Synthesis and Characterization of silver nanoparticles from *Pergularia daemia* leaf extract and its effect on oral microbiome

[1]Sindhuja Baskaran, [2]Rengaramanujam Jayaraman

[1]Research scholar, Dep of Microbiology,
Dr. N.G.P. Arts and Science College, Coimbatore, Tamilnadu, India, Pin- 641048.
[2]Professor, Department of Microbiology,
Dr. N.G.P. Arts and Science College, Coimbatore, Tamilnadu, India, Pin- 641048.

Abstract

Objective: Synthesis of metal nanoparticles by green chemistry method has been a major focus in recent years. Nanoparticles will have a high surface to volume ratio with unique properties compared to larger particles which are used in the field of medicine as an antimicrobial agent. The plant *Pergularia daemia* (veliparuthi) was reported to have antibacterial property, antidiabetic activity, wound healing and many more. So the plant based synthesis of silver nanoparticles can be used to treat dental carries caused by diverse microbial community like *Streptococci and Lactobacilli.*

Methodology: UV-Visible spectrophotometer, Fourier Transform Infrared Spectroscopy (FTIR), Scanning electron Microscopy (SEM), Energy-Dispersive X-ray Spectroscopy (EDAX) and X-ray diffraction (XRD) anlysis were used to confirm and characterize synthesized silver nanoparticles and to identify the compounds responsible for reduction of silver ions. Agar well diffusion method was used for studying the antibacterial activity of synthesized AgNPs.

Results: The synthesized silver nanoparticles were greenish yellow to dark brown in colour indicating the formation of AgNPs. Further confirmation was done by UV-Visible spectrophotometer (480 nm), FTIR (2924) signaling aromatic compounds act as the capping agent in AgNp's. The SEM showed spherical shaped particles in the size of 18nm, EDAX showed a strong signal of silver and the silver colloids conform in XRD. The silver nanoparticles showed antimicrobial activities against oral pathogens with highest inhibitory zone of 16mm in *Streptococcus mutans*.

Conclusion: It is evident that *Pergularia daemia* plant will be an alternative source to chemical synthesis of silver nanoparticles with better antimicrobial activity against the oral pathogens.

Keywords: *Pergularia daemia,* Oral pathogen, Silver nanoparticles, SEM and FTIR.

1. INTRODUCTION

The products obtained from the plants are used as medicine for a long period of time. As per the World Health Organization (WHO) nearly 80% of the world population uses the traditional plant medicines to cure disease (1). Plants provide us many important drugs to save life and they are used as the armamentarium of modern medicine (2). Recently a large number

of researches were focused on the plant based medicines (Ayurveda, unani, siddha) (3). The plant *Pergularia daemia* is known as uttaravaruni in sanskrit and veliparuthi in tamil (4-6). It belongs to Asclepiadaceae. This plant was reported to have the antibacterial property (7) antidiabetic (8) wound healing (9) anti-inflammatory properties (10) and used to treat different kinds of disease like sore eyes, cough, infantile diarrhea & biliousness (4-6). Since the plant has antibacterial properties it can be used to treat oral diseases.

In India plants were used to clean the teeth and to cure different types of oral diseases (11). It is also used as chewing sticks or tooth brush by Indian people. Though, many plants were checked for its antimicrobial properties but are not evolved into new products (12).The human mouth acts as a reservoir of large and diverse microbial community. In an average human mouth harbors nearly1010 bacteria in it, this includes *Streptococci, Lactobacilli & Staphylococci* (13). The most relevant clinical problem in mouth is the tooth cavity which is caused by a particular set of microorganisms. It includes bacteria, fungi, and viruses (14). The oral pathogens are considered as the major cause of oral cavities, periodontal diseases and many types of systemic diseases which includes cardio vascular diseases and diabetes (15&16). Since the oral pathogen has a great influence in systemic and oral health, there is a need to find a cure for this problem. Since the ancient times the food was stored using silver by Egyptian, Persians, Romans and Greeks (17). The application of silver in the utensil making was mainly because of its antibacterial property.

Anything in nano size will have a high surface to volume ratio. So it has some unique properties than the same material in larger size (18). Silver nanoparticles are the most commonly used nanoparticles in field of medicine due to its unique properties and its applications as an antimicrobial agent (19-21). Silver nanoparticles can be synthesized by using three different methods like chemical, physical, and green synthesis (22, 23). Comparing these three techniques physical & chemical methods have certain disadvantages like high cost equipments, toxic chemicals, high temperature and pressure. But the green synthesis is more eco-friendly, easier and cheaper method for the synthesis of silver nanoparticles (24-26). Considering the medicinal properties of the *Pergularia daemia* plant and silver nanoparticles in the present study the silver nanoparticles were synthesized from the aqueous leaf extract and investigation of its activity against the oral microbiome.

