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Comparative antibacterial activity of *Aloe barbadensis miller* and *Azadirachta indica* against bacterial isolates obtained from acne patients

Musarrat Sharif[1*], Arif Malik[1], Mehwish Shaheen[1]

[1]*Institute of Molecular Biology and Biotechnology, University of Lahore, Lahore, Pakistan*

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Abstract

Propionibacterium acnes and *Staphylococcus epidermidis* are pus forming bacteria that trigger inflammation in acne while *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the supportive agents for acne. The present study was conducted to compare the anti-acne activity of *Azdirachta indica* and *Aloe barbadensis miller*. Various extracts of *Azadiracta indica* and *Aloe barbadensis miller* were tested for antimicrobial activity by disc diffusion method and well diffusion method. It is concluded that *Aloe barbadensis miller* extracts has greater zone of inhibition against *P. aeruginosa* and *S. aureus* in comparison to *Azadirachta indica* extracts. On the other hand *Aloe barbadensis miller* extracts and *Azadirachta indica* extracts showed the maximum zone of inhibition against acne causing agents e.g. *S. epidermidis* and *P. acnes*. So, *Azadirachta indica* has better choice for anti-acne activity as compared to *Aloe barbadensis miller*.

* **Corresponding Author:** Musarrat Sharif ✉ musarratsharif38888@gmail.com

Introduction

Azadirachta indica also known as 'Nimtree' and 'Indian lilac' is a tree that belongs to '*Milliaceae*' family. It is native to India, Pakistan, Nepal, Bangladesh and Srilanka. It has height of 15-20m and is fast growing tree. Leaves of the *Azadirachta indica* has length of 20-40cm and pinnate like structure. The flowers are 25cm long and are arranged in drooping form. *Azadirachta indica* tree grows in tropical and semi tropical regions and is drought resistant. The branches of *Azadirachta indica* are wide and diverse. The triterpenoids, phenolic compounds, carotenoids, steroids and ketones alkaloids, flavonoids are all chemical constituents of *Azadirachta indica* contain many bioactive compounds (Verkerk and Wright, 1993). Other compounds present in *Azadirachta indica* that have biological activity include nimbidic acid, azadirachtin salannin, volatile oils, meliantriol and nimbin (Ahana *et al*., 2005).

Azadirachta indica tree also known as the wonder tree for centuries in the Indian subcontinent. US Environmental Protection Agency has approved *Azadirachta indica* for use on food crops and is considered useful to humans, animals, birds, beneficial insects and earthworms. *Azadirachta indica* is used in dermatitis, eczema, acne, bacterial, fungal infections and other skin disorders.

It has demonstrated its effectiveness as a powerful antibiotic. Now a day, *Azadirachta indica* and its extracts are used in numerous herbal and allopathic medicines. Beside this *Azadirachta indica* contraceptives are also available in the market these days. *Azadirachta indica* extracts which have nimbinin, nimbandiol as active constituents; alcoholic extract of the leaves was found to possess a significant blood sugar lowering effect which is very useful against diabetes. It helps to support immune system and is used in cases of inflammatory skin conditions. Traditionally *Azadirachta indica* has been used for skin and blood purifying conditions. *Azadirachta indica* not only helps in curing diseases but it also provides us with the strength of fighting diseases by enhancing our immunity (Debjit Bhowmik *et al*., 2010).

Aloe barbadensis miller grows up to 10-12cm and short stemmed plant. It belongs to lily family i.e. *Liliaceae*. *Aloe barbadensis miller* plant leaves are green in color and structure is fleshy, sharp, thick and spiny leaves. The *Aloe barbadensis* miller plants are mostly green in warm tropical areas because they cannot survive at freezing temperatures. More than 250 species of *Aloe barbadensis miller* are grown all over the world out of which two species are grown commercially namely *Aloe arborescence* and *Aloe barbadensis* also known as *Aloe barbadensis miller* (Yates, 2002). Mostly phytochemicals are present in the *Aloe barbadensis miller* leaves such as polymannans, acetylated mannans, anthraquinone C glycosides, anthrones, anthraquinones such as emodin and various lectins (Boudreau and Beland, 2006). *Aloe barbadensis miller* is a typical xerophyte with thick fleshy, strangely circularized spiny leaves. It has been promoted for large variety of conditions and has come to play a prominent role as a contemporary folk remedy (Khurram Shahzad *et al.,* 2009). The use of natural remedies is highly approached in human health, in particular drugs and cosmetics with an ongoing search for novel biologically active botanical agents. Since many years ago *Aloe barbadensis miller* has been used therapeutically in skin infections. *Aloe barbadensis miller* leaves contain mucilaginous tissue in the center of the leaf which is called *Aloe vera* gel (AVG) used in cosmetics and some medicinal products. Its pharmacological actions include anti-inflammatory, anti-irritant, healing of wounds and antibacterial effects. *Aloe barbadensis miller* and some other herbal extracts that have antibacterial activity against *P. acnes* are used in Ayurvedic formulation (Zohreh *et al*., 2014).

The aim of the present study was to perform a comparison of anti-acne activity of *Azadirachta indica* and *Aloe barbadensis miller* extract by dilution in different solvents. Collection of acne samples from different male and female acne patients.

Materials and methods

Collection of acne sample

Fifty samples of acne pus were taken from female and fifty samples from male acne patients randomly. The samples were taken in the form of swabs for bacterial and fungal isolation. The samples were processed to the microbiology lab for further analysis.

Isolation and purification of acne sample

The samples (pus from acne) were collected with sterile precautions by using sterile cotton swabs. Swabs were placed in sterile container and transported to lab immediately. Then these cotton swabs were swabbed on the nutrient agar plates and incubated for 24 hrs at 37°C.

The purification of mixed bacterial colonies were done by streaking on the nutrient agar plates for 24 hrs at 37°C (Uraku *et al.,* 2012). Using a sterile microbiological loop, the inoculums were sub-cultured evenly on other selective and differential media's Mannitol Salt Agar (MSA), Eosin Methylene Blue (EMB), Salmonella Shigella agar (SS-agar), Pseudomonas Cetrimide agar, Blood agar and MacConkey agar from pure culture. All the plates were incubated aerobically in an incubator at 37°C for 24 hours by streak plate method. After incubation, plates were examined for growth. These sub-cultured plates were then used in the identification and characterization of the organisms by microscopically and Biochemically (Ayandele *et al.*, 2011).

Anaerobic culture*:* Samples were swabbed on Brucella blood agar enriched with 5 per cent sheep blood, 5 mg/l haemin, 1 mg/l vitamin K1, after incubation at 37°C in an anaerobic incubator (85% N_2, 10% H_2, 5% CO_2) for *P. acne* (Murali *et al.,* 2015).

Samples were swabbed on Sabouraud dextrose agar/Potato dextrose agar for the growth of fungi and incubated at 25°C for seven days.

The individual colonies of bacterial and fungal isolates were examined for their macroscopic traits such as color, size and morphology.

Microscopy

The microscopic morphology and arrangement of purified bacteria were examined under 100X lens using gram staining, spore staining, Ziehl-Neelsen staining and capsule staining (Cappucino and Sherman, 2007). The microscopic morphology and arrangement of fungi were mounted on Lacto phenol cotton blue stain and identification of the fungal species were performed with aid of binocular compound microscope (40X) adopting the techniques (Manandhar *et al.*, 1995; Cappucino and Sherman, 2007; Bruge *et al.*, 2003).

Characterization of isolated organism

Different biochemical tests such as catalase test, coagulase test, oxidase test, urease test, indole production test, methyl red test were done for confirmation of isolated bacterial cultures on species level according to protocols described previously (Cheesbrough, 2000; Cappucino and Sherman, 2007).

Preparation of various extracts of Azadirachta indica and Aloe barbadensis miller

Plant material Collection and Identification

*Azadirachta indica*fresh leaves and *Aloe barbadensis miller* leaves were collected randomly from semi-arid regions of Lahore and Muridkey, Punjab.

Preparation of various extracts of Azadirachta indica

Five kilogram leaves of *Azadirachta indica* were washed and dry in shade for three to four days. Dried leaves then crushed by pestle and mortal. The 10g was dissolved in 100 ml of different organic solvents including pure, ethanol, acetone and methanol and crude extracts were prepared by maceration.

The crushed macerated parts of *Azadirachta indica* were placed at magnetic stirrer at 37°C and equally mixed in organic solvents by using magnet for 30 minutes. The conical flasks of these extracts were covered by cotton plugs to avoid the solvent evaporation. The extracts were placed in shaking incubator at 250 rpm for 48 hours. After shaking,

they were filtered with muslin cloth. The filtered extracts of the *Azadirachta indica* leaves were centrifuged at 8000 rpm for 20 minutes. The supernatants were collected in sterile flask. The filtered extracts were stored at 4°C (Irshad *et al.,* 2011).

Preparation of various extracts of Aloe barbadensis miller

10g fresh *Aloe barbadensis miller* leaves with gel were dried in the oven at 50°C for 48 hours and then powder. In the process of maceration, 10g of crushed plant part were dissolved in 100 ml of organic solvent i.e. ethanol, methanol acetone distilled water and dimethyl sulfoxide (DMSO). The conical flask was covered by cotton plugs to avoid solvent evaporation. The extracts were placed in shaking incubator at 250 rpm for 48 hours. After shaking, it was filtered with muslin cloth. The filtered extract was centrifuged at 8000 rpm for 20 minutes.

The supernatant was collected in sterile flask. Then, it was stored at 4°C. The agar disc-diffusion assay was performed by diffusing the *Aloe barbadensis miller* extract from a paper disc that contains test microorganisms (Saba *et al.,* 2011).

Isolation of test microorganisms from acne patients

Plants were used to obtain various extracts for in vitro study of antimicrobial activity. Microbial strains of different bacteria were isolated from different acne patients. The strains isolated were aerobic bacteria such as *Pseudomonas aeruginosa* and *S. aureus*. Anaerobic bacterial strains included *Propionibacterium acnes*. The strains were preserved at 4°C in the form of 40% glycerol stocks.

Culture media preparation

Muller Hinton media was prepared according to the instructions given by the manufacturer as 38 g of MH media was dissolved in 1000 ml of distilled water. The media was stirred for one minute and sterilized in an autoclave at 121°C at 15 lb/in² pressure for 15 minutes. The media was cooled at room temperature and poured into sterilized petri plates.

Preparation of inoculums

Inoculation was done by taking loop full of test organisms on medium of Muller Hinton agar. The media was kept in an incubator for 24 hrs and the temperature was maintained at 37°C along with sterile conditions. Inoculum was prepared in normal saline and compared to 0.5 McFarland standards. A 0.5 McFarland standard is prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate ($BaCl._2 \cdot 2H_2O$), with 9.95 mL of 1% sulfuric acid (H_2SO_4).

