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Research Article

In vitro Antimicrobial Activity and Phytochemical Analysis of Leaves, Seeds, Bark, Flowers of *Pongamia pinnata* (Linn. Pierre) Against Human Pathogens

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ABSTRACT

Objective: The present study was undertaken with a aim to investigate the antimicrobial activity of the various parts of *Pongamia pinnata*. The parts of the plant selected for the study were flowers, bark, seeds and leaves.

Methods: All the selected plant parts were shed dried, turned into powder form and were subjected to soxhlet apparatus for the extraction of bioactive compounds using methanol as a solvent. The extracts in various concentrations were further employed to check their antibacterial activity against the bacterial pathogens by well diffusion method. Along with this the phytochemical analysis of the crude extracts was also carried out.

Results: The results showed that all the plant parts used in study showed antibacterial activity, among all the seeds and leaves extract showed the maximum zone of inhibition in most of the bacterial pathogens such as *Klebsiella pneumoniae* (26 mm), *Escherichia coli* (25 mm) and *Salmonella typhi* (25 mm), *Streptococcus pyogenes* (24 mm) at a concentration of 400 µg/ml. The flower and bark extracts can be used as an substitute source of antibacterial agent against bacterial pathogens.

Conclusion: The plant parts used in the study showed a potential antibacterial activity against pathogens, hence it can be used as a natural source of antibiotics. Further studies are needed to characterize the active bioactive compounds which are highly crucial for drug development.

Keywords: Medicinal plants, Plant extract, Antibacterial activity, Human pathogens

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INTRODUCTION

Although pharmaceutical industries have been serving the mankind from past decades, by the development of several antibiotics against various pathogenic microorganisms, but those are now acquiring resistance to the antibiotics. There are enormous antibiotics developing across the world but still there are several deaths occurring due to hospital acquired infections. In the year 2002, the estimated deaths due to hospital acquired infections in U.S hospitals were 98,987 (Klevens RM, *et al.*, 2007). The outbreak of the several bacterial infections along with antibiotic resistance is hazardous to mankind. Among all the bacterial epidemic diseases the most common are food poisoning, typhoid fever, pneumococcal infections, urinary tract infections etc. Globally 14.3 million cases of typhoid and paratyphoid were reported in 2017 (Stanaway JD, *et al.*, 2019). In the year 1997, a total of 773,530 cases were registered under urinary tract infections (Russo TA and Johnson JR, 2003). Due to this, the scientists all over the world are looking forward for alternative treatment for such diseases. From centuries before the establishment of modern science and technology, medicine was practised traditionally in the form of juices, extracts, infusions of different parts of plants and their products. One such plant is *pongamia pinnata* (L.) pierre which is very common in indian siddha and unani medicines.

Pongamia pinnata is a fast growing, deciduous legume tree, popularly being explored for the production of biodiesel across the globe. This tree is considered as the sure source of biodiesel for the upcoming generations. Apart from this, this multipurpose tree is also used as insect repellent, antiseptic etc. The plant is known to have several medicinal properties such as anticonvulsant activity (manigauha A, *et al.*, 2009), neuroprotective activity (Swamy AV, *et al.*, 2013), gstroprotective activity (Belagihally SM, *et al.*, 2011), anthelminthic activity (Nirmal SA, *et al.*, 2007), anti-inflammatory activity (Srinivasan K, *et al.*, 2001), antinociceptive activity (Srinivasan K, *et al.*, 2003), antidiabetic activity (Sikarwar MS and Patil MB, 2010), anti-lice activity (Samuel AJ, *et al.*, 2009), antihyperglycemic activity (Badole SL and Bodhankar SL, 2009), larvicidal activity (Kolli GR and Sundararajan R, 2013), antiviral activity (Rameshthangam PA and Ramasamy P, 2007), sunscreen activity (Shenoy PA, *et al.*, 2010), anti-arthritic activity (Gautam KR, *et al.*, 2013), antioxidant activity (Priya RS, *et al.*, 2016), antibacterial activity (Sajid ZI, *et al.*, 2012) etc. The present study was done to investigate the antimicrobial activity of various parts of the plant such as leaves, seeds, bark, flowers against several pathogenic bacteria those are found to be epidemic in the recent past years. In addition to this qualitative analysis for the presence of phytochemical constituents in all the plant parts was also performed.

