International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 08-01-2021; Accepted: 08-02-2021: Published: 16-02-2021
www.botanyjournals.com
Volume 6; Issue 1; 2021; Page No. 532-535

A brief review on phytochemistry and anti-bacterial activity of *Azadirachta Indica*

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Abstract
In the present review work we have reviewed various scientific research articles published on phytochemical investigation and antibacterial activity of *Azadirachta indica*. It is commonly known as neem, a tree belongs to family Meliaceae and in the genus *Azadirachta*. It is native to the Indian subcontinent and typically grown in tropical and semi-tropical regions. Neem consists of fresh dried leaves, fruits, flowers and bark of *Azadirachta indica* J. Juss. It is the traditional plant which has many therapeutic uses due to presence of wide range of phytoconstituents in it. The active phytoconstituents of Neem includes nimbin, nimbolide, nimbinene, nimbandiol, tannins, Azadirachtin and flavonoids, etc. Neem has many therapeutic uses like anti-viral, anti-bacterial, anti-fungal, antiseptic, stimulant and insect repellent agent etc. It is also used in the treatment of skin disease, ulceration of cow-pox, rheumatism, anorexia, nausea and intestinal worms. Many researchers have worked on antibacterial activity of various parts of neem in different solvents against various micro-organisms.

Keywords: neem, anti-bacterial activity, phytochemistry, nimbin, flavonoids

Introduction
Azadirachta indica is commonly known as neem, a tree belongs to family Meliaceae and in the genus *Azadirachta*. It is native to the Indian subcontinent and typically grown in tropical and semi-tropical regions. Neem consists of fresh dried leaves, fruits, flowers and bark of *Azadirachta indica* J. Juss. It is the traditional plant which has many therapeutic uses due to presence of wide range of phytoconstituents in it. The *Azadirachta indica* leaves are green in colour having typical odour, bitter taste. The Azadirachta indica flowers are white in colour having bitter taste. The fruits of neem are green (unripe) and yellow (ripe)in colour having ovoid shape, hard bony endocarp. The fruits have the solitary with a thick testa and embryo with foliaceous cotyledons in the axis of scanty endocarp. Therapeutically neem is used as anti-viral, anti-bacterial, anti-fungal, antiseptic, stimulant and insect repellent agent. It is also used in the treatment of skin disease, ulceration of cow-pox, rheumatism, anorexia, nausea and intestinal worms [1, 2, 3].

Phytochemistry of neem
Manpreet kaur *et al.*, have reported some phytoconstituents which are present in the *Azadirachta indica* which includes nimbin, 6-desacetylnimbinene, nimbinene, nimbandiol, nimbolide, quercetin, β-sitosterol, ascorbic acid, n-hexacosanol, nonacosane, amino acid in the leaves. They have reported Gedunin, 7-deacetoxy-7α- hydroxygedunin, azadiredione, azadirone, nimbiol, 17-epiazadiradione in fruits. Also they have reported triterpenoids, 1, 2-diepoxyazadiradione, 7-acetylneotrichilenone, 7-desacetyl-7-benzoylgedunin and azadirachtin in the seeds [1]. Idoko A *et al.*, have reported ethanolic and aqueous extracts of neem showed the presence of alkaloids, flavonoids, saponins, cyanogenic glycosides, phenols, tannins, steroids and reducing sugars [2].
The chemical structures of selected phytoconstituents of neem were presented in Figure 1.

