

Medicinal Plants on Acne inducing Bacteria

T Samanta¹, A Das¹

*Corresponding author:

T Samanta

¹Dept. of Physiology, Raja N.L. Khan Women's College, Midnapore, West Bengal, India.

Abstract

Acne is a cutaneous pleomorphic disorder of the pilosebaceous unit involving abnormalities in sebum production and is characterized by both inflammatory (papules, pustules and nodules) and non-inflammatory (comedons, open and closed) lesions. *Propionibacterium acnes* and *Staphylococcus epidermidis* are common pus-forming microbes responsible for the development of various forms of *Acne vulgaris*. The present study was conducted to evaluate antimicrobial activities of seven medicinal plants against acne-inducing bacteria. Acetonic and aqueous extracts of *Azadirachta indica* (leaves), *Curcuma longa* (root), *Aloe vera* (leaves), *Withania somnifera* (leaves), *Terminalia arjuna* (bark), *Ocimum sanctum* (leaves), *Santalum album* (wood) were tested for antimicrobial activities by agar diffusion, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods. The results from the agar diffusion method showed that five medicinal plants could inhibit the growth of acne-inducing bacteria. Among these *Azadirachta indica*, *Curcuma longa* and *Terminalia arjuna* had strong inhibitory effects. Based on MIC, the acetonic extracts of *Azadirachta indica* and *Curcuma longa* had the greatest antimicrobial effects. Taken together, the present study indicated that *Azadirachta indica* had a strong inhibitory effect on acne-inducing bacteria.

Keywords: Acne, *Acne vulgaris*, MIC, MBC.

Introduction

Commonly known as acne, is medically coined as *Acne vulgaris*. It is a skin infection marked by some pilosebaceous units which consist of a hair follicle and the associated sebaceous glands. Acne might be both inflammatory as well as non-inflammatory. Generally it is referred to as pimples, blemishes or acne. Most of the teenagers suffer by common acne (about 85%). [1] gave a data regarding the negative personal and social consequences caused due to the occurrence of acne in adolescence. The reason of this age group being the main target is the increasing amount of male sex hormones at this stage of puberty both in the males and females. The regions that are affected are the faces and the upper neck regions. There are various reviews or research articles regarding the relation between acne and depression. [2] concluded in their review that the dermatologists should be aware of the depressive symptoms of such patients. Similar type of work was also done on questionnaire basis by [3]. A review by [4] also supported the fact that emotional factors and acne do connect. [5] also deduced by their studies that *Acne vulgaris* has an effect on the patient's quality of life. Similar work was also done by [6]. [7] studied the mood characteristics and psychiatric morbidity of acne patients.

Histological studies on the acne have shown that the main cause of acne is the blockage of follicles. This blockage might be caused by a keratin plug or hyperkeratinization. [8] showed in their work that

the sebaceous glands enlarge and hence the sebum production increases. Moreover, they might form an open or closed comedo, the former is commonly termed as blackheads.

[9] showed in their work that severity of acne can be treated by a number of combination treatments. Such treatments might include the use of some antibiotics like erythromycin, tetracycline etc., externally that are bactericidal. Even oral ingestion can be done for the above mentioned antibiotics. In females even some hormonal treatments are common for controlling acne. [10] have shown in their work that erythromycin with or without Zinc can eradicate erythromycin-resistant *Propionibacteria in vivo*.

[11] showed in their studies that estrogen/progesterone combination or antiandrogen, cyproterone and estrogen are effective in reducing the androgenic hormone levels and thus acne. Other than these various other treatments like flattening of the pimples by directly injecting cortisone into large sized pimples or application of topical retinoids can be of great use. Whereas [12] showed by his work that there was no correlation between the androgen levels and the pattern, distribution, severity of acne or the irregular periods or the presence of hirsutism. It was deduced in his studies that these abnormalities were also observed in females with polycystic ovary syndrome and idiopathic hirsutism. [13] reported that insulin and tolbutamide possess effective role against acne. [14] showed the effect of estradiol and testosterone on acne. Works on testosterone has also been done by [15].

Other than the above mentioned treatments Vitamin A has also been reported by many workers as one of the effective measures

against acne. [16] used retinol to control severely inflammatory *Acne vulgaris* within 3 to 4 months.

[17] also supported the role of Vitamin A in the treatment of acne. According to [18] the Vitamin A has become indispensable if treatment of acne is considered.

[19] also studied the effect of oral administration of Vitamin A.

Other than Vitamin A there are studies with Vitamin B₆, as reported by [20], where this Vitamin has been shown to have an effect on testosterone and hence on acne.

Just as Vitamin A has been studied, [21] studied the role of Cytochrome P₄₅₀.

