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Efficacy of Padikara Parpam (Alum) with L-Ascorbic Acid Against Bacterial Pathogens

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ABSTRACT
Drug-resistant bacteria and unidentified infectious pathogens continue to pose serious risks to public health. Both the demand for traditional medical systems to be readied for Siddha preparation and their scientific documentation are expanding. Siddha ancient literature contains numerous polyherbal formulations that are used to treat a wide range of illnesses. In Siddha therapy, certain mineral formulations are therapeutically utilized to cure or prevent infectious diseases in addition to herbal formulations. The purpose of the current study was to examine the antibacterial properties and therapeutic efficacy of Padikara Parpam, a siddha mineral preparation with L- ascorbic acid. The outcome of the phytochemical analysis suggests that the medicine that combines Padikara Parpam and L-Ascorbic acid exhibits very little phytochemical ingredients. The combined drug of Padikara Parpam and L- Ascorbic acid demonstrates the greatest amount of biochemical ingredients, according to the study's findings. The combination drug has a distinct peak at 248 nm in the absorbance of 1.917. The PPLAA was found to be potentially active against the majority of the test microbes. The maximum antibacterial activity exhibited by PPLAA at 100 mg/ml against P. aeruginosa (28.5±0.25 mm) followed by S. aureus (27.7±0.78 mm), S. pyogens (26.9±0.92 mm) and E. coli (24.6±0.65 mm). The MIC values ranged from 0.39 mg/mL to 200 mg/mL. The MIC value for the PP and LAA against E. faecalis, P. mirabilis, A. baumannii, and S. maltophilia was >200 mg/ml. The evidence of antibacterial action against bacteria suggests that the siddha drug might be used to produce medications with a broad spectrum of activity.

Key words: *Padikara Parpam, L-Ascorbic Acid, Combination of Padikara Parpam and L-Ascorbic acid, Antibacterial activity*

INTRODUCTION:

Controlling of microbes is essential. There has been progress in understanding and controlling microbes in developing and developed countries, but drug-resistant bacteria and unknown infectious agents remain significant public health threats.[1] The scientific documentation of traditional systems of medicine is growing, as is the need for it to be prepared for Siddha preparation. Many polyherbal compositions are found in Siddha classical literature are used to cure a variety of ailments. In Siddha medicine, apart from herbal formulations some mineral formulations are clinically used in the treatment or prevention of infectious illnesses. Siddha herbo-mineral formulations have significant antibacterial activity against therapeutically relevant standard reference ATCC bacterial strains.[2] The current study was conducted to investigate the therapeutic efficacy of siddha mineral formulation Padikara Parpam along with L-ascorbic acid and its antibacterial activity.

Padikaram (alum) is a double sulphate that is created by combining a magnum, chromium, aluminium, or ferum sulphate with an alkaline metal or group sulphate such as ammonium, potassium, or sodium sulphate.[3] It is mostly used as water purification for domestic purpose. It is used as an antiseptic, styptic, and astringent in health purpose. Padikara Parpam is often used in siddha medicine to treat urinary tract infections, menorrhagia, hematuria, and urine frequency.[4] It is employed in a variety of therapeutic systems as an antipyretic, antiseptic, antispasmodic, and hemostatic agent. It is also employed in a variety of chemical compositions and dosage forms.[5] The study of synergistic interactions helps researchers not only in the discovery of novel phytomedicines or pharmacological combinations, but also in the avoidance of unnecessary harmful synergies. Medicinal combinations can help eliminate drug resistance.[6] The characterization of herbal medications is currently a major topic in the natural drug market. Appropriate standardization procedures, toxicological and

pharmacological examination of these products to fulfill the requirements for global usage.[7]
The scientific research of plants to discover their antimicrobial content is still in its early stages. In the United States and many other nations throughout the world, several surveys on antibacterial medicinal plants have been done. There have been several studies on antimicrobial activities from plant derived compounds. Hence, an attempt was made to study the phytochemical analysis of combined drug by using standard methods and also to identify the functional groups of combination drug of Padikara Parpam and L-Ascorbic acid by UV spectroscopy, FTIR and GCMS and to evaluate the anti-microbial activity.

MATERIALS AND METHODS:

Materials:

Padikara Parpam was procured from IMPCOPS pharmacy and L-Ascorbic Acid from SRL chemicals. The ATCC (American Type Culture Collection) bacterial isolates were procured from Microbiologics Inc., Clinical cultures were procured from VRR Diagnostics.

