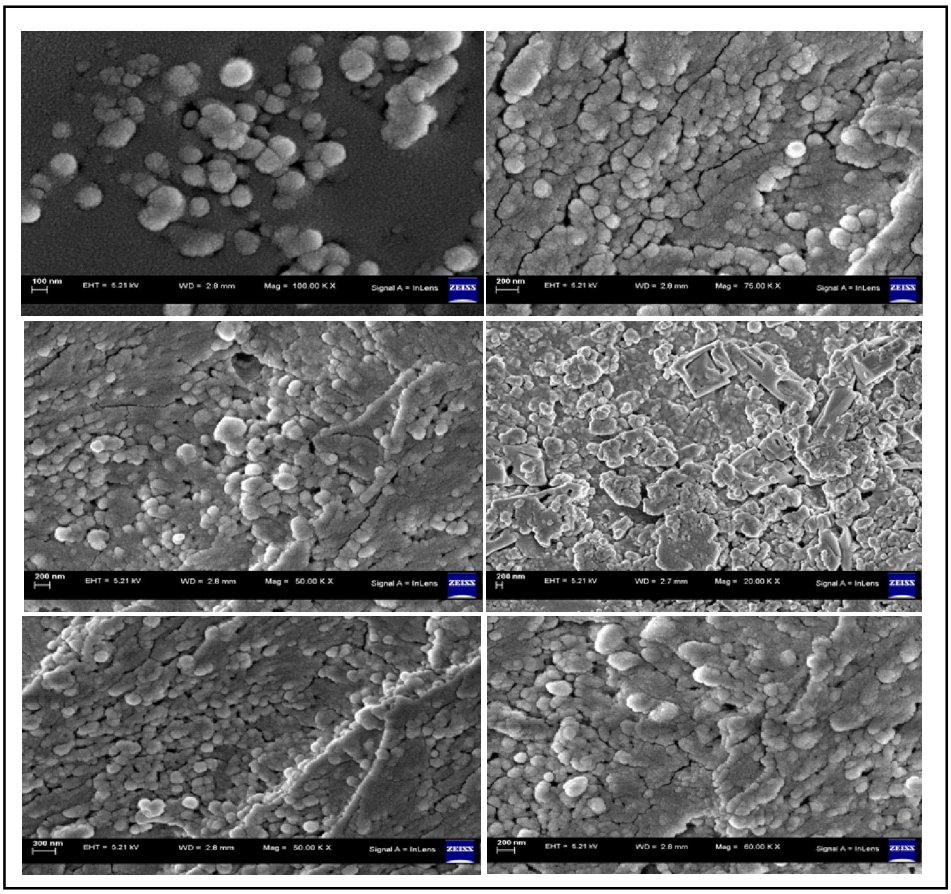

Figure 4: FESEM images of the synthesized AgNPs.

3.4 Transmission electron microscope (TEM)

Because it employs a stronger electron beam, transmission electron microscopy has a 1000 times greater resolution than scanning electron microscopy. The TEM delivers a great deal of atomic-level information. TEM pictures of silver nanoparticles are shown in Figure 5. The AgNPs produced had a medium surface area and a quasi-spherical shape with a size range of 20-500 nm (Dobrucka and Dlugaszewska, 2015). There were very few particles of varied sizes among the monodisperse particles. However, the TEM results matched the XRD results well. The Jeol JEM 2100 plus instrument was used for the TEM analysis (Gharpure *et al.*, 2022).

