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INVESTIGATION OF MEDICINAL PROPERTIES OF NEEM BARK EXTRACT

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ABSTRACT

The objective of this research was to prepare and characterize neem bark extract from Azadirachta indica. Bark was collected in mid-August, and the methanolic extract was analyzed for physical, chemical, antimicrobial properties, λmax, purity (UV spectroscopy), and structural features using FTIR.

KEYWORD: Neem, Antimircobial, Bark, Azadirachta indica.

INTRODUCTION

Neem extract comprises a diverse and complex combination of molecules, including hydrocarbons, phenolics, terpenoids, alkaloids, and glycosides. Azadirachta indica A. Juss is a valuable medicinal plant widely distributed in tropical regions such as Indonesia.

Belonging to the Meliaceae family, neem is a tropical medicinal plant with a long history of use. Its different parts are developed into remedies for treating a wide range of acute and chronic illnesses, particularly in Asia and Africa. The plant is distributed globally, including regions of Asia, Africa, America, and Australia. Scientifically known as Azadirachta indica A. Juss, neem has been valued since prehistoric times for its therapeutic properties. Because of its extraordinary usefulness, the United Nations has termed it the “tree of the 21st century,” and it is also recognized as a “tree for addressing global issues.”

Collection of plant - The bark of Azadirachta indica was collected from the botanical garden of Calcutta Institute of Pharmaceutical Technology and Allied Health Sciences, Banitabla,

Uluberia, Howrah, West Bengal, and was later authenticated by the Department of Botany, Vinodini PG College, Shekhawati University, Rajasthan.

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

Preparation of crude azadirachta indica bark exract –

Biological source: Azadirachata indica (family – Meliaceae)

Geographical source: West Bengal in India, Asia, Africa and other tropical parts of world.

Part used: Stem bark.

Collection – During mid-August, stem bark of Azadirachta indica was obtained and separated from the stem using a sharp knife. It was then cut into pieces and sun-dried for a week. The dried material was further reduced to a fine form using a cutter mill for extraction purposes.

Prepration of extract - Materials and apparatus: methanol, 250 mL separating funnel, sharp knife, distillation setup, filter paper, hot plate, distilled water, and cotton wool.
The finely cut bark material was introduced into a separating funnel, where methanol served as the extracting solvent at ambient temperature for a period of seven days. Subsequently, the extract was drained into a beaker through the outlet of the separating funnel.
This above process was repeated for four times.After that plant extract was distillated over five hours at 80 degree centigrade for concentrating the menstrum.
After distillation the menstrum was more concentrated in hot plate around 60 degree centigrade 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.

Characterization of Neem Bark Extract –
Physical property and chemical property evaluvation

Parameter	Reference	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.

In vitro Antimicrobial evaluation by zone of inhibition method - 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 preared 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: 250 ml. Beef Extract, Yeast Extract, sodium chloride, Agar, Nonabsorbent cotton and gauze to make cotton stoppers.

Composition of Nutrient Agar Media –

Composition	Percentage(%)
Agar	2
BeefExtract	1
YeastExtract	1
Nacl	0.5
Water	q.s

Procedure - Beef Extract, Yeast Extract, Nacl, Agar was firstly weighed to get 1%,1%,0.5%,2% in solution respectively to prepare 100 ml nutrient Agar media. It was added to 100 ml of distilled water and stirred to dissolve the yeast extract and beef extract and Nacl in volumetric flask and checked the pH between 6.0 to 7.0.Then Agar was added and after cotton plaguing the media was sterilized in autoclave and prepared the media.

Sterilization - Sterilization was done for glass apparatus in hot air oven for one hour at

160^0C.

Preparation of slant and subculture - Firstly the after sterilizing the test tube, agar nutrient media was melted at 45^0C and poured into the test tube at sixty 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 24 hours**.**

Determination of zone of inhibition - The antibacterial activity was determined by the agar well diffusion technique. A previously liquefied medium was inoculated with 0.2 ml of a bacterial suspension of uniform turbidity at ambient temperature. Approximately 20 ml of the inoculated culture medium was transferred into sterile Petri plates (8.5 cm in diameter), maintaining uniform layer thickness. Following complete solidification, wells of 6 mm diameter were aseptically prepared using a cork borer. The extract solution (1 mg/ml in distilled water) was then carefully introduced into the wells. The plates were allowed to stand for pre-diffusion for 30 minutes, followed by incubation at 37°C for 24 hours. The antibacterial activity was assessed by measuring the zone of inhibition using Hi-media.