Collection and Processing of Combination Drug:

The collected Padikara Parpam and L-Ascorbic Acid used to prepare combination drugs, which was carried out in a Central Research Lab, associated with a Tertiary Care Centre at Chennai. 100 mg of each were accurately weighed as 1:1 ratio and placed in a clean beaker. The mixture was then transferred to a 100 ml volumetric flask and filled to capacity with distilled water followed by filtration after thorough mixing. Then it was taken for analysis. The characterization of the chemical compound which is present in the combination drug was analyzed.[8]

Quantitative Determination of the Combination Drug:

Phytochemical Analysis:

Preliminary phytochemical analysis was done for the combination drug as per standard methods described by Brain and Turner (1975) and Evans et al., (1996).[9,10]

Biochemical Analysis:

Biochemical analysis was done for the combination as per standard methods described by Bharani (2009).[3]

UV-Visible Spectrographic Evaluation:

The mixture was centrifuged for 10min at 3000 rpm and filtered through Whatmann No.1 filter paper. The sample is diluted to a ratio of 1:10 in the same solvent. The mixture of Padikara Parpam with L-ascorbic acid was scanned at wavelength ranging from 200 to 800 nm using Shimadzu UV-1700, Japan model UV spectrometer and the characteristic peaks were detected. The UV-VIS peak values were recorded.

Fourier Transform Infrared Spectra:

The dried mixture of Padikara Parpam with L-ascorbic acid was used for FTIR analysis. 1mg of the dried mixture was encapsulated in 10 mg of KBr pellet, in order to make transparent test discs. The extract powder was loaded into an FTIR spectroscope (Shimadzu, Japan) with a scan range of 400 to 4000 cm^{-1}.

GC-MS Analysis:

Elite-I fused silica capillary column (30m 0.25mm ID 1df) made entirely of 100% dimethyl polysiloxane was used for the GC-MS analysis. The mixture of Padikara Parpam and L- Ascorbic acid was diluted in methanol and subjected to GC-MS analysis in a Perkin-Elmer GC clauses 500 system. For GC-MS detection, an electron ionization device with an ionizing energy of 70 eV was employed. Helium gas (99.99%) was used as the carrier gas with a split ratio of 10:1 and injector temperature of 250°C and ion source temperature of 280°C. The temperature was set to grow from 110°C (isothermal for 2 minutes) to 2000°C at a rate of 10°C per minute, then 5°C per minute to 280°C, and finally 9 minutes at 280°C. With a scan time of 0.5 seconds and fragment sizes ranging from 45 to 450 Da, mass spectra at 70eV were obtained. The GC ran for 36 minutes in its entirety. By comparing each component's average peak area to the total areas, the relative % quantity for each component was determined. Turbo mass was used to manage mass spectra and chromatograms.

Antimicrobial Activity:

The bacterial strains were Escherichia coli (ATCC 25922), Klebsiella pneumoniae (ATCC 700603), Pseudomonas aeruginosa (ATCC 27853), Staphylococcus aureus (ATCC 29213), Streptococcus pneumoniae (ATCC 49619), and Enterococcus faecalis (ATCC 29212) were employed for this investigation were used for this study.
The clinical specimens of bacteria used in this study were collected from the VRR Diagnostics, Chennai. Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis, Acinetobacter baumannii, Proteus mirabilis, and Stenotrophomonas maltophilia were used for this study.
These bacterial strains were kept at 5°C for further experiments after sub-cultured on Nutrient agar slants for12-24 hours.

Agar Well Diffusion Method:

The antibacterial activity of the Padikara Parpam (PP), L-ascorbic acid (LAA), and Padikara Parpam + L-ascorbic acid (PPLAA) by agar well diffusion method was assessed.[11]

In two different concentrations 50 mg/mL and 100 mg/mL, the antibacterial activities of the compounds were tested. (In Figures; 1-PP 50 mg/mL, 2-PP 100 mg/mL; 3-LAA 50 mg/mL, 4-LAA 100 mg/mL; 5-PPLAA 50 mg/mL, 6-PPLAA 100 mg/mL)

Minimum Inhibitory Concentration (MIC):

By using the Broth Macro Dilution method, the minimum inhibitory concentration (MIC) of PP, LAA, and PPLAA was found in Mueller Hinton Broth (MHB) for bacteria.[12]

RESULTS:

Qualitative Analysis:

Phytochemical Analysis:

The findings of the preliminary phytochemical analysis are provided in Table 1 and were used to determine the quality of the phytochemicals present in the combination medication of Padikara Parpam and L-Ascorbic acid. It means that only proteins and alkaloids, not other phytoconstituents, were detected in the experimental medication. The outcome suggests that the medicine that combines Padikara Parpam and L-Ascorbic acid exhibits very little phytochemical ingredients.

Biochemical Analysis:

The biochemical components of the combined drug of Padikara Parpam with L-Ascorbic acid are listed. Apparently, the experimental drug contains biochemical components including calcium, sulphate, chloride, iron- ferric, iron-ferrous, phosphate, albumin, reducing sugar, and amino acids, according to this information. The combined drug of Padikara Parpam and L- Ascorbic acid demonstrates the greatest amount of biochemical ingredients, according to the study's findings.

