Intercontinental Journal of Pharmaceutical Investigations and Research (ICJPIR)

ICJPIR |Vol.12 | Issue 3 | Jul - Sept -2025

www.icjpir.com

ISSN: 2349-5448 DOI: https://doi.org/10.61096/icjpir.v12.iss3.2025.159-169

Review

Comprehensive review on anti-microbial activity of *Azadirachta indica* (Neem) Bark extract and Gum extract

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Published on: 04 Aug 2024

Published by:
Futuristic Publications

Abstract

The present study focuses on the preparation, characterization, and antimicrobial evaluation of *Azadirachta indica* (Neem) bark extract and its chemically modified derivative-acrylamide-grafted neem gum polysaccharide (NGP-g-Am). Neem bark, collected in mid-August, was processed to obtain a methanolic extract, which was subsequently characterized through physical and chemical evaluations, UV-visible spectrophotometry for λ max and purity determination, and structural elucidation using FTIR spectroscopy. The extract exhibited notable antimicrobial activity against selected bacterial and fungal strains, validating the traditional medicinal use of neem bark. In parallel, neem gum polysaccharide (NGP) was chemically modified via microwave-assisted free radical polymerization using acrylamide, guided by a three-level full factorial design. The resulting graft copolymer (NGP-g-Am) was extensively characterized using UV-visible spectroscopy, FTIR, scanning electron microscopy (SEM), contact angle measurements, biodegradability assessment, hemocompatibility testing, and pH-dependent swelling behaviour. Molecular docking studies revealed that both NGP and NGP-g-Am exhibit strong binding affinities to Toll-like receptor 4 (TLR-4), suggesting a possible mechanism for their observed antimicrobial activities. The enhanced biological performance of the grafted polymer compared to native neem gum highlights its potential application in biomedical fields, especially as a biocompatible antimicrobial agent. This review consolidates current findings on the dual approach of using both natural and modified neem-based materials for antimicrobial purposes, offering valuable insights into the development of sustainable and effective bioactive compounds.

Keywords: Neem, Antimicrobial, *Azadirachta indica*, Chopped bark, Biomolecules, Neem gum, Hydrogel, Biodegradable, Swelling behaviour, Neem tree, Root bark, Minimum Inhibitory concentration (MIC).

INTRODUCTION

Plants are nature's gift by God to man for his beneficial herbal exploits in diver applications including herbal traditional medicine, antimicrobial, antifungal, biogas production, biofertilizers and antiseptic. Plant chemicals are referred to as phytochemicals. Several research works have identified thousands of these different plant chemicals, which were found in vegetables, fruits, beans, whole grains, nuts and seeds. Phytochemicals are chemical compounds produced by plants, generally to help them thrive or fight against predators or pathogens. The name comes from the Greek word "Phyto" which means plant. Some phytochemicals have been used as traditional medicine, as poison and as nutrients. Phytochemicals which are naturally contained in plants with known beneficial roles in the body have been classified as essential nutrients in diet, for the body's normal physiological functions. Drug resistance is a serious global problem, and spread of resistance poses additional challenges for clinicians and the pharmaceutical industry. Use of herbal medicines in the developed world continue to rise because they are rich source of novel drugs and their bioactive principles form the basis in medicine, nutraceuticals, pharmaceutical intermediates and lead compounds in synthetic drugs. Screening medicinal plants for biologically active compounds offers clues to develop newer antimicrobial agents. These compounds after possible chemical manipulation provide new and improved drugs to treat the infectious diseases and for the development of synthetic drugs. Neem has been used for thousands of years as medicine. The botanical name of the neem plant is *Azadirachta indica* A. Juss. Neem has been widely used by humans since prehistoric times to treat various diseases. The plant is extraordinary, and it is referred to as "21st-century trees" by the United Nations. Neem is also referred to as "a tree for solving global problems" because it has many benefits, and it can treat various diseases. Azadirachta indica Juss (AI) is a large ever green tree This plant is abundantly found in every part of India and has been used "Sarva Roga Nivarini". All parts of the plant have been used for medicinal purposes including fruits, seeds, leaves, roots and barks. This tree has been screened for antioxidant activity and it was found that various parts like leaves, seeds, flowers, stem bark and root bark could promote high antioxidant activity *Azadirachta Indica* (A. Indica) belongs to the family Meliaceae, commonly known as neem, commonly found in Pakistan, India, Nepal and Bangladesh. Different effects posed by these products include microbial growth inhibition, antioxidant activity and genetic/molecular pathways modulation. Various Phyto-ingredients of *Azadirachta indica* including limonoids, nimbin and nimbolide, and due to these constituents Neem plays a satisfactory role in treatment of infectious diseases. It is used in traditional medicine as a source of many therapeutic agents. A. indica (leaf, bark and seed) are known to contain antibacterial, antifungal activities against different pathogenic microorganisms and antiviral activity against vaccinia, chikungunya, measles and coxsackie B viruses. Different parts of neem (leaf, bark and seed oil) have been shown to exhibit wide pharmacological activities including; antioxidant, antimalarial, antimutagenic, anticarcinogenic anti-inflammatory, antihyperglycemic, antiulcer and antidiabetic properties. The biological activities are attributed to the presence of many bioactive compounds in different parts.

