ANTIBACTERIAL EFFICACY OF *ALOE VERA* AND *PONGAMIA GLABRA* AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

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Introduction

The use of herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. The World Health Organization (WHO) estimates that 80 percent of the populations of some Asian and African countries presently use herbal medicine for some aspect of primary health care. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available. The annual global export value of pharmaceutical plants in 2011 accounted for over US$2.2 billion[1].

As the plants represent an extraordinary reservoir for exploration of new drugs in the battle of disease, the present study has been planned to find out the antimicrobial potential in the extracts of plants. *Aloe vera* (L.) Burm.f. (*Aloe barbadensis* Miller) is a perennial succulent xerophyte, which develops water storage tissue in the leaves to survive in dry areas of low or erratic rainfall. The innermost part of the leaf is a clear, soft, moist, and slippery tissue that consists of large thin-walled parenchyma cells in which water is held in the form of viscous mucilage.[2] Therefore, the thick fleshy leaves of aloe plants contain not only cell wall carbohydrates such as cellulose and hemicelluloses.[3]

Aloe vera leaves contain phytochemical under study for possible bioactivity, such as acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones, anthraquinones, such as emodin, and various lectins[4-6].

The objectives of the present study are:

1. To prepare the extract from the leaves of *Aloe vera* and *Pongamia glabra* with alcohol and water
2. To screen the antibacterial activity of *Aloe vera* and *Pongamia glabra* leaf extracts
3. To estimate the alkaloid content of *Aloe vera* and *Pongamia glabra* leaf extracts
4. To compare the antibacterial activity of alcoholic and aqueous extracts of *Aloe vera* and *Pongamia glabra*

Materials and Methods

Isolation and identification of test bacteria[7]

Milk samples from 4 mastitis cattle and one fecal sample from calfdiarrhea were screened for the presence of bacteria by cultivation, isolation, and identification. The samples were withdrawn with an inoculating loop aseptically and streaked on blood agar, nutrient agar and mac-conkey agar culture media plates in primary, secondary, and tertiary fashion in order to obtain isolated colonies of bacteria.

These petri plates were incubated for 24 hr at 37°C. Following incubation, the plates were observed for colonial characteristics and hemolytic zones on blood agar plates. If more than one type of colony appeared on agar plates, the different colonies were selected out and subculture separately for obtaining the pure culture of the bacterial isolates. The pure isolates were taken on nutrient agar slants and preserved in a refrigerator at 4°C until subjected to further biochemical characterization.

Preparation of alcoholic extracts[8]

The alcoholic extract was prepared by soxhlet extraction. In this process the dried powdered form of plant material was extracted using ethyl alcohol. The concentrated active constituents from plant material were kept in sterilized test tubes stored in refrigerator at 4°C for further use. The traces of ethanol were removed by keeping the tubes at 50°C for 1 hr.

Aqueous extraction[8]

Aqueous extraction was carried out by decoction process. This was carried out by boiling in hot water, in this process one part of dried powder plant and 5 part of sterilized water were taken in a boiling water flask and boiled for 15 min. after boiling the extract was filtered through a Whatmann filter paper No.1, autoclaved at 121°C for 15 min and kept in clean and sterilized test tubes and stored at 4°C till further use.

Quantitative estimation of alkaloids[9]

Five gram of each sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 48 hours. After filtration, the extracts were concentrated on a water bath to $1/4^{th}$ of the original volume. Concentrated ammonium hydroxide was added in drops to the extract until the precipitation was complete. The whole solution was collected, washed with dilute ammonium hydroxide and then filtered. The residue obtained was dried and weighed.

Demonstration of antibacterial activity

Antimicrobial activity was measured using the standard method of disc diffusion on agar and the MIC was calculated using dilution method[10].

Results and Discussion

In this study, highest alkaloid content was found in *Aloe vera* (Table 3.1). It was reported that Aloin, also known as Barbaloin, is a bitter, yellow-brown colored compound noted in the exudate of at least 68 *Aloe* species at levels from 0.1 to 6.6% of leaf dry weight (making between 3% and 35% of the total exudate) and in another 17 species at indeterminate levels[11].

It was reported that the antibacterial activity was more for the *Aloe vera* leaf extracts which might be due to its high alkaloid content. The anti bacterial activity varied according to the amount of alkaloid present (Table 3.2).

The antibacterial activity of alcoholic/aqueous leaf extracts of *Pongamia glabra* showed activity against all test organisms. It also showed more pronounced activity for gram-positive bacteria than gram-negative bacteria (Table 3.3) The percentage inhibition was higher for direct aqueous extracts than alcoholic extracts of *Pongamia glabra* (Plate 1 and 2). The anti microbial activity of karanj oil probably due to inhibition of cell membrane synthesis[3].

