ANTIMICROBIAL ACTIVITY OF BIOACTIVE COMPOUNDS AGAINST TARGET PATHOGENS

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Abstract

Azadirachta indica has been used for millennia as a traditional remedy for a multitude of human ailments. Currently, the extensive antimicrobial activities of *A. indica* are being explored through research in the fields of dentistry, food safety, bacteriology, mycology, virology, and parasitology. The agar diffusion test was used to check the antimicrobial activity of the *A. indica* extract along with *Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli* and *Salmonella typhi* pathogens. The values of Zone of Inhibition were tabulated according to target pathogens. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal *Concentrations* (MBC) values were also recorded. *A. indica* extract of high antimicrobial activity against *Salmonella typhi* but was less active *Bacillus subtilis*. Moreover, the various ongoing studies and the diverse properties of *A. indica* discussed herein may serve as a guide for the discovery of new antimicrobials.

Keywords: *Azadirachta indica*, antimicrobial activity, MIC, MBC, pathogens.

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1. INTRODUCTION

Azadirchta indica (Neem tree) is related to the family *Meliaceae*. It is tropical evergreen tree at altitudes between sea level and 700 m, the best growth for Neem is in 9.5-37°C, it could tolerate 50°c but it could not grow in less than 4°C, and the annual rainfall (450 - 1200mm). It has been used in ayurvedic medicine for more than 4000 years due to its medicinal properties, Most of the plant such as fruits, seed, leaves, bark and root contain compounds which provided to be antiseptic, antiviral, antipyretic, anti-Inflammatory, antiulcer and antifungal. *Azadirachta indica* is used in India and Africa to make all sort of consumer products, such as pesticides and insect repellents, soaps, cosmetics, toothpaste, antiseptic, gargle, ointment, poultries, lubricants, fertilizers, fuel for oil lamps, rope, glue and tannin from bark fibre etc. It is important to mention that because Neem products are used for human consumption and medication, exposure to Neem in the process of treating plants with Neem oil poses no threat to humans or other animals. Moreover, Neem is not harmful to beneficial insects, affecting only those insects feeding on plants treated with Neem. Since most predator insect does not also feed on plants, they are not harmed by the presence of Neem. This impliesthat there is product safety. Neem *(Azadirachta indica)* is also environmental friendly in the sense that it biodegrade in a matter of weeks when exposed to sunlight or in soil. Furthermore, apart from the above mentioned uses of Neem *(Azadirachta indica*) seed oil, numerous tests have shown Neem oil to be effective as an insecticide, miticide, fungicide, nematocides and as an insect antifeedents and repellents. (Ukaoma et al., 2019).

Azadirachtaindica L. (neem) shows therapeutics role in health management due to rich source of various types of ingredients. The most important active constituent is azadirachtin and the others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin. Leaves contain ingredients such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol (Ali, 1993; Hossain et al., 2011; Kokate et al., 2010). Quercetin and ß-sitosterol, polyphenolic flavonoids, were purified from neem fresh leaves and were known to have antibacterial and antifungal properties (Govindachari et al., 1998) and seeds hold valuable constituents including gedunin and azadirachtin.

2. MATERIAL AND METHODS

2.1 Preparation of extracts

Prepared plant extract were collected from Reeva Herbal Pvt. Ltd, Ahmedabad. Decoction method with PEG for plant extraction. Prepared plant extract dilute with distilled water in 5:1 ratios before testing it for antibacterial activity (Daswani et al,. 2011).

2.2 Qualitative Phytochemical Screening (Tyagi and Agarwal, 2017; Roghini and Vijayalakshmi, 2018).

2.2.1 Test for Carbohydrates: The presence of carbohydrates was confirmed when 2 ml of extract was treated with 1 ml of Molisch's reagent and few drops of concentrated sulphuric acid which resulted in the formation of purple or reddish color.

2.2.2 Test for Tannins: To 1 ml of extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

2.2.3 Test for Saponins: 2 ml of extract, 2 ml of distilled water were added and shaken in a graduated cylinder for 15 min lengthwise. It resulted in the formation of 1 cm layer of foam that indicated the presence of saponins.