Antimicrobial activity has been investigated for neem leaves, bark and seed, but there are no studies on the comparative evaluation of aqueous extract of leaves, bark and seed. Hence, the current study was designed to investigate the comparative antimicrobial activity of neem leaves, bark and seed aqueous extract against human pathogenic bacteria and fungi. A number of factors such as, thickness and uniformity of the gel, size of the inoculum, temperature and pH that affect the accuracy and reproducibility of the agar diffusion method were also taken into consideration to obtain reliable results.

Gum is a versatile byproduct formed as a consequence of certain metabolic mechanisms in plants and trees. Plant derived gum is either water soluble or absorbs water to form a viscous solution. Neem gum contains mannose, fructose, glucosamine, arabinose, galactose, xylose and D-glucuronic acid. Neem's gum, which is used in a number of industries has been economically exploited. Neem tree is being grown on a large-scale basis for using all its parts, no wonder it is called a 'Universal Tree', having a cure for almost everything. There hasn't been much research on using neem gum as a tablet binder or a suspending agent for pharmaceutical suspensions. Hence, in the current research, neem gum was selected to evaluate its binding and suspending potentials using aceclofenac as a model drug. Neem gum, a water-soluble polysaccharide (NGP), is naturally obtained from exudates of *Azadirachta Indica* Recently, various researches have been carried out to investigate possible applications of graft copolymers in wound dressing, vascular prostheses, cartilage and bone replacement, and wound healing and as biomembrane. So hemocompatibility of prepared NGP-Gam. Researchers have reported the various uses of Neem seeds, fruits, oils, leaves, bark and root as general antiseptic, antimicrobial, and treatment of disorders (such as urinary, diarrhoea, fever, bronchitis, skin disease, septic sores, hypertension, infected burns and inflammatory disease). Screening of phytochemicals involves the extraction, screening and identification of bioactive substances (plant chemicals) in plants. Some of the phytochemicals were found in plants include; tannins, flavonoids, alkaloids, phenols, glycosides, carotenoids, antioxidants, steroids and saponins. These bioactive ingredients in *A Indica* are present in different detectable concentrations

Biochemistry, Pharmacology and Botany have their interest increased in phytochemical screening of plants for the presence of phytochemicals, for the development of medicine, pesticides and germicide functions.

Feng and Isman doubtlessly appreciate the difficult and expensive process involved in the screening, isolation and identification of plants' secondary metabolites produced in large quantity to be commercialized. He pointed out that at least nine neem limonoids have demonstrated an ability to block insect growth, affecting a range of species that includes some of the deadliest pests of agriculture and human health. In a phytochemical study carried out by Harry-Asobara and Eno-Obong, other parts of the plants while greater percentage of tannin was observed in Neem bark [1,2].

