



**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

**EVALUATION OF ANTIMICROBIAL ACTIVITY AND MIC OF TRADITIONALLY
USED MEDICINAL PLANT *AZADIRACHTA INDICA* AGAINST OPPORTUNISTIC
HUMAN PATHOGENIC MICROORGANISMS**

GIRI RP^{1*}, GANGAWANE AK², AND GIRI SG³

1: Ph.D. Scholar, Parul Institute of Applied Sciences, Parul University, Limda, Waghodia Road, Vadodara
- 391760

2: Deputy Registrar (Academic), Parul University, Limda, Waghodia Road, Vadodara - 391760.

3: Assistant Professor, Parul Institute of Applied Sciences, Parul University, Limda, Waghodia Road,
Vadodara - 391760

***Corresponding Author: Mr. Rudra Prasad Giri: E Mail: rgiri22@gmail.com**

Received 12th April 2020; Revised 26th May 2020; Accepted 20th July 2020; Available online 1st Feb. 2021

<https://doi.org/10.31032/IJBPAS/2021/10.2.5339>

ABSTRACT

Antimicrobial activity in leaf extract of neem (*Azadirachta indica*) against human pathogenic microorganism: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Candida albicans*. Antimicrobial activities of aqueous, methanol and ethyl acetate extracts of neem leaves were used. Varying concentration of each extracts 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.625 mg/ml prepared in DMSO and tested using well diffusion method and also checked for MIC. When compared with ampicillin-20mg/ml, the Ethyl Acetate extract shows highest zone of inhibition followed by methanol extract and aqueous extract on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Candida albicans* in an ascending order.

Keywords: *Azadirachta indica*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*,
Escherichia coli, *Enterococcus faecalis*, *Candida albicans*, microbiota

INTRODUCTION

Neem (*Azadirachta indica*) is a very popular tree in India. Neem is originated in Assam in northeast India, and Myanmar. Later, it was naturally allocated throughout much of the Indian subcontinent, particularly in drier areas. Neem name was originated from the Sanskrit Nimba, and it was known as the curer of all illness. The neem tree was intimately connected with the routine life of Indians. Neem tree is belonged to Meliaceae family. The taxonomy of neem is Plantae (kingdom), rutales (order), azadirachta (genus), indica (species). It is commonly called 'Indian Lilac' or 'Margosa'. Neem is one of the prime members of Traditional medicine. But validation and quality control of herbal medicine is quite critical to establish therapeutic efficacy and safety in healthcare use along with implementation of quality assurance is an important area in India [1]. Neem plant is widely cultivated throughout the world due to its adaptive to various climate conditions [2]. It is one of the most considered medicinal plants and is used for health.

Neem tree is familiar as 'the village pharmacy' in India. Neem is referred as "a tree for solving global problems" because it has many benefits and it can treat various diseases [3]. The plant product or natural

products show an important role in diseases prevention and treatment. Neem is one of the most strong blood purifiers, detoxifiers and immune system boosters known. Herbal remedies are known to cure many infectious diseases throughout the history of mankind. Thus, the discovery of medicinal plants as antimicrobial agents is useful in expanding the wide varieties of antibiotics available [4]. Neem leaves serve as antibacterial, antifungal, antiviral, anti-inflammatory, antimalarial, antioxidant, antiulcer, antimutagenic, antifertility, herbal contraception through spermicidal and non-hormonal activities without any side effect [5]. In Gambia and Ghana, neem leaves are made as tea drink to stop pregnancy at the age of the first 2-3 weeks [6]. The antibacterial properties of neem leaves have also been used for controlling airborne bacterial contamination in residential properties [7]. Neem leaf extract is used as an herbal remedy and as a source of many therapeutic agents in different countries. *Azadirachta indica* is used for the treatment of diabetes because of its potential anti-diabetic properties [8].