2.2.4 Test for Alkaloids: To 2 ml of extract, 2 ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagentwere added. Presence of green color or white precipitate indicates the presence of alkaloids.

2.2.5 Test for Flavonoids: To 2 ml of extract, 1 ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

2.2.6 Test for Glycosides: To 2 ml of extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

2.2.7 Test for Glycosides: To 2 ml of extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

2.2.8 Test for Quinones: To 1 ml of extract, 1 ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.

2.2.9 Test for Phenols: 2 ml of distilled water followed by few drops of 10% ferric chloride was added to 1ml of the extract. Formation of blue or green color indicates presence of phenols.

2.2.10 Test for Terpenoids: 0.5 ml of the extract was treated with 2 ml of chloroform and conc. sulphuric acid. Formation of red brown colour at the interface indicates the presence of terpenoids.

2.2.11 Test for Cardiac Glycosides: To 0.5 ml of the extract, 2 ml of glacial acetic acid and few drops of ferric chloride were added. This was under layered with 1 ml of conc. sulphuric acid. Formation of brown ring at the interface indicates the presence of cardiac glycosides.

2.2.12 Ninhydrin Test: To 2 ml of the fruit extract few drops of 0.2% ninhydrin reagent was added and heated for 5 min. Formation of blue colour indicates the presence of amino acids.

2.2.13 Test for Coumarins: 1 ml of 10% sodium hydroxide was added to 1ml of the extract. Formation of yellow colour indicates the presence of coumarins.

2.2.14 Test for Anthraquinones: To 1 ml of fruit extract few drops of 10% ammonia solution was added, appearance of pink color precipitate indicates the presence of anthraquinones.

2.2.15 Test for Steroids: To 1 ml of fruit extract equal volume of chloroform is added and a few drops of concentrated sulphuric acid added appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids

2.2.16 Test for Phlobatannins: Few drops of 2% hydrochloric acid were added to 1ml of the extract. Appearance of red colour precipitate indicates the presence of phlobatannins.

2.2.17 Test for Anthracyanine: To 1 ml of the extract was added 1 ml 2N sodium hydroxide and heated for 5 min at 100 °C. Formation of bluish green color indicates the presence of anthocyanin.

2.3 Bacterial Cultures

Themicroorganisms used were as follows, *Pseudomonas aeruginosa*(NCIM 2036), *Bacillus subtilis*(NCIM 2250), *Escherichia coli* (NCIM 2109) and*Salmonella typhi*. The stock cultures were maintained at 4°C on slopes of Nutrient agar and sub cultured for 24 hrs before use. (Sundaram*et al.,* 2011).

2.4 Antibiotics

Hi-media antibiotics used in the study were kanamycin (1000mcg), Rifampicin (15mcg), vancomycin (5mcg), Gentamicin (10 mcg), penicillin (10units), Penicillin-G (2units), Streptomycin (25mcg), Tetracycline (30 mcg), Erythromycin (60mcg), Colistin (methane sulphonate) (10mcg).

2.5 Preparation of Inoculums

Direct colony suspension method of choice organisms, e.g. *Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli* and *Salmonella typhi* colonies are taken directly from plate into distilled water. The suspension ought to match or even exceed the density regarding the 0.5 McFarland standards (Andrews, 2001). These types of suspensions need to be employed inside of 30 minute of preparation.

2.6 Preparation of the McFarland Standard

Add 0.05ml of $BaCl_2$ (1% w/v $BaCl_2$.$2H_2O$) to 9.95 ml of H_2SO_4 (1% v/v) along with continual mixing. Disperse the standard are hand out with the help of the same dimensions and volume screw cap tubes those utilized in expanding the particular broth cultures. Then close screw cap tube for the stop damage by evaporation. Retailer protected against gentle

at 37°C. Energetically agitate the turbidity standard over a vortex mixing machine just before use. Standards might be stored for up to 6 months after which period they must be dumped. (Andrews, 2001)