Table 1: Phytochemical and Biochemical constituents of combined drug

Sl. No	Constituent	Quality	Constituents
1	Phytochemical	Present	Alkaloids, Proteins
		Absent	Tanins, Saponins, Flavonoids, Alkaloids, Steroid, Quinones, Terpenoids, Cardiac glycosides, Phenols
2	Biochemicals	Present	Calcium, Sulphate, Chloride, Iron-(Ferric, Ferrous), Phosphate, Albumin, Reducing sugar, Amino acid
		Absent	Chloride, carbonate, zinc, tannic acid

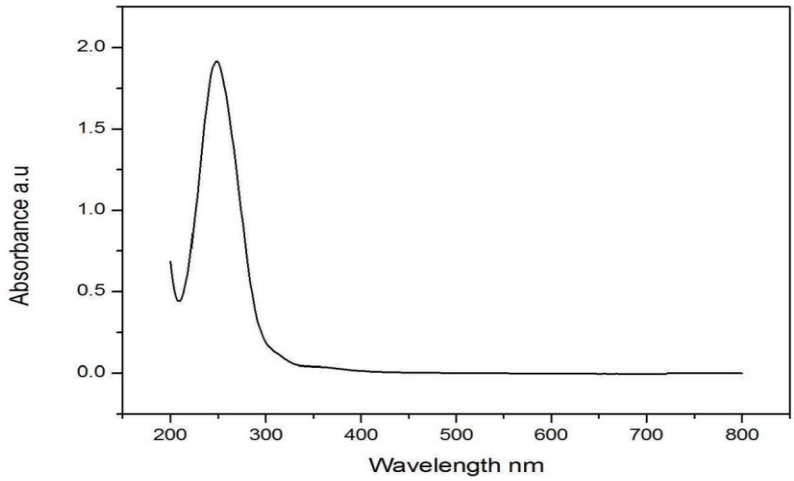
Quantitative Analysis:

Ultraviolet Spectroscopy (UV Spectroscopy):

The UV-visible spectrum of the sample was recorded and analysed using UV spectrophotometer in the range 200-800 nm. Constant deviation spectrograph emission spectrum has been recorded. Figure 1 shows the UV– Vis absorption spectrum of combination of Padikara Parpam and L-ascorbic acid. The combination drug has a distinct peak at 248 nm in the absorbance of 1.917.

Figure 1: The UV spectrum of Padikara Parpam with the combination of L- Ascorbic acid (Fourier Transform Infrared Spectra)

A total of 7 components were revealed from the FTIR analysis of combined drug of Padikara



Parpam with L-Ascorbic acid. The FTIR spectrum revealed that the drug combination showed the presence of peaks at 415.67, 469.67, 594.09, 1080.16, 1666.53, 3636.84, and 3661.92 cm^{-1} (Figure 2 and table 2).

The vibrations observed at 3661.92 cm-1 and 3636. 84 are attributed to O- H stretching vibration of primary alcoholic groups present in the glucose unit of combined drug of Padikara Parpam with L-Ascorbic acid.

The peaks showed at 1666.53 cm^{-1} is attributed to C-C stretching band mode of the alkanes of combined drug. The appearance of that band at 1080.16 cm^{-1} could be attributed to the induced vibration excitation of N-N covalent bonds. Peak at 594.09 cm^{-1} is attributed to the N-H wagging due to amine group. Peak at 469.67 and 415.67 cm^{-1} are attributed to the C-I bending vibrations of the combined drug of Padikara Parpam.

Figure 2: The FTIR spectrum of Padikara Parpam with the combination of L- Ascorbic acid

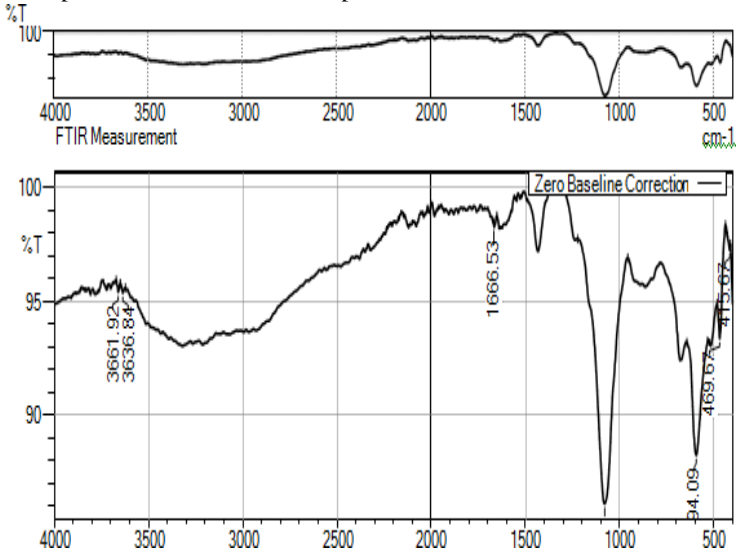


Table 2: The FTIR spectrum of combined drug (Padikara Parpam and L-Ascorbic acid)

