



## Evaluation of antibacterial activity and phytochemical investigation of *Azadirachta indica* L. against certain bacterial species

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ARTICLE INFO	ABSTRACT
<p>Received : 11 December 2021                      Revised : 16 February 2022                      Accepted : 21 February 2022</p> <p>Available online: 17 April 2022</p> <p><b>Key Words:</b>                      Active components                      Agar well diffusion                      Antibacterial activity  <i>Azadirachta indica</i>                      Phytochemical screening</p>	<p>Historically, plants have been a source of herbal medicines and are used for treating various human ailments. <i>Azadirachta indica</i>, commonly known as neem, is a multipurpose tree with a wide range of health benefits. Various parts of neem tree have been shown to exhibit antimicrobial effects against a wide variety of microorganisms. The present study was carried out for screening of active components and antibacterial activity of leaves and bark extracts of <i>A. indica</i>. Ethanol and methanol extracts of leaves and bark of <i>A. indica</i> were tested against Gram positive and Gram negative bacteria viz. <i>Escherichia coli</i>, <i>Pseudomonas aeruginosa</i>, <i>Klebsiella pneumoniae</i>, <i>Staphylococcus aureus</i>, and <i>Bacillus cereus</i>. The agar well diffusion method was used for testing the antibacterial activity. Results revealed that methanol extract of bark and ethanolic extract of leaves exhibited significant antibacterial activity. Methanolic extract of bark of <i>A. indica</i> showed maximum antibacterial activity against <i>B. cereus</i> and <i>K. pneumoniae</i> (16 mm) followed by <i>p. aeruginosa</i> (15.6 mm), <i>E. coli</i> (15.3 mm) and least was found against <i>S. aureus</i> (15 mm). Whereas ethanolic extract of leaves exhibited maximum activity against <i>P. aeruginosa</i> (11.3 mm), followed by <i>K. pneumoniae</i> (11 mm), <i>S. aureus</i> (10.3 mm), <i>E.coli</i> (7 mm) and no activity was observed against <i>B. cereus</i>. Phytochemical screening of plant extracts gave positive results for alkaloids, flavonoids, saponins and tannins.</p>

### Introduction

Since time immemorial medicinal plants, have always been used for treatment of human ailments. Plants possess therapeutic properties and therefore play an essential role in maintaining human health free from disease as well as in healthy state. Ancient, scared holy book such as Rigveda and various Samhitas written by ancient rishis had made significant contribution to the Indian medicine system. Both these ancient literatures provide information regarding the therapeutic properties of medicinal plants and their uses. Rigveda seems to be oldest compilation written between 3500 B.C. to 1600 B.C. Atharveda was written around 1200B.C. Further works of Charka and Sushruta namely Charaka Samhita and Sushruta Samhita respectively were written about 1000B.C. and contain a realistic and clear account of therapeutics

of Ayurveda, a traditional healthcare system of Indian medicine. The discovery of antibiotics in 20<sup>th</sup> century have completely transformed humanity's approach to infectious diseases and substantially reduced the threat passed by infectious disease. However, the emergence of drugs resistant microorganisms reversed the advances of the previous 50 years of research. The drug resistant microorganisms have complicated the treatment of infectious diseases in immune compromised AIDS and cancer patients (Davies, 1994). Under such circumstances, it becomes necessary to find out some suitable substitute of modern medicines from drug plants. Various plant has been discovered which have medical significance like antimicrobial, antibacterial, antioxidant, anti-helminthic, anti-cancerous, ant-inflammatory, anti-fungal activity

etc. These plant medicine not only cure diseases but also enhance our immunity. Medicinal plant contains a wide variety of secondary metabolites such as tannins, alkaloids, flavonoids etc. Phytochemicals that occur naturally in plants are referred to as secondary metabolites. These phytochemicals possess antimicrobial properties and therefore serve as an effective, cheap, and safe antimicrobial in treatment of infection.