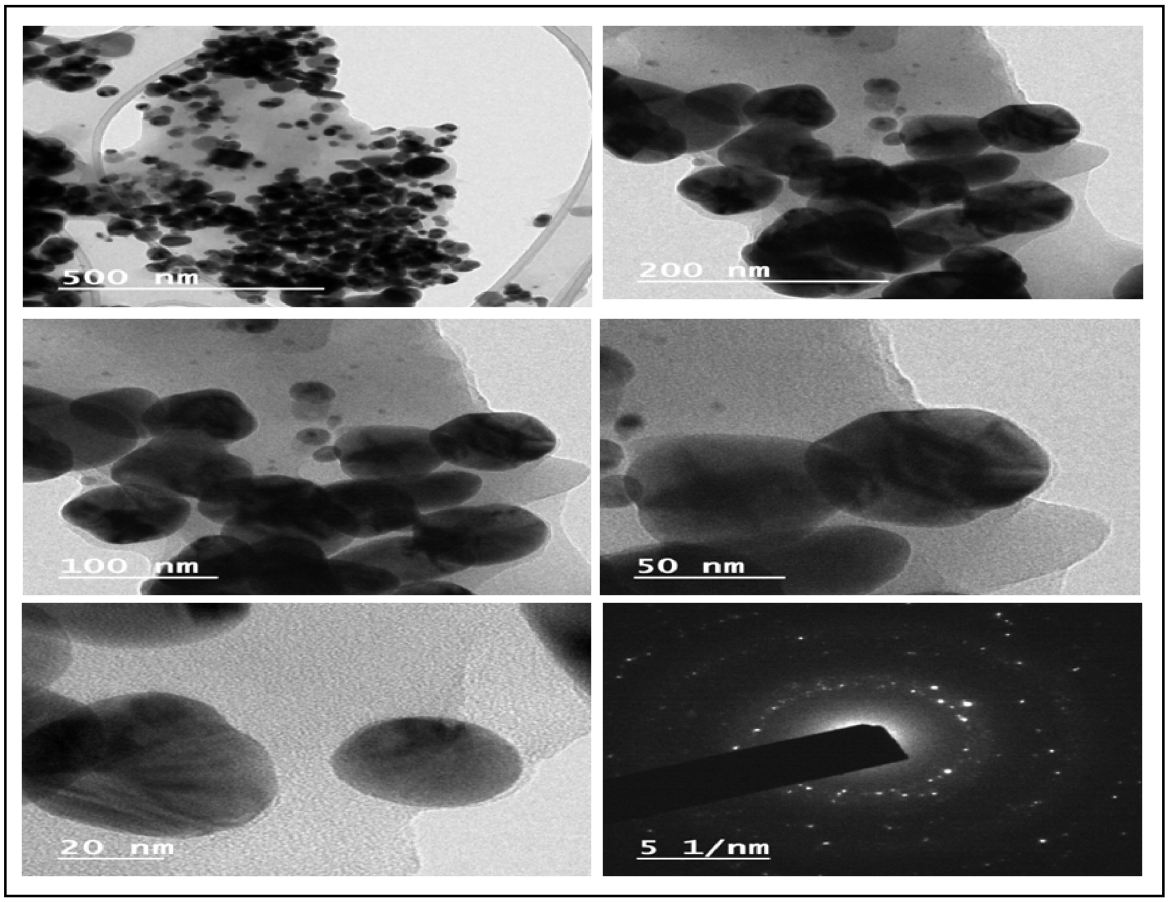

Figure 5: TEM images showing the presence of spherical AgNPs recorded with different magnifications.

3.5 The energy dispersive X-ray analysis (EDS)

The Bruker XFlash 6130 EDS instrument was used to do the analysis. The EDS investigation indicates that the particles are in fact metallic AgNPs and are crystalline in form (Figure 6). The presence of extracellular organic moieties on the surface of the metallic nanoparticles is indicated by the presence of carbon, oxygen, boron, iron, nitrogen, and copper (Dhandapani *et al.*,2016). The optical absorption peak of the biosynthesized metallic silver nanoparticles typically ranges from 3 to 4 Kev. Germany's Carl Zeiss type Supra 55 EDS instrument was used for the analysis.

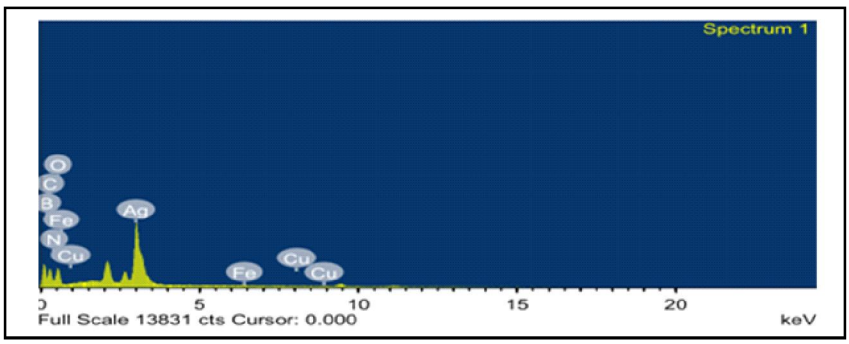

Figure 6: EDS spectrum of the synthesized AgNPs.