2. MATERIALS AND METHODS

Collection and Identification of plant materials

The *P*ergularia *daemia* leaves were collected from Karumathampatty, Coimbatore.

Preparation of extract

The fresh leaves were washed with distilled water and shade dried. Then the dried leaves were grained to make fine powder and it is used for the aqueous leaf extraction by soxhlet extraction method.

Qualitative Analysis of Phytochemicals

The freshly prepared leaf extract was subjected to qualitative phytochemical analysis and identified the presence of various phytoconstituents like alkaloids, flavanoids, terpenoids, steroids, tannins, proteins, phenols, saponins, glycosides, amino acids, reducing sugars, and carbohydrates. The above phytoconstituents were tested as per the standard methods (27&28).

Antimicrobial Activity of Aqueous leaf Extract

The antimicrobial activity of the leaf extract was evaluated using agar well diffusion technique. Lyophilized oral pathogen of 6 cultures were acquired from MTCC. Bacterial cultures, *Streptococcus mutans, Lactobacillus acidophilus*, *Enterococcus fecalis, Porphyromonas gingivalis* and fungal cultures *Candida albicans*, *Candida tropicalis*were used for the antimicrobial evaluation. Fresh microbial broth cultures were prepared and its concentration is adjusted to 0.5 OD which is equivalent to 0.5 Mac farland units (approximately 108 CFU/ml). The inoculums were seeded on sterile plates of freshly prepared Muller hinton agar media. The wells were made using gel puncture. Chloramphenicol disc (30mg/disc) is used as positive control. The antimicrobial activity of leaf extract was checked by adding the aqueous leaf extract in different concentrations (5, 10, 15 & 20ml).The plates were incubated at 37ºC for 24 hrs(for bacterial growth) and in room temperature for 2 to 3 days (for fungi growth). After incubation the plates were observed for zone of inhibition (32).

Synthesis of Silver Nanoparticles

Synthesis of AgNPs was carried out by the method of Singh *et al.* (2014) with some modifications of silver nitrate were mixed in a ratio of 1:4, respectively, and allowed to stand at room temperature for 24 h. The change in colour from slightly greenish yellow to dark brown of the above solution indicated the formation of AgNPs. The solution was subjected to centrifuge at 12,000 rpm for15 min at room temperature and finally washed (three times) with distilled water to remove unbounded capping materials. Finally, synthesized AgNPs were dried and powdered for further use.

Characterization of silver nanoparticles

Five different techniques were used to characterize the synthesized AgNPs.

UV–visible spectroscopy

The initial characterization for the formation of AgNPs was performed using UV–visible spectrophotometer (Shimadzu UV-2450). Spectrophotometric absorbance peak was observed at specific wavelength (300-700nm) which may be due to surface plasmon resonance (SPR) phenomenon exhibited by silver nanoparticles, indicated the production of AgNPs. Distilled water was used as the blank (29).

FTIR analysis

FTIR spectroscopy used to identify the biomolecules which are involved in reduction of silver nitrate to silver (30). FTIR spectroscopy analysis for synthesized AgNPs was

performed using attenuated total refection (ATR)-Fourier transform infrared spectroscopy (FTIR). The percentage transmittance of FTIR spectrum was recorded between the wavelength ranges 4000–400 cm−1 at resolution 4 cm−1.

Scanning electron microscopy (SEM)

Among the various electron microscopy techniques, SEM is a surface imaging system capable of resolving different particle sizes, size distribution, nanoparticle shapes, and surface morphology of integrated particles in micro and nanoscale (31).The limitation of SEM is its inability to resolve the internal structure, but it can provide valuable information regarding the purity and the degree of particle aggregation. SEM analysis for synthesized nanoparticles was performed at Karunya University, Tamil Nadu, India.

Energy Dispersive Analysis of X rays EDAX

EDAX is an analytical technique used for basic analysis or chemistry of a sample. It is based on the investigation of a sample by the interactions between electromagnetic radiation and the object, analyzing the X-rays emitted by the subject in response to being struck by electromagnetic radiation.

X-ray Diffraction spectroscopy

The nanoparticles prepared in this study are characterized by XRD analyses to verify the crystal and nano structure of silver particles. X-ray differentiation is based on monochromatic X-rays and the creative interference of a crystal sample.