Voluminous information on plants as antimicrobial agents against human pathogens are available. Large no. of workers reported antimicrobial activity of drug plants (Mahida and Mohan, 2006; Rajasekaran *et al.*, 2008; Alipour and Khan Mohammadi, 2011; Sudhir *et al.*, 2012; Buzayan and El-Garbulli, 2012; Ibrahim and Abu salem, 2014; Al- Mariri and Safi, 2014; Francine *et al.*, 2015) *Azadirachta indica*, commonly called as neem (margosa) belongs to family Meliaceae. Neem is the traditional medicinal plant of India. It is an evergreen tree, commonly grown in various parts of the Indian subcontinent. The plant is regarded as “village dispensary” in India because of the use of all its parts for various ailments in the indigenous system of medicine. Every part of the tree has been used as household remedy for treatment of various human ailments since ancient times (Rajasekaran *et al.*, 2008). Neem is also called as “Arista” in Sanskrit, a word that means Imperishable, Perfect & Complete (Girish and Shankara, 2008). A large number of biologically active compounds have been isolated from *A. indica* such as Flavonoids, flavonoglycoside, dihydrochalcones, tannins etc. Twigs of neem are widely used as toothbrush for its anti pyorrhoeal property. Neem oil possesses antifungal, antimicrobial and antiseptic properties and are used for treatment of chronic skin diseases, leprosy and ulcers. The objective of the study is to investigate the antibacterial potential of leaves and bark extracts of *A. indica* and to determine the phytochemical constituents present in the *A. indica* bark and leaves extract.

## Material and Methods

**Collection of Plant material and preparation of Plant extracts:** The Leaves and bark of *Azadirachta indica* were collected from from the Greenland Nursery in chunni kalan, Distt.

Fatehgharh Sahib.. The plant materials ( leaves and bark ) were thoroughly washed and dried in shade. After proper drying, the leaves and bark of *A. indica* were grinded to form coarse powder. Extraction of test drug plant material i.e leaves and bark were done in different extractants (methanol and ethanol). 40 gram of grinded plant material was extracted using 300ml of extraction solvent for 24-48 hrs in Soxhlet extractor. Finally the extract obtained after extraction was subjected to filtration through sterile filter paper whatman no.1. Solution was evaporated to dryness to get final volume of 40 ml under controlled temperature conditions. The final concentration of the extract was made at level in which 1 ml of extract solution represented 1 gm of powdered plant material (Barreto *et al.*, 2012). Extract solution thus obtained was designated as 100% concentrated drug solution. This 100% extract solution was further diluted with distilled water to obtain 75%, 50% and 25% concentrations.

**Collection of Test organisms:** The bacterial test organisms used in the present investigation are *Staphylococcus aureus* (MTCC code 3160), *Pseudomonas aeruginosa* (MTCC code 3542), *Escherichia coli* (MTCC code 443), *Klebsiella pneumoniae* (MTCC code 9544) and *Bacillus cereus* (MTCC code 430). All the culture were collected from Microbial Type Culture collection (MTCC) of IMTECH Chandigarh, India.

**Testing for antibacterial activity:** The agar well diffusion method was used for evaluation of antibacterial activity of plant extracts (Bell and Grudy, 1968).

### Addition of inoculums to culture medium

Nutrient agar medium was seeded with inoculums i.e (Nutrient broth culture of test organisms) 2ml of bacterial suspension was added into 100 ml of molten and cooled Nutrient agar medium. The flask was rotated gently for uniform distribution of test organisms.

### Preparation of agar plates:

The inoculated culture medium was then poured into sterile petri plates in Laminar Air Flow and allowed to solidify completely.

### Pouring of extract:

Sterile cork borer of 8mm in diameter was used to make 5 wells in the set of each petriplates, with 4 well in the periphery and one well in the center. 0.1ml (100µm) solution from each differ concentration i.e (100%, 75%, 50% and 25%) of

plant extract was added to four peripheral wells. Central wells were filled with 0.1ml solution of control.

Methanol, Ethanol and Sterilized water were used as control for methanolic, ethanolic and aqueous extract. These petriplates were then incubated at 37°C for 24 hours. After incubation the diameter of zones of inhibition were measured and tabulated for each test bacterial strain. Each sample was assayed in triplicate and value was measured and recorded. Inhibition zone was measured in millimetres with the ruler. It was measured from center of the well to the edge of the area with no growth (zero growth) and was multiplied by two. Further average value of inhibition zones was calculated. Effective inhibition zone was calculated by deducting the well size (cork borer size) from average value of inhibition zone.

**Phytochemical analysis:** The qualitative phytochemical screening of plant extracts was carried out for alkaloids, flavonoids, saponins, tanins and glycosides.

**Test for alkaloids by Mayer's reagent:** 1 ml of extract was treated with few drops of Mayer's reagent. Formation of white precipitates indicates the presence of alkaloids (Sheel *et al.*, 2014).

**Test for Saponins:** About 1ml of extract was diluted separately with distilled water to 20ml and shaken in graduated cylinder for 15 minutes. 1cm layer of foam indicates the presence of saponins (Sheel *et al.*, 2014).