Disc diffusion method

Test microorganisms were inoculated in Muller Hinton agar plates by spread plate method for making smooth lawn. Small discs of filter paper having a diameter of approximately 6mm were soaked in 15µl of plant extracts and placed on the inoculated petri plates. The discs were pressed gently to ensure complete contact with the media. The plates were kept in an incubator for 24hrs at 37°C. Each plate was examined after 24hrs for the zone of inhibition. The diameters of the zones of complete inhibition were measured including the diameter of the disc where azithromycin was used as positive control.

Well Diffusion method

The antibacterial activities of different strains were determined by modified agar well diffusion method. In this method, nutrient agar plates were seeded with 0.2 ml of 24 h broth culture of *Propionibacterium acnes,* a causative organism for *Acne vulgaris*. The agar plates were allowed to solidify. A sterile 8 mm borer was used to cut wells of equidistance in each of the plates. 0.5 ml of formulations, herbal extracts and marketed clindamycin gel were introduced into the wells at randomly. The plates were incubated anaerobically at 37°C for 24 hours. The antibacterial activities were evaluated by measuring the zones of inhibition (in mm).

Results and discussion

Collection of acne samples from different males and females patients

Acne samples were collected from different number of males and females patients. Bacterial isolates from

acne samples were *S. epidermidis, S. aureus, P. aeruginosa* and *P. acnes*. *S. epidermidis* have greater percentage in females than male's patients. *S. aureus* was more percentage in males than female's patients. *P. aeruginosa* was more in males than female's patients. Frequency of males and females patients is given below in the Table 1.

Table 1. Bacterial isolates and frequency of male and female patients.

Sr. no.	Bacterial isolates	Frequency of males patients (n=50)		Frequency of females patients (n=50)	
		Frequency	Percentage	Frequency	Percentage
1	*S. epidermidis*	25	50%	38	76%
2	*S. aureus*	45	90%	15	30%
3	*P. aeruginosa*	25	50%	10	20%
5	*P. acnes*	25	50%	25	50%

Table 2. Bacterial isolates results.

Tests	Bacterial isolates results			
	Staphylococcus epidermidis	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Propionibacterium acnes*
Morphology on nutrient agar	Abundant opaque golden growth, round	Abundant thin white growth with green colour	Slimy light greenish shade translucent raised growth	NA
Oxygen requirement	Aerobic	Aerobic	Aerobic	Anaerobic
Gram staining	Gram +ve cocci, cluster	Gram +ve, cocci, Cluster	Gram –ve Rods	Gram +ve, branched and unbranched rods, coccoid forms
Growth on MSA	White growth pink media	Yellow growth yellow media	No growth	NA
Growth on EMB	No growth	No growth	Mucoid green colour	NA
Growth on SS Agar	No growth	No growth	No growth	NA
Growth on cetrimide	No growth	No growth	Yellowish green color	NA
Growth on Blood agar	NA	NA	NA	Convex, semi opaque, and glistering
Catalase	+ve	+ve	+ve	+ve
Coagulase	-ve	+ve	-ve	-ve
Oxidase	-ve	-ve	+ve	-ve
Indole	-ve	-ve	-ve	+ve
Methyl red	+ve	+ve	-ve	+ve
Citrate	-ve	-ve	+ve	-ve
Urease	+ve	-ve	+ve	-ve
H_2S	+ve	-ve	-ve	-ve
Nitrate	+ve	+ve	+ve	+ve

Muddu *et al.* (2015) determined the bacteria involved in acne vulgaris and also determine the in vitro antibiotic sensitivity of aerobic isolates. In this study acne affected individuals were young males, especially students, with pustules being the commonest presentation. Males were more affected than females. Pustules (76.25%) were the commonest presentation. Students (71.25%) were more commonly affected. Among aerobic isolates (71.24%), *Staphylococcus epidermidis* (54.38%) was the most common organism. Among the anaerobes, *Propionibacterium acnes* (55.17) were the most common. *Staphylococcus epidermidis* was more the dominant organism.

Morphological and biochemical test were performed for the confirmation of isolated samples e.g. *S .epidermidis, S. aureus, P. aeruginosa* and *P. acnes*, *S. epidermidis* given below in the Table 2.

As per Table 3, it is concluded that 20g ethanol extract of *Azadirachta indica* showed maximum zone of inhibition against *Propinibacterium acnes* that is

19.3mm. Acetone showed second best results against *Propionibacterium acnes* in 20g and 10g extracts that is 18.8mm and 18mm. Almost all the extracts in 1gm showed zone of inhibition relative to the +ve control was within the range. So, all the extracts in 20g produce best results when compared with standard and 10g extract results were also acceptable according to standard results.

Table 3. Zone of inhibition of various extracts of *Azadirachta indica* against *Propionibacterium acnes*.

Zone of inhibition of various extractsof *Azadirachta indica* extracts against *Propionibacterium acnes*			
Solvent (100ml)	*A. indica* (1gm)	*A. indica* (10gm)	*A. indica* (20gm)
	Zone of inhibition		
Ethanol	11mm	14.7mm	18.8mm
Methanol	8.6mm	10.5mm	12.2mm
Acetone	16.4mm	18mm	18.2mm
DMSO	16mm	17.6mm	18.5mm
Water	9mm	18mm	19.3mm
+ve control	17mm	17mm	17mm

Table 4. Zone of inhibition of various extract of *Azadirachta indica* against *Pseudomonas aeruginosa*.

Zone of inhibition of various extract of *Azadirachta indica* against *Pseudomonas aeruginosa*			
Solvent 100ml	*A. indica* (1gm)	*A. indica* (10gm)	*A. indica* (20gm)
	Zone of inhibition		
Ethanol	7.8mm	8.8mm	17mm
Methanol	9mm	9.5mm	14.8mm
Acetone	9mm	12.3mm	15.3mm
DMSO	0	12mm	20mm
Water	6.6mm	10.4mm	13.5mm
+ve control	10mm	10mm	10mm

Kalpesh *et al.* (2012) studied that development and evaluation of herbal anti-acne formulation**.** In this study leaves of *Azadirachta indica* were cut into small pieces. Fruits of nutmeg were crushed to make powder. Desired quantities of herbal drugs were weighed and were individually added to the conical flask containing five times volume of 1:1 water-ethanol mixture.

Table 5. Zone of inhibition of various extracts of *Azadirachta indica* against *S.aureus*.

Zone of inhibition of various extracts of *Azadirachta indica* against *S. aureus*			
Solvent 100ml	*A. indica* (1gm)	*A. indica* (10gm)	*A.indica* (20gm)
	ZONE OF INHIBITION		
Ethanol	0	10.3mm	16.8mm
Methanol	0	10.2mm	15mm
Acetone	9mm	13mm	15mm
DMSO	0	12mm	15.3mm
Water	0	0	21.5mm
+ve control	12mm	12mm	12mm

Contents were allowed to boil on water bath under reflux condition for about 30 min. Contents were filtered out and residues were again boiled with five times volume of 1:1 water-ethanol mixture on water bath under reflux condition for about 15 min. Contents were filtered out and filtrates were combined. Filtrate was allowed to evaporate in evaporating pan until the desired concentration of the extract was obtained**.** Inhibitory zone of the *Azadirachta indica* was measured 8mm**.**

Table 6. Zone of inhibition of various extracts of *Azadirachta indica* against *S. epidermidis*.

Zone of inhibition of various extractsof *Azadirachta indica* against *S. epidermidis*			
Solvent 100ml	*A.indica* (1gm)	*A.indica* (10gm)	*A. indica*(20gm)
	ZONE OF INHIBITION		
Ethanol	9.4mm	16.8mm	18mm
Methanol	8.6mm	13.5mm	17.4mm
Acetone	9mm	15mm	17.3mm
DMSO	9.3mm	10.8mm	24mm
Water	9mm	12.3mm	17.8mm
+ve control	15mm	15mm	15mm

Table 7. Zone of inhibition of various extracts of *Aloe barbadensis miller* against *P. acnes*.

Zone of inhibition with different conc. of *Aloe barbadensis miller* powder against *Propionibacterium acnes*			
Solvent 100ml	*A.vera* (1gm)	*A.vera* (10gm)	*A.vera* (20gm)
	ZONE OF INHIBITION		
Ethanol	9mm	14.3mm	19mm
Methanol	10.6mm	13mm	15.1mm
Acetone	9.3mm	16mm	17.6mm
DMSO	0mm	12.6mm	14.2mm
Water	7.6mm	11.3mm	15mm
+ve control	13mm	13mm	13mm

Charde *et al.* (2014) research on the development and evaluation of herbal formulation for the treatment of acne. In vitro antibacterial activity was performed against *P. acnes, S. epidermidis and S. aureus,* using agar well diffusion method. The measured zones of inhibitions of the prepared formulations were compared with standard antibiotic (Clindamycin) and standard marketed topical herbal formulation. Results shown the zone of inhibition was 20mm with extract of *Azadirachta indica* 20mg per ml. According to the Table, it is concluded that 1g of *Azadirachta indica* extract of methanol and acetone solvent extract produce the same zone of inhibition against that is 9mm. Ethanol and methanol extract of 10g *Azadirachta indica* showed similar zone of inhibition that was 9mm.

Table 8. Zone of inhibition of various extracts of *Aloe barbadensis miller* against *Pseudomonas aeruginosa*.

Zone of inhibition of various extracts of *Aloe barbadensis miller* against *Pseudomonas aeruginosa*			
Solvent 100ml	*A. vera* (1gm)	*A.vera* (10gm)	*A.vera* (20gm)
	ZONE OF INHIBITION		
Ethanol	14.2mm	16.3mm	18.2mm
Methanol	9mm	9.4mm	9.5mm
Acetone	9.5mm	14mm	18mm
DMSO	0mm	18mm	18.7mm
Water	8.4mm	11.6mm	15mm
+ve control	9mm	9mm	9mm

The maximum zone of inhibition of 20mm of diameter was produced by 20g of *Azadirachta indica* extract of DMSO solvent; on the other hand 1g of DMSO extract has no zone of inhibition. 20g of *Azadirachta indica* extract in methanol and acetone showed the results that was 14.8mm and 15.3mm. The second best results were produced by 20g *Azadirachta indica* extract of ethanol that 17mm.

Table 9. Zone of inhibition of various extracts of *Aloe barbadensis miller* against *S. aureus*.