Antimicrobial activity of synthesized AgNp's

The antimicrobial activity of the synthesized AgNp's was evaluated using agar well diffusion technique for above mentioned oral pathogens. The antimicrobial activity of synthesized AgNp's was checked by adding the synthesized AgNp's in different concentrations (5, 10, 15 & 20µl). The plates were incubated at 37ºC for 24 hrs (for bacterial growth) and in room temperature for 2 to 3 days (for fungi growth). After incubation the plates were observed for zone of inhibition (33)

RESULTS

Qualitative Analysis of Phytochemicals

The qualitative analysis of aqueous leaf extract of *P. daemia* for phytochemicals resulted in the presence of alkaloids, flavanoids, terpenoids, steroids, tannins, proteins, phenols, saponins, glycosides, amino acids, reducing sugars, and carbohydrates.

Fig.1: *Pergularia daemia*leaf.

Table 1: Qualitative Phytochemical analysis of *Pergularia daemia*.

S. No	Name of the Phytocompounds	Name of the Test	The results of various extracts		
			Aqueous	Methanol	Chloroform
1	Alkaloid	Mayer's	+++	++	_
		Wagner	+++	_	_
2	Flavonoid	NaOH	++	_	_
3	Terpenoids	Chloroform + sulphuric acid	+	+	–
4	Steroids	Choloroform+ Acetic acid+ H_2SO_4	+	++	+
5	Tannins	Ferric chloride	++	+++	–
6	Phenol	$Fecl_3$	–	+	++
7	Carbohydrate	Molish	_	++	_
		Benedict's	–	++	_
8	Proteins	Million's	_	+	_
		Biuret	_	+	_
9	Glycosides	Borntrager's	+++	++	+++
		Legal's	+++	++	+++
10	Reducing sugar	Benedict's	++	+	++
11	Aminoacids	Ninhydrin	+	++	_
12	Saponin	Foam test	++	++	+

++++ - present in high amount; ++ - present in moderate amount;
\+ - present in low amount; - - absent

Antimicrobial activity of aqueous leaf extract:

The antimicrobial activity of aqueous leaf extract was determined against 11 different strains of oral pathogens. The (ZOI) was measured in millimeters (mm). The results were tabulated. The zone of inhibition of the microbes increased with the increase in concentration of the aqueous leaf extract.

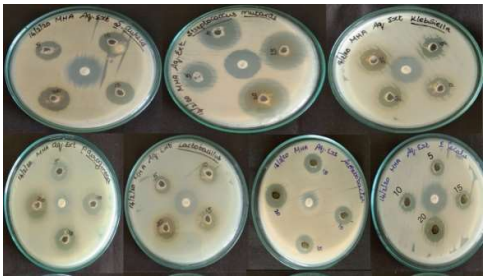

Fig. 2: Antimicrobial activity of aqueous leaf extract

Table 2: Antimicrobial activity of *P. daemia*.

Organism	Zone if inhibition in different extract (mm)		
	Chloroform 100 µl	**Methanol 100 µl**	**Aqueous 100 µl**
Streptococcus mutans	20	28	32
Lactobacillus acidophilus	13	19	23
Enterococcus faecalis	20	25	29
Porphyromonas gingivalis	20	23	29
Candida albicans	15	2	26
Candida tropicalis	14	2	26

s

Synthesis of Silver Nanoparticles

The $AgNO_3$were synthesized, dried, powdered and stored for further use.

Fig. 3: Synthesized Silver Nanoparticles

Characterization of silver nanoparticles

UV Visible Spectroscopy

The solution was kept in room temperature for 24 hrs before measuring the absorbance value to check the synthesized nanoparticles stability. The UV Spectrophotometry analysis showed the maximum absorbance at 480nm.

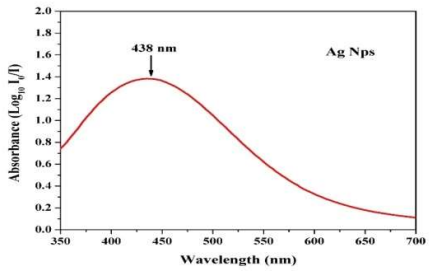

Fig.4 : UV Visible spectroscopy graph of synthesized AgNPs

FTIR Analysis

The FTIR spectra (fig 9) showed the peaks at 3434, 2924, 2853 , 1625, 1384, 1078, and 597 cm-1 which indicates the stretching vibrations of N-H strectching,C-H of aromatic compounds, N-H of amines, O-H of phenols,C-N of aliphatic acids,alkenes halogens and alkyl halides. The aromatic compounds present in it will act as the capping agent in AgNp's.