MATERIALS AND METHODS:(BARK)

Fig 1 : Neem Leaves and Bark

Determination of antimicrobial activity

The aqueous extracts of bark of *A. Indica* were screened for antimicrobial activity by agar well diffusion method. Agar surface was cut with the help of sterile cork borer having a diameter of 6.0 mm size. All bacterial and fungal strains were grown in nutrient broth (NB) and Sabouraud dextrose broth (SDB) for 4-6 hours at specified temperatures. The turbidity of the broth culture was adjusted to 0.5 McFarland units. This gives a suspension containing approximately 1-2 x 10 power (6) colony forming units (CFU)/ml (Mackie & Mac Cartney 1996). An aliquot (0.02 ml) of microbial culture was added to molten MHA at 45°C and poured into the petri plate. After solidification of the agar, appropriate wells were made on agar surface by using sterile cork borer (3 wells per 90 mm diameter plate). Different concentrations of the extracts were prepared using dimethyl sulfoxide (DMSO) and 50µl of each concentration was added to the wells. Bacterial cultures were incubated at 37°C for 24 hours and fungal cultures at 25ºC for 48 hours. Antimicrobial activity was determined by measuring the zone of inhibition surrounding the well. The assays were carried out under aseptic conditions. Ciprofloxacin (5µg/disc) and Amphotericin B (100µg/disc) were used as positive controls for bacteria and fungi respectively and DMSO as a negative control. Each concentration included duplicates and the results are average of two independent experiments.

Collection of plants

Azadirachta indica bark were collected from botanical garden Calcutta Institute of Pharmaceutical Technology and Allied Health Sciences, Banitabla, Uluberia, Howrah, West Bengal and then authenticated from department of botany, Vinodini PG college, Shekhawati university, Rajasthan**.**

Materials

Methanol, distilled water and agar were used to be analytical grade**.**

Preparation of crude *Azadirachta indica* bark extract

Biological source: Azadirachta indica (family – Meliaceae)
Geographical source: West Bengal in India, Asia, Africa and other tropical parts of world.
Part used: Stem bark
Collection: In the mid of august the stem bark was collected from the *Azadirachta indica* tree. After collecting the bark was peeled from the stem with a sharp knife and chopped into pieces which were sun dried for seven days. After drying the bark was cut more finely using cutter mill which was used for preparing extract.

Preparation of extract

Materials and equipments used: methanol, 250ml separating funnel, sharp knife, distillation apparatus, filter paper, hot plate, distilled water, cotton wool. Firstly, the finely chopped bark was kept into a separating funnel using methanol as menstrum at room temperature for seven days after that plant extract was collected into a beaker by opening the outlet of separating funnel [6,7,8]. This above process was repeated for four times. After

that plant extract was distilled over five hours at 80^0C for concentrating the menstrum. After distillation the menstrum was more concentrated in hot plate around 60^0C with continuous hand stirring till the after-cooling crystal was formed. The plant extract was filtered through filter paper. The filtrate was again concentrated till crystal was formed. The crystal was separated on filter paper. After drying, the crude extract was collected into plastic container and has been stored at room temperature till its use [9,10].

Table 1 : Physical Property And Chemical Property Evaluation

Parameter		**Observed**
Physical appearance	Brown powders	Brown powders
SOLUBILITY		
Distilled water	+	+
Methanol	+	+
Ethanol	+	+
(+) soluble, (-) insoluble		
Chemical test for terpenoid		
Procedure	**Observance**	**Result**
Salkowski test: Powdered drug was treated with chloroform and few drops of sulphuric acid	It was given yellow colour which was changed to red colour	Extract may contain terpenoid which is biologically antibacterial

Characterization of neem bark extract

Selection of microorganism and growth medium for testing the antimicrobial activity the *Staphylococcus aureus* ATCC 29737 (IPRS) and *Escherichia coli* ATCC 25992 was selected and done test for prepared crude *Azadirachta Indica* bark extract in the laboratory. Growth medium - General nutrient agar media

Preparation of nutrient agar media

Materials

Electronic balance, pH paper or pH meter with standard buffers Volumetric flask: 250ml. Beef Extract, Yeast Extract, sodium chloride, Agar, Non-absorbent cotton and gauze to make cotton stoppers.

Table 2: Composition of nutrient agar media

Composition of Nutrient Agar Medium	
Composition	**Percentage (%)**
Agar	2
Beef extract	1
Yeast extract	1
NaCl	0.5
Water	q.s

Sterilization

Sterilization was done for glass apparatus in hot air oven for one hour at 160^0C.