Zone of inhibition of various extracts of *Aloe barbadensis miller* against *S. aureus*			
	A.vera (1gm)	*A.vera* (10gm)	*A.vera* (20gm)
	ZONE OF INHIBITION		
Solvent 100ml	1mg	10mg	20mg
Ethanol	0	0	0
Methanol	0	0	11
Acetone	0	0	8.9
DMSO	0	0	0
Water	0	0	0
+ve control	42	42	42

Table 10. Zone of inhibition of various extracts of *Aloe barbadensis miller* against *S. epidermidis.*

Zone of inhibition of various extracts of *Aloe barbadensis miller* against *S. epidermidis*			
Solvent 100ml	*A. vera* (1gm)	*A. vera* (10gm)	*A. vera* (20gm)
	ZONE OF INHIBITION		
Ethanol	0	7.8	13.2
Methanol	0	0	11.5
Acetone	0	0	11.6
DMSO	0	6.2	9
Water	0	6	8.3
+ve control	24	24	24

Autade *et al.* (2015) investigated effect of *Azadirachta indica* extract against opportunistic bacterial and fungal pathogens associated with AIDS**.** There were the comparative results of the zones of the inhibition formed by the acetone and chloroform extract prepared from different *Azadirachta indica* plant parts against bacterial and fungal opportunistic pathogens tested by disc diffusion assay. *Azadirachta indica* leaf extract 30mg, showed highest zone of inhibition (20mm) against *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Table 11. Synergistic statistical results of *Aloe barbadensis miller* against acne causing bacteria with various solvents.

Solvent /bacteria	*P. acnes*	*S. epidermidis*	*S. aureus*	*P. aeruginosa*	Mean
		01 mg			
Ethanol	9	0	0	14.2	5.8
Methanol	10.6	0	0	9	4.9
Acetone	9.3	0	0	9.5	4.7
DMSO	0	0	0	0	0
Water	7.6	0	0	8.4	4
+ve control	13	24	42	9	22
		10 mg			
Ethanol	14.3	7.8	0	16.3	9.6
Methanol	13	0	0	9.4	5.6
Acetone	16	0	0	14	7.5
DMSO	12.6	6.2	0	18	9.2
Water	11.3	6	0	11.6	7.225
+ve control	13	24	42	9	22
		20mg			
Ethanol	19	13.2	0	18.2	12.6
Methanol	15.1	11.5	11	9.5	11.775
Acetone	17.6	11.6	8.9	18	14.025
DMSO	14.2	9	0	18.7	10.475
Water	15	8.3	0	15	9.575
+ve control	13	24	42	9	22

As per Table 5, it is concluded that the solvent extract of ethanol, methanol and DMSO and water of 1g and 10g *Aloe barbadensis miller* showed no zone of inhibition. On the other hand 20g of *Aloe barbadensis miller* extract in water produce maximum diameter of zone of inhibition that is 21.5mm.

Table 12. Synergistic statistical results of *Azadirachta indica* against acne causing bacteria with various solvents.

Conc.	Bacteria/ solvents	*P. acnes*	*S. epidermidis*	*S. aureus*	*P. aeruginosa*	Mean
1mg	Ethanol	11	9.4	0	7.8	7.05
	Methanol	8.6	8.6	0	9	6.55
	Acetone	16.4	9	9	9	10.85
	DMSO	16	9.3	12	0	9.325
	Water	9	9	0	6.6	6.15
	+ve control	17	15	12	10	13.5
10mg	Ethanol	14.7	16.8	10.3	8.8	12.65
	Methanol	10.5	13.5	10.2	9.5	10.925
	Acetone	18	15	13	12.3	14.575
	DMSO	17.6	10.8	12	12	13.1
	Water	18	12.3	0	10.4	10.175
	+ve control	17	15	12	10	13.5
20mg	Ethanol	18.8	18	16.8	17	17.65
	Methanol	12.2	17.4	15	14.8	14.85
	Acetone	18.2	17.3	15	15.3	16.45
	DMSO	18.5	24	15.3	20	17.15
	Water	19.3	17.8	21.5	13.5	18.025

Table 13. Statistic results of Comparative anti-acne activity of *Azadirachta indica* and *aloe barbadensis Miller*.

Bacteria	Plant	N	Mean	Std. Deviation	Std. Error
Pseudomonas aeruginosa	*Aloe barbadensis miller*	18	8.3500	4.40204	1.03757
	Azadirachta indica	18	7.0667	3.45781	.81501
S. aureus	*Aloe barbadensis miller*	18	7.0000	16.10626	3.79628
	Azadirachta indica	18	3.5000	5.17301	1.21929
S. epidermidis	*Aloe barbadensis miller*	18	4.0000	9.20358	2.16930
	Azadirachta indica	18	10.0500	2.35103	.55414
Propionibacterium acnes	*Aloe barbadensis miller*	18	8.2500	4.23713	.99870
	Azadirachta indica	18	13.6500	3.97792	.93760

All the other extract of 20g *Aloe barbadensis miller* should relatively same results between 15mm to 16.5mm that is according to the results of +ve control. 10g *Azadirachta indica* extract produce zone of inhibition between 10.3mm to 12mm. Autade *et al.* (2015) investigated effect of *Azadirachta indica* extract against opportunistic bacterial and fungal pathogens associated with AIDS**.**

There were the comparative results of the zones of the inhibition formed by the acetone and chloroform extract prepared from different *Azadirachta indica* plant parts against bacterial and fungal opportunistic pathogens tested by disc diffusion assay. *Azadirachta indica* leaf extract 30mg, showed highest zone of inhibition (20mm) against *Pseudomonas aeruginosa* and *Staphylococcus aureus* followed by (16mm) for *E. coli* and *Klebsiella pneumoniae* respectively.

Adnan *et al.* (2011) studied in vitro bactericidal and bacteriostatic potential of ingredients of traditional medicine obtained from kacha area (river indus)

district d.i.khan, KPK, against human bacterial pathogens The most active extract found was *Azadirachta indica* leaves which represented widest zone of inhibition of 16(±0.05) mm and minimum inhibitory concentration 0.19mg/ml against *Klebsiella pneumoniae*.

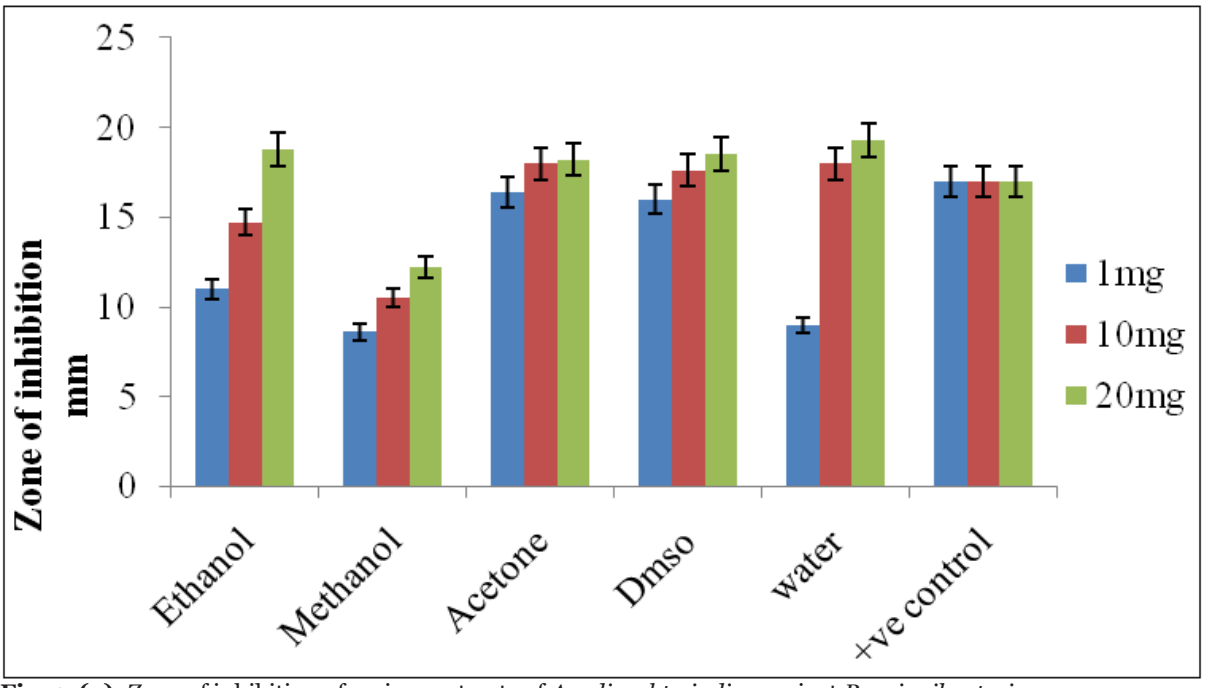
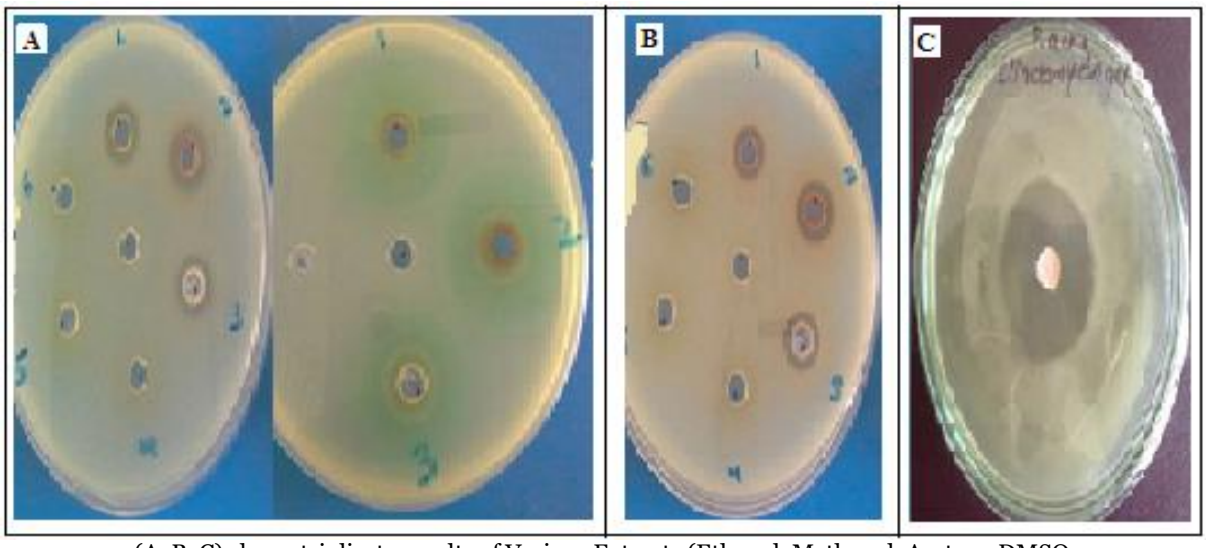


Fig. 1. (a). Zone of inhibition of various extracts of *Azadirachta indica* against *Propionibacterium acnes*.



(A, B, C) shows triplicate results of Various Extracts (Ethanol, Methanol, Acetone DMSO

Fig. 1(b). Zone of inhibition of various extract of *Azadirachta indica* against *P. acnes*.