**Test for flavonoids:** Take sample extract in a test tube and add few drops of conc. H<sub>2</sub>SO<sub>4</sub>. If flavonoids present in sample, yellow colour appears in solution.

**Test for tannins:**

To 1ml of the extract, few drop of 1% (w/v) Ferric chloride FeCl<sub>3</sub> solution were added. A green or brown color indicated the presence of tannins (Joshi *et al.*, 2013).

**Test for glycosides by Keller-killiani test:**

To the test tubes containing 2ml of extract and 1ml of glacial acetic acid, 3 drops of 5% (w/v) ferric chloride and concentrated sulphuric acid were added and observed, appearance of reddish brown color at the junction of 2 layers and bluish green in upper layer indicates the presence of glycosides (Shukla *et al.*, 2013).

## Results and Discussion

The antibacterial activity of *A. indica* leaves and bark (methanol and ethanol) extracts was evaluated against both Gram positive and Gram negative bacterial strains. The qualitative phytochemical analysis was carried out for detection of alkaloids, flavonoids, glycosides, tannins and saponins in plant extracts.

**Antibacterial activity:** Antibacterial activity of methanol and ethanol extracts of *A. indica* was studied against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Bacillus cereus*.

### Antibacterial activity of Methanolic extract of leaves of *Azadirachata indica* (Table-1)

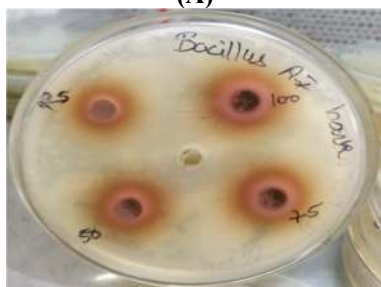
The maximum antibacterial activity at 100% concentration was recorded against *E.coli* (9mm), *Bacillus cereus* (7.6mm), *Pseudomonas aeruginosa* (6.3mm), *Klebsiella pneumoniae* (6mm) followed by *Staphylococcus aureus* (5.6mm). At 75% maximum inhibitory effect was found against *E. coli* (7.6mm) and minimum against *P. aeruginosa* (3.6mm) whereas at 50% concentration maximum inhibition was found against *E.coli* (6mm), *S. aureus* (2.3mm), followed by *K. pneumoniae* (2.6mm). No activity was found against *B.cereus* and *P. aeruginosa* at 50% concentration. No activity was observed in 25% concentration.

### Antibacterial activity of ethanol extract of leaves of *Azadirachata indica* (Table-1; Photoplate-2)

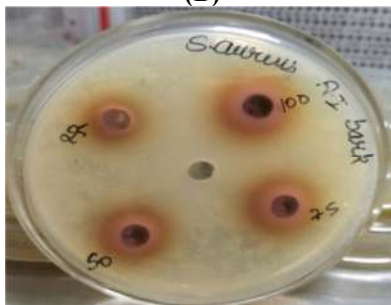
The maximum antibacterial activity at 100% concentration was recorded against *P. aeruginosa* (11.3mm) followed by *K. pneumoniae* (11mm) *S.aureus* (10.3mm) and *E.coli* (7mm). 75% concentration showed maximum inhibition against *P. aeruginosa* (9.6mm) and minimum against *E. coli* (6mm). While at 50% concentration maximum activity was found against *P. aeruginosa* (7.6mm) and minimum activity was observed against *K. pneumoniae* (3mm). At 25% Concentration maximum antibacterial activity was found against *S.aureus* (5mm) and minimum against *E.coli* (4mm). *K pneumoniae* exhibited no activity at 25 %



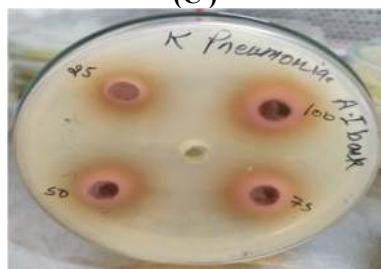
(A)



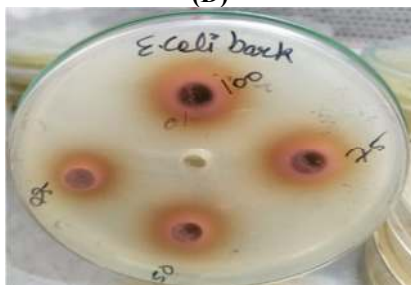
(B)



(C)



(D)



(E)

**Photoplate 1: Antibacterial activity of methanolic extract of bark of *Azadirachta indica* against: (A) *P.aeruginosa* (B) *B.cereus* (C) *S.aureus* (D) *K.pneumonia* (E) *E.coli***

concentration. All extract concentration did not exhibited any inhibitory activity against *B. cereus*.