Preparation of slant and subculture

Firstly, after sterilizing the test tube, agar nutrient media was melted at 45^0C and poured into the test tube at 60^0C degree angle and waited for solidification. After solidification prepared slant was kept into freeze. Prepared slant was inoculated by moving the loop gently up the surface of the agar in a snake-like fashion but was not to gouge the agar surface. Slant cultures were incubated for 24 hours and bacterial growth was observed.

Preparation of inoculums

For evaluation of antibacterial activity, fresh culture of bacteria was suspended in sterile water to obtain a uniform suspension of microorganism up to 24hour.

Determination of zone of inhibition

Antibacterial activity was checked by agar well diffusion method. In this method a previously liquefied medium was inoculated with 0.2 ml Bacterial suspension having a uniform turbidity at room temperature. 20 ml of culture medium was poured into the sterile Petri dish having an internal diameter of 8.5 cm. Care was taken for the uniform thickness of the layer of medium into plates. After complete solidification of liquefied inoculated

medium, the wells were made aseptically with cork borer having 6mm diameter. In each of these plates, extract solution (1mg/1ml stock solution prepared in distilled water) was placed carefully. Plates were kept for pre diffusion for 30 minutes. After normalizing to room temperature; the plates were incubated at 37ºc for 24 hrs for bacteria. After incubation period was over, the zone of inhibition was measure with help of Hi- media, measured with determination of λ max.

Table 3 : Zone of Inhibition of Extract

Microbial strains	**Zone of inhibition (mm)** ***Azadirachta indica* Bark Extract**
Staphylococcus aureus ATCC 29737	12
Escherichia coli ATCC 25992	0

Determination of λ max

Determination of λ max in methanol & ethanol and percentage of purity of *Azadirachta indica* bark extract using UV spectroscopic method (shimadzu-1700). An absorption maximum of extract was determined by UV spectroscopic method using shimadzu 1700 UV/Visible spectrophotometer. It had been found that the λ max for extract in methanol was 278.5 nm and in ethanol was 282 nm.

Procedure

Firstly 10 mg extract was weighed and was dissolved into 10 ml methanol and ethanol respectively to prepare 1mg/ml stock solution means 1000 µg/ml stock solution. From this stock solution 5 ml solution taking was diluted to 50 ml solution to prepare 100 µg/ml solutions, from this solution several dilutions were done and λ max for extract was estimated.

Calculation

Stock solution concentration - 1000 µg/ml 1 ml stock solution of extract contains equivalent to 1000 µg extract 5 ml stock solution of extract contains equivalent to 5 x 1000 = 5000 µg extract 5 ml stock solution of extract was dissolved in 50 ml solvent, so concentration becomes diluted from 1000 µg/ml to 100 µg/ml. From 100 µg/ml solution following concentration was prepared to estimate the Lamda maximum (λ max) of extract _

1. 1ml to 10ml = 10 µg/ml
2. 2ml to 10ml = 20 µg/ml
3. 3ml to 10ml = 30 µg/ml
4. 4ml to 10ml = 40 µg/ml
5. 5ml to 10ml = 50 µg/ml
6. 6 ml to10ml = 60 µg/ml

Table 4 : Results of UV analysis

Solvent employed	**LAMDA MAXIMUM (λ MAX) IN NANOMETER (nm)**			
	Reference	**Observed**		
			Concentration of extract in µg/ml	**Absorbance**
Methanol	279	278.5	50	0.1688
Ethanol	Not found	282	20	0.0881

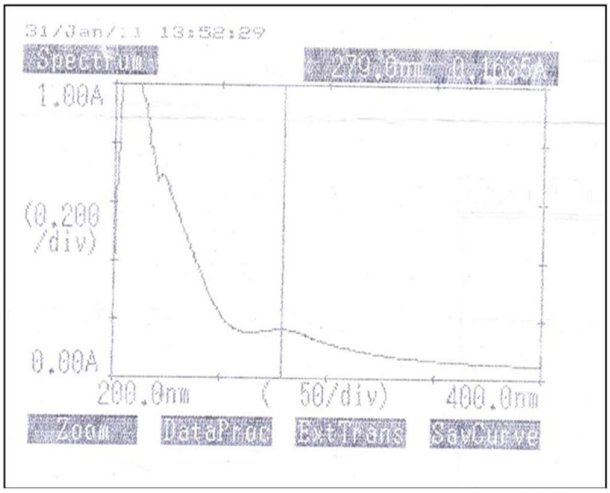

Fig 2 : UV lamda maximum (λ max) of neem bark extract in methanol at 278.