As per Table 6, it is concluded that *Azadirachta indica* extract of 20g ethanol and DMSO showed the maximum antibacterial activity against *S. epidermidis* as they produce 18mm and 24mm respectively.20g all the solvent produce good results as compared to the standard antibiotic.1g *Azadirachta indica* solvent extracts have results within the range of +ve control but in 10g of ethanol also has good results as compared to standard antibiotic. This showed that *Azadirachta indica* extract results have maximum antimicrobial activity against *S. epidermidis*. Farhat *et al.* (2013) proposed the study of antibacterial effect of some selected essential oils and medicinal herbs against acne causing bacteria. *Azadirachta indica*

extracts has 16mm zone of inhibition against *S. epidermidis* and *Propionibacterium acnes*. In the present study 3 essential oils and 2 extracts were examined for antimicrobial activity against *P. acnes* and *S. epidermidis*. Amongst the 3 essential oils, results showed that Zone of Inhibition of Cinnamon oil was found to be highest against *S. epidermidis* and *P. acnes* followed by Tea tree oil and Rosemary oil when compared with standard i.e. Clindamycin.

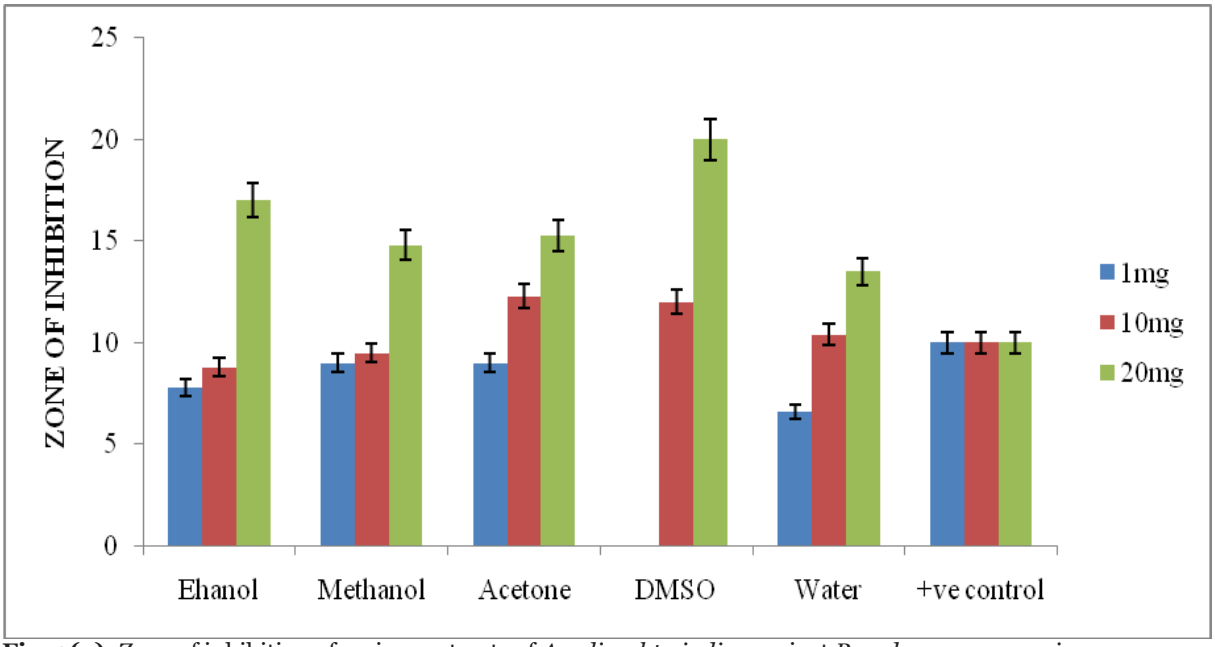
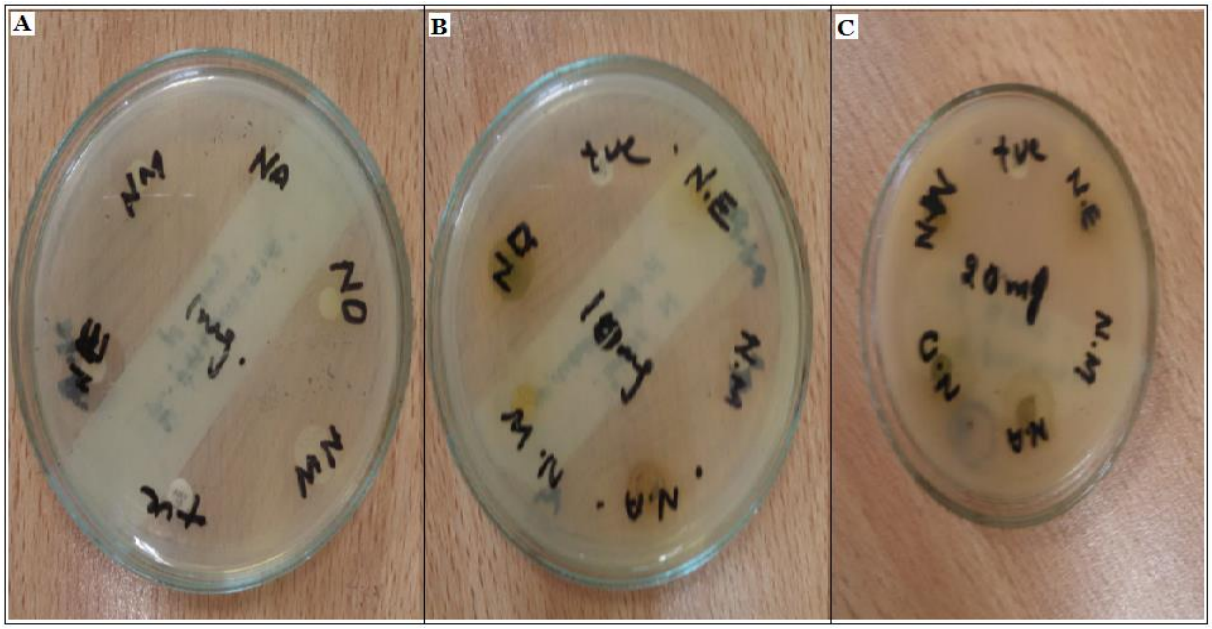


Fig. 2(a). Zone of inhibition of various extracts of *Azadirachta indica* against *Pseudomonas aeruginosa*.



(A, B, C) shows triplicate results of Various Extracts (Ethanol, Methanol, Acetone DMSO &Water

Fig. 2(b). Zone of inhibition of various extracts of *Azadirachta indica* against *Pseudomonas aeruginosa*.

This indicates that Rosemary oil shows least antibacterial activity.

Results of various extracts of Aloe barbadensis miller

As per Table 7, it is concluded that 20g ethanol extract of *Aloe barbadensis miller* has maximum zone of inhibition against *Propinibacterium acnes* that is 19mm.

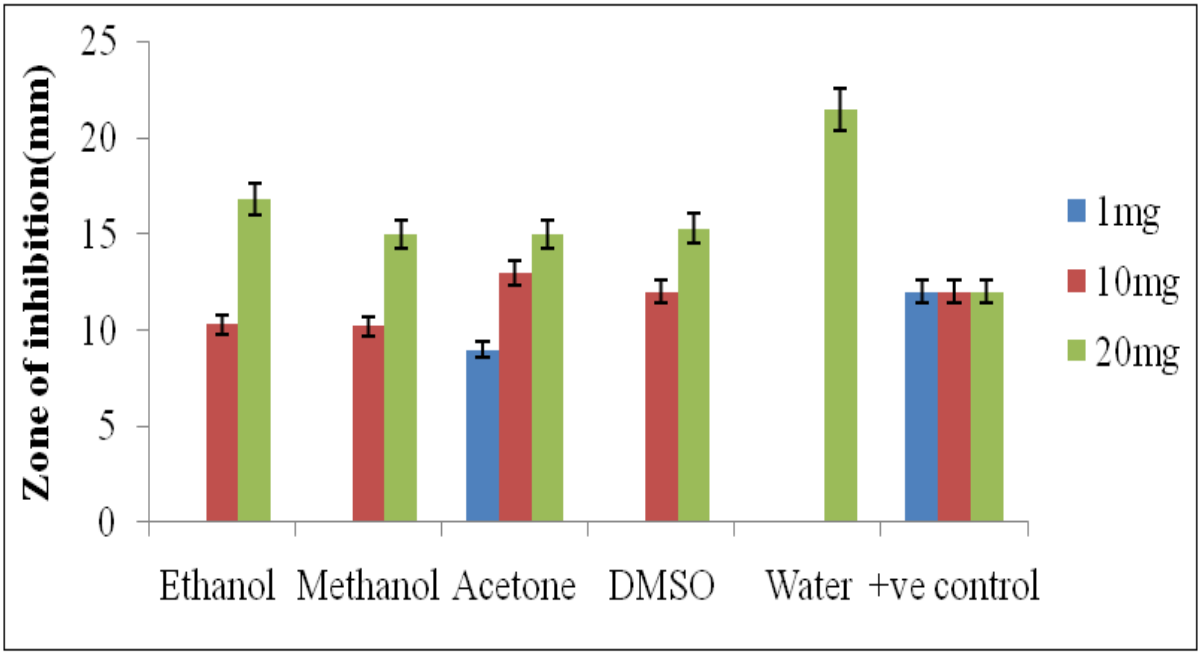
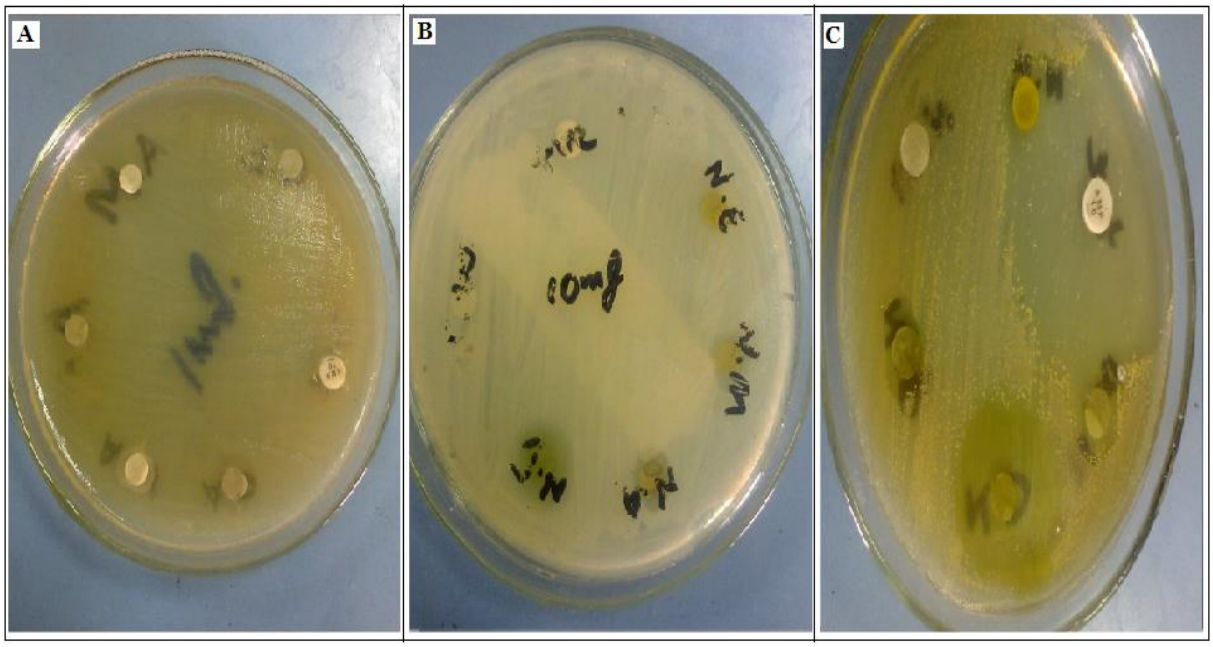


Fig. 3(a). Zone of inhibition of various extracts of *Azadirachta indica* against *S. aureus*.