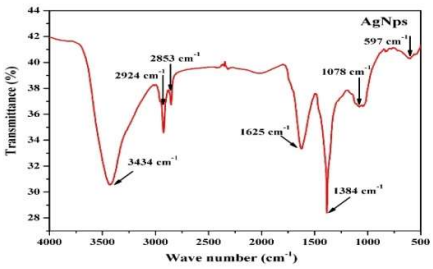

Fig. 5: FTIR graph of synthesized AgNPs

Scanning Electron Microscopy (SEM)

The synthesized silver nanoparticles size and shape will greatly influence their bioactivity and surface area (32). The SEM analysis showed that the nano particles have high density. The SEM images show that the AgNp's were Spherical shaped particles and are agglomerated. The size of AgNp’s is 18nm and is crystalline in nature.

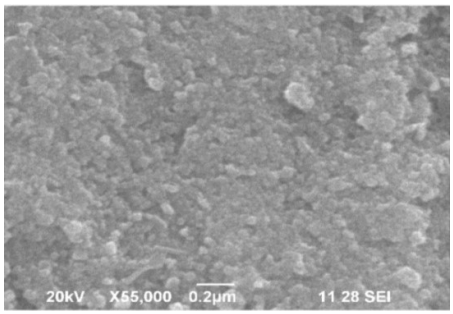

Fig. 6: SEM image of synthesized AgNPs

EDAX

The EDAX profile showed a strong signal of silver along with a weak oxygen peak which is due to the biomolecules that bound to the AgNP's surface, indicating the presence of elemental silver formed by the reduction of silver ions. Cl peaks are also seen which is due to Cl grid on which the samples was coated.

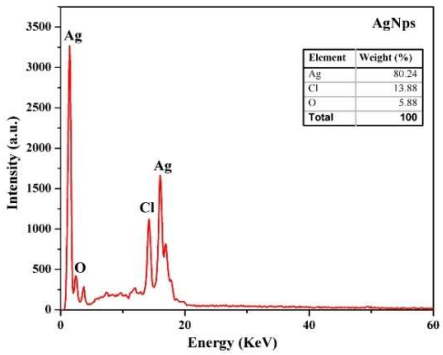

Fig.7: EDAX graph of synthesized AgNPs

X-ray Diffraction (XRD)

The XRD results confirming the existence of silver colloids in the sample. The Braggs reflections were observed in the XRD pattern at 20. These Braggs reflections clearly indicated the presence of (311), (111), (200) sets of lattice planes and further on the basis that they can be indexed as face-centered-cubic (FCC) structure of silver. Hence XRD pattern thus clearly illustrated that the silver nanoparticles formed in this synthesis are crystalline in nature.

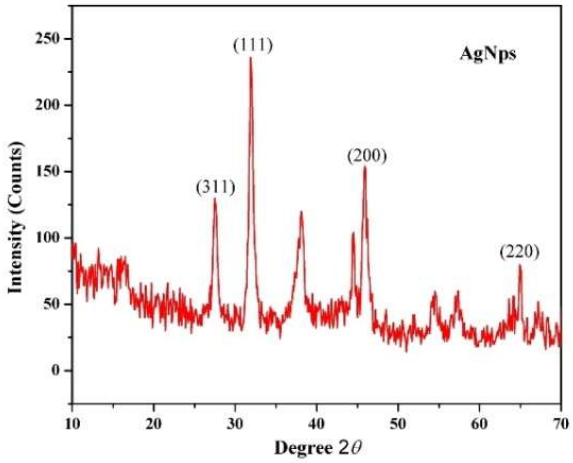

Fig.8: XRD graph of synthesized AgNPs

Antimicrobial activity of synthesized AgNp's

The antimicrobial property of the synthesized AgNp's was determined against 11 different strains of oral pathogens. The Zone of Inhibition (ZOI) was measured in millimeters (mm). The results were tabulated. It is found that the zone of inhibition of the microbes increased with the increase in concentration of the synthesized AgNp's.

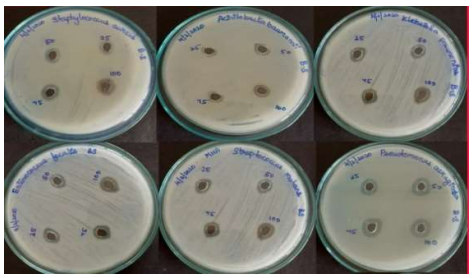

Fig.9: Antimicrobial activity of synthesized AgNPs.