METHODOLOGY

Collection of plant material

The fresh plant parts (leaves, seed, bark, flower) of *P. pinnata* were collected betweenMay and June 2019 from the local region of hennur cross, Bangalore, Karnataka, India. The fresh plant parts were washed under running tap water, shed dried for 7 weeks and pulverized into fine powder using a kitchen blender and stored in labelled airtight PTC bottles refrigerated at 4°C for further use (Morsy N, 2014).

Extraction of the plant samples

The shed dried plant materials were extensively extracted using Soxhlet apparatus. About 20 g of each powdered sample was filled in the thimble and extracted separately with 200 ml of methanol for 22 hours at temp of 50°C-60°C (temperature should be less than boiling point of the solvent, boiling point of methanol is 64.7°C) until the solvent in the siphon tube becomes colour-

less. After the complete extraction, the solvent used in the extraction was recovered and the extracts were concentrated in vacuum under reduced pressure using a rotatory evaporator until the dry extracts were recovered. The extracts were further stored in sterile petri plates at 4°C for further usage (Inala MS, *et al.*, 2015).

Preliminary phytochemical analysis of plant samples

All the extracts were tested for the presence of bioactive chemical compounds according to the following standard protocol as described by Nagy morsy (Chopade VV, *et al.*, 2008).

Test for alkaloids:

Dragandroff's test: 3 ml of the crude extract was taken in test tube and few drops of dil. HCL was added, then dragandroff's reagent was added to the mixture. The formation of red precipitate was taken as positive for the presence of alkaloids.

Test for flavonoids:

Lead acetate test: The crude extract was taken in a test tube and a few drops of lead acetate was added to it. The formation of yellow coloured precipitate was taken as positive for the presence of flavonoids.

Test for tannins:

Braymer's test: 2 ml of the crude extract was taken in a test tube along with 2 ml of distilled water and few drops of 10% $Fecl_3$ was added to it. The formation of green-black precipitate was taken as positive for the presence of tannins.

Test for saponins:

Froth test: 2 ml of the crude extract was taken in a test tube and 4 ml of distilled water was added to it, mixed well and shaken vigorously. The formation of stable froth was taken as saponins.

Test for terpenoids:

Salkowski test: 2 ml the crude extract was taken in a test tube and few drops of chloroform was added to it, then 1 ml of conc. sulphuric acid was added to it. The formation of a reddish-brown complex was taken as positive for the presence of terpenoids.

Test for steroids: 0.5 ml of crude extract was taken in a test tube and mixed with 2 ml of acetic anhydride followed by 2 ml of conc. sulphuric acid. The colour change from violet to green or blue was taken as positive for the presence of steroids.

Test for glycosides:

Keller-Kellani test: 5 ml of the crude extract was taken in a test tube and 2 ml of glacial acetic acid was added to it containing a drop $fecl_3$ solution, then 1 ml of conc. Sulphuric acid was added to it. The formation of greenish blue coloured complex was taken as positive for the presence of glycosides.

Test bacterial strains: The bacterial strains used in the present study were procured from microbial type culture collection centre, IMTECH, Chandigarh, India. The following bacterial strains were used: (1) *Bacillus cereus* (MTCC NO-430), (2) *Klebsiella pneumoniae* (MTCC NO-432), (3) *Streptococcus pyogenes* (MTCC NO-442), (4) Salmonella typhi (MTCC NO-735), (5) *Escherichia coli* (MTCC NO-405) *(Table 1)*.

Table 1: Bacterial strains with their MTCC NO

Test bacterial strains	MTCC NO.	Accuracy: 95%	Accuracy: 95%
Bacillus cereus	430	Accuracy: 95%	Accuracy: 95%
Klebsiella pneumoniae	432	Accuracy: 95%	Accuracy: 95%
Streptococcus pyogenes	442	Accuracy: 95%	Accuracy: 95%
Salmonella typhi	735	Accuracy: 95%	Accuracy: 95%
Escherichia coli	405	Accuracy: 95%	Accuracy: 95%

Culturing of bacterial strains and standardization of the inoculum: The culture media used for the antibacterial assay was obtained from HIMEDIA Laboratories Pvt. Ltd, Mumbai, India. All the bacterial strains were inoculated on nutrient agar slants and incubated at 37°C for 24 hours which served as stock (Al Muqarrabun LM, *et al.*, 2013). Sub culturing of the bacterial strains for antibacterial susceptibility test was done by taking a loopful of culture inoculated in Luria Bertani broth under aseptic conditions and incubated at 37°C for 4-5 hours. The turbidity of bacterial cultures was standardized to 0.5 McFarland standard unit to attain a final bacterial suspension of 1.5×10^8 CFU/cell.