Peak	Intensity	Correction Intensity	Base(H)	Base(L)	Mid-IR spectrum regions
415.67	97.28	0.32	428.20	412.77	Finger print region
469.67	93.37	2.60	483.18	438.81	Finger print region
594.09	88.24	4.99	644.24	534.29	Finger print region
1080.16	86.09	11.22	1223.85	956.71	Finger print region

1666.53	98.27	0.26	1669.42	1652.06	Double bond region
3636.84	95.37	0.33	3649.38	3623.34	Single bond region
3661.92	95.40	0.46	3672.53	3651.31	Single bond region

GCMS Analysis of Combined Drug Padikara Parpam and L- Ascorbic Acid

In the present study of the combined drug of Padikara Parpam with L- Ascorbic acid showed the presence of many essential compounds like Phthalic anhydride, 1,2- Benzenedicarboxylic acid, Phthalic anhydride, n-Hexadecanoic acid, Tridecanoic acid, N- Methyl-1 adamantaneacetamide, Stigmastan-3,5,22- trien and Stigmasta-5,22-dien-3-ol, acetate depicted in figure 3 and table 3.

Figure 3: GCMS Analysis of Padikara Parpam with the Combination of L- Ascorbic acid

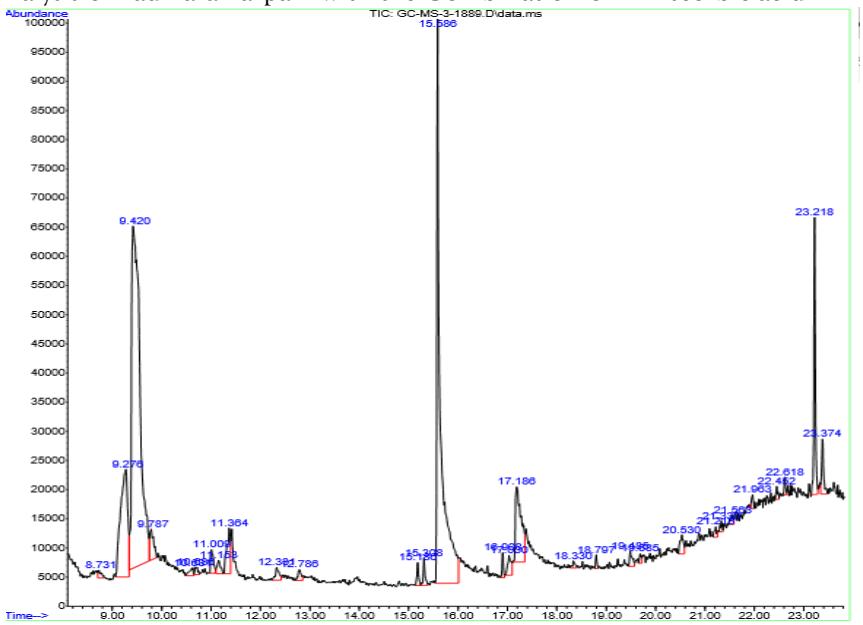


Table 3: High peaks of GC- MS analysis of phytocomponents in combined drug

Peak No	Name of the compound	Ref no	CAS no
Peak Number: 3. At 9.420 min. Area: 697133. Area % 37.57.	Phthalic anhydride	22799	000085-44-9
	1,2-Benzenedicarboxylic acid	34830	000088-99-3
	Phthalic anhydride	22800	000085-44-9
Peak Number: 14. At 15.586 min. Area: 479040. Area % 25.82.	n-Hexadecanoic acid	102726	000057-10-3
	n-Hexadecanoic acid	102724	000057-10-3
	Tridecanoic acid	70149	000638-53-9
Peak Number: 29. At 23.218 min. Area: 102593. Area % 5.53.	N-Methyl-1- adamantaneacetamide	64771	031897-93-5
	Stigmastan-3,5,22-trien	193576	1000214-18-1

	Stigmasta-5,22-dien-3-ol, acetate	208067	054482-54-1

Antimicrobial Activity:
The CLSI Standard reference bacterial strains such as Escherichia coli (ATCC 25922), Klebsiella pneumoniae (ATCC 700603), Pseudomonas aeruginosa (ATCC 27853) (figure 4 a) and Staphylococcus aureus (ATCC 29213), Streptococcus pneumonia (ATCC 49619), and Enterococcus faecalis (ATCC 29212) (figure 4b) were isolated using Blood agar for subculture the gram- positive bacterial strains and MacConkey agar for subculture the gram- negative bacterial strains.

