

**FORMULATION AND EVALUTION OF ANTI MICROBIAL
ACITIVITY OF TRANSDERMAL PATHES OF ETHANOLIC
EXTRACT OF PONGAMIA PINNATA SEED**

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ABSTRACT

The anti microbial acivity of ethanolic extract of dries seed of pongamia pinnata(karanja), is a popular growing tree in India, Africa, china. The plant are used as therapeutic agent such as anti inflammatory, anti cancer, anti diarrial, anti arthritis etc, The pongamia pinnata ethanolic extract was prepared maceration process the preliminary phytochemical screening was carried out by standard protocols. The antimicrobial efficacy of seeds of pongamia pinnata was carried by Kirby Bauer Agar Well Diffusion method at concentration of 100ug against selective Gram positive pathogens such as *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*. Gram negative *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*. The results in the study indicate that the anti microbial activity depends upon their type of bacteria used for the study. This study evaluate the anti microbial activity of pongamia pinnata seed extract. The crude extract of the plant showed significant activity

against tested organisms. This study also showed that the plant could be a potential source for new anti microbial agent.

KEYWORDS: Anti microbial activity, Transdermal patch, Pongamia pinnata. Screening of anti microbial activity.

INTRODUCTION

Pongamia pinnata is popularly known as karanja in Hindi, India Beech tree in English and kanuga in telugu belongs to the family Fabaceae.^[1] *Pongamia pinnata* exhibits many pharmacological activity. The Indian system of traditional medicine Ayurveda and Siddha use *pongamia pinnata* to treat various kinds of diseases including diabetes mellitus.^[2] All parts of the plant treatment of tumors, piles, skin diseases, itches, abscess, painful rheumatic joints, wounds, ulcers fodder green manure.^[3] More recently, the effectiveness of biomedicines from *pongamia pinnata* has been reported specifically as antimicrobial and therapeutic agents.^[4] The *pongamia pinnata* seed oil can be converted to biodiesel by transesterification method. The activities such as Anti inflammatory activity^[5], Anti ulceric^[6], wound healing property^[7] were reported. The literature survey on as elite medicinal plant *pongamia pinnata* showed that it is a potential medicinal plant. Since, there is no report on antimicrobial activity of seed extract of *pongamia pinnata*; the present investigation was conducted to find the anti microbial properties.

MATERIALS AND METHODS

List of chemicals

Table no 1: list of chemicals.

S.no	Chemical name	Company name
1.	Dimethylsulfoxide(DMSO)	Nice
2.	Glycerine	Marck
3.	Distilled water	Marck
4.	Hydroxylpropyl methyl cellulose(HPMC)	Nice

Collection of *pongamia pinnata* seeds

The *pongamia pinnata* seeds were collected from in and around Perambalur. collected seed are authenticated by Botanist, Department of botany, National college, Trichy. Then the seeds are cleaned properly and shade dried at room temperature.

Cold maceration process seed of *pongamia pinnata*

The collected, Cleaned and shade dried seed are subjected to the size reduction and Sieved. Then the *pongamia pinnata* extract are prepared by cold maceration process. About 40 gm of dry powdered *pongamia pinnata* are taken with 250 ml of 70% (W/V) Ethanol are maceration for week in a round bottom flask with occasional shaking.

The flask was kept in the dark to avoid effect of the light on the active constituents of the pongamia pinnata. Then the extract are filtered through a muslin cloth after a week of maceration. The extract are concentrate till dryness. The use of water bath maintain the room temperature the extract are heated for evaporation till the gryness.

Formulation of transdermal patches

Take 0.20 g of HPMC or pectin in the beaker and add 0.3ml of DMSO and 0.3ml glycerine stirrer well using then add 0.10g of extract and stirrer properly by using magnetic stirrer then add required amount of distilled water. Strirrer continuously in the magnetic stirrer for 30 minutes to dispersion the chemicals. Then pour it in the petri dish and wait for 24 hrs.^[8,9]

Table no. 2: Formulation for transdermal patches.

S. no.	Ingredients	Formulation			
		F1	F2	F3	F4
1.	seed extract (mg)	10	10	10	10
2.	Pectin(mg)	20	40	-	-
3.	HPMC(mg)	-	-	20	40
4.	DMSO(ml)	0.3	0.3	0.3	0.3
5.	Glycerine(ml)	0.3	0.3	0.3	0.3
6.	Disilled water(ml)	q.s	q.s	q.s	q.s

EVALUTION OF ANTI MICROBIAL ACTIVITY OF TRANSDERMAL PATCH MICRO ORGANISMS AND CULTURE MEDIA

Bacterial cultures such as, *Staphylococcus aureus*, *Bacillus subtilis*, *E.coli*, *Psudomona*, and *Candida albicans* were obtained from Eumic analytical Lab and Research Institute, Tiruchirappalli. Bacterial strains were maintained on Nutrient agar slants (Hi media) at 4°C.

INOCULUM PREPARATION

Bacterial cultures were sub cultured in liquid medium (Nutrient broth) at 37°C for 8h and further used for the test (10^5 - 10^6 CFU /ml). These suspensions were prepared immediately before the test was carried out.^[10, 11, 12, 13]

PREPARATION OF CULTURE MEDIA

NUTRIENT AGAR MEDIUM

Nutrient agar medium is one of the most used medium for several routine bacteriological purposes.