The inhibitory zone obtained for each microorganism showed that the antibacterial activity was more for gram-positive bacteria than gram-negative bacteria. The antibacterial activity was more for aqueous extracts than alcohol

extracts of *Aloe vera*. (Table 3.4), (Plates 3 & 4) because of high diffusion rate of the aqueous extracts through aqueous agar media. The extracts revealed more inhibitory activity for Gram positive bacteria than Gram negative bacteria. Our results of antibacterial activity are in conformity to earlier findings in which alcoholic extract of *Aloe vera* was found to be acting against all test pathogens[12].

Among the bacteria and fungi tested, *Aloe vera* possesses more inhibitory effect on the *S. aureus.* This result could be responsible for the popular use of *Aloe vera* gel and leaf to relieve many types of gastrointestinal irritations since *S. aureus* form part of the normal microbial flora of the skin, upper respiratory tract and intestinal tract[13].

Table 3.1: Percentage of alkaloid content

Name of the species	Alkaloid content %
Aloe vera	27.90
Pongamia glabra	5.38

Table 3.2: The antibacterial screening of alcoholic and aqueous extracts

Species	Extract	Relative inhibition of growth							
		B.s.	*B. c.*	*S. a.*	*S. e.*	*P. a.*	*E. c.*	*K. p.*	*P. v.*
Pongamia glabra	Alcohol	+	±	-	±	±	±	-	-
	Aqueous	+	+	±	±	±	±	±	-
Aloe vera	Alcohol	+	+	±	±	±	±	±	±
	Aqueous	+	+	+	+	+	±	±	±

Note:
+: >1.5 mm;
±: ≥ 0.7 mm;
-: 0.5 mm

Gram-positive : *B.s.: Bacillus subtilis,*
B.c.: Bacillus coagulans,
S.a - Staphylococcus aureus,
S.e.: Staphylococcus epidermis
Gram-negative : *P.a.: Pseudomonas aeruginosa,*
E.c.: Eschericia coli,
K.p.: Klebsiella pneumonia,
P.v.: Proteus vulgaris

Table 3.3: Antibacterial screening of alcoholic/aqueous extracts of *Pongamia glabra*

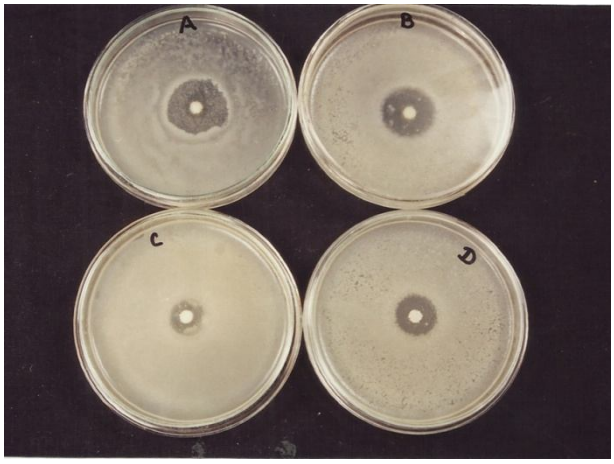
Species	Part Used	Name of the Microorganisms	Diameter of zone of inhibition (cm)	
			Alcohol Extract	Aqueous Extract
Pongamia glabra	Leaf	**Gram-positive:**		
		Bacillus subtilis	1.5	1.9
		Bacillus coagulans	1.2	1.8
		Staphylococcus aureus	0.8	1.4
		Staphylococcus epidermis	0.9	1.4
		Gram-negative:		
		Pseudomonas aeruginosa	1.0	1.2
		Eschericia coli	0.4	1.0
		Klebsiella pneumonia	0.7	0.9
		Proteus vulgaris	0.3	0.4

Table 3.4: Antibacterial screening of alcoholic/aqueous extracts of *Aloe vera*

Species	Part Used	Name of the Microorganisms	Diameter of zone of inhibition (cm)	
			Alcohol Extract	Aqueous Extract
Aloe vera	Leaf	**Gram-positive:**		
		Bacillus subtilis	1.7	4.2
		Bacillus coagulans	1.7	3.8
		Staphylococcus aureus	1.2	3.6
		Staphylococcus epidermis	1.0	3.4
		Gram-negative:		
		Pseudomonas aeruginosa	1.4	2.6
		Eschericia coli	1.2	1.6
		Klebsiella pneumonia	1.0	1.4
		Proteus vulgaris	0.9	1.2

