Subscription Agency
Exploring and Distributing Knowledge Globally

Journal of Advancement in Pharmacognosy
Volume 3 Issue 2, 2023
ISSN: 2582-8401

Evaluation of Antibacterial Effect of Herbal Formulation on Skin Infection causing Bacteria through *In - vitro* Methods

Tanishka Agrawal,[1,2] Priyanka Pandey[1*], Wasim Raja[1]
[1]Central Laboratory Facility, Chhattisgarh Council of Science and Technology, Raipur, (Chhattisgarh)
[2]School of Biological and Chemical Sciences, MATS University, Raipur (Chhattisgarh)

Corresponding Author
Email Id: priyankapandey2907@gmail.com

ABSTRACT

The present review reveals that the plant combination of Azadirachta indica and Ocimum sanctum (Neem and Tulsi) extract is found to have therapeutic uses in treating various ailments. A detailed research work in the characterization and standardization is strongly required for this potential plant in developing its various formulations, which can ultimately be beneficial for humans as well as animals. Further studies are warranted to explore much depth about both plants known by the name "The tree of life". Even though dermatology is characterized by an enormous range of disease/reaction patterns, prevalence surveys suggest that the bulk of skin diseases belong to fewer than ten categories. A methanol extract of Azadirachta indica and Ocimum leaves was examined for its ability to inhibit the growth of Staphylococcus aureus, Staphylococcus epidermidis, Cutibacterium acnes, Corynebacterium granulosum. In the present study, two extractions have been developed containing hydro-alcoholic extract of Ocimum sanctum and Azadirachta indica, which have been reported for their antimicrobial activity. The outcomes shown that the antibacterial property of Azadirachta indica and Ocimum sanctum lwaf extract combination inhibit the growth of microorganisms special on skin disease causing batteries in a dose-dependent manner.

Keywords*: Phytochemical, Antimicrobial, Azadirachta indica, Ocimum sanctum, methanolic, Bacteria, E. Coli, seeds, antibiotics, Skin diseases.*

INTRODUCTION

Skin diseases are a major health problem affecting a high proportion of the population in India (Abolfotouh and Bahamdan, 2000). Skin diseases can place a heavy emotional and psychological burden on patients that may be far worse than the physical impact (Ayer and Burrows, 2006). Increased consciousness especially among the youth of their body and beauty further aggravates their anxiety (Bajaj et al., 2009). Many factors determine the pattern and prevalence of cutaneous diseases among the youth such as gender, race, personal hygiene, quality of skin care, environmental milieu and diet (Dunwell and Rose, 2003). In some instances, patients appear to produce their skin lesions as an outlet for nervous tensions arising from interpersonal conflicts and/or unresolved emotional problems (Obasi and Naguib, 1999).

Even though dermatology is characterized by an enormous range of disease/reaction patterns, prevalence surveys suggest that the bulk of skin diseases belong to fewer than ten categories (Dogra and Kumar, 2003). Such observations are useful in developing educational and preventive health programs for the benefit of university students. Their proper management at

Journal of Advancement in Pharmacognosy
Volume 3 Issue 2, 2023
ISSN: 2582-8401

earlier stages with education of students is important to prevent disfiguring complications and psychological sequelae later in life (Bajaj et al., 2009).

Antibacterial as well as antiviral activity of a molecule is completely associated with the compounds that provincially kill bacteria and virus or slow down their rate of growth, without being extensively toxic to nearby tissues. Most recently discovered antimicrobial agents are modified natural compounds and this modification is done through chemical mode, for example, b-lactams (penicillins), carbapenems, or cephalosporin. Pure natural products, such as aminoglycosides, and other entirely synthetic antibiotics, for example, sulfonamides, are also frequently used. The antimicrobial agents could be classified as the agents that can either be bactericidal, which kill bacteria, or bacteriostatic, which slow down the growth of bacteria. Antibacterial agents are the most important in fighting infectious diseases. But, with their wide use as well as abuse, the appearance of bacterial resistance toward antibacterial agents has become a major problem for today's pharmaceutical industry. Resistance is most commonly based on developmental processes taking place, for example, antibiotic therapy, that leads to inheritable resistance. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines which have made large contributions to human health and well-being. Although many drugs that come from trees generally have been replaced by more potent synthetic ones, trees remain a source for some drug ingredients (Thomson, 1978). Medicinal plants have become important for the treatment of different disease conditions, such as diabetes, malaria, anemia for a long time now (Fola, 1993), but the potential of higher plants as source for new drugs is still largely unexplored (Udupa, 2006). Systematic screening of them may result in the discovery of novel effective compounds (Tomoko et al., 2002).