Neem has a tremendous effect on chronic skin conditions. Acne, psoriasis, eczema, ringworm and even stubborn growth are

among the affect that can clear up easily when high quality, organic neem oil is used. Neem oil and leaves has been applicable in siddha medicine for thecure of skin diseases [9]. The paste of the neem leaves with turmeric powder is applied externally on small pox eruption, viral infection, itching sole of the foot, cracking of the heels, and is also used to cure boils and burns. The paste of the flowers if consumed regularly stops burning of the eyes, watery eyes, giddiness, vomiting, tastelessness, and heart burn [10]. However public interest towards herbal herbal remedies increasing but many of them have not undergone careful scientific assessment, and some have the potential to cause serious toxic effects and major drug-to-drug interactions [11]. A source of antimicrobial activity has been reported from many plants therefore these plants may use as a source of effective and powerful drugs in different countries in the world [12].

Crude extracts of the neem plant *Azadirachta indica* which were earlier determined to have enduring antimicrobial activity were investigated for their rate and area of bacterial killing. Different concentrated extract solutions reacted to the Minimum inhibitory concentration of type culture strains of *Staphylococcus aureus*, *Escherichia coli*; *Pseudomonas aeruginosa*;

Enterococcus faecals and *Candida albicans* were used.

Each and every parts of the neem tree – leaves, flowers, seeds, fruits, root and bark have been used traditionally for the treatment of inflammations, infection, fever, skin disease, and dental disorders [13]. All parts of the neem tree have been utilized as a traditional medicine for house-hold remedies against various human ailments, from antiquity [14]. Ayurveda evaluated neem as a cure for various ailments, predominantly due to its great antimicrobial activity. It is now served as a valuable source of unique natural products, for the development of industrial products.

Leaf extracts of neem were found to have a potent inhibitory action on a wider spectrum of microorganisms, including *C. albicans* [15]. The hot and acetone extracts of the bark, leaf and seeds of the plant differ in their inhibiting property of fungi.

In a study antidermatophytic activity of the aqueous and ethanolic extracts of neem leaves was investigated against 88 clinical isolates of dermatophytes by agar dilution. The ethanolic extract was found to be more active inhibiting of the isolates [16].

It has been suggested that the actions of antimicrobial agents could be adequately distinguished by certain mechanisms such as

selective inhibition of bacterial cell wall synthesis, attachment to receptors and inhibition of transpeptidation enzymes, thereby blocking the final stage of peptidoglycan synthesis [17].

It is observed that the marketed herbal products contained herbs, which had confirmed data for their antimicrobial activity, still most of them failed to produce any pharmacological results such things need to be checked by manufacturer and prescriber to get expected effect [18].

Neem has active ingredients those shows chemo preventive effect towards various tumours via modulation of numerous cell signalling pathways. But study should be performed in animal model to understand mechanism of action in the diseases management [19].

The Methanol neem extract contains a photochemical substance, such as phenolic and flavonoids, which may inhibit the growth of foodborne bacteria. Neem leaf extract displayed antibacterial activity against some bacteria due to the high contents of antioxidant, which confirm the presence of a bioactive compound against these bacteria [20].

Investigation shows that the crude extracts of neem showed significant antioxidant activity; thus, these extracts could be used as natural

antioxidants for the preparation of medicines to treat difference disease [21].

The crude extract *Azardirachta indica* leaves possesses significant antibacterial activity on isolated fish pathogens. The susceptibility test was regulated on isolates using antibiotics, selected based on their importance to human medicine and on fish production [22].