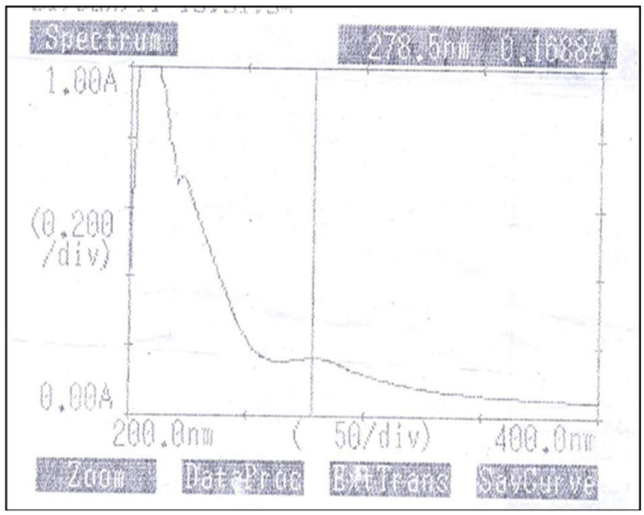

Fig 3 : UV absorbance of neem bark extract at 279nm

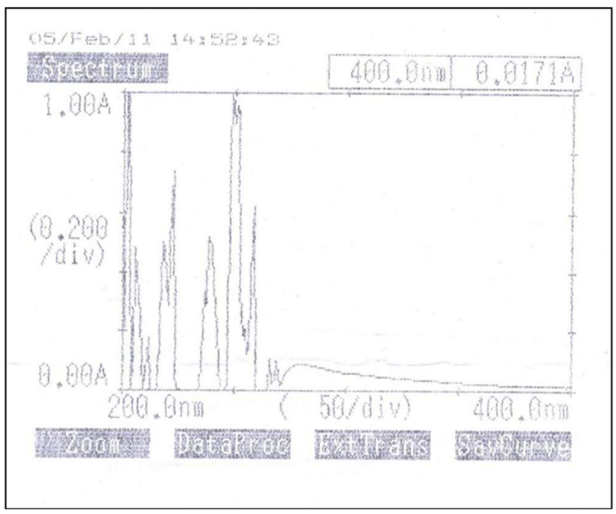

Fig 4 : UV lamda maximum (λ max) of neem bark extract in ethanol at 282 nm

Calculation of %purity of prepared extract

Value for prepared extract was being taken as reference data from U.S. patent (date of patent-May 7, 1985, patent no.-4515785, sheet 3 of 3) is 110 at 279 nm in methanol. [4]

Table 5 : UV analysis for determining percentage purity

Concentration in µg/ml	Concentration in %w/v	Observed absorbance at 279 nm	$E^{1\%}_{1cm}$ value of reference at 279 nm
50	0.005	0.1685	110

Calculation

% Purity = Observed absorbance/'E' value * 100/concentration

Conversion of µg/ml to % w/v

% w/v * 10,000 = µg/ml

So, 50 µg/ml = 50/10000%w/v

= 0.005%w/v

% Purity = 0.1685/110*100/0.005

= 30.63%w/w

RESULTS AND DISCUSSIONS

Prepared neem bark extract was prepared in methanol, color of this extract was brown crystalline powder, was soluble in water, methanol, ethanol as this extract was prepared in methanol, so it forms intermolecular hydrogen bonding between different molecules to be soluble, antimicrobial activity was checked against *Staphylococcus aureus* ATCC 29737 (IPRS) and *Escherichia coli* ATCC 25992, but zone of inhibition was found only against *Staphylococcus aureus* ATCC 29737 (IPRS) was 12 mm, solution inhibited growth of microbes around well of nutrient media . λ max and absorbance was determined using different concentration of solution for extract that in a optimized same concentration, λ max in methanol was 278.5 nm and absorbance in methanol was found 0.1688, λ max in ethanol was 282 nm and absorbance was found 0.0881. Absorbance was measured at 279 nm of particular concentration of extract solution was 0.1685 to calculate percentage of purity of neem bark extract that was estimated at 0.005% w/v concentration, 279 nm is 30.63% w/w using reference value at 279 nm of neem bark extract. FTIR study was done to confirm structure of neem bark extract with reference IR spectrum data and the main peaks were found at 3437.88, 2847.17, 2933.53, 1730.03, 1715.56, 1455.19, 1379.97 cm^{-1} of sample which was identical to reference spectra data. [4]