Neem (*Azadirachta indica*) commonly called ‘India Lilac’ or ‘Margosa’, belongs to the family Meliaceae, subfamily Meloideae and tribe Melieae. Neem is the most versatile, multifarious trees of tropics, with immense potential. It possesses maximum useful non-wood products (leaves, bark, flowers, fruits, seed, gum, oil and neem cake) than any other tree species. Various parts of the neem tree have been used as traditional Ayurvedic medicine in India. Neem oil and the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, and constipation and also as a general health promoter. Neem oil finds use to control various skin infections. Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations and phthisis (Girish and Shankara, 2008).

Tulsi is an aromatic shrub in the basil family Lamiaceae (tribe ocimeae) that is thought to have originated in north central India and now grows native throughout the eastern world tropics (Bast et al., 2014). Within Ayurveda, tulsi is known as “The Incomparable One,” “Mother Medicine of Nature” and “The Queen of Herbs,” and is revered as an “elixir of life” that is without equal for both its medicinal and spiritual properties. Within India, tulsi has been adopted into spiritual rituals and lifestyle practices that provide a vast array of health benefits that are just beginning to be confirmed by modern science. This emerging science on tulsi, which reinforces ancient Ayurvedic wisdom, suggests that tulsi is a tonic for the body, mind and spirit that offers solutions to many modern-day health problems (Singh et al., 2010).

In the present study, 2 extractions have been developed containing hydro-alcoholic extract of *Ocimum sanctum and Azadirachta indica*, which have been reported for their antimicrobial,

Journal of Advancement in Pharmacognosy
Volume 3 Issue 2, 2023
ISSN: 2582-8401

anti-inflammatory and antioxidant activities. The developed formulations were examined for antimicrobial activities against microorganism frequently involved in acne inflammation, P. acnes (Haman, 2008). Therefore, we have to carry out the Antibacterial Effect of Herbal Formulation like neem and tulsi on Skin Infection Causing Bacteria through *In-Vitro* Methods.

MATERIALS AND METHODS

Plant Resources: The *Ocimum sanctum and Azadirachta indica* leaf was obtained from a neighborhood herbal garden of Raipur, Chhattisgarh, dried for a few days in the shade, then powdered, and kept in airtight vials for future study.

Preparation of Extracts: *Ocimum sanctum and Azadirachta indica* leaf (20g) was extracted using a mixture of 50% methanol and Millipore water solvent, then the material was gathered and concentrated in a water bath at 40–50 C. An airtight container was used to keep the dry powder.

Microorganisms: Gram-positive microbes among the microorganisms examined were Bacillus subtilis, Bacillus cereus, Bacillus frimicutes, and the Gram negative bacteria Escherichia coli, Entrobacter, Klebsiella, and Escherichia coli. The National Chemical Laboratory (NCL) in Pune, India provided the bacterial strains used in this investigation. The bacteria were cultivated at 37 degrees Celsius in nutrient broth and maintained at 4 degrees Celsius on nutrient agar slants.

Antibacterial Assay: The agar disc diffusion method was used to investigate the bactericidal activity of *Ocimum sanctum* and *Azadirachta indica* leaf extract at four different concentrations: 100, 75, 50, and 25 mg/ml (Nair et al., 2005). Following the preparation of Muller Hinton agar in accordance with the manufacturer's instructions, the plates were seeded with the appropriate microorganisms like *Staphylococcus aureus, Staphylococcus epidermidis, Cutibacterium acnes, Corynebacterium granulosum*. Discs with a diameter of 6 mm were produced using Whatmann filter paper No. 24 and sterilised. The discs were then impregnated with the extracts and the solvent DMSO Antibiotics for Gram positive and Gram negative bacteria include NX-Norfloxacin, OF- Ofloxacin, E-Erythromycin, and CFM-Cefixime. The control group used was bacteria. The zones of inhibition were measured using a measuring scale after the plates were incubated for 24 hours at 37 degrees Celsius. To assure accuracy, the aforementioned experiment was carried out three times.