Table 3: Antimicrobial activity of synthesized AgNPs

Organism	Zone Of Inhibition (mm)			
	5µl	10 µl	15 µl	20 µl
Streptococcus mutans	8	10	13	16
Lactobacillus acidophilus	8	9	10	13
Enterococcus faecalis	8	9	10	13
Porphyromonas gingivalis	9	11	12	14
Candida albicans	10	12	14	15
Candida tropicalis	8	9	10	13

DISCUSSION

In the present study, an eco friendly green synthesis approach isgiven. The green synthesis of AgNPs using *Pergularia daemia* leaf ectract to be eco-friendly, rapid and produced nanoparticles are fairly uniform in shape and size. Colour change occur due to surface plasmon resonance during the reaction with ingredients present in the *Pergularia daemia* leaf extract resulting in the formation of silver nanoparticles, which is confirmed by UV-Vis spectroscopy, FT-IR, SEM, EDAX and XRD. The FT-IR spectrum described the biological molecules which perform dual functions of stabilization and formation of silver nanoparticles in the aqueous medium. The formation of AgNpPs was confirmed by color change which is measured using UV–visible spectroscopy at 438nm.Further characterization with SEM and XRD shows that the synthesized AgNP's is in crystalline shape with a face centered cubic structure and with a size of 18nm. The FTIR study shows the functional groups of the synthesized AgNP's and the stretch of bonds. It also showed that the functional groups present in the leaf extract is acting as a capping agent. The EDAX spectra show that the AgNP's reduced from the *P. daemia* has the weight percentage of silver as 80.24%. SEM figures clearly depict that the coordinated silver nanoparticles are spherical. The synthesized AgNPs were spherical in shape. The mean size of AgNPs was found to be 18nm by SEM. Biosynthesized AgNPs showed significant antibacterial activity against oral pathogen (34).

CONCLUSION

The green synthesis on AgNpPs was done using the leaf extract of *Pergularia daemia* plant. It is a cost efficient, simple, safe and Eco friendly method. The plant extract and the AgNpPs have a good Antimicrobial activity against the oral pathogenic bacteria and fungi. The synthesized nanoparticles are more effective against oral pathogenic bacteria and fungi than any other extracts. However further investigations are required to conform the applications of these AgNP's.

ACKNOWLEDGMENT

The author feel thankful for providing appropriate characterization result from Karunya University, Tamil Nadu, India. The author also express her gratitude towards the host institution Dr.N.G.P Arts and Science College, Management, Principle, Deans, Head Of the Department, Guide and all other staffs for rendering all the facilities for support.

REFERENCE

1. WHO, IUCN, WWF, Guidelines on the conservation of medicinal plants. Switzerland: IUCN Gland; 1993.
2. Cragg G M, Newman D J, Sander K M. Natural products in drug discovery and development.J. Nat prod 1997;60:52_60.
3. Dahanukar S A, Kulkarni A R, Rege NN. pharmacology of medicinal plants and natural products. Indian J pharmacology 2000;32: S81_S118.
4. Kirthikar K R, Basu B D. Indian medicinal plants. Vol III. International Book distributors, Dehradun 1999; 1546_ 1617.
5. Nadkarani A K. Indian material medica vol I. popular prakashan pvt Ltd., Bombay 1976; 277-278.