**Antibacterial activity of methanolic extract of bark of *Azadirachta indica* (Table-2; Photoplate-1):** The maximum antibacterial activity at 100% concentration was recorded against *Bacillus cereus* and *Klebsiella pneumoniae* (16mm) followed by *Pseudomonas aeruginosa* (15.6mm), *E.coli* (15.3mm) and *Staphylococcus aureus* (15mm). At 75% maximum inhibitory effect was found against *B. cereus* (14.6mm) and minimum against *P. aeruginosa* and *E. coli* (13.6mm)

Maximum inhibition at 50% concentration was found against *S.aureus* (12mm), and minimum against *P. aeruginosa* (10.6mm) and *Bacillus cereus* (11.3mm). While at 25% concentrations maximum activity was revealed against *Bacillus cereus*, *S.aureus*, *P.aeruginosa*, *K. pneumoniae* (8.6mm) and minimum against *E.coli* (6.6mm)

**Antibacterial activity of ethanolic extract of bark of *Azadirachta indica* (Table-2)**

The maximum antibacterial activity at 100% concentration was recorded against *S.aureus* (8mm) followed by *P. aeruginosa* (7mm), *E. coli* (6mm), *B. cereus* and *K. pneumoniae* (5mm). Maximum inhibition at 75% was observed against *S.aureus* (6.6mm) and minimum inhibition was found against *K. pneumoniae* (3.6mm). 50% concentration exhibited maximum antibacterial activity against *S.aureus* (5mm) and minimum activity against *K. pneumoniae* (2mm). No activity was observed at 25% concentration.

**Results of Phytochemical analysis:** The preliminary phytochemical analysis of leaves extract revealed that methanolic and ethanolic extract were found rich in tannins, flavonoids and saponins. Alkaloids were only detected in ethanolic extract of leaves. Glycosides were found absent (Table-3). Similarly tannins, flavonoids and saponins were found in bark extracts (methanol and ethanol) of *A. indica*. Alkaloids and glycosides were found absent (Table-4). The phytoconstituents alkaloids, glycosides, flavonoids, saponins and tannins forms the defensive mechanism of the plants against pathogens.

The present findings revealed that methanolic bark extract of *A. indica* was superior and effective against all test bacterial strains. Whereas moderate activity was observed in ethanolic and methanolic extract of leaves. Ethanolic bark extract of *A. indica*

**Table 1: Antibacterial activity of different extracts of *Azadirachta indica* leaves**

EXTRACT TYPE	TEST ORGANISMS	EFFECTIVE ZONE OF INHIBITION (mm)			
		Extract Conc. 100%	Extract Conc. 75%	Extract Conc. 50%	Extract Conc. 25%
METHANOL	<i>E. coli</i>	9 mm	7.6 mm	6 mm	--
	<i>P. aeruginosa</i>	6.3 mm	3.6 mm	--	--
	<i>K. pneumoniae</i>	6 mm	5 mm	2.6 mm	--
	<i>S. aureus</i>	5.6 mm	4 mm	2.3 mm	--
	<i>B. cereus</i>	7.6 mm	4.3 mm	--	--
ETHANOL	<i>E. coli</i>	7 mm	6mm	5.3 mm	4 mm
	<i>P. aeruginosa</i>	11.3 mm	9.6 mm	7.6 mm	4.6 mm
	<i>K. pneumoniae</i>	11 mm	7.6 mm	3 mm	--
	<i>S. aureus</i>	10.3 mm	8 mm	6.3 mm	5 mm
	<i>B. cereus</i>	--	--	--	--
CONTROL		--			

Effective inhibition zone (mm)\*= Average value of inhibition zones of three replicates -Well size (-)= No activity  
 \*Effective inhibition zone is zone which is measured after deducting the cork borer size from from total inhibition zone.