RESULT

The initiation of microbial growth was considered as zero hour and further accordingly reading was taken. Our present study shows that antibacterial activity of 50% methanolic extract of *Azadirachta indica* against *Cutibacterium acnes* is best in 75% concentration after 12 hours (11.00mm zone of inhibition). Although 100% concentration is having mild effect as 7mm zone of inhibition. Whereas, antibacterial activity of 50% methanolic extract of *Ocimum sanctum* against *Cutibacterium acnes* is best in 75% concentration after 12 hours (10.1 mm zone of inhibition).

Although 100% concentration is having mild effect as 5mm zone of inhibition. Amongst the 3 formulations, i.e., *Azadirachta indica, Ocimum sanctum* and the combination of both, the best activity was shown by the combination formulation of *Ocimum sanctum* and *Azadirachta indica* against *Cutibacterium acnes* at 75% concentration after 12 hours of incubation (11.55

Journal of Advancement in Pharmacognosy
Volume 3 Issue 2, 2023
ISSN: 2582-8401

mm Zone of Inhibition). On the other hand, other bacteria show static growths in varying concentrations.

Table 1: The study of anti-bacterial activities of standard antibiotics using disk-diffusion method

SI	Bacteria Used	Zone of Inhibition (In Mm)			
		NX10	OF5	E15	CFM5
1	*Staphylococcus aureus*	37.00	32.00	15.00	28.00
2	*Staphylococcus epidermidis*	39.00	36.00	20.00	35.00
3	*Cutibacterium acnes*	39.00	36.00	20.00	35.00
4	*Corynebacterium granulosum*	35.00	39.00	40.10	32.00

Table 2: The study of anti-bacterial activities of *Azadirachta indica* using disk diffusion method (1st observation)

Si	Bacteria Used	Zone of Inhibition (In Mm)			
		25%	50%	75%	100%
1	*Staphylococcus aureus*	7	8	7.9	7.1
2	*Staphylococcus epidermidis*	10	7.5	6.5	8
3	*Cutibacterium acnes*	7	8	11	10
4	*Corynebacterium granulosum*	7.1	9.5	9	9.2

Table 3: The study of anti-bacterial activities of *Azadirachta indica* using disk diffusion method (2nd observation)

Si	Bacteria Used	Zone of Inhibition (In Mm)			
		25%	50%	75%	100%
1	*Staphylococcus aureus*	5	7.5	6.8	7.1
2	*Staphylococcus epidermidis*	7.8	7.2	6.9	8.1
3	*Cutibacterium acnes*	5.1	7.5	9	8.5
4	*Corynebacterium granulosum*	5	8	10	8

Table 4: The study of anti-bacterial activities of *Azadirachta indica* using disk diffusion method (3rdobservation)

SI	Bacteria Used	Zone of Inhibition (In Mm)			
		25%	50%	75%	100%
1	*Staphylococcus aureus*	5.5	8	6.5	7.5
2	*Staphylococcus epidermidis*	8	7.8	7	8.5
3	*Cutibacterium acnes*	7.2	8.5	12	9
4	*Corynebacterium granulosum*	6	8.5	9.2	9

Journal of Advancement in Pharmacognosy
Volume 3 Issue 2, 2023
ISSN: 2582-8401

Table 5: The Study of Antibacterial activities of *Azadirachta indica* using Disk Diffusion method (Mean)

SI	Bacteria Used	Zone of Inhibition (In Mm)			
		25%	50%	75%	100%
1	*Staphylococcus aureus*	5.83	7.83	7.07	7.23
2	*Staphylococcus epidermidis*	8.6	7.5	6.8	8.2
3	*Cutibacterium acnes*	6.43	8	10.67	9.17
4	*Corynebacterium granulosum*	6.03	8.67	9.4	8.73