Antibacterial susceptibility test by well diffusion method: The antibacterial potential of the different crude extracts was investigated by the help of well diffusion method. The MHA (Mueller Hinton Agar) plates were prepared by pouring 30 ml of sterilized Mueller Hinton agar media into the sterilized Petri plates under aseptic conditions to make an agar layer of 6-7 mm thick (Arote SR, *et al.*, 2009). The Petri plates were left undisturbed for 30 min for solidification. The bacterial inoculum containing 1.5×10^8 cells to be tested was uniformly spread on the surface of agar plates using a sterile cotton swab. Following this, 4 wells in Petri plates having diameter of 10 mm each but 20 mm apart are cut out using a sterile gel puncher (Sangwan S, *et al.*, 2010). Prior to the introduction of the extracts into the wells, the extracts were dissolved in DMSO (Dimethyl Sulfoxide., $(CH_3)_2SO$). 20 µl of different concentrations (50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml, 250 µg/ml and 400 µg/ml) of crude extracts was then introduced into the wells by the help of micropipette under aseptic conditions. DMSO serves as control in one out of the 4 wells in each Petri plates. The petri plates are kept at room temperature for 1 hour for the diffusion of the extract into the agar medium and incubated at 37°C for 24 hours (Arote SR and Yeole PG, 2010). After the end of incubation period, the plates were checked for the zone of inhibition and its diameter was measured in mm. The same was repeated thrice for each concentration for concordant values and the corresponding values were noted and the mean of the three was calculated. The mean of all the readings are summed up in Tables 2-5 respectively (Bajpai VK, *et al.*, 2009).

RESULTS

The methanolic extracts of various parts of *Pongamia pinnata* were analysed for the presence of antimicrobial activity against 5 bacterial pathogens. The experimental results obtained from the present study are as follows:

Antimicrobial activity of flower extracts

The methanolic extract of flower showed least activity as compared to the extracts of other plant parts (Kesari V, *et al.*, 2010). However, the highest inhibition was shown in *Bacillus cereus* (18 mm) at a concentration of 400 µg/ml and *Klebsiella pneumoniae* (17 mm) at a concentration of 400 µg/ml. The other pathogens too showed zone of inhibition but was less as compared to *Bacillus cereus* and *Klebsiella pneumoniae (Table 2)*.

Antimicrobial activity of seed extracts

The methanolic extract of seed showed the highest activity among all the plant parts. The possible reason behind these may be the presence of several essential oils trapped in these seeds (Punitha R, *et al.*, 2006). The highest inhibition was shown in *Klebsiella pneumoniae* (26 mm) at a concentration of 400 µg/ml and *Escherichia coli* (25 mm) at a concentration of 400 µg/ml. The 2nd highest zone of inhibition was shown in other pathogens such as *Bacillus cereus* (24 mm), *Salmonella typhi* (24 mm), both at a concentration of 400 µg/ml and least was shown in *Streptococcus pyogenes* (21 mm) *(Table 3)*.

Table 2: Antimicrobial activity of different concentrations of flower extract against various bacterial pathogens

Test organisms	Control (DMSO)	Different concentrations of flower extract in (µg/ml)					
		50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml	400 µg/ml
		Diameter of zone of inhibition (mm)					
Bacillus cereus	-	15	12	12	16	18	19
	-	15	13	15	14	19	19
	-	14	15	12	18	19	18
Mean	-	14.6	13.3	13	16	18.6	18.6
Escherichia coli	-	15	14	16	16	18	17
	-	14	12	14	16	17	16
	-	14	15	13	17	18	16
Mean	-	14.3	13.6	14.3	16.3	17.6	16.3
Klebsiella pneumoniae	-	17	13	15	12	15	17
	-	12	15	18	14	17	16
	-	10	12	14	16	19	18
Mean	-	13	13.3	15.6	14	17	17
Salmonella typhi	-	15	15	15	16	19	17
	-	15	16	14	14	15	17
	-	14	17	18	12	14	15
Mean	-	14.6	16	15.6	14	16	16
Streptococcus pyogenes	-	12	14	12	13	15	15
	-	12	14	14	14	15	16
	-	13	11	14	14	14	17
Mean	-	12.3	13	13.3	13.6	14.67	16