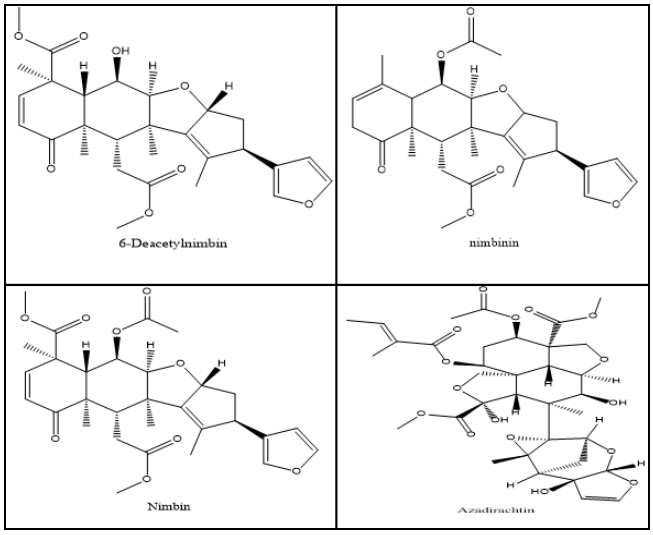

Fig 1: Chemical Structures of Selected Phytoconstituents of Neem

Anti-bacterial activity of neem
The antibacterial activities of neem reported by many researchers were reviewed as follows:
Munazza Sharif *et al.*, have reported the antibacterial activity of neem against the pathogenic bacteria *V. vulnificus*. They have taken the minimal inhibitory concentration of 150µg/ml. At this concentration they got the results against anti-bacterial activity of neem against *V. vulnificus* using sterile disc method and well diffusion method. The well diffusion method showed the zone of inhibition (15.3±0.5 mm) at the concentration of 150µg/ml and there is no zone of inhibition in the control well method. The anti-bacterial activity comparison study was performed neem loaded sterile disc and antibiotic disc. The study showed the zone of inhibition of 21.6±1.5 mm for neem and tetracycline shows the zone of inhibition of 21.6±1.5 mm [3].
R. Manivasagaperumal *et al.*, have reported antibacterial

activity using agar well diffusion method. The bacterial inoculums were spreaded on the media when nutrient agar was inoculated with the micro-organisms. The plant extract is filled in the wells by punching the agar. At 37° for 18hours plates were incubated and by measuring the zone of inhibition the anti-bacterial activity is determined. The different extracts antibacterial potential is determined by their zones of inhibition [4].

R C Patil *et al.*, have reported antibacterial activity using the disc diffusion method. To determine the anti-bacterial activity of aqueous, ethanol, methanol and acetone extracts they have used the modified paper disc diffusion method. The turbidity produced in the inoculums is compared with the standard i.e. McFarland standard. Over the nutrient agar plate inoculums were spreaded using sterile cotton swab to get uniform microbial growth. Ampicillin 10mcg/disc used as positive control. The plates were incubated at 37°c for 18hours. By measuring the diameter of zone of inhibition the anti-bacterial activity is determined. They have classified the antibacterial activity as strong (>20mm), moderate (16-19mm) and mild (12-15mm) and less than 12mm was taken as inactive [5].

Alka Sahrawat *et al.*, determined the anti-bacterial activity of all the extracts of the neem leaves against the *E. coli* strains by disc diffusion method. The benzene extract of the neem leaves shows the inhibition growth against E. coli (maximum growth 46.56% and minimum growth 39.98%). The acetone extract of the neem leaves shows the inhibition growth against *E. coli* (maximum growth 58.77% and minimum growth 50.40%). The toluene extract of the neem leaves shows the inhibition growth against *E. coli* (maximum growth 15.25% and minimum growth 0%). The ethylacetate extract of the neem leaves shows the inhibition growth against *E. coli* (maximum growth 40.55% and minimum growth 33.20%). The ethanol extract of the neem leaves shows the inhibition growth against *E. coli* (maximum growth 33.85% and minimum growth 29.99%). The butyl alcohol extract of the neem leaves shows the inhibition growth against E. coli (maximum growth 41.15% and minimum growth 32.88%) [6].

Zabta k. Shinwari *et al.*, determined the anti-bacterial activity of neem against *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Shigella sonnei, Klebsiella pneumonia, Salmonella paratyphi, Salmonella typhimurium, Listeria monocytogenes, Yersinia pestis, Pseudomonas aeruginosa* [7].

Faiza Aslam *et al.*, determined the anti-bacterial activity of neem leaves extract (50mg, 75mg) by the inhibition of the growth of *S. aureus, Corynebacterium bovis* and *E. coli*. The strains of these bacteria are sensitive against +ve control Chloramphenicol [8].

Hamidah Jaafar Sidek *et al.*, carried out the anti-microbial activity using the disk diffusion assay towards three microbes those are *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Three paper discs were used for the assay. Ampicillin (10 µg) is used as positive control. Second paper disc is soaked in ethanol and used as negative control. Third paper disc was soaked in neem extract and inhibition of anti-bacterial effect is determined. On the nutrient agar media the 0.2ml of broth culture was transferred. The plates were incubated at 37°c for 24hours. By measuring the diameter of zone of inhibition the anti-bacterial activity is determined [9]. Aamir F *et al.*, determined the anti-bacterial activity using ampicillin as positive control. The plates were incubated at 32°c for 48 hours [10].

Raja Ratna Reddy *et al.*, used the *Staphylococcus aureus, Enterococcus faecalis* (Gram positive) and *Pseudomonas aeruginosa, Proteus mirabilis* (Gram negative) for the anti-bacterial study. The bacterial strains were maintained in Muller Hinton Agar (MHA, pH 7.2) at 37±1° C. All the bacterial strains were found to be sensitive ti the ciprofloxacin. Neem leaves extract shows its anti-bacterial activity against all the concentration of bacteria [11].

Taqdees Malik *et al.*, performed the anti-bacterial activity of neem leaves extract against the *E. coli, Pseudomonas*, and *S. aureus* using different methods such as Agar well diffusion, antibiotic discs sensitivity test, Minimum inhibitory concentration, minimum bactericidal concentration. The neem ethanolic extract gives the zone of inhibition against the *Pseudomonas* of 23mm, *E. coli* of 25mm and *S. aureus* of 26mm [12].