Table 6: The study of anti-bacterial activities of *Ocimum sanctum* using disk diffusion method (1st observation)

SI	Bacteria Used	Zone of Inhibition (In Mm)			
		25%	50%	75%	100%
1	*Staphylococcus aureus*	5	6	5.5	8.5
2	*Staphylococcus epidermidis*	5	8	6	5.5
3	*Cutibacterium acnes*	6.5	6.8	10.1	5
4	*Corynebacterium granulosum*	6.5	6	8.5	9

Table 7: The study of anti-bacterial activities of *Ocimum sanctum* using disk diffusion method (2nd observation)

SI	Bacteria Used	Zone of Inhibition (In Mm)			
		25%	50%	75%	100%
1	*Staphylococcus aureus*	5.1	5.8	6	7
2	*Staphylococcus epidermidis*	6	8.5	6.5	7
3	*Cutibacterium acnes*	7	6	9	5.5
4	*Corynebacterium granulosum*	7.2	8	7	8.5

Table 8: The study of anti-bacterial activities of *Ocimum sanctum* using disk diffusion method (3rd observation)

SI	Bacteria Used	Zone of Inhibition (In Mm)			
		25%	50%	75%	100%
1	*Staphylococcus aureus*	5.5	6	5.2	8
2	*Staphylococcus epidermidis*	5	8.2	7	6
3	*Cutibacterium acnes*	6.8	7	11	6
4	*Corynebacterium granulosum*	7	7.2	8	8.2

Journal of Advancement in Pharmacognosy
Volume 3 Issue 2, 2023
ISSN: 2582-8401

Table 9: The study of anti-bacterial activities of *Ocimum sanctum* using disk diffusion method (Mean)

SI	Bacteria Used	Zone of Inhibition (In Mm)			
		25%	50%	75%	100%
1	*Staphylococcus aureus*	5.2	5.93	5.57	7.83
2	*Staphylococcus epidermidis*	5.33	8.23	6.5	6.17
3	*Cutibacterium acnes*	6.77	6.6	10.03	5.5
4	*Corynebacterium granulosum*	6.9	7.07	7.83	8.57

Table 10: The study of anti-bacterial activities of *Azadirachta indica* and *Ocimum sanctum* formulation using disk diffusion method (1st observation)

SI	Bacteria Used	Zone Of Inhibition (In Mm)			
		25%	50%	75%	100%
1	*Staphylococcus aureus*	6	7	6.7	7.8
2	*Staphylococcus epidermidis*	7.5	7.75	6.25	6.75
3	*Cutibacterium acnes*	6.75	7.4	11.55	7.5
4	*Corynebacterium granulosum*	6.8	7.75	8.75	9.5

Table 11: The study of anti-bacterial activities of *Azadirachta indica* and *Ocimum sanctum* formulation using disk diffusion method (2nd observation)

Si	Bacteria Used	Zone of Inhibition (In Mm)			
		25%	50%	75%	100%
1	*Staphylococcus aureus*	6.5	6.97	6.73	7.17
2	*Staphylococcus epidermidis*	7.67	7.25	7.25	7.65
3	*Cutibacterium acnes*	7.08	7.73	12.85	7.9
4	*Corynebacterium granulosum*	6.93	7.98	9.08	9.57