2.6.1 Adjustment to the Density of Organism Suspension of the 0.5 McFarland Standards

Add sterile distilled water for adjust density of bacterial suspension equal to the 0.5 McFarland standard compare with white background with contrast black line. (Andrews, 2001)

2.7 Antibacterial Sensitivity Testing

2.7.1 Kirby-Bauer Disc Diffusion Method

Antibacterial activities of the different antibiotics were determined using the agar disc diffusion by Kirby-Bauer method.Kirby-Bauer method is recommended by the National Committee for Clinical Laboratory Standards (1993) and the World Health Organization (WHO). Sterile Petri plates containing Mueller-Hinton agar (Hi-media) used for the assays and 100 µl standardized inoculum (which has been adjusted to be able to 0.5 McFarland standard), has been spread using a sterile glass spreader by spread plate method. The standard Hi-media antibiotic discs were aseptically placed above sterile Mueller-Hinton agar plates seeded with respective test organisms. The plate incubates for 5 min at 37˚C for the diffusion of compound. The plates were incubated inverted position at 37˚C or 24 hrs. At the end of incubation inhibition zone formed around the disc were assessed in mm (millimeter) and the results were recorded.

2.7.2 Agar-well Diffusion method

The antimicrobial activity of the different extracts of the plant was assayed by agar well diffusion method. 100 µl standardized inoculum (which has been adjusted to be able to 0.5 McFarland standard), has been inoculate in Mueller-Hinton agar (Hi-media).The plant extracts of 5,10,15,20,25,30,35,40,45 and 50µl concentrations were added in agar well. The plates were then incubated at 37°C for 24 h. The plates were incubated inverted position at 37˚C or 24 hrs and each extract was tested on three repeat plates. At the end of incubation inhibition zone formed around the well were assessed in mm (millimeter) and the results were recorded.

2.8 Minimum inhibitory concentration (MIC) & Minimum Bactericidal concentration (MBC)

Nutrient broth method test was carried out to check the antimicrobial activity of test solution against given microorganisms. 0.1 ml standardized inoculum (which has been adjusted to be able to 0.5 McFarland standards) has been inoculate in nutrient broth. The Nutrient-broth were incubated in incubator at 37℃ for 24 hrs and then observed the turbidity. The tubes that showed no turbidity in the MIC test were taken and a100 µl from each tube was spread on Nutrient agar plate. The nutrient agar plates were incubated for 24 h at 37°C and the absence of growth was observed. The concentration of the extracts that showed no growth was recorded as the Minimum Bactericidal Concentration (MBC).

3. RESULT

The present systematic examination shows the phytochemical analysis, antimicrobial activity of the extract of Neem.

3.1 Phytochemical analysis

The Phytochemical analysis with the extract of Neem showed the presence of Flavonoids, Terpenoids and Quinones (Table 1).

Table1: Qualitative Phytochemical Analysis of Neem Extract

Sr. No.	Test Name	Neem
1	Carbohydrates	-
2	Tannin	-
3	Saponins	-
4	Alkloids	-
5	Flavonoids	+

6	Glycosides	-
7	Quinones	+
8	Phenols	-
9	Terpenoids	+
10	CardiacGlycosides	-
11	Ninhydrin	-
12	Coumarins	-
13	Anthraquinones	-
14	Steroids	-
15	Phlobatannins	-
16	Anthraacyanine	-