Neem is now commercially marketed in India by many manufacturer, we expect a good brand always produce a quality product, but question arises when we find many complain related to quality issue of herbal product. In reality we can easily find large no of substandard neem product available in the market. Synthetic medicine has a standard dose but herbal medicine manufacturers are not strict to any particular strength. Side effects of neem has already been reported which can't be neglect when it comes for human consumption. So a standard MIC for different crude leaf extract is required to be established before evaluating efficacy or effectiveness of marketed neem formulation. As use of neem internally or externally is considered to be good hence efficacy of pure neem extract on human opportunistic pathogenic microorganism member of microbiota i.e. *Staphylococcus aureus* (skin infections, respiratory infections),

Pseudomonas aeruginosa (cystic fibrosis and traumatic burns), *Escherichia coli* (serious food poisoning), *Enterococcus faecalis* (nosocomial Infection), *Candida albicans* (candidiasis) are required to be investigated.

MATERIALS AND METHODS

Plant sample: *Azadirachta indica* leaves were collected from local area in Vadodara, Gujarat. The leaves were washed in normal water, rinsed in sterile distilled water and dried for 5-6 days under room temperature. The dried leaves were then blended to powder with a clean kitchen blender and stored in air tight glass containers until required for preparation.

Preparation of Leaf Extracts: 50 grams leaf powder of *Azadirachta indica* was weighed into three separate conical flasks. In each conical flask 150 ml of extractant (Water, Methanol and Ethyl acetate) added [23]. The mixtures were properly agitated and allowed to stand at room temperature for 3 days. The solutions were then filtered using muslin cloth and each filtrate was poured into 100ml glass container separately for evaporating. The filtrates were concentrated to dryness by placing on heating mantle at 40°C to dried-off the remaining extractant and stored at 4°C for future use [24].

Microorganism Preparation: Human opportunistic pathogenic microorganism

present in human microbiota were selected for the study, they are *Staphylococcus aureus*, *Enterococcus faecalis* (Gram positive), *E.coli*, *Pseudomonas aeruginosa* (Gram negative) and *Candida albicans* fungi. All selected microorganism were obtained from Pathological Laboratories of Parul Sevashram Hospital, Vadodara, Gujarat.

The bacterial strains were maintained in Nutrient Broth (at pH 7.4±0.2) at 36±1°C and fungi *Candida albicans* was maintained in Potato dextrose broth (at pH 5.1±0.2) at 25-30°C. All culture media was procured from HiMedia Laboratories and prepared as per given procedure. The stock culture slants were maintained at 4°C in the laboratory of Parul Institute of Applied Sciences.

Antimicrobial Assay of Plant Extracts: Antimicrobial assay of water, methanol and ethyl acetate extracts of neem leaves were performed by agar well diffusion method in Muller Hinton agar (MHA) plates. An aliquot (0.02 ml) of microbial culture was added to molten MHA at 45°C and poured into the petriplate. After solidification of the agar, eight wells of 8mm were bored in the inoculated media with the help of sterile cork borer (8mm). 500, 250, 125, 62.5, 31.25, 15.625mg/ml concentrations of the extracts were prepared using dimethyl sulfoxide (DMSO) respectively [23]. 100µl of each

concentration was added to the wells. Bacterial cultures were incubated at 37°C for 24 hours and fungal cultures at 25°C for 48 hours. Antimicrobial activity was determined by measuring the zone of inhibition surrounding the well along with Minimum inhibitory concentration (MIC), which is defined as the lowest concentration of the compound that inhibits the growth of microorganisms [25, 26].

The assays were carried out under aseptic conditions maintaining required biohazard level. Ampicillin (20mg/ml) was used as positive controls and DMSO as a negative control. Each concentration included duplicates and the results are average of two independent experiments.

RESULT AND DISCUSSION

Antimicrobial activity of the extract: The results of the antimicrobial activity of the extract on the test organisms are shown in **Tables 1, 2, and 3**. The plant extract showed different degrees of inhibitions on the test organisms. The ethyl acetate extract had more activity on the test organisms than the other solvent extractions.

Minimum inhibitory concentration (MIC) of the extract: The results of the MIC of the extracts on the test organisms are shown in **Table 4**. The MIC of the three extracts was 125 mg/ml for the organisms respectively.