3.6 XRD analysis

Rigaku Japan's Smart Lab was used as the instrument specification for the XRD analysis. By using an X-ray diffractometer, the crystallinity of produced Ag nanoparticles was examined. Figure 7. displays the XRD pattern. It was produced using a CuK radiation source (= 1.5406 A°) in the 2° range of 20-80°. The XRD pattern of the biogenic (water extract of *W. floribunda* leaves) generated AgNPs, showed four major peaks. 50 Kv and 60 mA were used to run the X-ray tube (Deka *et al.*, 2021). The Gonio scan axis has a [2°] movement range of 0.5 to 170 and a Cu anode material. At 2° = 32.3439, 38.1962, 44.4344, 54.8829, 64.5862, 77.4115, and 81.5886, the XRD pattern shows seven peaks. The silver nanoparticles crystallise in a FCC form, according to the XRD measurements (Qinqin *et al.*, 2016). The FCC's extraordinarily high peak.

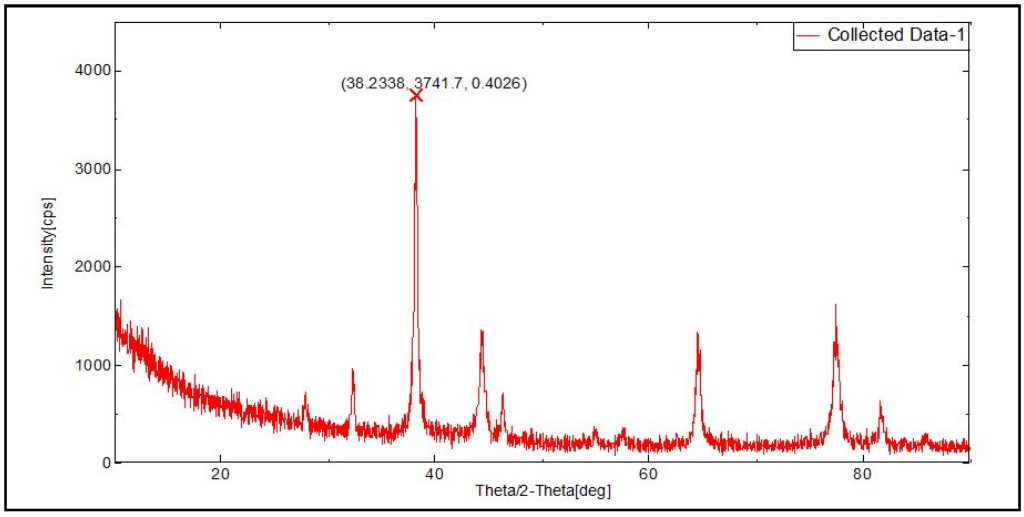

Figure 7: XRD pattern of the synthesized Ag NPs.

3.7 Anti-inflammatory activity

The LACSMI BIOFARMS PVT. LTD. Passaydan, Survey No.28/3/21 Samarth colony, Pimple Naka, Pune (Delivery note No. A-106/ 12/ 03/2022) provided the wistar rat weighing around (160-200 g). The wistar rats are starved for 12 h. before the use. Polypropylene cages were prepared for the rats and a regular rodent feed and water were provided. The animals fasted for 12 h before the experiment and were not given any food or water. After that, it is ensured that it is suitable for human consumption. The *W. floribunda* has one of the most important medicinal plants because of its anti-inflammatory activity, has an aqueous extract of AgNPs dose size depends on the weight of the animal and previous literature (Moldovan *et al.*, 2014). The doses may be easily calculated using the information provided in this study.

3.8 Drugs

The acquisition of wistar rats weighing between 160 and 200 g. Purchased from their respective companies, diclofenac (Pharma Cure Laboratories Garha, Jalandhar) and carrageenan soy lecithin (Indore, Madhya Pradesh, India) were utilised in the study.