Clinically important bacterial strains such as Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis, Acinetobacter baumannii, Proteus mirabilis, and Stenotrophomonas maltophilia were isolated using Blood agar for subculture the gram- positive bacterial strains and MacConkey agar for subculture the gram- negative bacterial strains (Figure 4c).

Figure: 4a, 4b, & 4c Isolated Gram- negative bacterial strains (ATCC) and clinically important collected bacterial strains

Figure 4a

Figure 4b

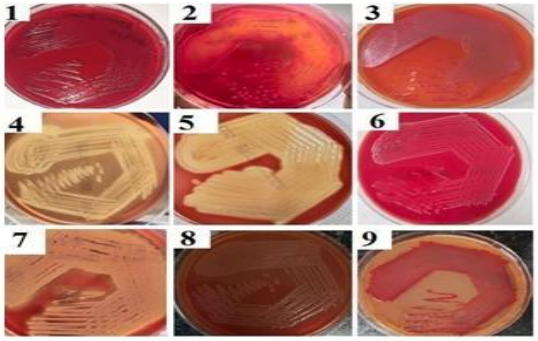
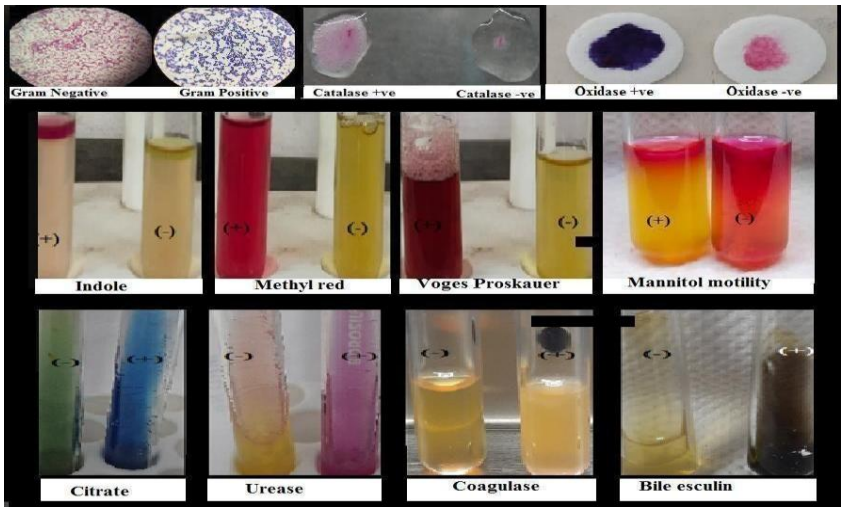


Figure 4c

Identification of Bacterial Isolates:
Isolated Gram positive and Gram negative bacterial strains were further identified by preliminary test and biochemical test (Figure 5).
Figure 5: Biochemical identification of bacteria

The Effect of Padikara Parpam (PP), L-Ascorbic Acid (LAA) and Combined Drug of Padikara Parpam with L-Ascorbic Acid (PPLAA) On Antibacterial Activity:



Antibacterial activity of Padikara Parpam, L-ascorbic acid and combined drug of Padikara Parpam with L-Ascorbic acid was tested against G +ve and G –ve bacteria like Escherichia coli (ATCC 25922), Klebsiella pneumonia (ATCC 700603), Pseudomonas aeruginosa (ATCC 27853), Staphylococcus aureus (ATCC 29213), Streptococcus pneumonia (ATCC 49619), Enterococcus faecalis (ATCC 29212) and Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis, Acinetobacter baumannii, Proteus mirabilis, and Stenotrophomonas maltophilia. The inhibitory impact of the ethanol extracts at 50 and 100 mg/ml concentrations varied in result.

Antibacterial Activity of Individual and Combined Drug of Padikara Parpam, L-Ascorbic Acid on Gram Negative Bacteria of ATCC Culture:

Gram-negative bacterial growth was inhibited by PP, LAA and PPLAA although a greater susceptibility of E. coli was apparent. The LAA showed a minimum activity against K. pneumonia in 50 mg/ml concentration. Out of three G –ve bacteria tested, E. coli and P. aeruginosa have susceptible both concentrations of drugs.

The 100mg/ml of PPLAA exhibited high antibacterial activity against E. coli (20.5±0.3), K. pneumoniae (19.5±0.3), and P. aeruginosa (17.6 ±0.2). The antibacterial activity of PP, LAA and PPLAA were shown in Figure 6 and table 4.