Table 3: Antimicrobial activity of different concentrations of seed extract against various bacterial pathogens

Test organisms	Control (DMSO)	Different concentrations of seed extract in (µg/ml)					
		50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml	400 µg/ml
		Diameter of zone of inhibition (mm)					
Bacillus cereus	-	19	18	19	19	23	25
	-	16	18	18	19	21	24
	-	17	18	17	20	21	25
Mean	-	17.3	18	18	19.6	21.6	24.6
Escherichia coli	-	21	22	22	23	22	25
	-	20	23	24	23	24	25
	-	20	22	23	22	24	26
Mean	-	20.3	22.3	23	22.6	23.3	25.3
Klebsiella pneumoniae	-	19	21	22	23	25	27
	-	23	20	22	24	24	27
	-	22	24	23	22	25	26
Mean	-	21	21.6	22.3	23	24.67	26.3
Salmonella typhi	-	20	20	21	22	21	24
	-	19	20	18	22	20	23
	-	20	21	23	23	22	25
Mean	-	19.6	20.3	20.6	22.3	21	24
Streptococcus pyogenes	-	17	17	19	20	20	22
	-	15	17	18	22	19	21
	-	18	19	20	22	20	21
Mean	-	16.6	17.6	19	21.3	19.6	21.3

Antimicrobial activity of bark extracts

The methanolic extract of leaves showed an intermediate activity between extracts of flower and seed (Brijesh S, *et al.*, 2006). The highest zone of inhibition was obtained in *Escherichia coli* (21 mm) and *Klebsiella pneumoniae* (22 mm), both at a concentration of 400 µg/ml.The remaining 3 pathogens also showed zone of inhibition like in as *Bacillus cereus* (18 mm), *Salmonella typhi* (20 mm) and *Streptococcus pyogenes* (19 mm) at a concentration of 400 µg/ml respectively *(Table 4)*.

Antimicrobial activity of leaves extracts

The methanolic extract of leaves too showed highest activity similar to seed extract. However the leaves extract unlike seed extract showed maximum zone of inhibition in *Salmonella typhi* (25 mm) and *Streptococcus pyogenes* (24 mm), both at a concentration of 400 µg/ml (Scott PT, *et al.*, 2008). The other bacterial pathogens too showed various zone of inhibition at different concentrations but they too showed their maximum inhibition at 400 µg/ml *(Table 5)*.

The qualitative study showed the presence of various phytochemcial constituents in various parts of the plant (Rajeshkumar S, 2016). The results of phytochemical analysis showed that seed holds a number of important phytochemicals such as alkaloids, flavonoids, tannins, saponins, steroids, terpinoids etc. The crude extract of bark too showed the presence of phytochemical constituents (Menpara D and Chanda S, 2014). The crude extract of leaves and flower showed positive result for alkaloids, flavonoids, tannins, saponins, steroids, terpinoids, but showed negative result for the presence of glycosides *(Table 6)*.

Table 4: Antimicrobial activity of different concentrations of bark extract against various bacterial pathogens

Test organisms	Control (DMSO)	Different concentrations of bark extract in (µg/ml)					
		50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml	400 µg/ml
		Diameter of zone of inhibition (mm)					
Bacillus cereus	-	15	17	16	16	16	18
	-	17	15	15	21	20	18
	-	17	18	17	18	23	19
Mean	-	16.3	16.6	16	18.3	19.6	18
Escherichia coli	-	17	18	16	20	19	22
	-	16	16	17	16	16	20
	-	14	14	19	18	20	22
Mean	-	15.6	16	17.3	18	18.3	21.3
Klebsiella pneumoniae	-	12	15	19	20	20	22
	-	14	18	20	20	19	22
	-	13	14	17	20	22	22
Mean	-	13	15.6	18.6	20	20.3	22.3
Salmonella typhi	-	18	18	20	17	20	21
	-	18	17	18	19	17	20
	-	20	19	16	19	20	21
Mean	-	18.6	18	18	18.3	19	20.3
Streptococcus pyogenes	-	14	15	17	19	17	20
	-	15	15	16	21	20	19
	-	17	15	15	18	20	18
Mean	-	15.3	15	16	19.3	19	19