Table 1: Inhibition of paw edema in percentage of AgNPs (n = 06) compared with standard (diclofenac)

Dose	0 Min	30 Min	60 Min	90 Min	120 Min	180 Min	240 Min	300 Min
01 mg/kg	1.29	15.59	30.53	42.64	63.96	73.33	50.81	34.93
5 mg/kg	1.52	19.35	38.42	43.15	68.02	78.10	57.30	41.61
10 mg/kg	1.94	25.16	41.58	48.98	69.04	78.57	60.54	42.69
STD 5 mg/kg	0.65	32.80	48.42	60.91	68.34	80.48	76.14	70.27

3.9 Ethical considerations

The experimental procedure and protocol as per the Amruthwahini College of Pharmacy, Sangamner, Dist., A. Nagar and Maharashtra approved the proposal for the animal activity study. Using a model of carrageenan-induced rat paw edema, it confirmed the "Guidelines for care and use of animals in scientific research" (Indian National Science Academy 1998, Revised 2000) (AVCOP/IAEC/2021-22/1153/ 26/01). The rats were divided in to three groups (n = 6) after receiving doses of 1, 5 and 10 mg/kg p.o. of the aqueous extract of AgNPs and distilled water (control). The average weight of wistar rats was in between 140-190 g which is used to calculate the dose size. Diclofenac (1mg/kg) was administered as a standard (Nde *et al.*, 2021). Carrageenan (0.1 ml, 1%) was injected into the right hind paw sub planter in each rat.The injection volume of carrageenan was determined using a plethysmometer (Medicaid System, Mode No. PTH-707, New Delhi, India) at 0, 30, 60, 90, 120, 180, 240, and 300 min. After each interval, the following formula is used to calculate the percentage inhibition (PI) of edema: PI = 1-Vt/Vc X 100, where Vt and Vc are the volumes used for the comparison between the turkey and the edema control (Phatangare and Gaje, 2017).The aqueous extract of AgNPs *W. floribunda* produced noticeably better results (p - 0.05), demonstrating its anti-inflammatory properties.

Table 2: The results of a typical subcutaneous injection of diclofenac. Values are the mean ± SEM of 6 animals, with **p<0.01and ***p<0.001compared to the control (normal saline); p<0.001compared using the Tukey-Kramer test. Comparison of all column pairs and one-way analysis of variances

Treatment (mg/kg)	0 Min	30 Min	60 Min	90 Min	120 Min	180 Min
Control	1.55 ± 0.014	1.87 ± 0.015	1.91 ±0.013	1.98 ± 0.009	2.00 ± 0.015	2.10 ± 0.013
STD (05 mg/kg)	1.54 ± 0.023	1.25 ± 0.023	0.98 ± 0.016	0.77 ± 0.021	0.63 ± 0.022	0.41 ± 0.011
01 mg/kg	1.53 ± 0.077	1.57 ± 0.026	1.32 ± 0.013**	1.13 ± 0.025*	0.71 ± 0.011***	0.56 ± 0.027**
05 mg/kg	1.52 ± 0.088	1.50 ± 0.039	1.17 ± 0.033**	1.12± 0.02**	0.63 ±0.019***	0.46 ± 0.02**
10 mg/kg	1.52 ± 0.006	1.16± 0.018	1.11± 0.025**	1.05 ±0.022***	0.61 ± 0.02***	0.45 ±0.023***

Table 3: Data of dose dependent Anti-inflammatory activity of stevioside (n=06)