(A, B, C) shows triplicate results of Various Extracts (Ethanol, Methanol, Acetone DMSO &Water)

Fig. 3(b). Zone of inhibition of *Azadirachta indica* powder against *S. aureus*.

Acetone showed second best results against *Propionibacterium acnes* in 20g and 10g extracts that is 17.6mm and 16mm. Almost all the extracts in 1gm produced zone of inhibition relative to the +ve control except DMSO that showed zero zone of inhibition. So, all the extracts in 20g produce best results when compared with standard and 10g extract results were also acceptable according to standard results. In vitro antimicrobial activity was performed by Nikunj *et al.* (2010) against *P. acnes*. In this method agar well diffusion method was performed and measured the zones of inhibition. Results shows that *Aloe barbadensis miller* gel has 8mm zone of inhibition in ethanol extract against *P. acnes*. Sawarkar *et al.* (2010) was performed in vitro antibacterial activity against *Propionibacterium acnes* (*P. acnes*). *Aloe barbadensis miller* gel 10mg per ml has 8.5mm inhibitory zone of inhibition.

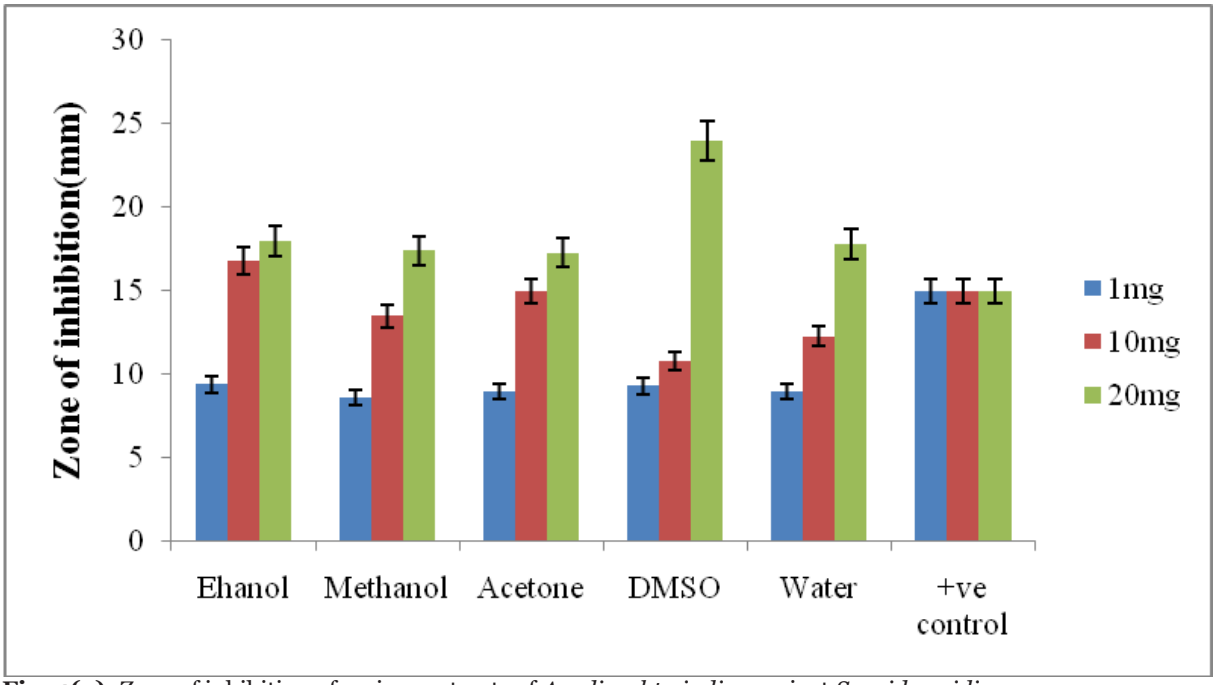
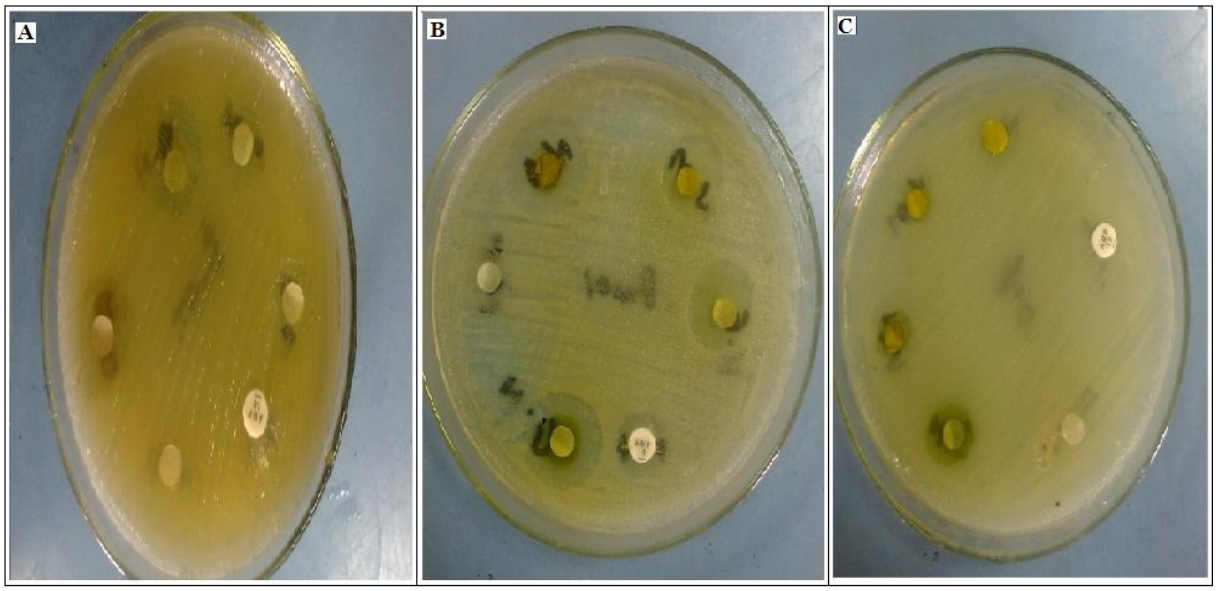


Fig. 4(a). Zone of inhibition of various extracts of *Azadirachta indica* against *S. epidermidis*.



(A, B, C) shows triplicate results of Various Extracts (Ethanol, Methanol, Acetone DMSO &Water)

Fig. 4(b). Zone of inhibition of *Azadirachta indica* powder against *S. epidermidis*.

As per Table 8, it is concluded that 20g ethanol and DMSO extracts showed the maximum zone of inhibition against *P. aeruginosa* that is 18.7mm. Second best results were showed by 20g extract of acetone and 10g extract of DMSO that is that is 18mm.1g extract of all the solvent shows results according to the standard except DMSO. +ve control has been shown 9mm zone of inhibition.

Except 1gm DMSO and water extracts all the solvent extracts produce zone of inhibition that complied with zone of inhibition of +ve control azithromycin antibiotic disc. Lalitha *et al.* (2012) was aimed to examine the antimicrobial activity of Dimethyl sulfoxide (DMSO) crude extracts of *Aloe barbadensis Miller*gel against the selected bacterial and fungal pathogens *of Escherichia coli, Klebsiella pnemoniae, Proteus mirabilis, Psedomonas aeruginosa, Staphylococcus aureus, Aspergillus niger, Candida albicans* and *Penicillium* spp**.**