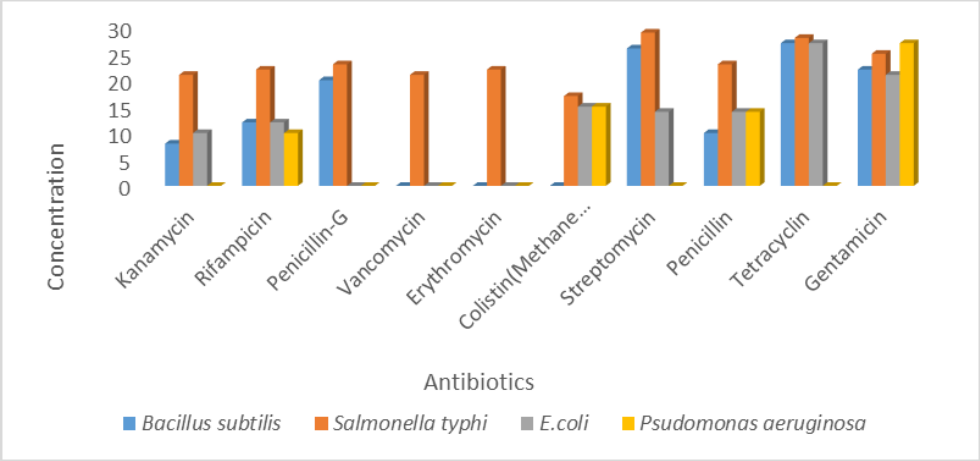
(+ = Positive, - = Negative)

3.2 Antibacterial Sensitivity Testing

3.2.1 Kirby-Bauer Disc Diffusion Method

The significant antibacterial activities of the *Bacillus subtilis*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Escherichia coli* organisms were comparable to the standard Hi-media antibiotic disc by Kirby-Bauer disc diffusion method. The results show the antibiotic sensitivity testing against the *Bacillus subtilis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Escherichia coli* microorganisms. The gram positive bacterium *Bacillus subtilis* was a sensitive to Tetracyclin and Streptomycin whereas showed intermediate to Penicillin-G and Gentamicin antibiotic. Whereas showed resistant to Vancomycin, Erythromycin, and Colistin (Methane Sulphonate). Gram negative bacterium *Salmonella typhi* was a sensitive to Streptomycin, Tetracyclin, Penicillin-G, Kanamycin, Rifampicin, Vancomycin, Erythromycin, Penicillin, and Gentamicin. Whereas showed intermediate to Colistin (Methane Sulphonate). Second gram negative bacterium *Escherichia coli* was a sensitive to Tetracyclin and Gentamicin whereas showed intermediate to Colistin (Methane Sulphonate), Streptomycin, Penicillin. Whereas showed resistant to Penicillin-G, Vancomycin, and Erythromycin. Third gram negative bacterium *Pseudomonas aeruginosa* was a sensitive to Gentamicin whereas showed intermediate to Colistin (Methane Sulphonate) and Penicillin. Whereas showed resistant to Kanamycin, Penicillin-G, Vancomycin, Erythromycin, Streptomycin and Tetracyclin. (Fig. 1)

Fig.1: Antibiogram results of Gram Positive and Gram Negative organisms with Standard Hi-Media Antibiotics



3.2.2 Antimicrobial activity well Diffusion Method

Generally, Plant extracts are rich in antimicrobial compounds. The antimicrobial activity against most of the target organisms. Agar well diffusion method was used to check the antimicrobial activity of extracted plant samples. With the help of this test we determined if the culture we were using had antibacterial property or not. (Fig. 2 & 3)

Fig.2: Antibacterial Activity of Neem Extract of Gram Positive *Bacillus subtilis* organism (Diameter of inhibition zone in mm*)

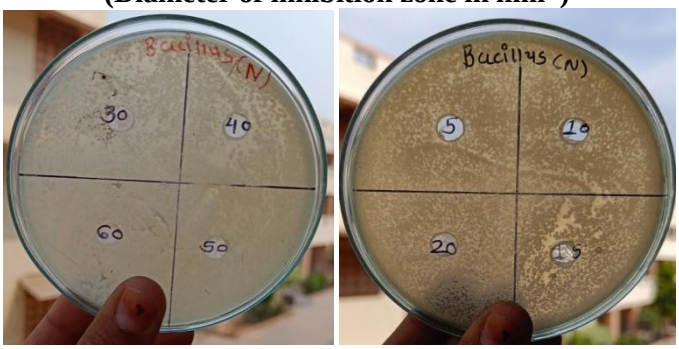


Fig.3: Antibacterial Activity of Neem Extract of Gram Negative organism (Diameter of inhibition zone in mm*)