Figure 6: Antibacterial activity against E. coli, K. pneumoniae, & P. aeruginosa

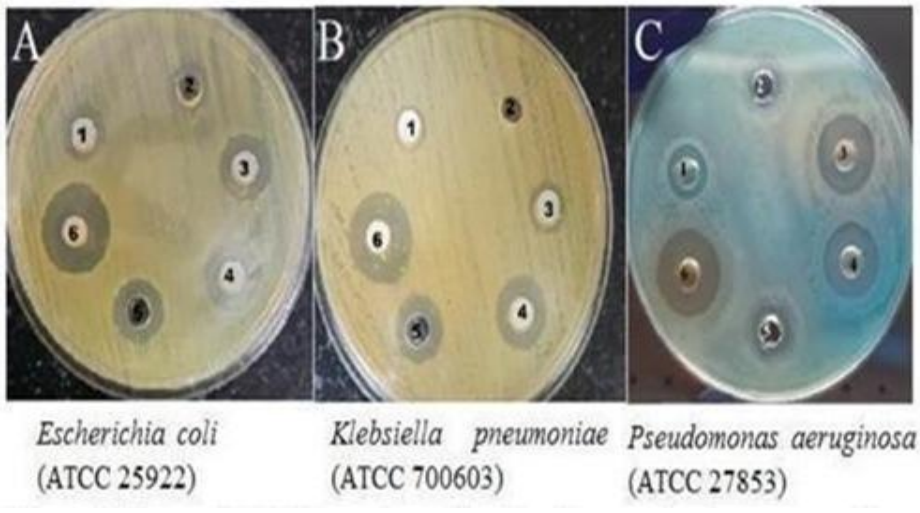


Table 4: Zone of inhibition of individual and combined drug against G Negative bacterial (ATCC)

Name of bacteria	PP		LAA		PPLAA	
	50 mg/ml	100 mg/ml	50 mg/ml	100 mg/ml	50 mg/ml	100 mg/ml

E.coli	13.7 ± 0.2	16.4 ±0.1	10.3±0.1	15.0 ±0.2	12.2± 0.2	14.8± 0.7
K. pneumonia	10.3±0.5	13.5±0.6	8.3±0.1	14.3±0.2	10.0±0.5	12.8±0.1
P. aeruginosa	12.6±0.67	20.5±0.3	12.0±0.3	19.5±0.3	15.1±0.7	17.6±0.2
Values represented mean ± SD of three independent experiments with three replicates each						

Antibacterial Activity of PP, LAA and PPLAA on Gram Positive Bacteria of ATCC Culture:

Gram positive bacterial growth was inhibited by PP, LAA and PPLAA although a greater susceptibility of S. pneumonia was apparent. The LAA showed a minimum activity against S. aureus in both 50 mg/ml and 100 mg/ml concentrations. Out of three G positive bacteria tested, S. pneumonia have susceptible both concentrations of all three drugs.

The drug, PPLAA at 100 mg/ml has shown very strong antibacterial action against S. pneumonia (24.3±0.5 mm), E. faecalis (20.3±0.7 mm) and S. aureus (19.1±0.5 mm). Figure 7 and table 5 displayed the bactericidal activity of PP, LAA, and PPLAA.

Figure 7: Antibacterial activity against E. coli, K. pneumoniae, & P. aeruginosa

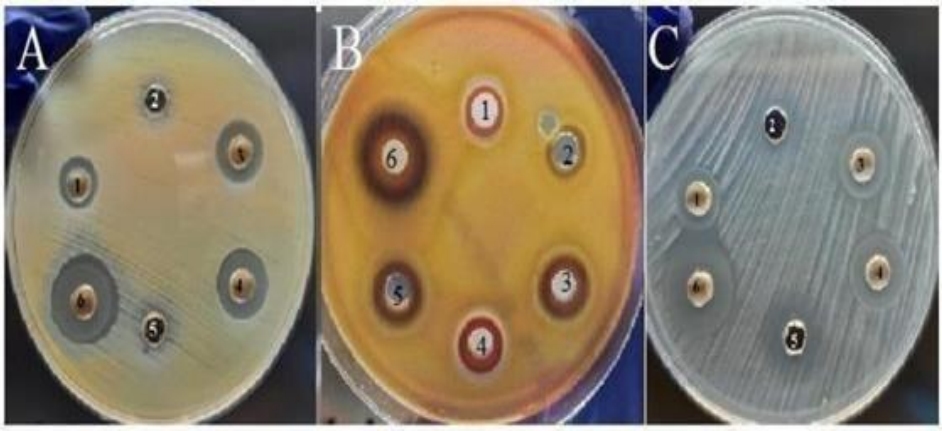


Table 5: Zone of inhibition of individual and combined drug against G positive bacterial (ATCC)

Name of bacteria	PP		LAA		PPLAA	
	50 mg/ml	100 mg/ml	50 mg/ml	100 mg/ml	50 mg/ml	100 mg/ml
S. aureus	11.2 ± 0.2	14.5 ±0.3	04.2 ±0.2	05.1 ±0.1	14.1±0.3	19.1± 0.5
S. pneumonia	11.5±0.3	16.3±0.6	12.6±0.7	17.2±0.6	15.4±0.7	24.3±0.5
E. faecalis	12.7±0.7	16.7±0.2	03.5±0.1	11.3±0.2	14.3±0.6	20.3±0.7
Values represented mean ± SD of three independent experiments with three replicates each						