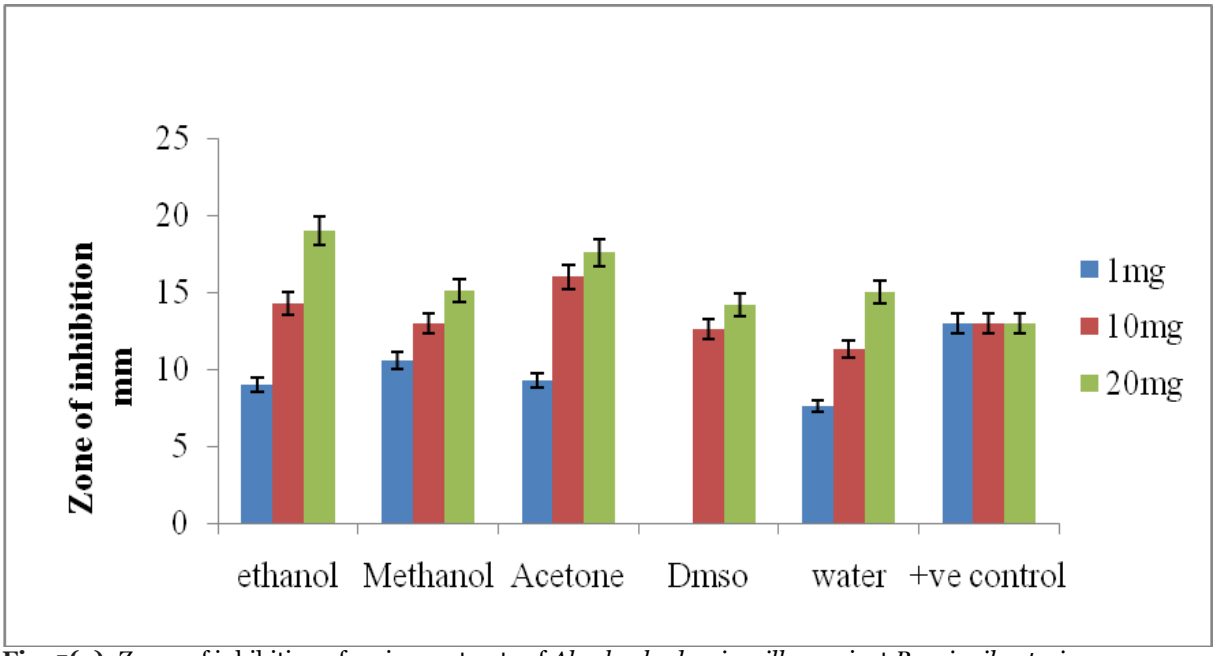
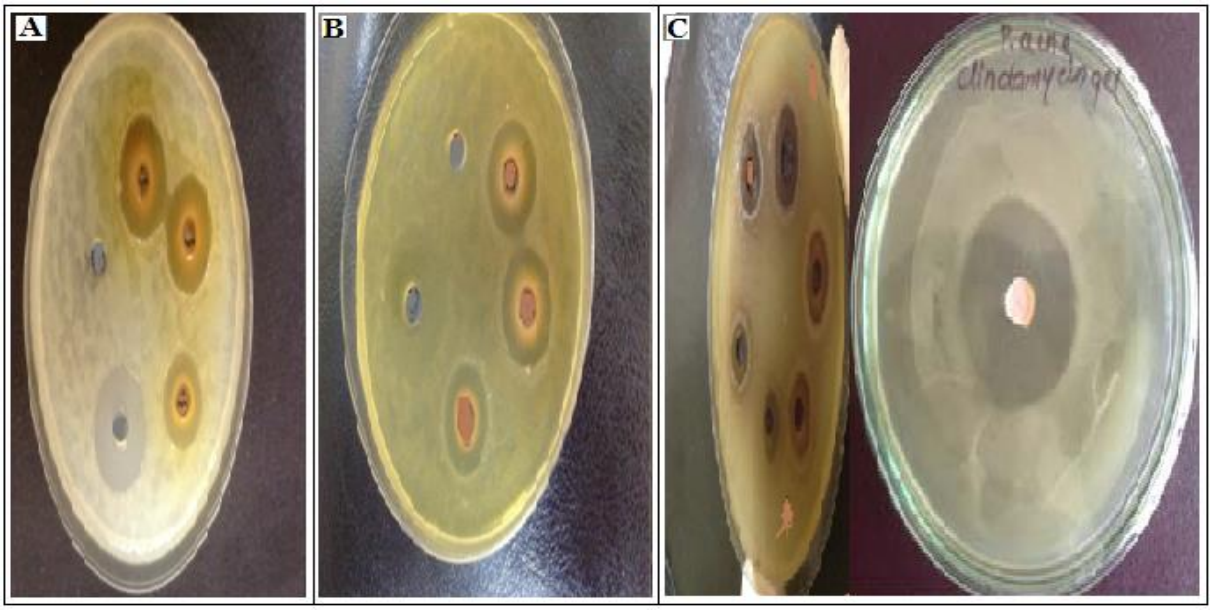


Fig. 5(a). Zones of inhibition of various extracts of *Aloe barbadensis miller* against *Propionibacterium acnes.*



(A, B, C) shows triplicate results of Various Extracts (Ethanol, Methanol, Acetone DMSO &Water)

Fig. 5(b). Zone of inhibition of various extracts of *Aloe barbadensis miller* against *Propionobacterium acnes.*

The study shows that the *Aloe barbadensis miller* gel in the conc.200µg/mL has maximum zone of inhibition 10 mm for *Pseudomonas aeruginosa* of Gram negative bacteria. As per Table 9, *Aloe barbadensis miller* powder extracts of 20g in methanol and acetone showed the antibacterial activity against *S. aureus* as they produce zone of inhibition of 11mm and 8.9mm respectively. All the other solvent extract of *Aloe barbadensis miller* showed no antibacterial activity as they produce no zone of inhibition. Rather than the +ve control clindamycin showed good results against *S. aureus* as compared to *Propionibacterium acnes.* Agarry *et al.* (2005) compared the antimicrobial activities of ethanolic extracts of *A. vera* gel and leaf against *S. aureus*, *P. aeruginosa*, *Trichophyton mentagraphytes*, *T. schoeleinii*, *M. canis* and *C.albicans*. Antimicrobial susceptibility test showed that both the gel and the leaf inhibited the growth of *S. aureus*.

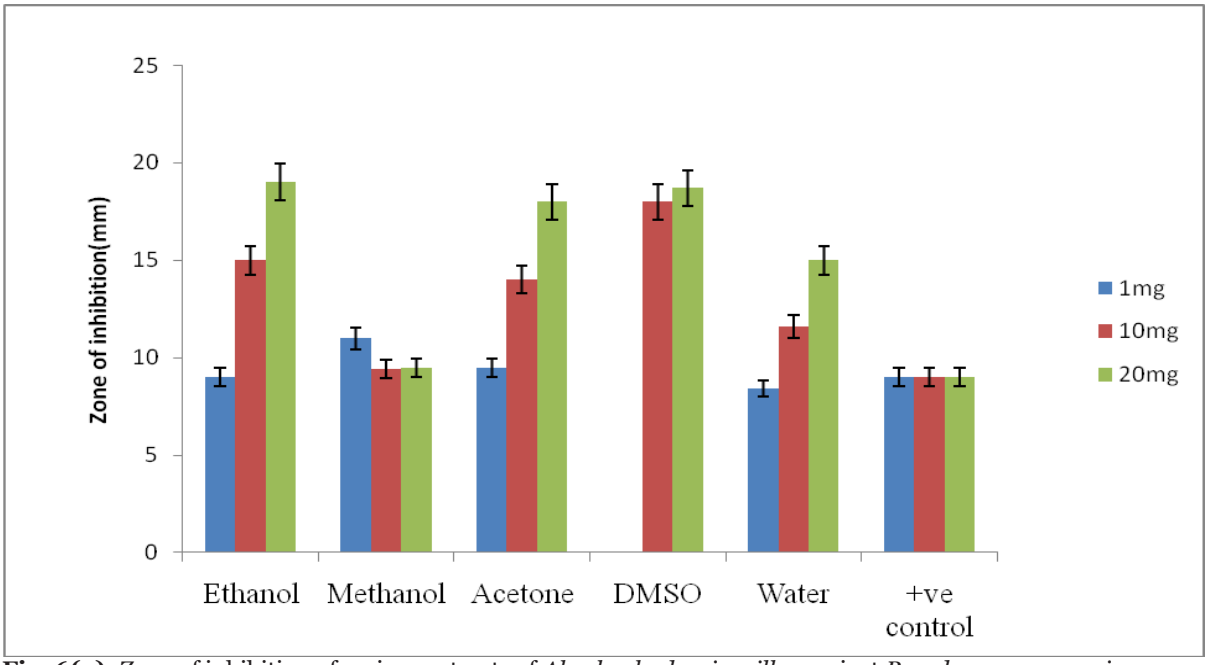
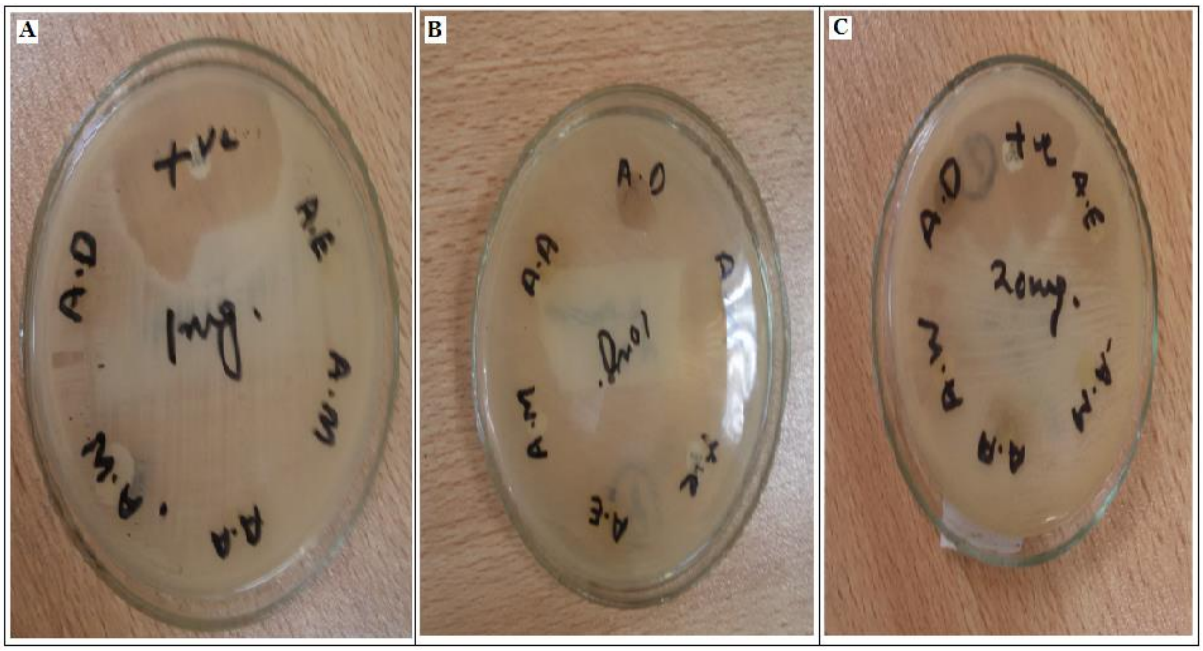


Fig. 6(a). Zone of inhibition of various extracts of *Aloe barbadensis miller* against *Pseudomonas aeruginosa*.



(A, B, C) shows triplicate results of Various Extracts (Ethanol, Methanol, Acetone DMSO &Water)

Fig. 6(b). Zone of inhibition of various extracts of *Aloe barbadensis Miller* against *P. aeruginosa*.

Only the gel inhibited the growth of *T. mentagrophytes*, while the leaf possesses inhibitory effects on both *P. aeruginosa* and *C. albicans*. Thiruppathi *et al.* (2010) conducted a study to determine the antimicrobial activity of *A. vera* juice with different solvents viz., hexane, ethyl acetate, petroleum ether and ethanol against Gram positive bacteria (*B. subtilis*, *S. aureus*), Gram negative bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa*). The result showed that more antimicrobial activity in ethyl acetate (1-9 mm) and ethanol extract (7-12mm). The least inhibitory effect on petroleum ether extract was 2 mm. As per Table 10, it is concluded that 1gm conc. of all the solvent extracts of *Aloe barbadensis miller* showed no zone of inhibition against *S. epidermidis*. Methanol and acetone in 10g of solvent extract showed no zone of inhibition as well. So, *Aloe barbadensis miller* has no antibacterial activity in

these concentrations. Other solvents extracts of 10g *Aloe barbadensis miller* showed zone of inhibition that was in range of +ve control. 20g *Aloe barbadensis miller* extract of all solvent produce maximum zone of inhibition that is 13.2mm.