Fig.3 (a) *Salmonella typhi*

Fig.3 (b) *Escherichia coli*

Fig.3(c) *Pseudomonasaeruginosa*

The Neem extract showed no zone of inhibition against gram positive organism *Bacillus* and gram negative organism *Salmonella typhi, Escherichia coli and Pseudomonas aeruginosa.* (Table 2)

Table 2: Zone of Inhibition against Gram Positive organism and Gram Negative organism

Sr. No.	Neem Extract Concentration (µl)	Name of Organism			
		Salmonella typhi	***Bacillus subtilis***	***E. coli***	***Pseudomonas aeruginosa***
1	5	-	-	-	-
2	10	-	-	-	-
3	15	-	-	-	-
4	20	-	-	-	-
5	30	-	-	-	-
6	40	-	-	-	-
7	50	-	-	-	-
8	60	-	-	-	-

(- =No Zone of Inhibition)

3.3 Minimum Inhibitory Concentration (MIC)

The Neem extract showed an antibacterial action against *Bacillus subtilis* organism with MIC value 6ml (Table 3).

Table3: Minimum Inhibitory Concentration (MIC) of Extracts from Neem against *Bacillus subtilis*

Name of organism *Bacillus subtilis*	
Extract (ml)	**Neem**
1	+
2	+
3	+
4	+
5	+
6	-
7	-
8	-
9	-
10	-

Name of organism *Bacillus subtilis*	
Extract (ml)	**Neem**
5.0	+
5.1	+
5.2	+
5.3	+
5.4	+
5.5	+
5.6	+
5.7	-
5.8	-
5.9	-
6.0	-

(+=growth, -=No growth)

The Neem extract showed an antibacterial action against *Salmonella typhi* organism with MIC value 5ml (Table 4).

Table 4: Minimum Inhibitory Concentration (MIC) of Extracts from Neema against *Salmonella typhi*

Name of organism *Salmonella typhi*	
Extract (ml)	**Neem**
4	+
4.1	+

4.2	+
4.3	+
4.4	+
4.5	-
4.6	-
4.7	-
4.8	-
4.9	-
5	-

Name of organism *Salmonella typhi*	
Extract (ml)	**Neem**
1	+
2	+
3	+
4	+
5	-
6	-
7	-
8	-
9	-
10	-

(+= growth,-=No growth)

The Neem extract showed an antibacterial action against *Escherichia coli* with MIC value 6 ml (Table 5).

Table 5: Minimum Inhibitory Concentration (MIC) of Extracts from Neem against *Escherichia Coli*

Name of Organism *E. coli*	
Extract (ml)	**Neem**
1	+
2	+
3	+
4	+
5	-
6	-
7	-
8	-
9	-
10	-

Name of organism *E. coli*	
Extract (ml)	**Neem**
4	+
4.1	+
4.2	+
4.3	+
4.4	+
4.5	+
4.6	+
4.7	-
4.8	-
4.9	-
5	-

(+=growth, -=No growth)

The Neem extract showed an antibacterial action against *Pseudomonas aeruginosa* with MIC value 5ml (Table 6).

Table 6: Minimum Inhibitory Concentration (MIC) of Extracts from Neem against *Pseudomonas aeruginosa*

Name of organism *Pseudomonas aeruginosa*	
Extract (ml)	**Neem**
1	+

2	+
3	+
4	+
5	-
6	-
7	-
8	-
9	-
10	-

Name of organism *Pseudomonas aeruginosa*	
Extract (ml)	**Neem**
4	+
4.1	+
4.2	+
4.3	+
4.4	+
4.5	+
4.6	+
4.7	+
4.8	-
4.9	-
5	-

(+=growth, -=No growth)

The Neem extract showed an excellent antibacterial action against *Salmonella typhi, Pseudomonas aeruginosa and Escherichia coli* organism with MIC value 5ml but where moderate antibacterial action against *Bacillus subtilis* with MIC value 6ml.

3.4 Minimum Bactericidal Concentration (MBC)

The Neem extract showed an antibacterial action against *Bacillus subtilis* with MBC value 5.9ml (Table7).