Other than hormones, Vitamins etc even metallic ions like Zinc has gained a lot of importance in the treatment of acne.

[22] showed in his work that the effect of zinc alone and zinc together with Vitamin A was no less on acne. The study also showed the effect of tetracyclines with zinc.

[23] studied the effect of oral administration of zinc sulphate in acne vulgaris patients.

Apart from all these studies, it can thus be concluded that there is a need of a prompt identification and treatment of acne [24]. Studies on the effects of some plant extracts on acne vulgaris has been reported by some scientists like [25] and hence this study is an attempt to contribute in one such area.

Materials And Methods

Acne sampling

Pus from acne was taken from a group of patients using sterile cotton swab into 4 conicals for each patient, each containing 25ml of Nutrient Broth. The conicals were then kept in rotary shaker at 37°C for 24 hours.

The viability of microorganisms was maintained by regular transfer into freshly prepared Nutrient Broth.

Plant Materials

The plant materials used in the following study are *Azadirachta indica*, *Curcuma longa*, *Terminalia arjuna*, *Withania somnifera*, *Santalum album*, *Aloe vera* and *Ocimum sanctum*.

Extractions

Healthy mature leaves, bark, roots etc. of the respective plants in trial were taken.

Leaves were surface sterilized with 70% alcohol and then rinsed with sterilized distilled water. All the leaves were air dried and then homogenized to fine powder with the help of mixer grinder. The powder was stored in air tight bottles. Similarly the other respective parts were also sterilized.

Aqueous extract

Ten grams of powdered plant materials (leaves, roots and barks) were soaked in 100ml of water and kept on a rotary shaker for 24

hrs at 37°C. Thereafter, it was filtered with the help of filter paper (Whatman No. 1) and then centrifuged in 5000g for 15 min. The supernatant was collected and tested against microorganisms.

Acetone extract

Ten grams of powdered plant materials were mixed with 100ml of acetone and kept on a rotary shaker for 24 hrs at 37°C. Thereafter, it was filtered with the help of filter paper (Whatman No. 1) and then centrifuged in 5000g for 15 min. The supernatant was collected and tested against microorganisms.

Antimicrobial Susceptibility Test

Acetone and aqueous extracts of all collected plants were tested against acne-inducing organisms.

Susceptibility testing was done by the following three methods:

Agar Well Diffusion (Kirby-Bauer method).

Minimum Inhibitory Concentration (MIC).

Minimum Bactericidal Concentration (MBC).

All the extracts were first tested for screening using Agar Well Diffusion techniques. Then only active antimicrobial properties containing extracts can be tested to determine their MIC and MBC.

Agar Well Diffusion

Sensitivity of microorganisms can be examined by agar diffusion method. The sensitivity of antimicrobial agents (extracts) against test organisms was calculated by measuring the zone of inhibition produced due to the diffusion of extractions through the agar.

It has been found that the diameter of inhibition zone is directly proportional to the concentration of antimicrobial agents (extracts), upon a limit. Zone may vary due to diffusibility of antimicrobial agents (extracts), size of inoculants and type of medium.

Mueller-Hinton agar was prepared from a commercially available dehydrated base according to the manufacturer's instructions. Mueller-Hinton agar (3.9%) was taken and mixed with hot distilled water and autoclaved. After autoclaving, it is allowed to cool at 45 °C to 50°C. The freshly prepared and cooled medium was then poured into glass, flat-bottomed Petridishes on a plane, horizontal surface to give a uniform depth of approximately 4mm.

Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension for taking the inoculum. The Mueller-Hinton Agar plates were inoculated using swab streak. The lid left ajar for 3 to 5 minutes.

Using a 4mm cork borer, wells were made in all the plates. Different extracts were added into the groove with one blank of each. In Blank, only acetone was added. Plates were incubated for 18-24 hrs at 35 °C -37°C aerobically in an incubator.

After 16 to 18 hours of incubation, each plate was examined. If the plate was satisfactorily streaked and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured,

including the diameter of the disc. Zones are measured to the nearest whole millimeter, using sliding calipers as a ruler, which is held on the back of the inverted Petri plate.

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of an antimicrobial agent is defined as the maximum dilution of the product that will still inhibit the growth of a test microorganism. There is different degree of inhibition in different microorganisms with reference to particular antimicrobial agents. The main principle of this method is that, identical inoculations are inoculated in the tubes of media containing different concentrations of plant extract. The turbidity of the test sample is measured by spectrophotometer with respect to blank.

Mueller-Hinton medium was prepared.

The extracts were diluted. Each extract was serially diluted using water into 1/10, 1/20, 1/40, and 1/80.