S.No.	Weight of animal	Fraction in dose	0 min test sample	30 min test sample	60 min test sample	90 min test sample	120 min test sample	180 min test sample	240 min test sample	300 min test sample
I	170		1.50	1.55	1.22	1.13	0.87	0.68	0.98	0.92
II	185		1.54	1.61	1.27	1.16	0.89	0.69	0.99	0.95
III	190		1.50	1.68	1.33	1.17	0.79	0.63	0.91	0.89
IV	170	**01 mg/kg**	1.56	1.44	1.28	1.03	0.81	0.61	0.92	0.91
V	160		1.53	1.59	1.27	1.22	0.85	0.69	0.94	0.85
VI	180		1.52	1.54	1.26	1.18	0.77	0.68	0.88	0.90
	Mean ⟶		1.52	1.56	1.27	1.14	0.74	0.61	0.9	0.86
	Std. error of mean		0.057	0.023	0.014	0.023	0.022	0.023	0.017	0.0126
	Standard deviation		0.014	0.058	0.035	0.056	0.056	0.056	0.042	0.0310
	% Inhibition		**1.94**	**16.13**	**33.16**	**42.13**	**62.44**	**70.95**	**51.35**	**34.93**
I	150		1.47	1.45	1.22	1.15	0.75	0.59	0.83	0.88
II	180		1.49	1.48	1.24	1.12	0.81	0.54	0.84	0.81
III	160	**05 mg/kg**	1.48	1.43	1.08	1.02	0.71	0.55	0.81	0.84
IV	170		1.46	1.47	1.16	1.15	0.69	0.59	0.71	0.78
V	160		1.44	1.54	1.19	1.19	0.72	0.51	0.82	0.76
VI	150		1.50	1.70	1.22	1.06	0.74	0.58	0.80	0.83
	Mean ⟶		1.47	1.51	1.15	1.1	0.74	0.56	0.80	0.81
	Std. error of mean		0.013	0.045	0.023	0.025	0.017	0.023	0.010	0.015
	Standard deviation		0.033	0.045	0.023	0.025	0.017	0.023	0.010	0.036
	% Inhibition		**5.16**	**18.82**	**39.47**	**44.16**	**62.44**	**73.33**	**56.76**	**41.61**
I	160		1.11	1.53	1.03	1.05	0.76	0.67	0.84	0.83
II	170		1.41	1.44	1.06	1.03	0.77	0.63	0.81	0.92
III	180		1.40	1.52	1.16	0.94	0.79	0.59	0.89	0.77
IV	160		1.39	1.55	1.17	1.06	0.73	0.49	0.79	0.73
V	170		1.51	1.59	1.19	1.04	0.62	0.41	0.71	0.67
VI	180		1.49	1.51	1.13	0.94	0.59	0.43	0.72	0.81
	Mean ⟶		1.38	1.50	1.12	1.01	0.71	0.53	0.79	0.78
	Std. error of mean		0.021	0.008	0.020	0.017	0.031	0.036	0.030	0.032
	Standard deviation	**10 mg/kg**	0.052	0.020	0.050	0.042	0.076	0.089	0.075	0.077
	% Inhibition		**10.97**	**19.35**	**41.05**	**48.73**	**63.96**	**74.76**	**57.30**	**42.69**

3.10 Graphical representation of 1mg/kg, 5 mg/kg and 10mg/kg with comparison of standard siclofenac (5 mg/kg)

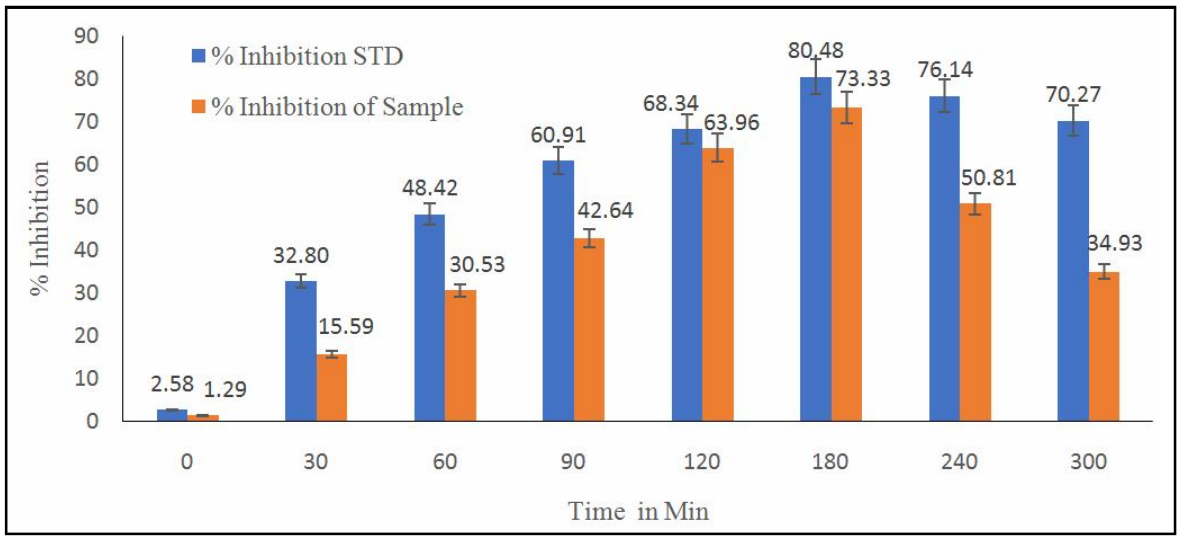

Figure 8: The% inhibition of sample with standard (1mg/kg).