6. Anonymous. Indian Medicinal plants.Vol IV Orient Longman Ltd, Hyderabad 1995; (9)236-38.
7. Senthil kumar M, Gurumoorthi P, Janardhanan k. Antibacterial potential of some plants used by tribals in Marudhamalai hills, Tamil Nadu, Nat. prod.Radiance 2005; 4:27-34.
8. Wahi A K, Ravi J, Hemalatha S and Singh P N. Antibacterial activity of *Daemiaextensa*. J. Nat. Remedie 2002; 2:80-3.
9. Kumar B , Yadav D K, Govindharajan R, Pushpangadan P. Wound healing activity of *Pergularia daemia* (forsk) chiov pharmacognosy 1:12-Ethnopharmacol 2006;4.
10. Bhaskar V H, Balakrishnan N.Analgesic, anti_inflammatory and antipyretic activities of *Pergularia daemia* and Carissa carandas.Daru 2009; 17:168-174.
11. Srivastava J, Lambert, Vietmeyer N. Medicinal plants;An expanding role in development _Washington DC: World Bank Technical paper 1996; P. 320.
12. Balandrin M F, Klocke J, Wurtele E S, Bollinger WH. Natural plant chemicals; sources of industrial and medicinal material science 1985; 228:1154_60.
13. Kenneth T. Todar's online Textbook of bacteriology, The bacterial flora of Humans, Madisons, Wisconsin 53706: 2008-2012.
14. Wade, W G. The oral microbiome in hesalth and disease. Pharmacol Res 2013; 69,137_143.
15. zarco, M F, Vess, T J and Ginsburg, G S.. The oral microbiome in health and disease and the potential impact on personalized dental medicine. Oral Dis 2012; (18)109-120.
16. He J, Li Y, Cao Y, Xue, J, Zhou,X. The oral microbiome diversity and its relation to human diseases. Folia microbial (Praha) 2015; (60)69-80.
17. Mody V V, Siwale R, singh A, Mody H R. Introduction to metallic nanoparticles. J pharm Bioall sci 2010; (2)282-289.
18. Saha J, BegumA, Mukherjee A, Kumar S. A novel green synthesis of silver nanoparticles and their catalytic action in reduction of methylene blue dye. Sustain Environ Res.
19. Ahmed S, Ahmed M, Swami B L, Ikram S. A review on plant extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise journal of advanced research 2016; 7(1):17-28.
20. Lopes C R B, Courrol L C. Green synthesis of silver nanopartricles with extract of mumusops coriacea and light. Journal of Luminiscences 2018;(199): 183-187.
21. Bendale Y, Bendale V, Paul S. Evaluation of cytotoxic activity of platinum nanoparticles against normal and cancer potential through induction of apoptosis, Integrative medicine research. 2017;6(2) :141 _8.
22. Saratale R G, Kuruppusamy I, Saratale G D, Pugazhendhi A, Kumar G, Park Y, etal. A comprehensive review on green nanomaterials using biological systems: Recent perception and their future applications. Colloids and surfaces: Bio interfaces 2018;(170): 20-35.
23. oves M, Aslam M, Rauf M A, Quayyum S Qari H A, Khan M S, etal. Antimicrobial and anticancer activities of silver nanoparticles synthesized from the root hair extract of phoenix dactylifera. Material science and engineering 2018;(89):429-43.
24. Agarwal H, Venkat Kumar S, Rajeshkumar S. A review on green synthesis of zinc oxide nanoparticles an ecofriendly approach. Resource-efficient technologies. 2017;3(4):406-413.
25. Chen J, Liu X, Wang C, Yin S S, Li X L, Hu WJ, etal. Nitric oxide ameliorates zinc oxide nanoparticle induced phytotoxicity in rice sedlings. Journal of hazardous materials. 2015; (297):173-182.

26. Khan S T, Mussarat J, AI-Khedhairy A A. Countering drug resistance, infectious diseases and species using metal and metal oxides nanoparticles: current status. Colloids and surfaces B, Biointerfaces 2016; (146):70_83.
27. Kokate C K, khandelwal K R, Pawar A P, Gokhale S B. Practical Pharmacognony 3rd ed., Nirali Prakashan Pune: 1995; p.137.
28. Trease and Evans. Textbook of pharmacognosy 12th ed., ELBS Publications. 1989; pp.49, 126, 132_137,205, 248.
29. Prabhu S, Poulose E K. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. International nano letters 2012; 2(1), 32.
30. Singh K, Panghal M., Kadyan S, Chaudhary U, Yadav J P. Antibacterial activity of synthesized silver nanoparticles from Tinospora cordifolia against multi drug resistant strains of Pseudomonas aeruginosa isolated from burn patients. Journal of Nanomedicine & Nanotechnology 2014; 5(2), 1.
31. Ozturk B Y, Gursu B Y, Dag I. Antibiofilm and antimicrobial activities of green synthesized silver nanoparticles using marine red algae Gelidium corneum. Process Biochemistry 2010; (89):208-219.
32. Zhang X F, LiuZ G, Shen W, Gurunathan S. Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. International journal of molecular sciences 2016; 17(9)1534.
33. Perez C, Pauli, M, Bezevque P. An antibiotic assay by agar well diffusion method. Acta Biol Med Exp 1990;(15):113-115.
34. Lekeshmanaswamy M, Anusiyadevi K. Biosynthesis of silver nanoparticles using Pergularia daemia leaf extract and its enhanced antibacterial activity against gram negative bacteria (Escherichia coli) Journal of matpr 2020; 499.