Table 1: Inhibition zone diameter of aqueous extract of neem leaf (mm)

Test organism	Concentration of extract (mg/ml)						Positive control (Ampicillin) 20mg/ml	Negative control(DMSO)
	500	250	125	62.5	31.25	15.625		
<i>Staphylococcus aureus</i>	20	17	15	12	09	06	15	-
<i>Pseudomonas aeruginosa</i>	15	12	08	05	-	-	13	-
<i>E. coli</i>	19	16	14	12	10	08	14	-
<i>Enterococcus faecalis</i>	13	10	09	06	-	-	22	-
<i>Candida albicans</i>	18	16	13	11	07	-	15	-

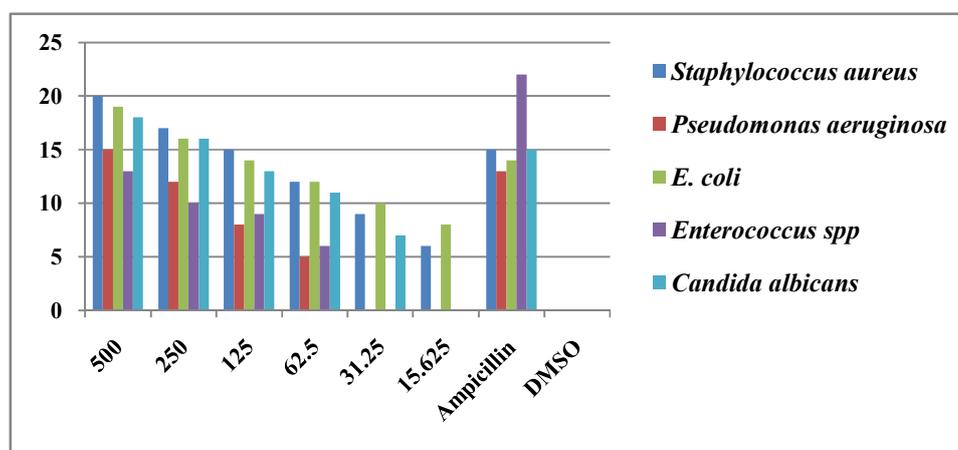


Figure 1: Inhibition Effect of Different Aqueous Extract of Neem Leaf

Table 2: Inhibition zone diameter of Methanolic extract of neem leaf (mm)

Test organism	Concentration of extract (mg/ml)						Positive control (Ampicillin) 20mg/ml	Negative control (DMSO)
	500	250	125	62.5	31.25	15.625		
<i>Staphylococcus aureus</i>	18	16	13	09	08	-	32	-
<i>Pseudomonas aeruginosa</i>	14	09	07	05	-	-	25	-
<i>E. coli</i>	18	16	14	13	10	08	19	-
<i>Enterococcus faecalis</i>	15	11	08	-	-	-	22	-
<i>Candida albicans</i>	20	17	15	14	12	10	17	-