The dilutions made for each plant extract is showed in Table: 1.

Table 1: The dilutions made for each plant extract

Dilution	Water (ml)	Extract (ml)
1/10	9	1
1/20	9.5	0.5
1/40	9.75	0.25
1/80	9.875	0.125

Two sets of 1-4 tubes were labeled and arranged in two rows. First row for the control, organism free and second row for the test organism. One ml of diluted extract was added into corresponding one tube. One ml of inoculum was added to only one test set. Thus 21 sets were prepared for the tested organism and two extracts. Seven tubes were taken for acne organism's control, containing broth and organism. One test tube was taken as blank, containing only broth.

All the tubes were then incubated for 18-24 hrs at 35 °C -37°C aerobically in a rotary shaker of an incubator.

Optical Density of the cultures was measured at 660nm using Spectrophotometer 106 (SYSTRONICS). MIC was taken as the lowest concentration of the test compound which inhibited the appearance of visible growth.

Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of antibiotic required to kill an organism. It can be determined from broth dilution MIC tests by sub culturing to agar media without antibiotics. Antimicrobials are usually regarded as bactericidal if the MBC is no more than four times the MIC.

Results And Discussion

In the present investigation, antimicrobial potentials of 7 ethnomedicinal plants were examined. Among them 5 plant extracts showed antimicrobial activity against acne inducing bacteria (Table: 2). Plant extracts were most active in this series, of which extracts of *Azadirachta indica*, *Curcuma longa*, *Terminalia arjuna*, *Withania somnifera*, *Santalum album* showed highest zone of inhibition against the tested microorganism. Among these, the acetone extract of *Azadirachta indica* showed the highest zone of inhibition (20mm) followed by *Curcuma longa* (18mm).

Table 2: The inhibition zone diameter obtained by agar diffusion method:

SAMPLE	PLANTS	Acetone(mm)	Aqueous(mm)
A	<i>Azadirachta indica</i>	20	17
B	<i>Curcuma longa</i>	18	15
C	<i>Terminalia arjuna</i>	15	12
D	<i>Withania somnifera</i>	12	10
E	<i>Santalum album</i>	11	09
F	<i>Aloe vera</i>	04	04
G	<i>Ocimum sanctum</i>	04	04

The growth of acne-inducing bacteria was completely inhibited by 5 plant extracts and weakly inhibited by 2 plant extracts (FIG.1).

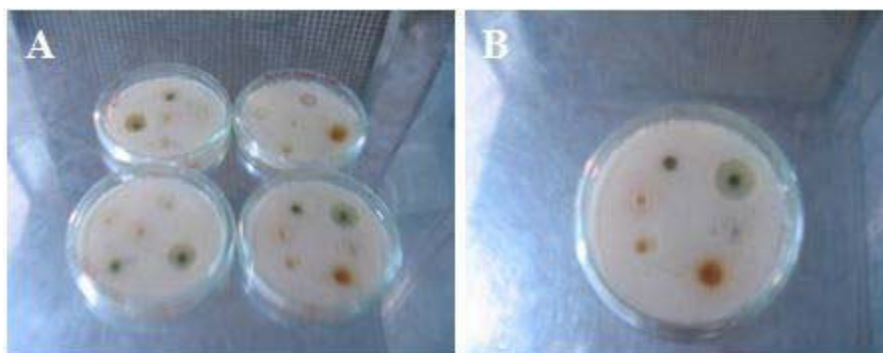


Figure. 1: Clear zone representing the inhibition of the growth of the acne-producing organism being studied,

A: Plates showing the inhibition zone of the acne producing bacteria with some of the plant extracts put to test; B: A plate showing the inhibition zone of the acne producing bacteria with some of the plant extracts put to test

The MIC and MBC were determined against the tested microorganism (FIG.2).



Figure. 2: MIC of the tested plant extracts.

The MIC (Table: 3) exhibited significant at 1/80, 1/40 dilutions and followed by 1/20, 1/10 dilutions. The MBC (Table: 4) exhibited no significant result at any dilution.