Table 7: Minimum Bactericidal Concentration (MBC) of Extracts from Neem against *Bacillus subtilis*

Name of organism *Bacillus subtilis*	
Extract (ml)	**Neem**
5.6	Lawn Growth & No Inhibition
5.7	Growth & No Inhibition
5.8	Growth & No Inhibition
5.9	No Growth & Inhibition
6	No Growth & Inhibition

The Neem extract showed an antibacterial action against *Salmonella typhi* with MBC value 4.8ml. (Table 8)

Table 8: Minimum Bactericidal Concentration (MBC) of Extracts from Neem against *Salmonella typhi*

Name of organism *Salmonella typhi*	
Extract (ml)	**Neem**
4.5	Lawn Growth & No Inhibition
4.6	Growth & No Inhibition
4.7	Growth & No Inhibition
4.8	No Growth & Inhibition
4.9	No Growth & Inhibition
5	No Growth & Inhibition

The Neem extract showed an antibacterial action against *Escherichia coli* with MBC value 4.9ml. (Table 9)

Table 9 : Minimum Bactericidal Concentration (MBC) of Extracts from Neem against *Escherichia coli*

Name of organism *Escherichia coli*	
Extract (ml)	**Neem**
4.6	Lawn Growth & No Inhibition
4.7	Growth & No Inhibition
4.8	Growth &No Inhibition
4.9	No Growth & Inhibition
5	No Growth & Inhibition

The Neem extract showed an antibacterial action against *Pseudomonas aeruginosa* with MBC value 4.9ml. (Table 10)

Table10: Minimum Bactericidal Concentration (MBC) of Extracts from Neem against *Pseudomonas aeruginosa*

Name of organism *Pseudomonas aeruginosa*	
Extract (ml)	**Neem**
4.7	Lawn Growth & No Inhibition
4.8	Growth & No Inhibition
4.9	No Growth & Inhibition
5	No Growth & Inhibition

The Neem extract showed an excellent antibacterial action against *Salmonella typhi* with MBC value 4.8ml and good antimicrobial action against *Pseudomonas aeruginosa and Escherichia coli* organism with MBC value 4.9ml and but where a moderate antibacterial action against B*acillus subtilis* with MBC value 5.9ml.

4. DISCUSSION

Ali reported, in this study, the ethanolic extract of neem leaf exhibited antibacterial

activity against the MDR bacteria of poultry. Further in vivo study need to be carried out in poultry to observe its antibacterial efficacy (Ali et al., 2021).

Khan reported, neem is commonly considered as medicinal tree inIndia. Its products are extensively used in Ayurveda and Unani medicinesbecauseofitsantimicrobialproperties. Inoldentimesitwasusedashousehold remedy against various diseases. Now trends and awareness for using neem plant products as medicine and disinfectants are increasing. In this study alcoholic leaf extract of *A. indica*, exhibits antimicrobial effects against the target pathogens *Enterococcus, Staphylococcus aureus, Pseudomonas* and *E. coli*. It is a simple and inexpensive method for the better sanitization with no toxic effects (Khan etal., 2021).

Herrera-Calderon reported, *Azadirachta indica* (Neem) plant acts as a medicinal plant have been found effective in the treatment of bacterial, fungal, viral and other diseases and revealed the antibacterial, antifungal, antiviral, antimalarial, antiulcer and other biological activities. Due to increasing antibiotic resistance in microorganisms and side effects of synthetic antibiotics neem plant are now growing popularity in the treatment of many infections. Neem plant is considered as clinically effective and safer alternatives to the synthetic antibiotics. Extensive research in the area of isolation and characterization of the active principles of neem plant is essential so that better, safer and cost effective drugs for curing various diseases and infections can be developed (Herrera-Calderon et al .,2019).