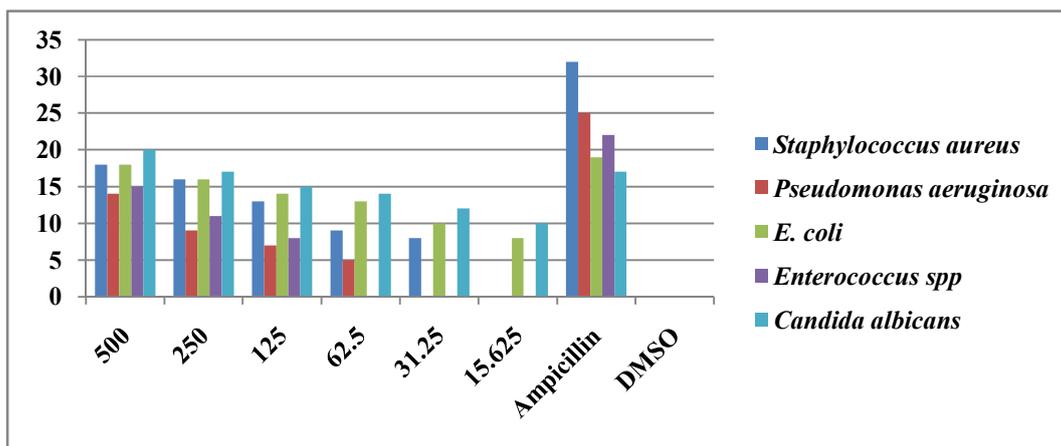


Figure 2: Inhibition Effect of Methanolic extract of neem leaf (mm)

Table 3: Inhibition zone diameter of Ethyl Acetate extract of neem leaf

Test organism	Concentration of extract (mg/ml)						Positive control (Ampicillin) 20mg/ml	Negative control (DMSO)
	500	250	125	62.5	31.25	15.625		
<i>Staphylococcus aureus</i>	17	15	14	12	09	07	32	-
<i>Pseudomonasaeruginosa</i>	16	14	12	09	-	-	25	-
<i>E. coli</i>	19	17	16	13	10	09	14	-
<i>Enterococcus faecalis</i>	13	09	04	-	-	-	18	-
<i>Candida albicans</i>	18	15	13	12	10	08	17	-

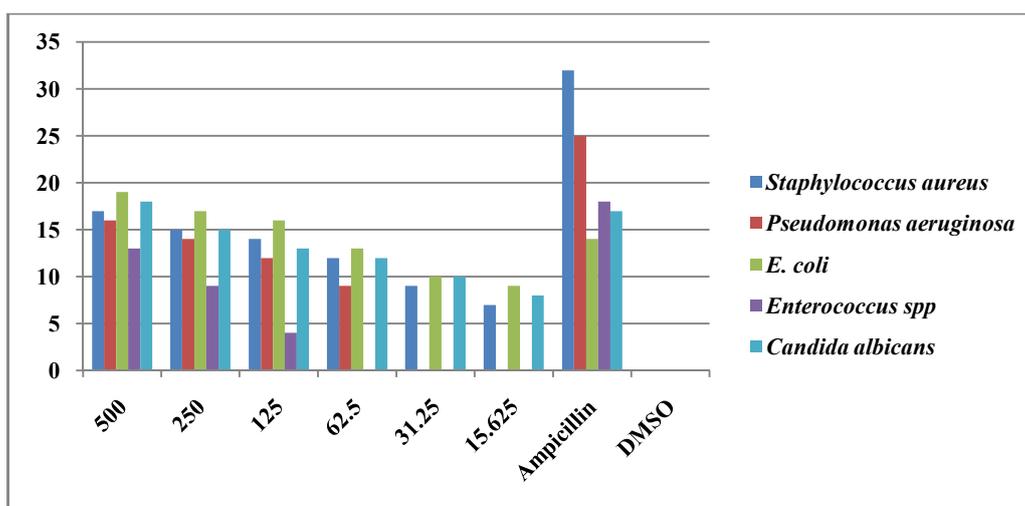


Figure 3: Inhibition Effect of Ethyl Acetate extract of neem leaf

Table 4: Minimum Inhibitory Concentration (mg/ml) Of Neem Leaf Extract On Test Organisms

Test organism	Minimum Inhibitory Concentration (mg/ml)		
	Water Extraction	Methanol Extraction	Ethyl acetate extraction
<i>Staphylococcus aureus</i>	15.625	31.25	125
<i>Pseudomonasaeruginosa</i>	62.5	62.5	62.5
<i>E. coli</i>	15.625	15.625	15.625
<i>Enterococcus faecalis</i>	62.5	125	125
<i>Candida albicans</i>	31.25	15.625	15.625