Antibacterial Activity of PP, LAA and PPLAA against Clinically Isolated Bacterial Strains:
The results of antibacterial activity of PP, LAA and PPLAA were represented in Figure 8 and table 6. The PPLAA was found to be potentially active against the majority of the test microbes. The maximum antibacterial activity exhibited by PPLAA at 100 mg/ml against P. aeruginosa (28.5±0.25 mm) followed by S. aereus (27.7±0.78 mm), S. pyogenes (26.9±0.92 mm) and E. coli (24.6±0.65 mm). There is no activity registered against K. pneumonia, P. mirabilis and A. baumannii when they treated with LAA at 50 mg/ml and also 100 mg/ml of LAA was given nil effect against K. pneumonia. The drug, PP has registered the moderate activity against all pathogens. The maximum activity of PP was found at 100 mg/ml against P. aeruginosa (21.6±0.23 mm) and S. aereus (21.5±0.12 mm).

Figure 8: Zone of inhibition of individual and combination drug against clinically isolated bacterial strains

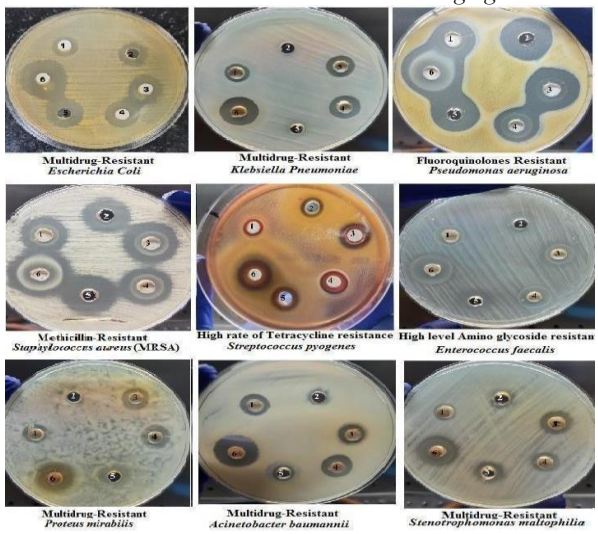


Table 6: Zone of Inhibition of Individual and Combined Drug against Clinical Pathogens

Name of bacteria	PP		LAA		PPLAA	
	50 mg/ml	100 mg/ml	50 mg/ml	100 mg/ml	50 mg/ml	100 mg/ml
E. coli	12.1±0.25	16.7±0.11	13.3±0.25	17.1±0.35	17.5±0.61	**24.6±0.65**
K. pneumoniae	11.0±0.17	15.4±0.13	0.0±0.0	0.0±0.0	14.2±0.35	18.9±0.28
P. aeruginosa	18.2±0.10	21.6±0.23	21.2±0.17	22.4±0.58	23.6±0.86	**28.5±0.25**
S. aureus	18.8±0.10	21.5±0.12	17.1±0.10	22.1±0.61	22.6±0.56	**27.7±0.78**
S. pyogens	12.4±0.12	16.8±0.17	12.7±0.12	18.2±0.35	18.8±0.29	**26.9±0.92**
E. faecalis	12.2±0.38	14.5±0.23	11.2±0.38	14.4±0.28	14.3±0.33	18.6±0.34
P. mirabilis	11.7±0.27	14.4±0.20	0.0±0.0	14.1±0.25	14.5±0.27	17.7±0.58
A. baumannii	11.6±0.11	13.4±0.12	0.0±0.0	10.0±0.17	13.5±0.35	18.1±0.68
S. maltophilia	10.4±0.59	11.3±0.37	10.1±0.10	11.2±0.14	13.8±0.27	15.1±0.27

MIC/MBC of PP, LAA and PPLAA against Gram Negative Pathogens (ATCC):
Table 7 shows the results of a screening of the MICs of PP, LAA, and PPLAA against pathogenic G-ve

bacteria. The MIC values for bacteria ranged from 0.39 to 200 mg/ml. When compared to the other two test substances, the PPLAA produced the best results. The MIC for E. coli, K. pneumonia, and P. aeruginosa was 50 mg/ml of PPLAA, whereas the MIC for these three organisms was 100 mg/ml of PP. The least value of MIC was registered in these organisms at 200 mg/ml of LAA.