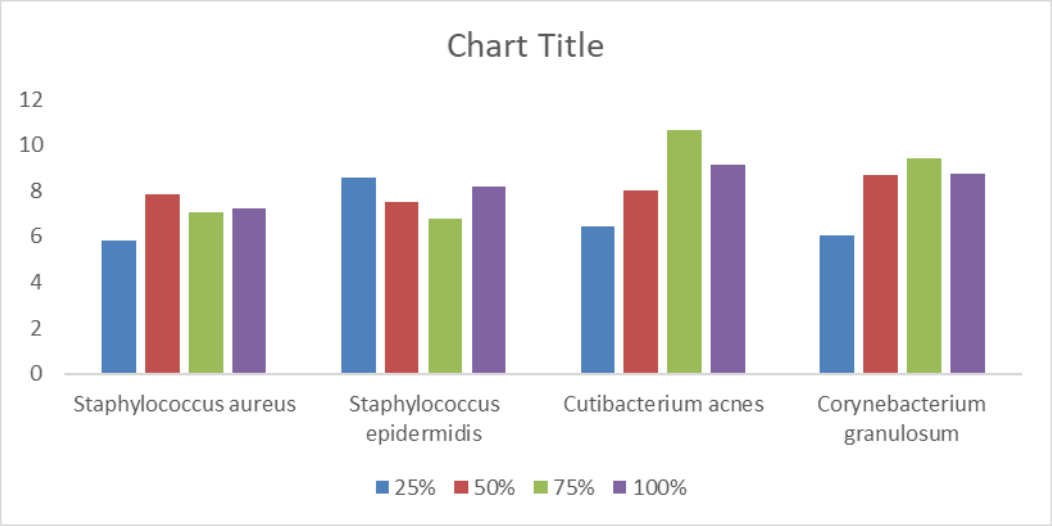
Table 12: The study of anti-bacterial activities of *Azadirachta indica* and *Ocimum sanctum* formulation using disk diffusion method (3rdobservation)

Si	Bacteria Used	Zone of Inhibition (In Mm)			
		25%	50%	75%	100%
1	*Staphylococcus aureus*	7	6.8	6.5	7.2
2	*Staphylococcus epidermidis*	7.5	6.8	7.5	8
3	*Cutibacterium acnes*	6.5	7.8	14	8.2
4	*Corynebacterium granulosum*	6.5	8	9.5	10

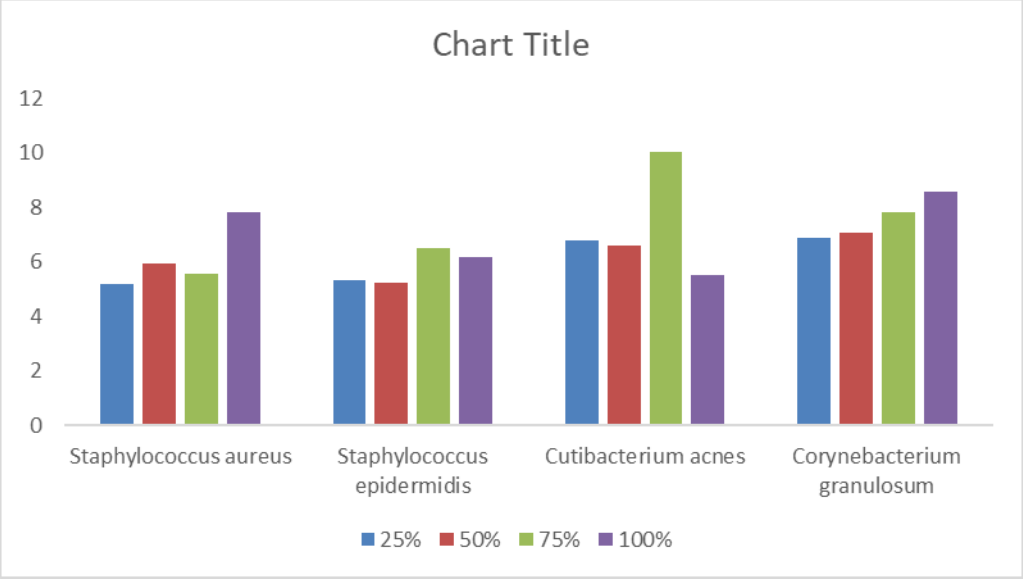
Journal of Advancement in Pharmacognosy
Volume 3 Issue 2, 2023
ISSN: 2582-8401

Table 13: The study of anti-bacterial activities of *Azadirachta indica* and *Ocimum sanctum* formulation using disk diffusion method (Mean)