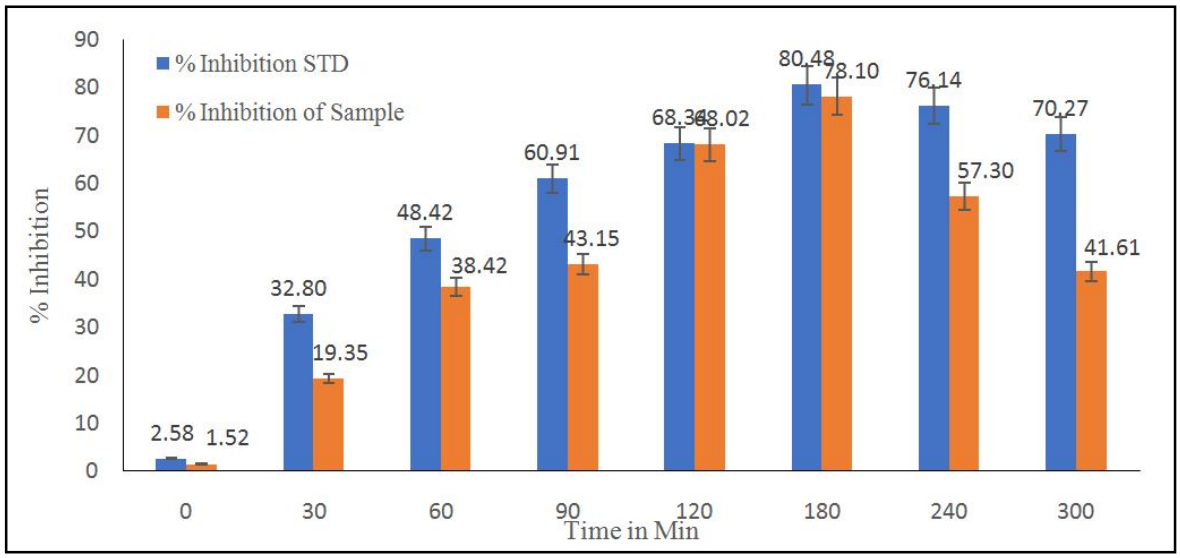

Figure 9: The% inhibition of sample with standard (5 mg/kg).

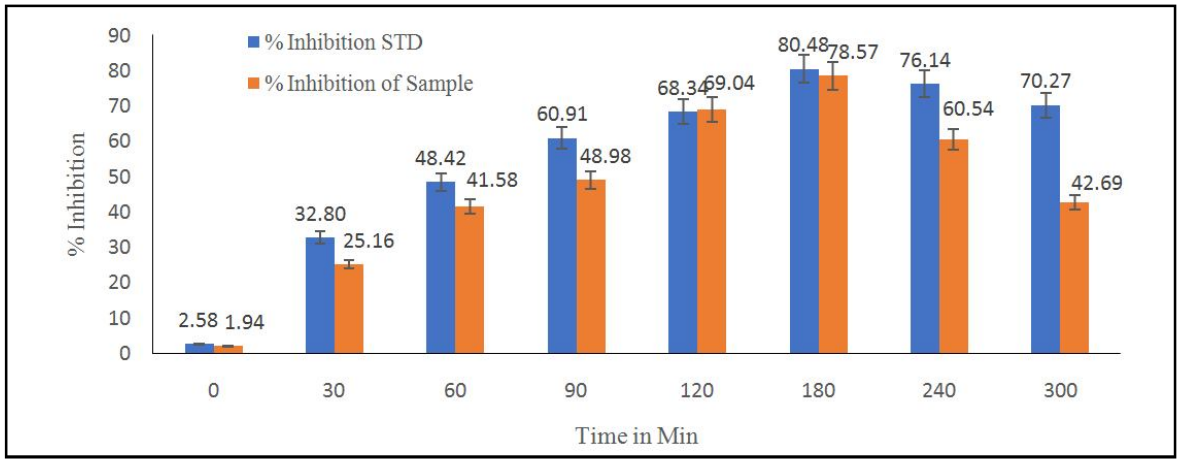

Figure10: The% inhibition of sample with standard (10 mg/kg).