S. Cesa *et al.*, done the anti-bacterial activity of neem oil against eight *H. pylori* strains. All the strains showed the minimal inhibitory concentration of 128 µg/mL and one showed the MIC value of 64 µg/ml [13].

Bharat Pokhrel *et al.*, determined the anti-bacterial activity of neem. The diameter of zone of inhibition was measured and it showed the maximum inhibition against the *S. aures* and minimum inhibition against the *E. coli*. There is increase in the zone of inhibition by increasing the concentration of the extract i.e. 22±3mm [14].

Abhijeet Balasaheb Shinde *et al.*, used the tetracycline and streptomycin as standard references and positive controls for the anti-bacterial activity. The plates were incubated at 37°c for 24hours. The diameter of zone of inhibition is measured against the tested bacteria to determine the anti-bacterial activity [15].

C. Rajasekaran *et al.*, used the eight strains of positive bacteria those are *Micrococcus glutamicus, Lactobacillus bulgaris, Streptococcus faecalis, Staphylococcus aureus, Bacillus stearothermophilus, Staphylococcus pyogenes, Micrococcus luteus, Bacillus cereusand* two strains of negative bacteria those are *Escherichia coli* and *Pseudomonas aeruginosa* to determine the anti-bacterial activity [16].

Sharmila Banu Gani *et al.*, using the agar gel disc diffusion method perfomed the anti-bacterial activity. Into the sterilized petri dish agar (muller hinton) was Pored and it is solidified. The plate is rotated approximately 600 time to ensure the distribution of inoculums. The gentamycine discs were used as positive and negative control. The petridishes were incubated at 37°c for 24hours. By measuring the clear zone of inhibition the anti-bacterial activity of gradient solvents is determined. Hexane, acetone and methanolic extracts were found very effective against the bacteria [17].

Nwakaeze Emmanuel *et al.*, performed the anti-bacterial activity of neem leaf extracts against the various pathogens. The minimal inhibitory concentration against the pathogens was found to be 25 mg/ml and 12.5 mg/ml [18].

Darmendra kumar yadav *et al.*, performed the anti-bacterial activity against various 21strains of pathogens. The neem extract were effective against the pathogens such as *Streptococcus mutans, S. salivarius and Fusobacterium nucleatum*, etc [19].

A.Akpuaka *et al.*, done the extraction of the neem leaf using the n-hexane to determine its anti-bacterial activity. It gives the zone of inhibition of 17mm, 12mm, 5mm, 3mm and

11mm respectively [20]. Garima Pandey *et al.*, using agar well diffusion method peromed the anti-bacterial activity against the *Ecsherichia coli* and *Staphyloccus aureus*. Using muller hinton agar the test culture is inoculated and solidified. A 50µl DMSO is used as negative control and 10µg of streptomycin is used as positive control. The plates were then incubated at 37°C for 24 hr and the zone of inhibition is measured [21]. S. Susmitha *et al.*, performed the anti-bacterial activity of different solvents using muller hinton agar by cup diffusion method. The plates were incubated at 37°cfor 24h. Then the zone of inhibition is observed and measured [22]. Sharma Y *et al.*, done the anti-bacterial activity of various parts of neem against various bacteria. The extracts were done using the aqueous, methanol and ethanol and then these extracts were screened against the bacteria those are *E.coli* and *Bacillus amyloliquefaciens* by using agar diffusion method. The neem seed of aqueous extract showed the maximum anti-bacterial activity against *E.coli* and *Bacillus amyloliquefaciens* of 7mm. The neem leaf ethanolic extract showed the maximum anti-bacterial activity against *Bacillus amyloliquefaciens* of 31mm. The methanolic extract of neem seed and neem leaf showed the maximum anti-bacterial activity [23]. T. Revathi *et al.*, perfomed the anti-bacterial activity using the muller hinton agar by well diffusion method against *E. coli* and *K. aerogenes* bacteria (negative controls). The samples were loaded on the wells and kept overnight at 37°c. The anti-bacterial activity is determined by measuring the zone of inhibition [24].

Conclusion

The present review concludes that neem is the plant having many therapeutic uses due to presence of wide range of phytoconstituents. The active phytoconstituents of neem includes nimbin, nimbolide, nimbinene, nimbandiol, tannins, Azadirachtin and flavonoids, etc. Neem has many therapeutic uses like anti-viral, anti-bacterial, anti-fungal, antiseptic, stimulant and insect repellent agent etc. It is also used in the treatment of skin disease, ulceration of cow-pox, rheumatism, anorexia, nausea and intestinal worms. Out of reported activities, antibacterial activity is commonly reported by many researchers.

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