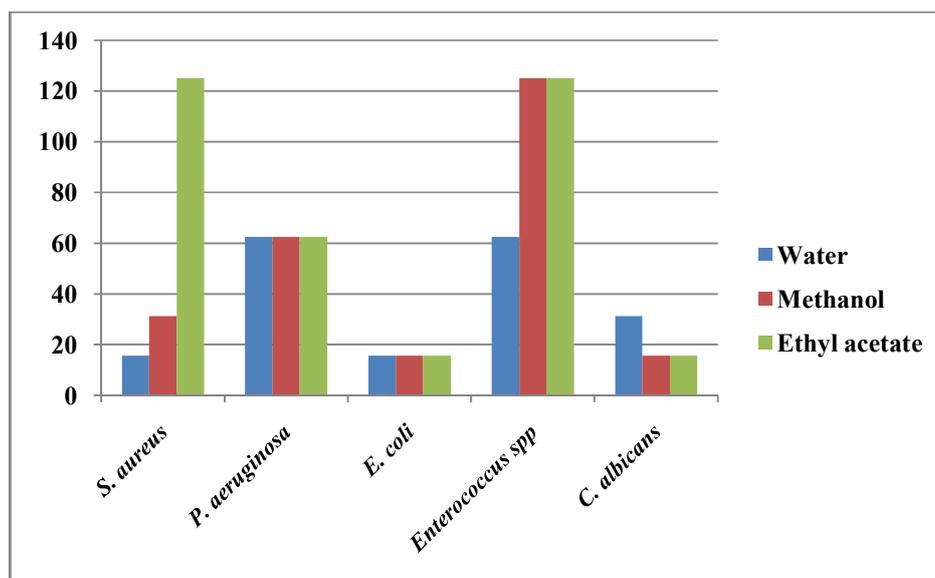


Figure 4: Minimum Inhibitory Concentration (mg/ml) Of Neem Leaf Extract on Test Organisms

In the present study, it has been found that three different extract of the Neem leaf had appreciable effect on the tested microorganisms (Table and Figure 1, 2, 3). This result shows, all neem leaf extract possess inhibitory effect but in different concentration level. Aqueous extract of the Neem leaves had shown appreciable inhibition effect on the tested microorganisms (Table 1). The antimicrobial potency of aqueous extracts of *A. indica* leaf found highly active in all tested concentration level. Where *Staphylococcus aureus*, *E. coli* and *Candida albicans* were

inhibited by aqueous extract of neem leaf in lower concentration level.

Both Methanolic and Ethyl acetate extraction shows good inhibition effect against all microorganisms however ethyl acetate extraction has shown better result. These results are supporting result of similar experiment performed by other researcher. On the basis of available maximum marketed strength highest strength of 500mg/ml have been selected.

The MIC values (Table 4, Figure 4) were found to range from 125mg/ml to 15.625mg/ml for ethyl acetate, methanol and

aqueous: thus indicating that evaluation of MIC is sufficient for measuring microbial activity. The result of MIC showed that ethyl acetate extract was more effective against the test organisms than other extract. The study showed that the standard antibiotics used as positive control compare well with the plant extract.

Staphylococcus aureus and *Enterococcus faecalis* are gram positive bacteria; *E.coli* and *Pseudomonas aeruginosa* are gram negative; and *Candida albicans* is a fungi and all are opportunistic human pathogen. However they all are member of normal human microbiota and rested by immunity but their growth above certain level can bring serious illness, as neem leaf have shown anti-microbial effect against them hence regular use of neem may keep our immunity compatible.

As many synthetic drug causes serious adverse effect so now a days large no of herbal products are marketed in India in different dosage form and strength; and becoming public's choice attraction because of ancient believe. The numerous reports of adverse effects and widespread sale of adulterated products and misleading health claims of these products demand proper regulations on botanical medicine [27]. Leaf, bark, flower etc. parts of *Azadaricta*

indica are widely used in the manufacturing of herbal products. If we look into the market we will find huge no of companies are manufacturing neem contain products and claiming their effectiveness in label but on the other hand large number of customer complained us to find the effect of pure neem on disease causing microbes through standard simple method [28]. We purchase several neem products from market for maintaining our good health and immunity. So in the scope of the study microorganism those are opportunistic human pathogen and member of human microbiota selected. Pure neem extract has shown appreciable result which need to be compared with marketed product.