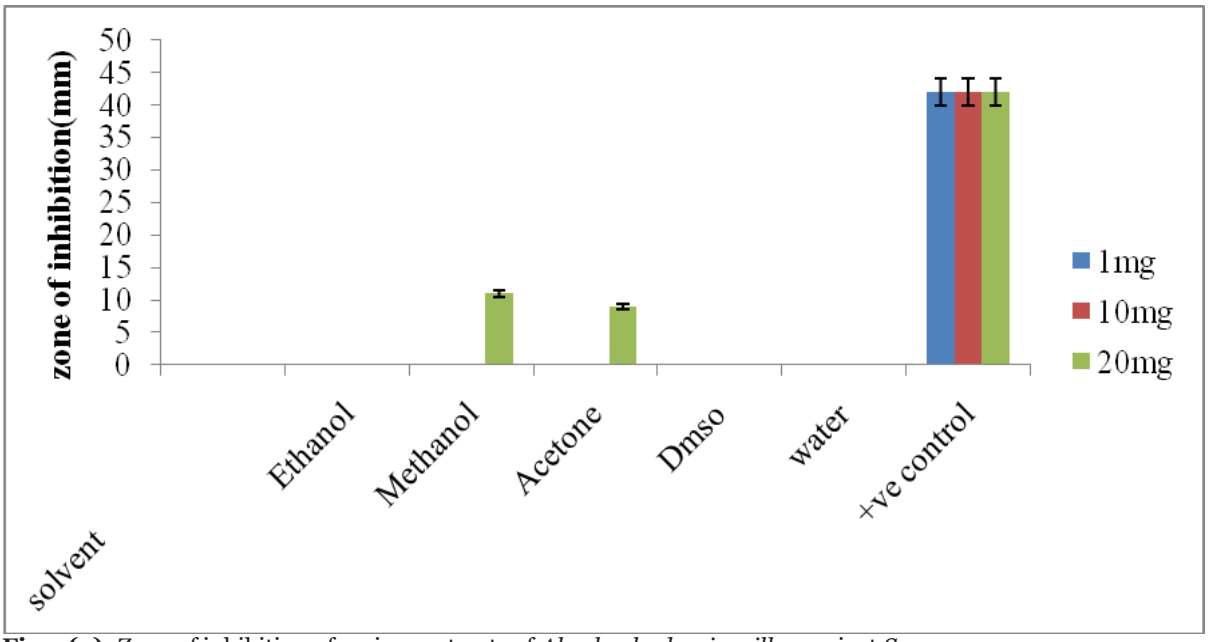
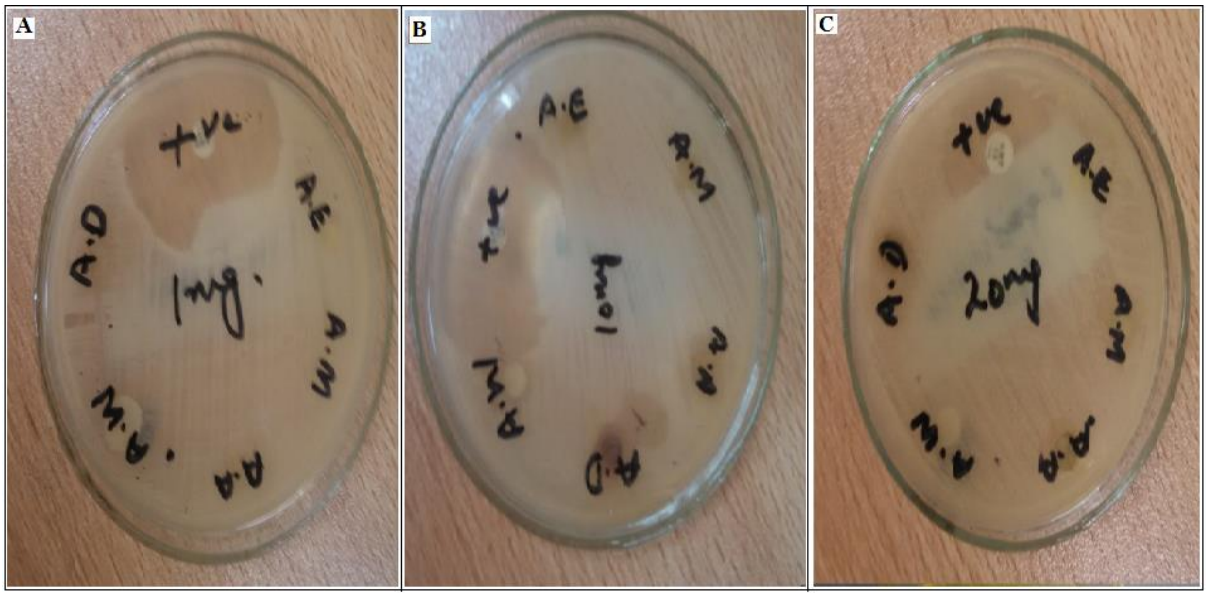


Fig. 7(a). Zone of inhibition of various extracts of *Aloe barbadensis miller* against *S. aureus.*



(A, B, C) shows triplicate results of Various Extracts (Ethanol, Methanol, Acetone DMSO &Water)

Fig. 7(b). Zone of inhibition of various extracts of *Aloe barbadensis miller* against *S. aureus.*

Udgire *et al.* (2014) studied antibacterial activity of *Aloe barbadensis miller* against skin pathogens. In this study disc diffusion method was used to test the antimicrobial activity. The maximum antibacterial activity was observed in methanol with maximum against *S. aureus* and *S. epidermidis* with zone of inhibition 12 and 11mm.

Comparison of anti-acne activity of Azadirachta indica and Aloe barbadensis Miller

Statistical analysis of anti-acne activity of *Aloe barbadensis Miller* and *Azadirachta indica* synergistic and comparative results of various solvents. As per Table 11, it is concluded that 1gm conc. of all the solvent extracts of *Aloe barbadensis*

miller showed no zone of inhibition against *S. epidermidis*. Methanol and acetone in 10g of solvent extract showed no zone of inhibition as well. So, *Aloe barbadensis miller* has no antibacterial activity in these concentrations. Other solvents extracts of 10g *Aloe barbadensis miller* showed zone of inhibition that was in range of +ve control. 20g *Aloe barbadensis miller* extract of all solvent produce better result against *P. acne* and *P. aeruginosa*.

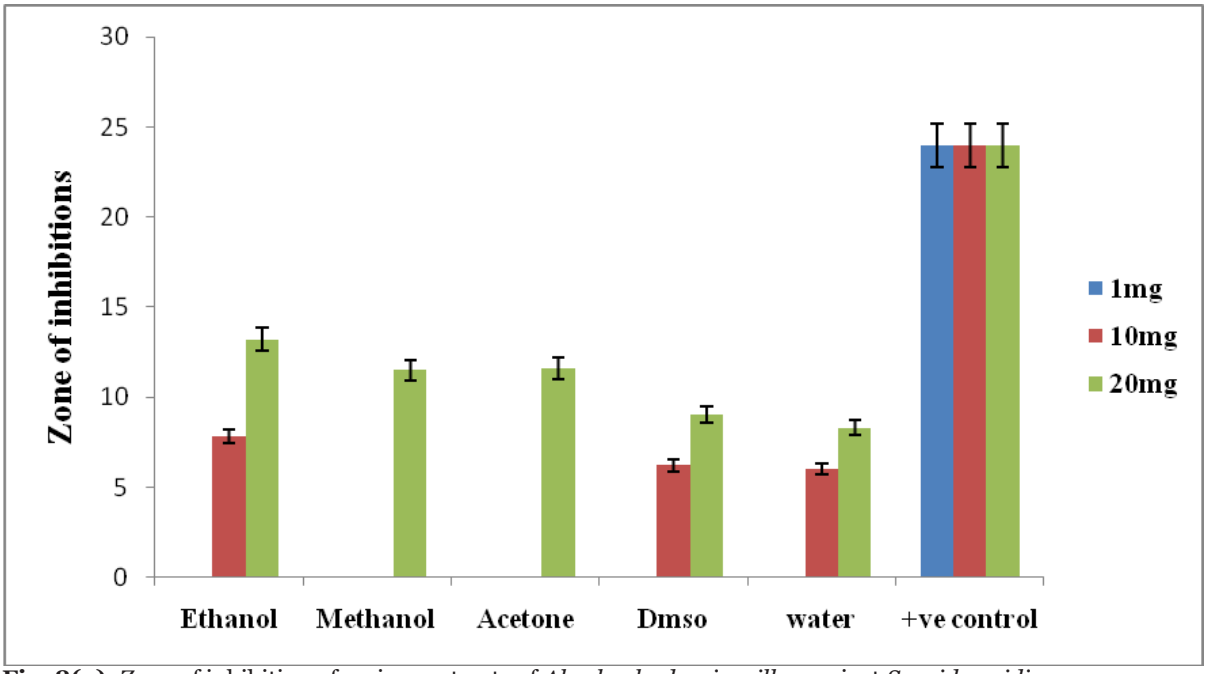
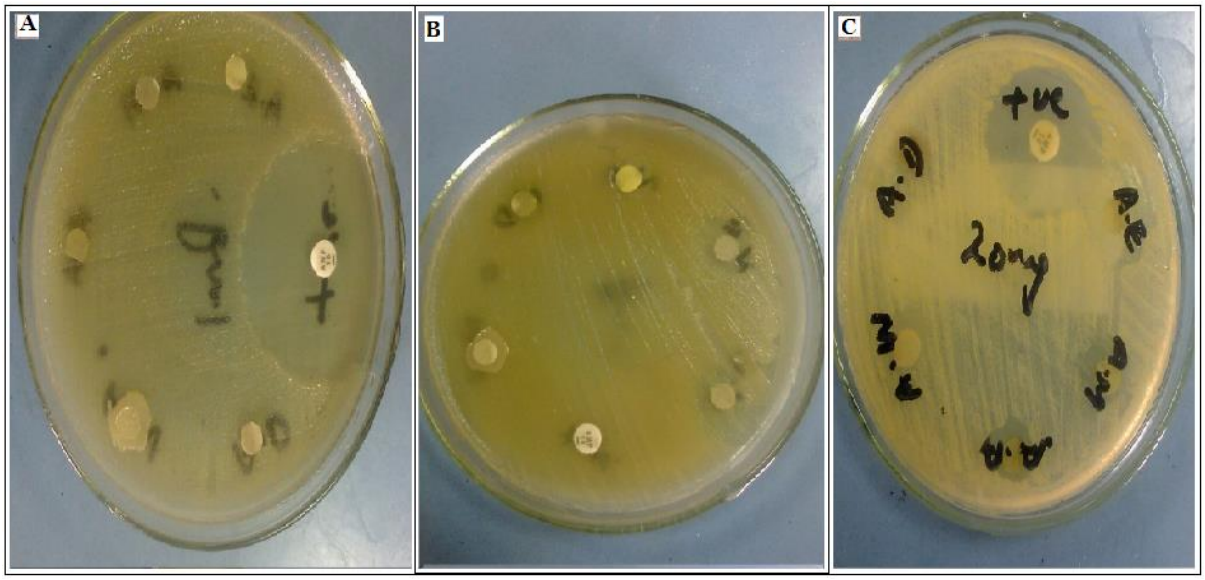


Fig. 8(a). Zone of inhibition of various extracts of *Aloe barbadensis miller* against *S. epidermidis*.