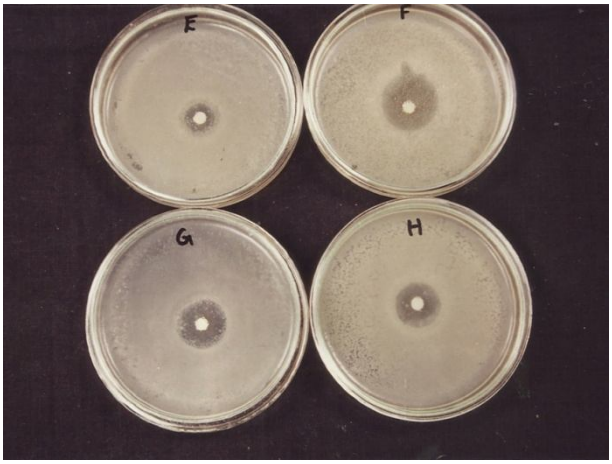
Plate 1

Disc Diffusion assay: Anti bacterial effect of alcoholic extract of *Pongamia glabra* against gram-positive organisms



A - *Bacillus subtilis*
B - *Bacillus coagulans*
C - *Staphylococcus aureus*
D - *Staphylococcus epidermis*

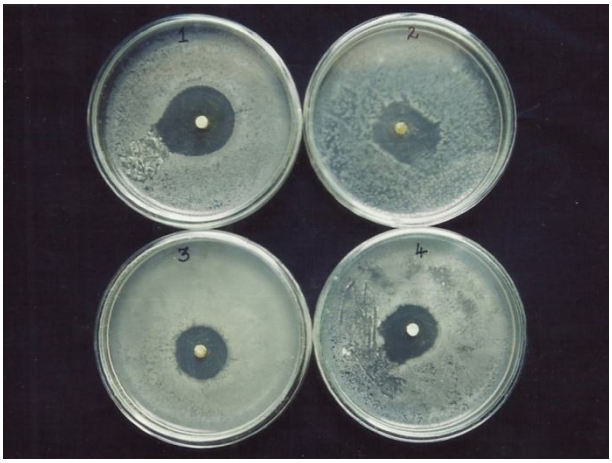
Anti bacterial effect of alcoholic extract of *Pongamia glabra* against gram-negative organisms



E - *Pseudomonas aeruginosa*
F - *Eschericia coli*
G - *Klebsiella pneumonia*
H -*Proteus vulgaris*

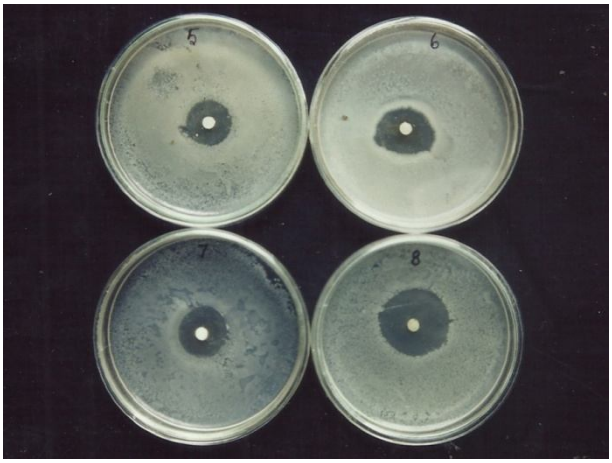
Plate 2

Anti bacterial effect of aqueous extract of *Pongamia glabra* against gram positive organisms



1. *Bacillus subtilis*
2. *Bacillus coagulans*
3. *Staphylococcus aureus*
4. *Staphylococcus epidermis*

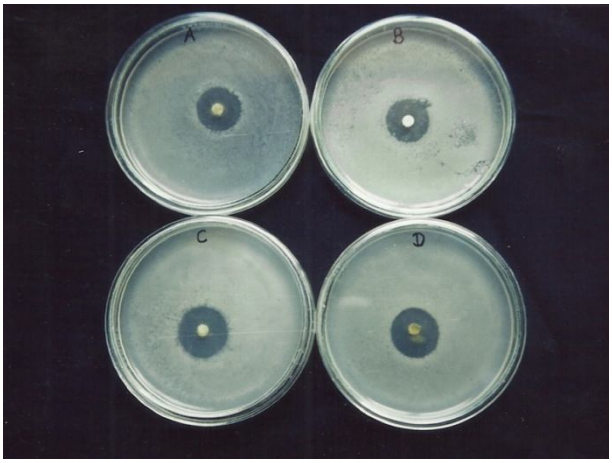
Anti bacterial effect of aqueous extract of *Pongamia glabra* against gram negative organisms