CONCLUSION

The results of the present study indicate pure crude neem extraction above 125mg/ml concentration is highly active in killing of tested microbes rather than powder form of neem leaf. Application of neem product in extracted form may be more effective in treatment of disease condition by specified microorganism. However as this in-vitro experiment has been performed in standard laboratory condition maintaining required level of safety to find out the efficacy but clinical trial require to predict the actual effectiveness of different neem extract in

treatment of disease. As it is now known neem has its side effect so maximum dose tolerability study for neem need to be performed. Along with marketed neem product are need to be tested for authenticity and efficacy. Unlike synthetic medicine herbal medicine contain several bio active compounds which put a researcher in more critical situation. So well formulated more research are required. As a range from 125mg/ml to 15.625mg/ml of MIC has been found effective, hence for marketed product concentration of 125mg/ml or above can be assumed to be effective.

REFERENCE

- [1] Mukherjee P K, Harwansh R K, Bahadur S, Banerjee S, Kar A, “Evidence Based Validation of Indian Traditional Medicine – Way Forward”, World J. Tradit. Chin. Med., 2, 1, (2016), 48–61.
- [2] Ashafa A O T, Orekoya L O and Yakubu M T, “Toxicity profile of ethanolic extract of *Azadirachta indica* stem bark in male wistar rats”, Asian pacific journal of tropical biomedicine., 2,10(2012),811-817.
- [3] Kumar V S and Navaratnam V, “Neem prehistory to contemporary medicinal uses to human kind”, Asian pacific Journal Tropical Biomedicine., 3, 7(2013), 505-514.
- [4] Zaidan M R S, Rain A N, Badrul A R, Adlin A, Norazah A, Zakiah I, “In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method”, Tropical biomedicine., 22, 2(2005), 165-170.
- [5] Tiwari S, Verna A K, Chakraborty S, Dharma K and Singh V C, “Neem and its potential for safe guarding health of animals and human”, A Review Journal of Biological Sciences., 14, 2(2014), 110-123.
- [6] Ketkar A Y, Ketkar C M, Jacobson M, S M and Schumutterer H, “Various uses of neem products”, The neem tree., <https://neemfoundation.org/the-neem-tree/>, (2005)518-558.
- [7] S A and Aslam J, “Study on the effect of neem leaves smoke in controlling air-borne bacteria in residential premises”, Current Research in Bacteriol., 1, 2 (2008), 64-66.
- [8] Dholi S K, Raparla R, Mankala S K, Nagappan K, “In vivo antidiabetic evaluation of neem leaf extract in alloxan induced rats”, J. App Pharm Sci., 01, 04, (2011), 100-105.
- [9] Thas JJ, “Siddha medicine-background and principles and the