Table 3: Determination of MIC of the tested plant extracts:

Plants	Dilutions							
	1/10		1/20		1/40		1/80	
	A Q	A C	A Q	AC	A Q	AC	A Q	AC
<i>Azadirachta indica</i>	G	N G	G	NG	N G	NG	G	NG
<i>Curcuma longa</i>	G	N G	G	G	N G	G	N G	NG
<i>Terminalia arjuna</i>	G	G	G	NG	G	NG	G	NG
<i>Withania somnifera</i>	G	G	N G	G	N G	G	N G	G
<i>Santalum album</i>	G	G	G	G	G	NG	G	NG
<i>Aloe vera</i>	G	G	G	G	G	G	G	G
<i>Ocimum sanctum</i>	G	G	G	G	G	G	G	G

NG= No Growth; G= Growth; AQ= Aqueous; AC= Acetone

Table 4: Determination of MBC of the tested plant extracts

Plants	Dilutions							
	1/10		1/20		1/40		1/80	
	A Q	A C	A Q	A C	A Q	A C	A Q	A C
<i>Azadirachta indica</i>	G	G	G	G	G	G	G	G
<i>Curcuma longa</i>	G	G	G	G	G	G	G	G
<i>Terminalia arjuna</i>	G	G	G	G	G	G	G	G
<i>Withania somnifera</i>	G	G	G	G	G	G	G	G
<i>Santalum album</i>	G	G	G	G	G	G	G	G
<i>Aloe vera</i>	G	G	G	G	G	G	G	G
<i>Ocimum sanctum</i>	G	G	G	G	G	G	G	G

G= Growth; AQ= Aqueous; AC= Acetone

The Gram character of the tested organism was also observed under 100X oil immersion and found it to be Gram-positive rod-shaped bacteria (FIG.3).

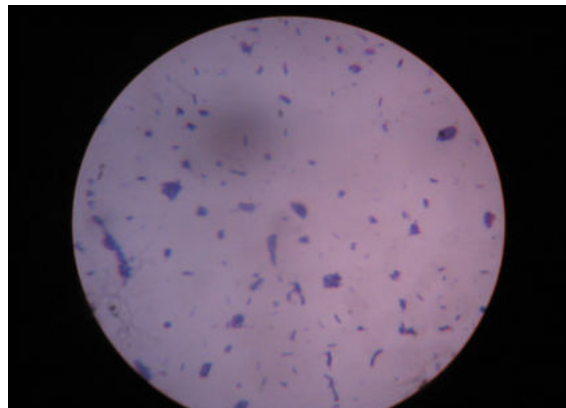


Figure. 3: Microscopic view (100X) showing Gram-character of the acne-producing organism studied.

Various Indian herbs like *Azadirachta indica*, *Curcuma longa* etc. contains many essential oils. Various studies have shown the antipyretic, antihelminthic and anti-inflammatory effects of such essential oils. It also helps in controlling the biliary secretion and purifies the blood. It is even a good remedy for splenic enlargement. Due to these pharmacological activities, these herb formulations might be used in the treatment of Acne.

Studies have even shown that these essential oils have a suppressing activity on *Propionibacterium acnes*. Vast studies have already been done regarding the efficacy of such Indian herbs. There are various studies that prove that topical together with oral use of these herbs are very useful against acne. The present study is thus based on such works together with some other plants which still needs to be explored.

Among the tested plants some have the potential to be used as traditional medicine. The results of the present study support the folkloric usage of the studied plants and suggested that some of the plant extracts possess compounds (hydrophobic) with antibacterial properties.

A combination of *Aloe barbadensis* (aloe vera), *Azadirachta indica* (neem), *Curcuma longa* (turmeric), *Hemidesmus indicus* (Indian sarsaparilla), *Terminalia chebula* (chebulic myrobalan), *Terminalia arjuna* (arjun), *Withania somnifera* (ashwagandha) and *Piper longum* (long pepper) was given orally combined with either a gel or cream of the same formula but without long pepper (which is used orally to increase absorption of other herbs) (Ghosh V.K. et al., 2011). In this screening, the extract of *Azadirachta indica* showed profound antimicrobial activity and that can be used as antimicrobial agents in new drugs for the therapy of acne. But water, petroleum ether and ethanolic extracts of *Azadirachta indica* exhibit moderate inhibitory activity against acne-inducing bacteria (Nand et al., 2012). Besides having antiplasmodial, antitrypanosomal, antioxidant effects, the extracts of *Azadirachta*

indica have some toxicological activities such as allergic, genotoxic and radiosensitizing effects in humans (Sunday E Atawadi and Joy C. Atawadi, 2009).

In the present study it has been found that the plant extracts showed satisfactorily MIC values but no significant MBC values were obtained. The reason behind this might be that the plant extracts used for this study were bacteriostatic but not bactericidal. This might also be due to insufficient concentration of plant extracts used.

Conclusion

Hence, from the present study it can be concluded that *Azadirachta indica* can be used for the treatment of acne together with the other plant extracts used in the study. Further studies are required for the

characterization of acne-inducing bacteria, for the identification of the secondary metabolites of the plants responsible for anti-acne activity and the concentration of the metabolite at which its activity would be least without showing any toxicity.

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