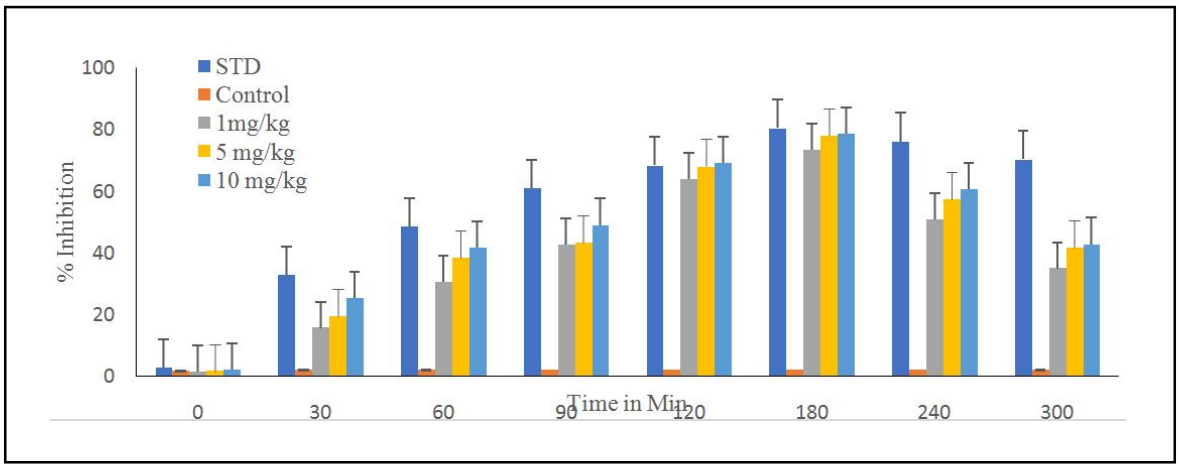

Figure 11: Comparative graph of standard, control and sample.

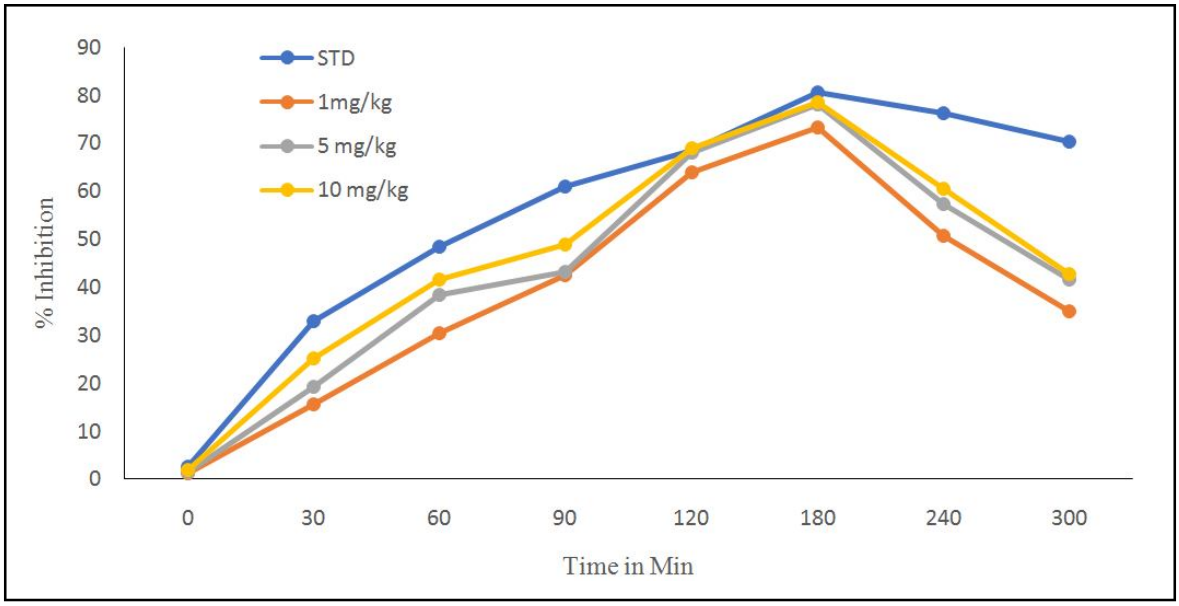

Figure 12: Release in histamine (1h), serotonin and bradykinin (2 h) and prostaglandins (3 h).