Table 5: Antimicrobial activity of different concentrations of leaves extract against various bacterial pathogens

Test organisms	Control (DMSO)	Different concentrations of leaves extract in (µg/ml)					
		50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml	400 µg/ml
		Diameter of zone of inhibition (mm)					
Bacillus cereus	-	10	10	14	14	17	18
	-	11	15	17	17	18	19
	-	10	15	16	18	20	20
Mean	-	10.3	13.3	15.6	16.3	18.3	19
Escherichia coli	-	11	12	12	18	17	20
	-	9	11	11	16	19	18
	-	10	12	13	15	18	20
Mean	-	10	11.6	12	16.3	18	19.3
Klebsiella pneumoniae	-	14	15	18	15	13	21
	-	13	17	18	17	18	20
	-	13	17	16	17	19	18
Mean	-	13.3	16.3	17.6	16.3	16.6	19.3
Salmonella typhi	-	15	16	19	22	23	25
	-	16	20	23	24	24	27
	-	19	18	25	20	26	25
Mean	-	16.6	18	22.3	22	24.3	25.6
Streptococcus pyogenes	-	16	18	20	21	19	22
	-	18	18	21	21	23	25
	-	20	21	23	23	24	25
Mean	-	18	19	21.3	21.6	22	24

DISCUSSION

The above results elucidated that various parts of *Pongamia pinnata* used for the present study has a wide range of antimicrobial activity (Dhandapani R and Sabna B, 2008). The zone of inhibition formed by the action of extracts obtained from dried plant parts showed that *pongamia pinnata* possess a broad-spectrum activity. Among all the plant parts, the seeds and leaves proved to a very efficient against most of the bacterial pathogens used in the present study (Singh RK, *et al.*, 1997). The bark and the flowers although showed a mild antimicrobial activity, still their extracts can be employed for bactericidal activity (Dwivedi D, *et al.*, 2017). With the increasing number of antimicrobial resistant microorganisms, and super bugs, where most of the commercial antibiotics are failed, the antimicrobial activity shown by medicinal plants as one of being used in the present study could prove to be very efficient in the pharmaceutical industry with an extra in-built benefit i.e., they don't possess any side effects or any allergic reactions in the body (Sahoo DP, *et al.*, 2010). The active presence of various phytochemicals constituents such as alkaloids, terpinoids, steroids, tannins etc might be the possible reason for such activities in several medicinal plants (Carcache-Blanco EJ, *et al.*, 2003). Still further studies need to be done such as quantitative and spectrophotometric analysis of all the phytochemicals present in various regions of the plant (Pandikumar P, *et al.*, 2011), to know the exact structure and conformation of the compounds present in the plant which are acting as a defensive mechanism to stop the growth of bacterial pathogens, so that in future these compounds can be used along/replaced in place of commercial available antibiotics which are now a day's not that efficient against many microbial diseases (Islam MA, *et al.*, 2017) *(Figures 1-7)*.