**Table 2. Antibacterial activity of different extracts of *Azadirachta indica* bark**

EXTRACT TYPE	TEST ORGANISMS	EFFECTIVE ZONE OF INHIBITION (mm)			
		Extract Conc. 100%	Extract Conc. 75%	Extract Conc. 50%	Extract Conc. 25%
METHANOL	<i>E. coli</i>	15.3 mm	13.6 mm	11.3 mm	6.6 mm
	<i>P. aeruginosa</i>	15.6 mm	13.6 mm	10.6 mm	8.6 mm
	<i>K. pneumoniae</i>	16 mm	14.3 mm	11.6 mm	8.6 mm
	<i>S. aureus</i>	15 mm	14 mm	12 mm	8.6mm
	<i>B. cereus</i>	16 mm	14.6 mm	11.3 mm	8.6 mm
ETHANOL	<i>E. coli</i>	6 mm	4.3 mm	2.3 mm	--
	<i>P.aeruginosa</i>	7 mm	6 mm	3 mm	--
	<i>K. pneumoniae</i>	5 mm	3.6 mm	2 mm	--
	<i>S. aureus</i>	8 mm	6.6 mm	5 mm	--
	<i>B. cereus</i>	5 mm	4 mm	2.3 mm	--
CONTROL	--				

Effective inhibition zone (mm)\*= Average value of inhibition zones of three replicates -Well size (-)= No activity  
 \*Effective inhibition zone is zone which is measured after deducting the cork borer size from from total inhibition zone.

**Table 3: Phytochemical screening of leaves extract of *Azadirachta indica***

Chemical Constituents	Test performed	Methanol extract	Ethanol extract
Alkaloids	Mayer's test	-	+
Tannins	Ferric chloride test	+	+
Flavonoids	Sulphuric acid test	+	+
Saponins	Foam test	+	+
Glycosides	Keller's Killiani test	-	-

- '+' indicates the presence of plant constituents.
- '-' indicates the absence of plant constituents.



**Table 4. Phytochemical screening of bark extract of *Azadirachta indica***

Chemical Constituents	Test performed	Methanol extract	Ethanol extract
Alkaloids	Mayer's test	-	-
Tannins	Ferric chloride test	+	+
Flavonoids	Sulphuric acid test	+	+
Saponins	Foam test	+	+
Glycosides	Keller's Killiani test	-	-

- '+' indicates the presence of plant constituents.
- '-' indicates the absence of plant constituents.

failed to exhibit inhibitory activity at 25% concentration. The zone of growth inhibition of bacteria corresponded to the drug concentration of plant material. A declined trend of inhibition zone was found with the dilution of extract.

The phytochemical analysis of plant extract was done in order to detect the phytoconstituents. Methanolic and ethanolic extracts of *A. indica* bark as well as methanolic extract of *A. indica* leaves showed the presence of tannins, flavonoids and saponins. Whereas alkaloids and glycosides were found absent in all three extracts. On the other hand, ethanolic extract of leaves were found rich in alkaloids, tannins, saponins, flavonoids and glycosides showed absence. More inhibitory activity in methanolic bark extract may be due to higher solubility of tannins, flavonoids and saponins in methanol extract. Moreover the amount of dissolved phytoconstituents may be higher in methanolic bark extracts as compared to other extracts. Previous studies have showed that *Azadirachta indica* extracts were found effective against a wide variety of microorganisms. Our results coincides with findings of previous researchers. Chaturvedi *et al.*, (2011) studied antibacterial activity of methanolic extract of Neem bark against *S. aureus*, *Klebsiella* and *Pseudomonas species*. The extracts were found inhibitory to *S. aureus*, *Klebsiella* and *Pseudomonas species*. Maragathavali *et al.*, (2012) reported that methanolic extract of leaf had inhibitory effect on the test organisms i.e. *E. coli*, *P. aeruginosa* and *S. aureus*. Singh *et al.* (2015) reported the antibacterial efficacy of methanolic extract of *A. indica* leaves against *S. aureus* Nigussie *et al.*(2021) evaluated the antibacterial



(A)



(B)



(C)



(D)



(E)

**Photoplate 2: Antibacterial activity of ethanol extract of leaves of *Azadirachta indica* against: (A) *P.aeruginosa* (B) *B.cereus* (C) *S.aureus* (D) *K.pneumoniae* (E) *E.coli***

activity of methanolic extract of leaves of *A. indica* against bacterial strains isolated from the wounds of lymphoedema patients i.e. *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Shewanella alage*. They found that methanolic extracts of *A. indica* leaves exhibited antimicrobial activity against selected bacterial isolates involved in wound infections.