Si	Bacteria Used	Zone of Inhibition (In Mm)			
		25%	50%	75%	100%
1	*Staphylococcus aureus*	06.50±1.01	07.10±0.8	07.00±0.56	06.50±0.8
2	*Staphylococcus epidermidis*	08.00±0.80	07.20±0.47	08.00±1.12	08.20±0.54
3	*Cutibacterium acnes*	07.00±0.12	08.00±.81	13.00±0.54	08.00±1.25
4	*Corynebacterium granulosum*	07.50±0.75	08.20±12	09.00±.98	09.20±0.75

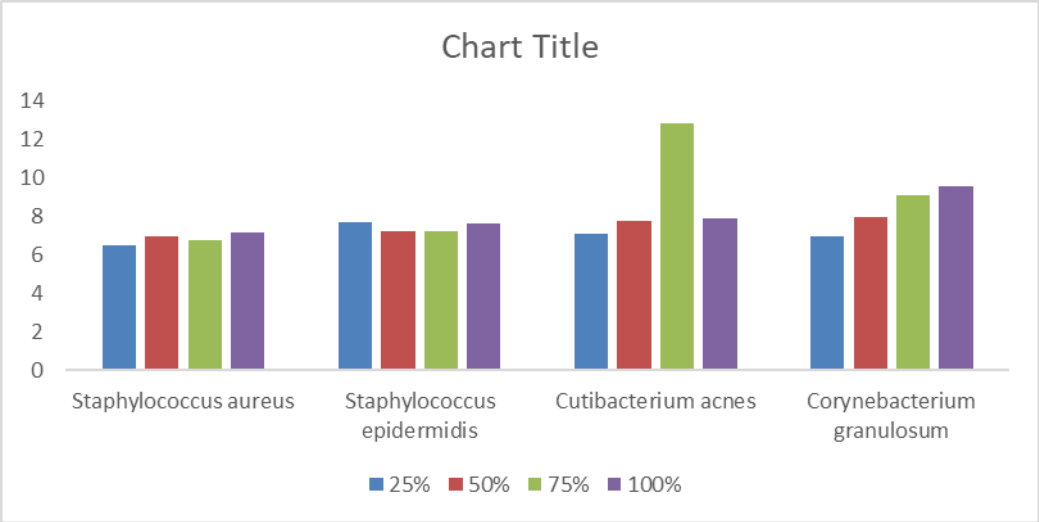


Graph 1: Showing the Mean of Zones of Inhibition of Azadirachta indica extract against different bacteria using Disk Diffusion method



Graph 2: Showing the Mean of Zones of Inhibition of Ocimum sanctum extract against different bacteria using Disk Diffusion method

Journal of Advancement in Pharmacognosy
Volume 3 Issue 2, 2023
ISSN: 2582-8401



Graph 3: Showing the Mean of Zones of Inhibition of Azadirachta indica and Ocimum sanctum formulation against different bacteria using Disk Diffusion method

DISCUSSION AND SUMMARY

The present review reveals that the plant combination of *Azadirachta indica and Ocimum sanctum* (Neem and Tulsi) extract is found to have therapeutic uses in treating various ailments. A detailed research work in the characterization and standardization is strongly required for this potential plant in developing its various formulations, which can ultimately be beneficial for humans as well as animals. Further studies are warranted to explore much depth about both plants known by the name “The tree of life”.

A medicinal plant is a plant that is used with the intention of maintaining health, to beadministered for a specific condition, for both, whether in modern medicine or in traditional medicine (Hall et al, 2012). Medicinal plants may provide three main kinds of benefits: health benefits to the people who consume them as medicines; financial benefits to people who harvest, process, and distribute them for sale; and society- wide benefits, such as job opportunities, taxation income and a healthier labor force (Hall et al., 2012). Although herbal plants have their vital uses but on the other hand, they are also toxic at certain levels if their concentrations are not measured. So it is important to estimate their potential and efficacy.