Table 7: MIC/MBC of PP, LAA and PPLAA against Gram Negative pathogens (ATCC)

Name of bacteria	**PP**			**LAA**			**PPLAA**		
	E. coli	K. pneumonia	P. aeruginosa	E. coli	K. pneumonia	P. aeruginosa	E. coli	K. pneumonia	P. aeruginosa
0.39 to 25.00 mg/mL	GS	GS	GS	GS	GS	GS	GS	GS	GS
50.00 mg/mL	GS	GS	GS	GS	GS	GS	NG	NG	NG
100.00 mg/mL	NG	GS	NG	GS	GS	GS	NG	NG	NG
200.00 mg/ml	NG	NG	NG	NG	NG	NG	NG	NG	NG
MBC value	>100	>100	>100	>200	>200	>200	>50	>50	>50

GS – Growth seen NG – No growth

MIC/MBC of PP, LAA and PPLAA against G Positive Pathogens (ATCC):

Table 8 shows the results of a screening of the MICs of PP, LAA, and PPLAA against pathogenic G positive bacteria. Bacterial MIC values ranged from 0.39 to 200 mg/ml. When compared to the other two test substances, the PPLAA produced the best results. The MIC for S. aureus, S. pneumoniae, and E. faecalis was 50 mg/ml of PPLAA, whereas the MIC for these three organisms was 100 mg/ml of PP. The least value of MIC was registered in these organisms at 200 mg/ml of LAA.

Table 8: MIC/MBC of PP, LAA and PPLAA against Gram positive pathogens (ATCC)

Name of bacteria	**PP**			**LAA**			**PPLAA**		
	S. aureus	S. pneumonia	E. faecalis	S. aureus	S. pneumonia	E. faecalis	S. aureus	S. pneumonia	E. faecalis
0.39 to 25.00 mg/mL	GS	GS	GS	GS	GS	GS	GS	GS	GS
50.00 mg/mL	GS	GS	GS	GS	GS	GS	NG	NG	NG
100.00 mg/mL	NG	GS	NG	GS	GS	GS	NG	NG	NG
200.00 mg/ml	NG	NG	NG	GS	NG	NG	NG	NG	NG
MBC value	>100	>100	>100	>200	>200	>200	>50	>50	>50

GS – Growth seen NG – No growth

MIC/MBC of PP, LAA and PPLAA against Clinical Pathogens:

Table 9 shows the results of a screening of the MICs of PP, LAA, and PPLAA against clinically isolated pathogenic microorganisms. The MIC values ranged from 0.39 mg/mL to 200 mg/mL. The MIC value for the PP and LAA against E. faecalis, P. mirabilis, A. baumannii, and S. maltophilia was >200 mg/ml. The best MIC value (>50 mg/ml) was found in PPLAA against E. coli, K. pneumoniae, P. aeruginosa, S. aureus, and S. pyogenes.

Table 9: MIC/MBC of PP, LAA and PPLAA against Clinical Pathogens

Name of bacteria	PP		LAA		PPLAA	
	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	>100	>200	>200	>200	>50	>100
K. pneumoniae	>100	>200	NA	>200	>50	>100
P. aeruginosa	>100	>200	>100	>200	>50	>100
S. aureus	>100	>200	>100	>200	>50	>100
S. pyogenes	>100	>200	>100	>200	>50	>100
E. faecalis	>200	>200	>200	>200	>100	>200
P. mirabilis	>200	>200	>200	>200	>100	>200
A. baumannii	>200	>200	>200	>200	>100	>200
S. maltophilia	>200	>200	>200	>200	>100	>200

NA – No activity

DISCUSSION:

The screening for phytochemicals offers fundamental information on the therapeutic value of the siddha medicine. Natural and synthesized alkaloids have medical use, including analgesic, antispasmodic, and bactericidal properties, antioxidant activity, and usage in renal disorders.[13,14] The present findings of bioactive component of PPLAA was in other siddha drug with the previous reports; which reported presence of numerous bioactive components with beneficial pharmacological activities.[15,16,17]

The results demonstrate that all of the test medicines displayed antibacterial activity that increased with increasing concentration. In the present investigation, PPLAA has shown good antimicrobial activity against both ATCC and clinically isolated pathogens. All tested concentrations showed considerable antibacterial activity but 100 mg/ml has shown the better activity followed by 50mg/mL. The highest sensitivity of PPLAA was registered against P. aeruginosa, E. faecalis, E. coli, S. aureus and S. pyogenes. These findings clearly show that the activity of 100 mg/mL was outstanding among the two concentrations employed in the research. It has been suggested that the presence of active ingredients in medications is the main reason of antibacterial activity.[18] Similar study was reported in Padikara Parpam showed the better anti-microbial activity of Padikara Parpam against Staphylococcus petrasii Subsp. pragensis.[4] Also Shobha et al., (2014) reported that the PDP has sensitive against the tested Enterococcus SPP (E. faecalis and E.faecium).[19] The existence of antibiotic efflux systems, which confer resistance to several antimicrobial drugs, may have a role in the high prevalence of multidrug resistance.[20] The evidence of antibacterial action against bacteria suggests that the siddha drug might be used to produce medications with a broad spectrum of activity. The current findings were concurrent with the early workers and this combination drug can be used as an antibacterial agent. Future studies with larger sample size are recommended.

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