- application for skin diseases”, Clinics in Dermatol., 26, 1, (2008) 62-78.
- [10] Shanmugham NK, “Dictionary of medicinal plants”, Nakkeeranpaddipakkam V ellitu., (1989), 686-687.
- [11] Dhamji H K and Chauhan A S, “Herbs now in arena of hyperlipidaemia”, Der Pharmacia Sinica., 2, .3, (2011), 51-59.
- [12] Srivastava J, Lambert J, Vietmeyer N, “Medicinal plants: an expanding role in development,” World Bank technical paper, 320, (1996), 21-36.
- [13] Subapriya R, and Nagini S, “Medicinal properties of neem leaves: A review” Curr. Med. Chem. Anticancer Agents. 5, 2, (2005), 149-156.
- [14] Koul O. Isman MB and Kelkar CM, “Properties and uses of neem, *Azadirachta indica*” Canadian Journal of Botany., 68, (1990), 1-11.
- [15] Mahmoud D A, Hassanein NM, Youssef KA, Abou Zeid MA, “Antifungal Activity Of Different Neem Leaf Extracts And The Nimonol Against Some Important Human Pathogens”, Brazilian Journal of Microbiology., 42,(2011), 1007-1016.
- [16] Venugopal P V, Venugopal T V, “Antidermatophytic activity of neem leaves in vitro”, Indian Journal of Pharmacology., 26(1994), 141-143.
- [17] Baggot J D, “Antimicrobial selection, administration and Dosage”, Journal of the South African Veterinary Association., 69, 4 (1998), 174-185.
- [18] Jan F, Hussain I, Muhammad N, Khan M A, Khan M A, Akbar M T and Ahmad W, “In-vitro evaluation of Antimicrobial Branded Herbal formulation for their efficacy”, J Clin Toxicol., 4, 2(2014), 1000188.
- [19] Alzohairy M A, “Therapeutics Role of *Azadirachta indica* (Neem) and Their Active Constituents in Diseases Prevention and Treatment”, Evidence-Based Complementary and Alternative Medicine, Hindawi Publishing Corporation., 2016, 11 (2016), 1-11.
- [20] Yehia H M, “Methanolic extract of neem leaf and its antibacterial activity against foodborne and contaminated bacteria on sodium dodecyl sulphate-polyacrylamide gel electrophoresis”, American-Eurasian J. Agric. And Environ. Sci., 16, 3 (2016) 598-604.

- [21] AL-Hashemi Z SS, Hossain M A, “Biological activities of different neem leaf crude extracts used locally in Ayurvedic medicine”, Pacific Science Review A: Natural Science and Engineering, 18, 2(2016), 128-131.
- [22] Kavitha M, Raja M, Kamaraj C, Karthik R R, Balasubramaniam V, Balasubramani G and Preumal P, “In vitro antimicrobial activity of *Azadirachta indica* leaves against fish pathogenic bacteria isolated from naturally infected *Dawkinsia filamentosa* (black spot barb)”, Medicinal & Aromatic Plants (Los Angeles), an open access journal., 6, 3(2017), 2167-0412.
- [23] Maragathavalli S, Brindha S, Kaviyarasi N S, B Annadurai B, Gangwar S K, “Antimicrobial Activity In Leaf Extract Of Neem (*Azadirachta indica* Linn.)”, International Journal of Science and Nature., 3, 1, (2012), 110-113.
- [24] Habila M P, Philip M W, Chidama S S, Wonder S K, “Antibacterial effects of crude extract of *Azadirachta indica* against *Escherichia coli*, *Salmonella spp* and *Staphylococcus aureus*”, International Journal of Medicine and Medical Sciences., 5, 1, (2013), 14-18.
- [25] Perez C, Paul M and BazerqueP, “Antibiotic Assay by Agar Well Diffusion Method,” ActaBiologiae et Medicine Experimentalis, 15, (1990), 13-135.
- [26] Reddy Y R R, Kumari C K, Lokanatha O, Mamatha S, Reddy C D, “Antimicrobial activity of *Azadirachta indica* (neem) leaf, bark and seed extracts”, International Journal of Research in Phytochemistry & Pharmacology., 3, 1, (2013), 1-4.
- [27] WHO “Research guidelines for evaluating the safety and efficacy of herbal medicine”, Manila: WHO Regional office for the Western Pacific., https://apps.who.int/iris/bitstream/handle/10665/207008/9290611103_en.pdf?sequence=1&isAllowed=y.,(1993) 1-86.
- [28] Giri RP, Gangawane A K., Giri S G, “Herbal Medicine and Indian Market”, Alochana Chakra Journal, 9, 5 (2020), 6241-6246.