3.11 Statistical analysis

Results are based on the average and standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used throughout the entire statistical analysis, which was then followed by the multiple Turkey's comparison test. A *p* value of 0.05 or lesser was considered statistically significant.

4. Discussion

The present study focuses on the green synthesis of AgNPs using *W. floribunda* Salisb leaf extract as anti-inflammatory agent. The anti-inflammatory properties of *W. floribunda* are reported in the pharmaceutical sciences and it is valuable in the field of natural product. So the aim was finding the anti-inflammatory potency of the AgNPs.The change in colour with incubation time indicated the formation of AgNPs (Thirumurugan *et al.*, 2010). The XRD pattern indicates seven peaks. The size of AgNPs was calculated by Debye scherer's formula. The silver nanoparticles crystallise in a FCC form, according to the XRD measurements (Qinqin, *et al.*, 2016).The green synthesized AgNPs were observed by UV-Vis spectrum and the absorption peak curve was obtained between 200 and 450 nm (Mohanty, 2018). FTIR bands are responsible for compounds like flavonoids and terpenoids and they may be held accountable for the effective stability and capping of produced AgNPs. These compounds are accountable for the production of AgNPs (Elbagory *et al.*,2019). The optical absorption peak of the biosynthesized metallic silver nanoparticles typically ranges from 3 to 4 Kev. (Dhandapani *et al.*, 2016). The TEM results matched the XRD results well. The AgNPs formed had a quasi-spherical shape with a size range of 20-500 nm (Dobrucka and Dlugazazewska, 2015) . In FESEM analysis, the obtained micrographs revealed that silver nanoparticles were spherical and particles form cluster (Ahmad *et al.*,2016).The images showed the synthesized nanoparticles in various magnifications 100 nm, 200 nm and 300 nm.

The evaluated plant extracts of the AgNPs were effective in reducing the carrageenan induced paw edema. This may support the popular use of plants in the treatment of inflammation (Meckes*et al.*, 2004). Both *in vivo* and *in vitro*, methods are available for the evaluation of anti-inflammatory agents. Among the *in vivo* methods, carrageenan

induced rat paw edema assay is believed to be one of the most reliable and most widely used. Oral route of administration of drug is a common approach to administer the drug (Winter *et al.*, 1962). Carrageenan is commonly used to induce the inflammation for the purpose of identifying substances with anti-inflammatory properties (Humbal *et al.*, 2019). The AgNPs, administered or injected locally in to sub-planter tissue of the right hind paw of each rat having dose size (1 mg/kg, 5 mg/kg,10 mg/kg) produces a severe inflammatory reaction (Yadav *et al.*, 2012). The diclofenac are used as standard (5 mg/kg). Carrageenan-induced edema is caused by the acute phase of inflammation, which is mediated by histamine, bradykinin, and prostagladins generated under the influence of cyclooxygenase (Borgi *et al.*, 2007).The swelling that appeared in the rat paw upon carrageenan injection is given at each inter after 1 h. The initial phase is attributed to the release of histamine and serotonin, with the later phase associated to the releasing of prostaglandin (Nayak*et al.*, 2010). The AgNPs extract of *W. floribunda* leaves shown a stronger anti-inflammatory profile and could be employed as an anti-inflammatory therapy.

5. Conclusion

The use of plant extracts in the green synthesis of silver nanoparticles has several advantages over conventional processes since they are beneficial, simple to improve and ecologically friendly. The AgNPs structural and morphological characteristics were investigated using UV-Visible, FTIR, TEM, FESEM, and XRD. Figures 2, 3 and 4 display TEM and FESEM micrographs with Ag-EDS. The produced Ag nanoparticles were spherical having an average size of 23.18 nm and were in the nanometer range. The EDS picture confirms that the sample contains Ag. EDS and FTIR confirmed that the Nano-Ag powder made using the biosynthetic technique was pure. The size of the Ag powder was validated by TEM and FESEM analyses.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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