Verma & Mehata reported, AgNPs have been successfully synthesized using a well-known medicinal plant Neem leaf extract. The synthesized AgNPs are crystalline in nature, poly dispersed and exhibit high energy SPR b and at around 400 nm and a strong PL at around 450 nm, depending on control able parameters. The synthesis is found to be efficient in terms of reaction time as well as stability of the AgNPs. The rate of synthesize is faster in case of Neem as compared to the other biological methods microbes, DNA etc. Thus, the rate of reaction of biological synthesis is comparable to that of the chemical methods. The synthesis process, i.e., formation of AgNPs critically depends on the pH, temperature, reactant concentration and reaction time. By changing the environmental parameters, the size and shape of the synthesized nanoparticles can be altered. Synthesis of AgNPs is enhanced with time at higher temperature and alkaline pH. Green synthesized AgNPs are found to have enhanced antibacterial activity against bacterial colony isolated from soil sample. Due to the enhanced antimicrobial activity of AgNPs, it is effectively used in the field of medicine as well as in food and cosmetic industries (Verma & Mehata, 2016).

Raut reported, *Azadirachta indica* extract is an important source of compounds having anti-microbial, anti-oxidant, anti-tumor, anti-malarial, anti-fungal, anti-inflammatory and anti-viral properties. The results indicated that using plant parts of neem had beneficial effect in controlling the pathogenic microbial organisms and thus can be used in therapeutic formulations in near future. (Raut et al., 2014).

Sinaga reported, overall, the results support partly the use of these medicinal plants as traditional remedies for treatment of some infections and also the potential of these plants for the development of modern antimicrobial agents. Most of the plant extract showed various inhibitory effects against some the microorganisms tested which was found to be ineffective against the four standard bacteria. The gradient extracts of seeds and bark of Neem also show dose depend an activity against the tested organisms. The antibacterial activities of Neem extracts may be due to polyphenolic compounds (flavonoids), alkaloids and higher terpenoids which are detected to be present in the plant (Sinaga et al., 2016).

Sharma reported, based on the above research it can be concluded that *Azadirachta indica* seeds can be a good resource for herbal drugs that can be used as a supplement for neurological diseases and after the comparative study it has been found the neem leaf and neem seeds have equal antibacterial activity against Gram +ve i.e. *E. coli* and Gram–ve i.e. *Bacillus amyloliquefaciens*. Their methanolic extracts are very much effective and useful against bacteria because they consists large amount of secondary

metabolites. It is expected that using natural products as therapeutics agents will probably not elicit resistance in microorganism. The presence of flavonoid of neem seeds has shown the future aspects to become the supplements for neurological disorder. Further work also includes the further purification of metabolites that are responsible for their nero protective effects and also for antibacterial activity. It is expected that neem seeds be used as therapeutic agents for patients with neurological disorders (Sharma et al., 2014).

Cesa reported, neem oil was characterized, beyond the limonoid content, in terms of the color characteristics and the phenolic fingerprint, showing the presence of a high content of benzoic acid. The great inhibitory efficacy against important enzymes, such as tyrosinase, correlated with the high content of benzoic acid, and lipase makes it an interesting matrix to be used in the prevention or care of hyper pigmentation problems and obesity. Moreover, antimalarial and anti-leishmanial activity were reported in addition to its safety profile against THP-1 cell line. On the other hand, be it tested, weak or no activity was evident against *H. pylori*, *Candida* spp. and *Malassezia furfur* strains. Overall, these findings on pure Neem oil open new scenarios for the recognition of this natural product for the treatment of different diseases and could rationally justify the ethnobotanical uses in traditional medicine (Cesaetal., 2019).

5. CONCLUSION

Neem is commonly considered as medicinal tree in India. Its products are extensively used in Ayurveda and Unani medicines because of its antimicrobial properties. In olden times it was used as house hold remedy against various diseases. Now trends and awareness for using neem plant products as medicine and disinfectants are increasing. In this study Neem extract of exhibits antimicrobial effects against the target Pathogens *Bacillus subtilis*, *Salmonella typhi*, *E. coli* and *Pseudomonas aeruginosa.* The Neem extract of high antimicrobial activity against *Salmonella typhi* but was less active *Bacillus subtilis*.

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