Material and Methods (GUM)

Fig 5 : Neem Gum

Crude neem gum polysaccharide (NGP) was purchased from a local shop of New Delhi, India. Gum was authenticated by Prof. D.K. Chauhan, Department of Botany, University of Allahabad, Allahabad, Uttar Pradesh. Crude gum was dissolved in sufficient amount of double distilled water and heated up to 40°C. After 2hr, gum solution was filtered through a double-fold muslin cloth to remove un-dissolved portion. Gum was precipitated by using ethyl alcohol and dried in an oven at 40°C. Further gum was powdered, passed through a 60# sieve, and stored in airtight polypropylene jars under desiccated condition Acrylamide (Am) and ceric ammonium nitrate (CAN) were procured from Merck Specialties Private Limited, Mumbai, India. Ethyl alcohol was supplied by S.D. Fine Chemicals, Mumbai, India. All the chemicals were used as supplied without any purification. In experiments, double distilled water was used.

Preparation of acrylamide-grafted neem gum Polysaccharide

NGP-derived graft copolymer was synthesized by free radical–induced microwave-assisted polymerization. Polysaccharide (1 g) was dissolved in 30ml of double distilled water. Acrylamide solution was separately prepared by dissolving 6g of acrylamide in 25 ml double distilled water. Prepared acrylamide aqueous solution was transferred into polysaccharide solution and stirred for 1 hr at 200 rpm. Various concentrations of ceric ammonium nitrate were prepared in 30ml double distilled water as depicted in Table 6. To initiate free radical formation, CAN solution was added in acrylamide-polysaccharide solution followed by stirring at 150 rpm for 30 min. The solution was kept aside for overnight and further irradiated using the microwave (100 W) for the different time periods using 30-s heating and 30-s cooling cycle. After completion of the microwave cycle, the mixture was allowed to come at room temperature followed by precipitation using acetone. Precipitate was further washed with 20%v/v ethanolic aqueous solution to remove un-reacted acrylamide and homopolymer. Graft copolymer was dried at 40°C in hot air oven until constant weight was obtained. Dried polymer was powdered using domestic mixer grinder, passed with a 20# sieve and stored in air tight container. In the present study, two-factor, three-level, full factorial design was used to optimize acrylamide grafting over NGP Concentration of CAN and number of microwave exposure were selected as independent variables, and swelling index (%) was selected as responses (dependent variables). Each independent variable, however, was investigated at three different levels: high level (+), medium level (0), and low level (−), as depicted in Table 6. Results of responses (dependent variables) were analyzed using Design Expert software (Version 7.0.0, Stat-Ease, Inc., Minneapolis).

Table 6 : Details of Independent Variables

Batch Code	Independent Variables	
	Concentration of CAN(%w/v)	No. Of Microwave Exposure
N1	0.5	16
N2	0.3	16
N3	0.4	16
N4	0.5	13
N5	0.3	13
N6	0.4	13
N7	0.5	10
N8	0.3	10
N9	0.4	10

Evidence of grafting

Grafting of NGP was proven by study based on the characterization of native and graft copolymer using UV-visible spectroscopy and Fourier transform infrared spectroscopy.

UV-visible spectroscopy method

Absorption maxima of native as well as grafted polymer were measured and validated. To validate both modified and un modified polymers, 10 mg of samples was taken and dis solved in 0.1 N HCl and phosphate buffer pH 7.4 separately. Samples were diluted and absorption maxima were measured accordingly. For intraday studies, samples were measured thrice a day at 7 am, 12 am, and 5 pm, and inter day studies was carried out by measuring absorption maxima at 12 am for 7 consecutive days. Results were shown as an average of triplicate studies with standard deviation.