5. *Pseudomonas aeruginosa*
6. *Eschericia coli*
7. *Klebsiella pneumonia*
8. *Proteus vulgaris*

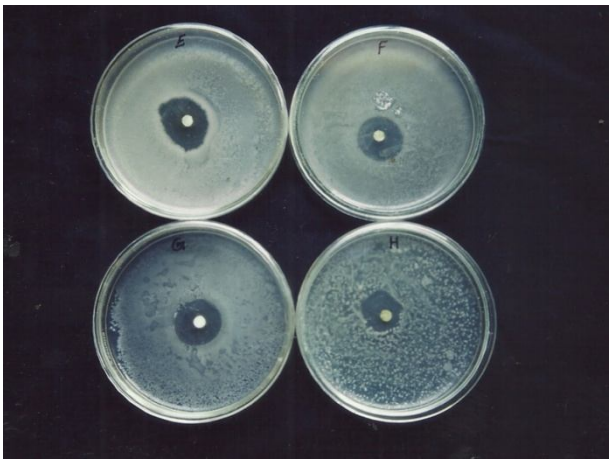
Plate 3

Anti bacterial effect of alcoholic extract of *Aloe vera* against gram-positive organisms



A - *Bacillus subtilis*
B - *Bacillus coagulans*
C - *Staphylococcus aureus*
D - *Staphylococcus epidermis*

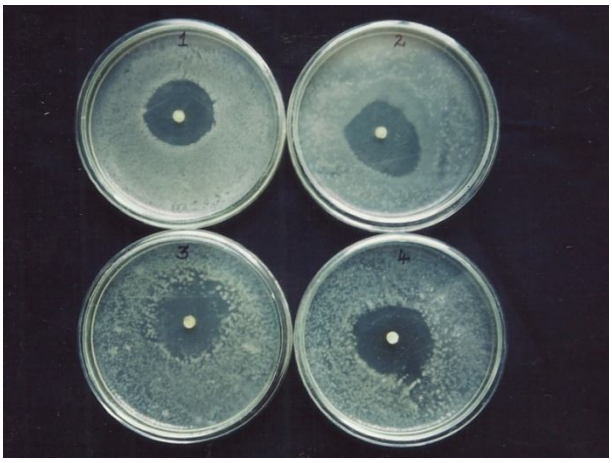
Anti bacterial effect of alcoholic extract of *Aloe vera* against gram-negative organisms



E - *Pseudomonas aeruginosa*
F - *Eschericia coli*
G - *Klebsiella pneumonia*
H - *Proteus vulgaris*

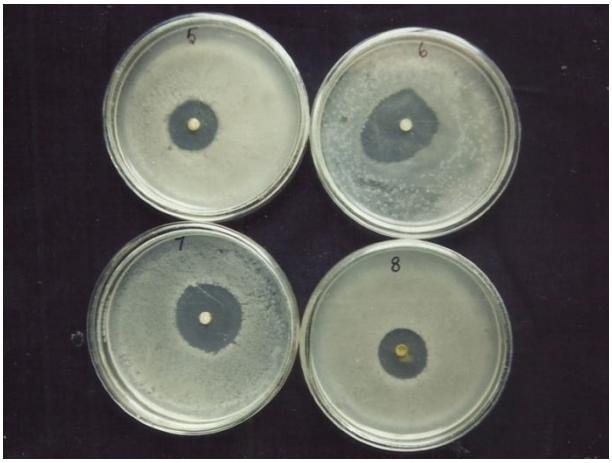
Plate 4

Anti bacterial effect of aqueous extract of *Aloe vera* against gram positive organisms



1. *Bacillus subtilis*
2. *Bacillus coagulans*
3. *Staphylococcus aureus*
4. *Staphylococcus epidermis*

Anti bacterial effect of aqueous extract of *Pongamia glabra* against gram negative organisms



5. *Pseudomonas aeruginosa*
6. *Eschericia coli*
7. *Klebsiella pneumonia*
8. *Proteus vulgaris*

Conclusion

Antimicrobial resistance is a global problem. Emergence of multidrug resistance has limited the therapeutic options. Hence monitoring resistance is of paramount importance. Antimicrobial resistance monitoring will help to review the current status of antimicrobial resistance locally, nationally and globally and helpful in minimizing the consequence of drug resistance. Hence the present study was aimed to focus the antibacterial properties of *Pongamia glabra* and *Aloe vera*.

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