(A, B, C) shows triplicate results of Various Extracts (Ethanol, Methanol, Acetone DMSO &Water)

Fig. 8(b). Zone of inhibition of various extracts of *Aloe barbadensis* miller against *S. epidermidis*.

Rubina *et al.* (2009) study showed that ethanol, methanol and acetone extracts of *Aloe vera* gel were studied for their antimicrobial activity against four Gram-positive and Gram-negative bacteria using agar well diffusion method. The extracts showed varied levels of antimicrobial activity against the tested pathogens. The ethanol and methanol extracts showed higher activity while acetone extract, showed

least or no activity against most of the tested pathogens. The antimicrobial activity of the *A. vera* gel extract to be dependent on the synergistic effect of different compounds. With the broad spectral antimicrobial effect of *A. vera* gel.

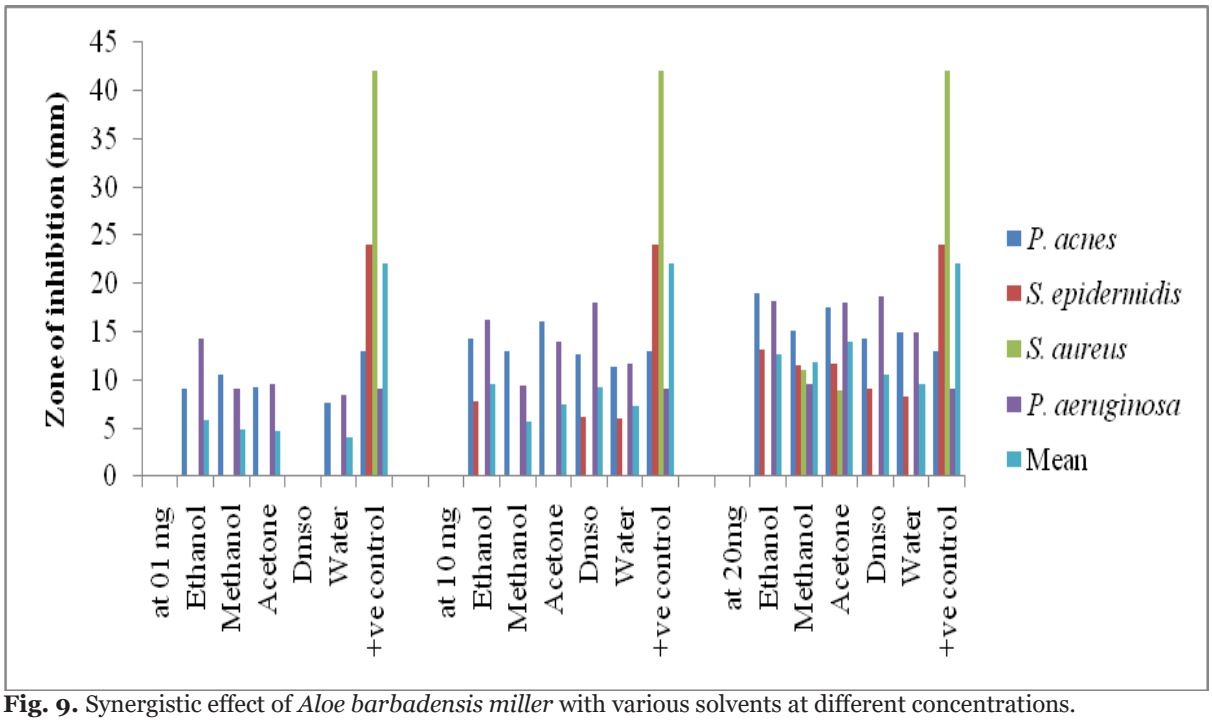


Fig. 9. Synergistic effect of *Aloe barbadensis miller* with various solvents at different concentrations.

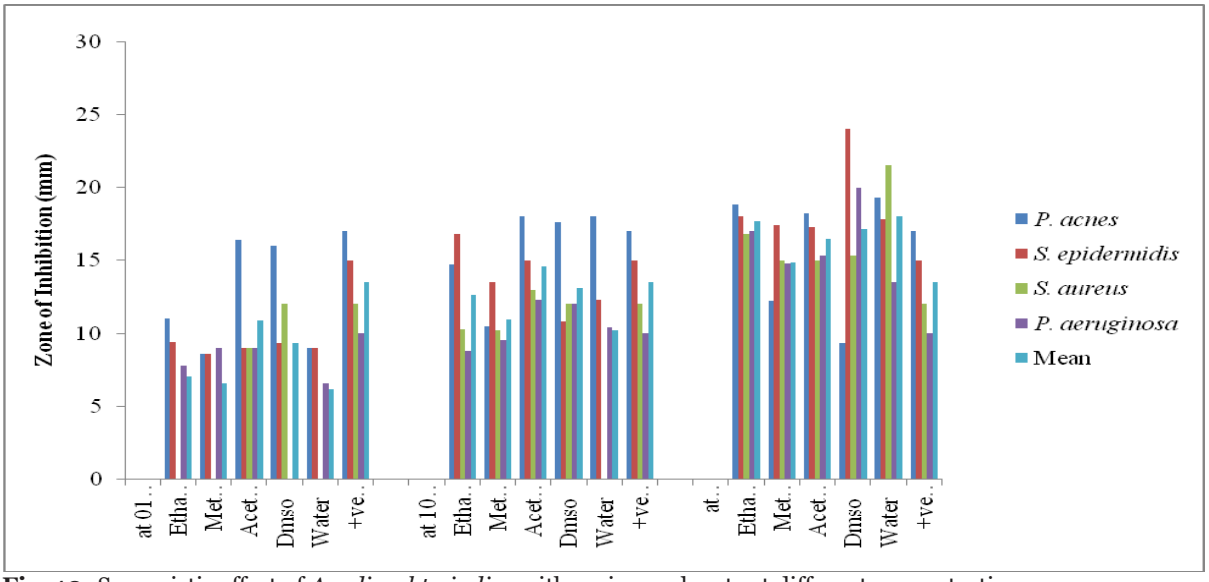


Fig. 10. Synergistic effect of *Azadirachta indica* with various solvents at different concentrations.

As per Table 12, it is concluded that 1gm conc. of all the solvent extracts of *Azadirachta indica* showed less zone of inhibition against *S. aureus*. 20g *Azadirachta indica* extract of all solvent produce better result against all tested bacterial isolates. The results in current study were similar to those in the study of Al-Emran *et al.* (2011) who reported that the results depict that leaf extracts of *Azadirachta indica* could be used as a potential source of antimicrobial agents against the bacterial strains tested.

But in Muna *et al.* (2019) showed opposite finding that only acetone extract of *Azadirachta indica* showed good antimicrobial properties.

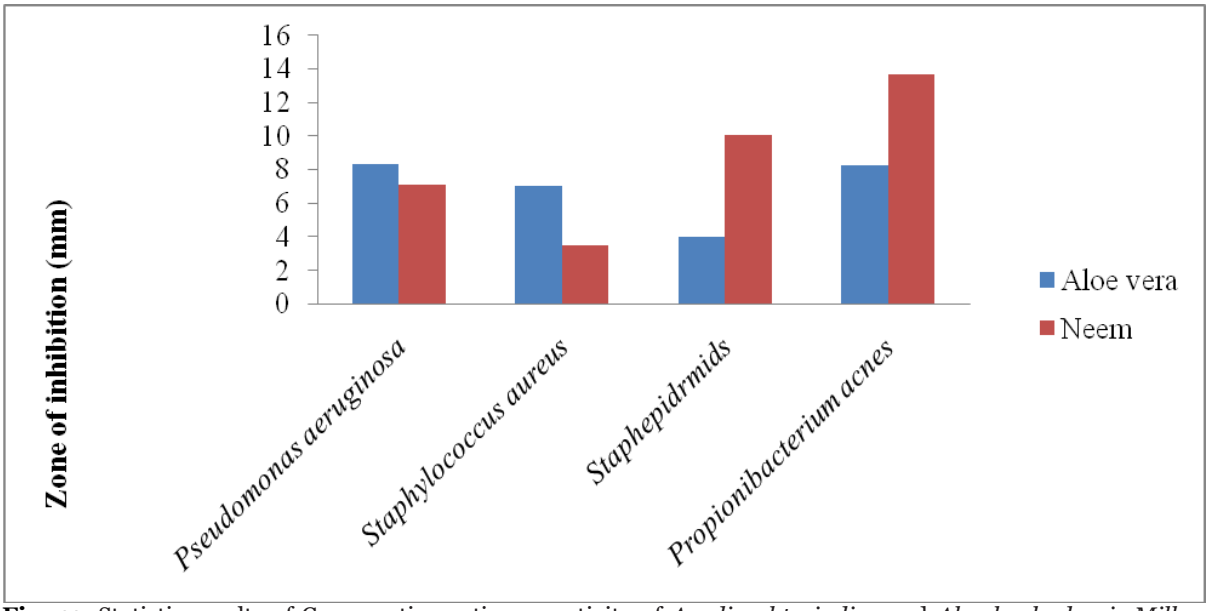


Fig. 11. Statistic results of Comparative anti-acne activity of *Azadirachta indica* and *Aloe barbadensis Miller*.

Conclusion

According to spss16 results, *Aloe barbadensis miller* showed greater zone of inhibition against *P. aeruginosa* and *S. aureus,* which are supportive organisms of acne. This showed that *Aloe barbadensis miller* extract is effective against these organisms in comparison to *Azadirachta indica*.

On the other hand, when compared the anti-bacterial activity of *Azadirachta indica* and *Aloe barbadensis miller* against acne causing organisms *Azadirachta indica* extracts showed the maximum zone of inhibition against *S. epidermidis* and *P. acnes*, as compared *to Aloe barbadensis miller* extracts. *P. acnes* is the major or important causative agent of acne, so *Azadirachta indica* would be the better choice or remedy against acne in acne patients as it showed maximum activity against *P. acnes* as compared to the *Aloe barbadensis miller*. So, concluded that *Azadirachata indiaca* has better anti-acne activity as compared to the *Aloe barbadensis miller*.

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