Ingredients : Grams/Litre
Peptone : 5gm

Beef extract	:	3gm
Agar	:	15gm
Sodium chloride	:	5gm
Yeast extract	:	1.5gm
p^H	:	7.0

After adding all the ingredients into the distilled water it is boiled to dissolve the medium completely and sterilized by autoclaving at 15lb psi pressure (121°C) for 15 minutes.

NUTRIENT BROTH

The nutrient broth was prepared by the same composition without agar. At the adding all the ingredients into the distilled water it is boiled to dissolve the medium completely and sterilized by autoclaving at 15 lb psi pressure(121°C) for 15 minutes.

ASSAY OF ANTIMICROBIAL ACTIVITY

Microbial inoculum preparation

The nutrient broth was prepared, then identified bacterial colonies were inoculated into the broth culture were used for antimicrobial activity.

KIRBY BAUER AGAR WELL DIFFUSION ASSAY

The nutrient agar medium was prepared and sterilized by autoclaving at 121°C 15 lbs pressure for 15 minutes then aseptically poured the medium into the sterile petriplates and allowed to solidify the Bacterial broth culture was swabbed on each petriplates using a sterile bud. Then wells were made by well cutter. The organic solvent used for extraction of plants is used as a negative control, gentamicin is used as a positive control. The samples (two concentration 50µg & 100µg) positive and the negative controls were added to each well aseptically.

This procedure was repeated for each Petri plates then the petriplates were incubated at 37°C for 24 hrs. After incubation the plates were observed for the zone of inhibition.

Table no. 3: Zone of inhibition of *pongamia pinnata* seed.

Name of the micro organism	Extract 100 μ l Added And Zone Of Inhibition (Mm/ML)			
	50 μ l	100 μ l	Negative Control	Positive Control
<i>Staphylococcus aureus</i>	15	20	0	25
<i>Streptococcus</i>	14	17	0	25
<i>Enterococcus faecalis</i>	20	30	0	25
<i>E.coli</i>	22	35	0	25
<i>Pseudomonas aeruginosa</i>	25	38	0	25
<i>Klebsiella pneumonia</i>	20	25	0	30

RESULT

Screening of antimicrobial activity

The prepared transdermal patch of various concentration and ethanolic extract of *pongamia pinnata* seed are exhibited for anti microbial activity against various microorganism such as Gram negative bacterial, viz *E.coli*, Gram positive bacteria *staphylococcus aureus*.

Screening of Anti microbial activity of *pongamia pinnata* seed

GRAM POSITIVE BACTERIA

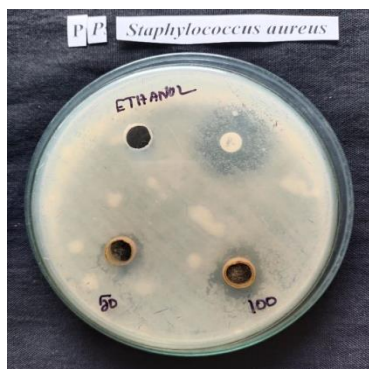


Figure No. 1: *Staphylococcus Aureus*.

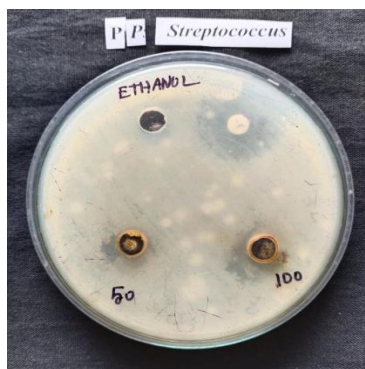


Figure No. 2: *Streptococcus*



Figure No. 3: *Enterococcus Faecalis*.

GRAM NEGATIVE BACTERIA

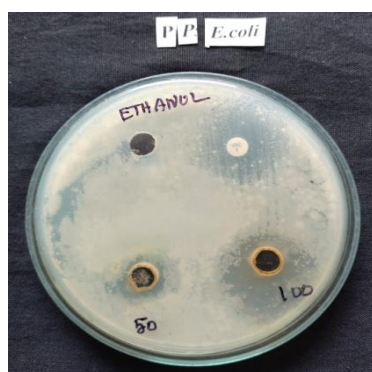


Figure. No. 4: *E.coli*.

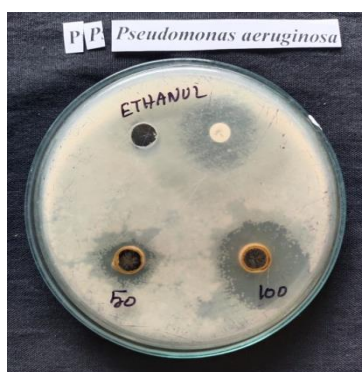


Figure no. 5: *Pseudomonas Aeruginosa*.

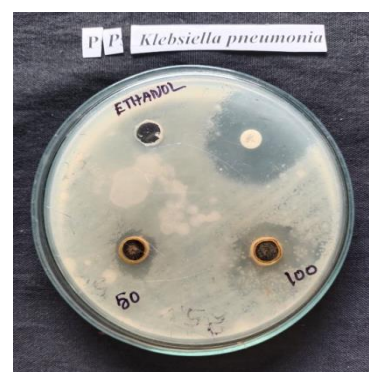


Figure no. 25: *Klebsiella pneumonia*.

CONCLUSION

The study determine the good anti microbial activity of the transdermal patch formulation containing the herbal extracts. These could make them potential anti microbial agents effective in the treatment of skin infections. The use of ethanolic extract produce a effective anti microbial property. The prepared formulation effective in bacteria. The zone of inhibition is more against bacteria agent. So the prepared transdermal patch have better antimicrobial property.

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