Our present study shows that the highest antibacterial activity is shown by 50% methanolic extract of formulation of *Ocimum sanctum* and *Azadirachta indica* against *Cutibacterium acnes* in 75% concentration after 12 hours of incubation (11.55 mm Zone of Inhibition). Although 50% concentration is having mild effect as 7.4mm Zone of Inhibition. Against *Corynebacterium granulosum*, 50% methanolic extract of *Azadirachta indica* in 50% concentration is having a good effect as 9.5mm. On the other hand, 50% methanolic extract of *Ocimum sanctum* in 50% concentration is having a mild effect against *Corynebacterium granulosum* as 6mm. On the other hand, other bacteria show static growths in varying concentrations.

The above observations suggest that different concentrations (50% 75% 100%) were having good antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Cutibacterium acnes* and *Corynebacterium granulosum*. Thus the extracts have varying activities against all microorganisms. In our study the extracts of *Azadirachta indica* and

Journal of Advancement in Pharmacognosy
Volume 3 Issue 2, 2023
ISSN: 2582-8401

Ocimum sanctum have shown highest antibacterial activity against *Cutibacterium acnes,* formerly known as *Propionibacterium acnes*. Although, it also showed activity against other mentioned bacteria as well at different concentrations.

Medicinal herbs as potential source of therapeutics has attained a significant role in health system all over the world for both humans and animals not only in the diseased condition but also as potential material for maintaining proper health. Research to find out scientific evidence for claims by tribal healers on Indian herbs has been intensified. Once these local ethno medical preparations are scientifically evaluated and disseminated properly, people will be better informed regarding efficacious drug treatment. Determining the biological activity properties of plants used in traditional medicine is helpful to the rural communities and informal settlements. Several authors are currently being undertaken to isolate the active compounds by bioassay-guided fractionation from the species that showed high biological activity during screening. Therefore, these scientific investigations may be utilized to develop herbal drugs for these diseases and improved health status. Neem and tulsi have been widely used because of their anti-inflammatory, antimicrobial and antioxidant activities. Thus further studies on the phytochemicals and properties of Tulsi and Neem can be done and it can be helpful in inventing a new drug against acne and other skin infections with negligible side effects, if so.

REFERENCES

1) Abolfotouh MA, Bahamdan K. Skin disorders among blind and deaf male students in Southwestern Saudi Arabia. Ann Saudi Med. 2000; 20:161–4.
2) Bajaj DR, Devrajani BR, Ghouri RA, Matlani BL. Pattern of skin disorders among adolescent female students at Hyderabad, Sindh. J Pak Assoc Derma. 2009; 19:79–85.
3) Bast F, Rani P, Meena D. Chloroplast DNA phylogeography of holy basil (Ocimumtenuiflorum) in Indian subcontinent. ScientificWorldJournal2014; 2014:847, 482.
4) Dunwell P, Rose A. Study of the skin disease spectrum occurring in an Afro-Caribbean population. Int J Dermatol. 2003; 42:287–9.
5) Girish K, Shankara BS. Electronic Journal of Biology. 2008; 4(3):102-111.
6) Hannan JM, Marenah L, Ali L, Rokeya B, Flatt PR, Abdel-Wahab YH. *Ocimum sanctum* leaf extracts stimulate insulin secretion from perfusd pancreas, isolated islets and clonal pancreatic beta-cells. J Endocrinol. 2006; 189:127–36.
7) Obasi OE, Naguib M. Dermatitis artefacta: A review of 14 cases. Ann Saudi Med. 1999; 19:223–7.
8) Singh S, Malhotra M, Majumdar DK. Antibacterial activity of *Ocimum sanctum* L.fixed oil. Indian J Exp Biol. 2005; 43:835–7.
9) Thomson WAR. (ed). Medicines from the Earth. McGraw-Hill Book Co, Maidenhead, United Kingdom, 1978.
10) Tomoko N, Takashi A, Hiromu T, Yuka I, Hiroko M, Munekazu I *et al.* Antibacterial activity of extracts preparated from tropical and subtropical plants on methicillin-resistant Staphylococcus aureus. J Health Sci.
11) Udupa SL, Shetty S, Udupa AL, Somayaji SN. Effect of *Ocimum sanctum* Linn.on normal and dexamethasone suppressed wound healing. Indian J Exp Biol. 2006; 44:49–54.