Fourier transform infrared spectroscopic method

IR spectral analysis of polymers was utilized to prove grafting. FTIR analysis was performed at Central Instrument Facilities, School of Medical and Allied Sciences, Galgotias University, India. Dried powdered gum sample was put on the analyzer plate of Bruker ATR equipment (Alpha, ECD-ATR). Spectra were analyzed in transmittance mode in the range of wave number 4000 to 600 cm with 66 scans and 2-cm resolution. Obtained spectra were recorded and interpreted to analyze functional groups present in the polysaccharide and graft copolymers.

Scanning electron microscope analysis

Surface properties of NGP and NGP-g-Am (N1) were carried out by scanning electron microscope (SEM) analysis. The surface properties of the native, as well as grafted polymers, were analyzed by using Zeiss EVO 18 analyzer. The powders were gold coated and mounted in sample holder.

Grafting parameters

Different grafting parameters such as % grafting (%G), % efficiency (%E), % grafting ratio (%Gr), and % conversion (%C) were determined using the following Eqs.1, 2, 3 and 4 respective

$$\%G = \frac{Weig \quad of\ graft\ co\ polymer - Weight\ of\ NG}{Weigh\ \ of\ NG}\ 100 \qquad (1)$$

$$\%E = \frac{(Weigh\ \ of\ graft\ co\ polymer - weight\ of\ NG)}{Weigh\ \ of\ acrylamide}\ 100 \qquad (2)$$

$$\%\ Gr = \frac{(Weight\ of\ graft\ copolymer - We \qquad of\ NG)}{Weigh\ \ of\ NG}\ 100 \qquad (3)$$

$$\%C = \frac{(Weight\ of\ graft\ co\ polymer - Weight\ of\ NG)}{Weight\ of\ NG!}\ 100 \qquad (4)$$

Swelling study

Swelling characteristics of the grafted polymer were investigated. In this study, 2 g of dried polymer was taken and transferred into Petri dish containing 25 ml of distilled water. After the fixed time (i.e., 12 hr), extra surface water was wiped off from the polymer surface and polymers were reweighted, which leads us to calculate swelling using

$$\text{Ps}\ (\%) = \frac{(Ws - Wd)}{Wd}\ 100$$

where Ws and Wd are the weights of swollen and dried polymer, respectively. Effects of pH on the swelling behaviour of polymer were also done using 1 N NaOH and 0.1 N HCl solvent [11, 12].

Student's t test analysis

It is a mathematical treatment for the analysis of mean value obtained for repeated experiments. In this experiment, Student's t test was done assuming a null hypothesis that there is no significant difference between the swelling index of graft copolymers in distilled water, 0.1 N HCl, and 1 N NaOH. To test the null hypothesis, 95% confidence level was taken into consideration**.**

$$V = \frac{1}{(1+Q)}$$

$$Q = \frac{(Ws - DP)}{(W \times Do)}$$

Where Ws is weight of solvent in swelled polymer, Dp is density of polymer (g/cm), Do density of solvent, and W is weight of polymer used in study [13].

Molecular docking for antimicrobial efficacy

Molecular docking studies were carried out to compare anti-microbial efficacy of native and graft copolymers. In present research, Auto Dock tools (ADT) and Auto dock docking program were used the antimicrobial efficacy of NGP and acrylamide graft copolymers of NGP.

In this research, docking calculations were carried out using Docking server. To minimize the energy of ligand molecule, the MMFF94 force field was used. Gasteiger partial charges were added to ligand atoms and non-polar hydrogen atoms were merged. Docking calculations were carried out on 1fyw-SIGNALING PROTEIN protein model. Auto Dock tools were used to add essential hydrogen atoms, Kollman united-atom-type charges, and solvation parameters. Affinity (grid) maps of 20×20×20Å grid points and 0.375Å spacing were generated using the Auto Grid program. Auto Dock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electro static terms, respectively. Docking simulations were per formed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method. Initial position, orientation, and torsions of the ligand molecules were set randomly. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250,000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å and quaternion and torsion steps of 5 were applied.