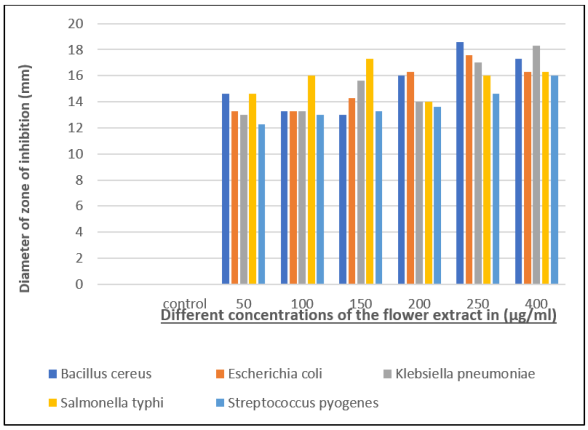

Figure 1: Antimicrobial activity of flower extract on bacterial pathogens

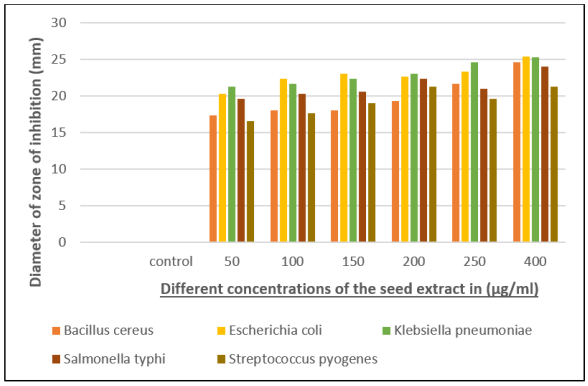

Figure 2: Antimicrobial activity of seed extract on bacterial pathogens

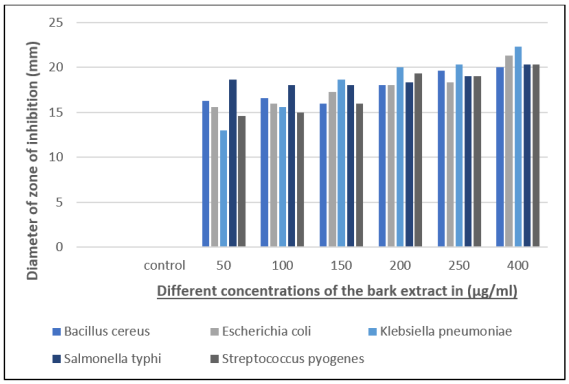

Figure 3: Antimicrobial activity of bark extract on bacterial pathogens

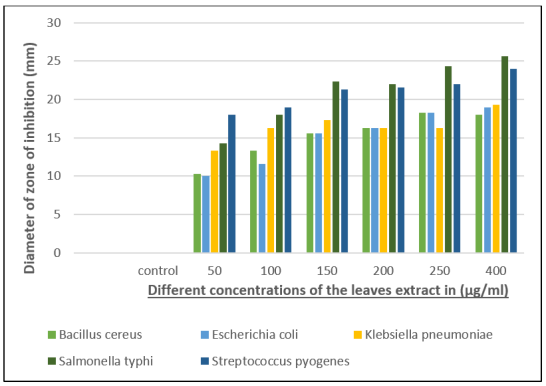

Figure 4:Antimicrobial activity of leaves extract on bacterial pathogens

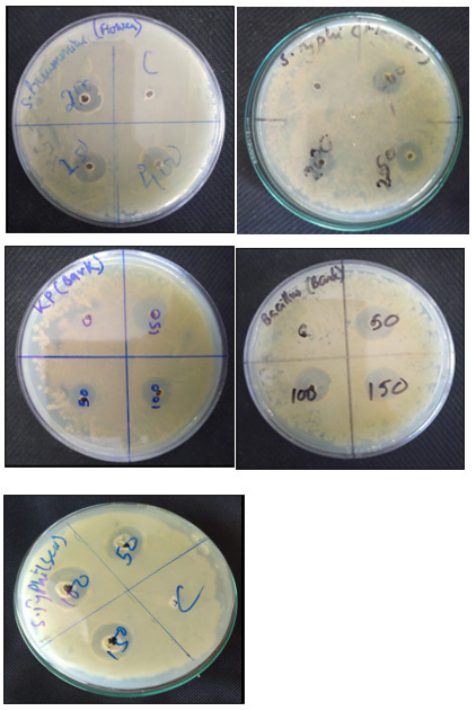

Figure 5: Zone of inhibition formed by the methanolic extracts of various plant parts of *Pongamia pinnata* on culture plate of bacterial pathogens

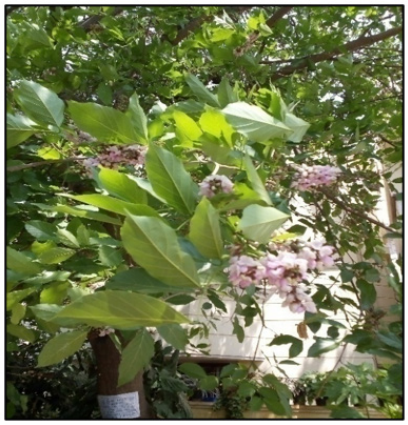

Figure 6: *Pongamia pinnata* tree

Figure 7: Seeds of *Pongamia pinnata*

CONCLUSION

Pongamia pinnata showed an appreciable anti-microbial activity and it remains important to discover new activities and leader compounds for drug development. In this context, the present study showed the antimicrobial activity of various parts of *Pongamia pinnata* against several bacterial pathogens. The extracts used in the study need to be further processed and may be used in large scale production for commercial and pharmaceutical applications in future.

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