### Conclusion

*Azadirachta indica* (neem) is the most versatile medicinal plant with therapeutic properties. It is rich in large number of bioactive compounds having antimicrobial, antioxidant, antiviral, antifungal and anti-inflammatory activities. From this study, it can be concluded that crude extracts of

*A. indica* (leaf and bark) have antibacterial activity against bacterial test strains. Our findings support the traditional medicinal usage of plant. Neem plant contain a number of active phytoconstituents having beneficial effects in control of pathogenic microbes and therefore can be used in preparation of herbal formulations in future.

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### Conflict of interest

The authors declare that they have no conflict of interest.

### References

- Alipour, M., & Khanmohammadi, O. (2011). Antimicrobial activity of plant extracts against oral and skin pathogens. *African journal of microbiology*, 5(19), 2909-29011.
- Al-mariri, A., & Safi, M. (2014). In vitro antimicrobial activity of several plant extract and oils against some Gram -negative bacteria. *Iranian Journal of Medical Science*, 39(1), 36-43.
- Barreto, M., Critchley, A.T., & Straker, C.J. (2002). Extracts from seaweeds can promote fungal growth. *Journal of Basic Microbiology*, 42(5),302-310.
- Buzayan, M. M., & El-Garbuli, F.R. (2012). Antimicrobial activity of medicinal aqueous plant extract against *Mycobacterium tuberculosis*. *Malaysian journal of microbiology*, 8(3), 03-206.
- Chaturvedi, M.P., Bag, A., Rawat, V., Jyala, N.S., Satyavali, V., & Jha, P.K.(2011). Antibacterial Effects of *Azadirachta indica* Leaf and Bark Extracts in Clinical Isolates of Diabetic Patients. *Nat J Integ Res Med*, 2, 5-9.
- Davies, J. (1994). Inactivation of antibiotics and the dissemination of resistant genes. *Science*. 264, 375-382.
- Francine, U., Jeanette, U., & Pierre, R.J. (2015). Assessment of antibacterial activity of Neem plant (*Azadirachta indica*) on *Staphylococcus aureus* and *Escherichia coli*. *JMPS*, 3(4), 85-91.
- Girish, K., & Shankar ,B.S. (2008).Neem- A Green Treasure. *Electronic Journal of biology*, 4(3), 102-111.
- Ibrahim, H. M., & Abu Salem, F. M. (2014). Antimicrobial activity of some medicinal plant extract. *International Scholarly and Scientist Research & Innovation*, 8(10), 1168-1173.
- Joshi, A., Bhojbe, M., & sattarkar, A. (2013). Phytochemical investigation of the roots of *Grewia microcos* Linn.. *Journal of Chemical and Pharmaceutical Research*, 5(7),80-87.
- Mahida, Y., & Mohan, J.S.S. (2006). Screening of Indian Plant extract of antimicrobial activity. *Pharmaceutical Biology*, 44(8), 627-631.
- Maragathavalli, S., Brindha, S., Kaviyarsai., N. S., Annadurai, B., & Gargwar, S. K. (2012). Antimicrobial activity of leaf extract of neem (*Azadirachta indica*). *International Journal of Science and Nature*, 3(1), 110-113.
- Nigussie, D., Davie, G., Legesse, B.A., Fekadu, A., & Makonnen, E. (2021). Antibacterial activity of methanol extracts of the leaves of three medicinal plants against selected bacteria isolated from wounds of lymphoedema patients. *BMC Complementary medicine and therapies* m2, 1-10.
- Rajasekaran, C., Meignamam, E., Vijaykumar, V., kalaivani, T., Ramya, S., Premkumar, N., Siva, R., & Jayakumaraj, R (2008). Investigation on antibacterial activity of leaf extract of *Azdirachata indica* A. Juss (Mediaceae); A Traditional medicinal plant of India. *Ethnobotanical leaflets*, 12, 1213-1217.
- Singh, B., Ahamad, A., & Pal, V. (2015). Evaluation of Antibacterial Activity and Phytochemical Screening of *Azadirachta indica* Leaves Extracts Against *Staphylococcus aureus*. *UK journal of pharmaceutical and biosciences*, 3(4), 43-47.
- Shukla, S., Mehta, A., & Bajpai, V.K. (2013). Phytochemical Screening and Anthelmintic and Antifungal Activities of Leaf Extracts of *Stevia rebaudiana*. *Journal of biological active products from nature*, 1(3), 56-63.
- Sheel,R., Nisha, K., & Kumar, J (2014). Preliminary Phytochemical Screening of Methanolic Extract Of *Clerodendron infortunatum*. *IOSR Journal of Applied Chemistry*, 7(1), 10-13.

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