Experimentation for antimicrobial efficacy

The antimicrobial activity of NGP and N1 was evaluated and compared using disc diffusion method. Test microorganism was obtained from the Department of Medical Lab Technology, School of Medical and Allied Sciences, Galgotias University, India, and comprised the gram-negative bacteria *Escherichia coli* and fungus *Aspergillus niger*. Test microorganisms were initially cultured on sterilized nutritive agar medium. *Escherichia coli* was cultured for 24 hr at 30 °C and *Aspergillus niger* for 48 hr at 30°C. NGP and N1 were dissolved and diluted in double distilled water to prepare 1, 0.5, and 0.25 mg/ml solutions. Solutions were poured onto 5-mm discs and incubated for the next 24 hr. Activity assays were replicated in triplicate. After incubation, inhibition zone was measured in millimetre and antimicrobial activity of N1 was compared with native polymer, i.e., NGP. [5]

RESULT

Synthesis of acrylamide-grafted NGP was carried out by free radical–initiated microwave-assisted polymerization reaction. CAN was a well-known reagent to initiate free radical polymerization. Initially, CAN starts to form NO_2 free radicals. These NO_2 radicals react with OH groups of NGP and initiate free radical formation within NGP.

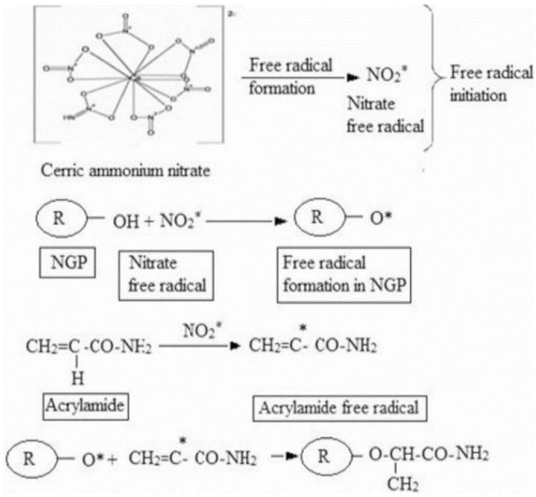

Fig 6 : Grafting Mechanism Of Acrylamide Over NGP

NO_2 free radicals also formed acrylamide free radicals when reacting with acrylamide. Process elicits that NO_2 free radicals do specifically not react either with NGP or with acrylamide but with both polymer (i.e., NGP) and monomer (i.e., acrylamide). Further, polymeric free radicals and monomer radicals react with each other to start grafting over the polymeric backbone. Polymer and monomer free radicals couple with each other via covalent bond. During the study, it was observed that CAN form nitrate free radicals even at room temperature (25°C) without microwave exposure, and it was proven by gel formation within solution when it was kept for overnight. Gel formation directly depended upon the concentration of CAN in respective solution. Further microwave exposure helps the solution to proceed better grafting (visually identified by gel formation) due to the generation of more free radicals. Microwave irradiation induced rapid energy transfer and shortening of the reaction time. [5]

CONCLUSION

This study successfully demonstrated the antimicrobial potential of both *Azadirachta indica* bark extract and its chemically modified derivative, acrylamide-grafted neem gum polysaccharide (NGP-g-Am). The crude bark extract exhibited broad-spectrum antimicrobial activity against *Staphylococcus aureus, Escherichia coli,* and *Aspergillus niger*, as confirmed by agar well diffusion assay, with zones of inhibition supporting the presence of bioactive compounds such as terpenoids. UV spectrophotometric analysis indicated maximum absorbance at 278.5 nm (methanol), further confirming extract stability and purity.

In the second phase, native neem gum polysaccharide (NGP) was successfully grafted with acrylamide using free radical–induced, microwave-assisted polymerization. The optimization of grafting conditions was achieved using a full factorial design based on CAN concentration and microwave exposure cycles, with swelling index as the response parameter. Structural confirmation and evidence of grafting were validated through UV-Vis, FTIR, and SEM analyses, revealing significant changes in spectral patterns and surface morphology of the graft copolymer compared to the native gum.

Further, molecular docking studies showed stronger interaction potential of the grafted polymer (N1) with the target protein model (1FYW), indicating enhanced antimicrobial efficacy. This was supported by in vitro antimicrobial assays, where N1 demonstrated superior inhibition zones against *Escherichia coli* and *Aspergillus niger* compared to the native NGP.

Overall, the study confirms that both the *Azadirachta indica* bark extract and its acrylamide-grafted polysaccharide form (NGP-g-Am) possess promising antimicrobial properties. The grafted polymer not only

improves the functional properties of the natural gum but also offers potential for use in biomedical and pharmaceutical applications as a novel bioactive material.

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