Zone of inhibition of extract

Microbial strains	**Zone of Inhibition(mm)**
	Azadirachta indica BARK EXRACT
Staphylococcus aureusATCC29737	12
Escherichia coli ATCC 25992	0

Determination of λmax - The determination of λmax in methanol and ethanol, as well as the percentage purity of Azadirachta indica bark extract, was carried out using UV spectrophotometry (Shimadzu-1700). The absorption maxima of the extract were recorded using a Shimadzu-1700 UV/Visible spectrophotometer. The λmax values were observed at 278.5 nm in methanol and 282 nm in ethanol.

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 was done and λ max for extract was estimated.

Calculation – Stock solution concentration - 1000µg/ml

1 ml stock solution of extract contains equivalent to 1000µg extract.

5 ml stock solution of extract contains equivalent to 5 * 10000 = 5000µg extract.

5 ml stock solution of extract was dissolved in 50ml solvent,so concentration becomes Diluted from 1000µg/ml to100µg/ml.

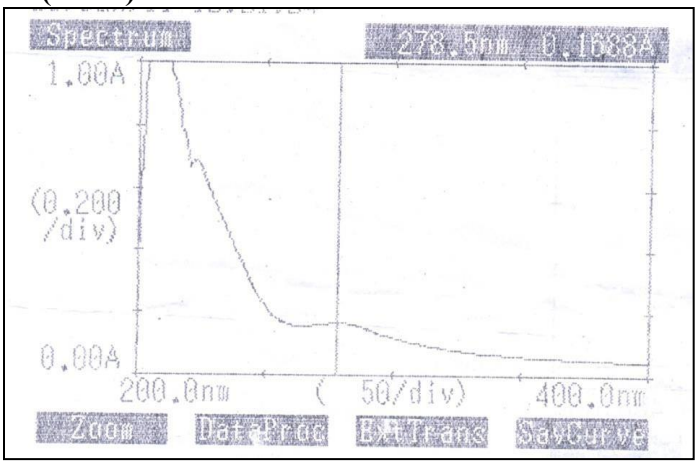
From 100 µg/ml solution following concentration was prepared to estimate the Lamda maximum (λmax) of extract –

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

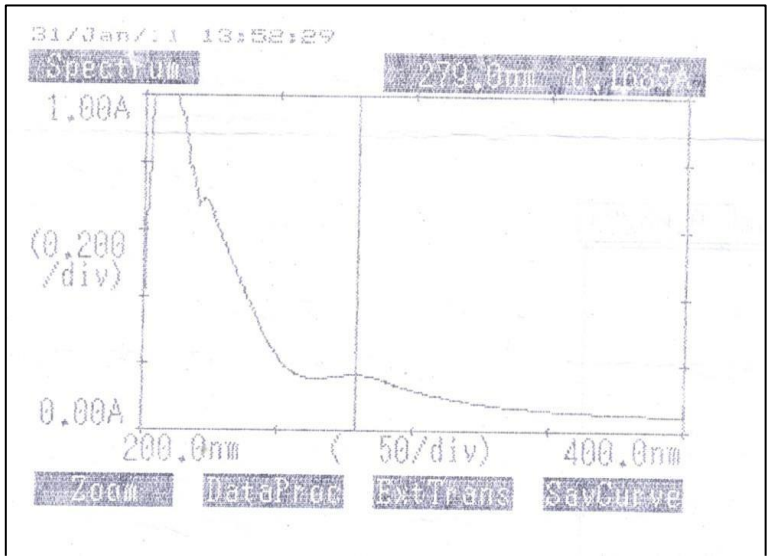
Result of UV analysis -

Solvent employed	**Lamda maximum (λmax) in nanometer (nm)**			
	Reference	**Observed**		
			Concentration of extractin µg/ml	**Absorbance**
Methanol	279	278.5	50	0.1688
Ethanol	Not found	282	20	0.0881

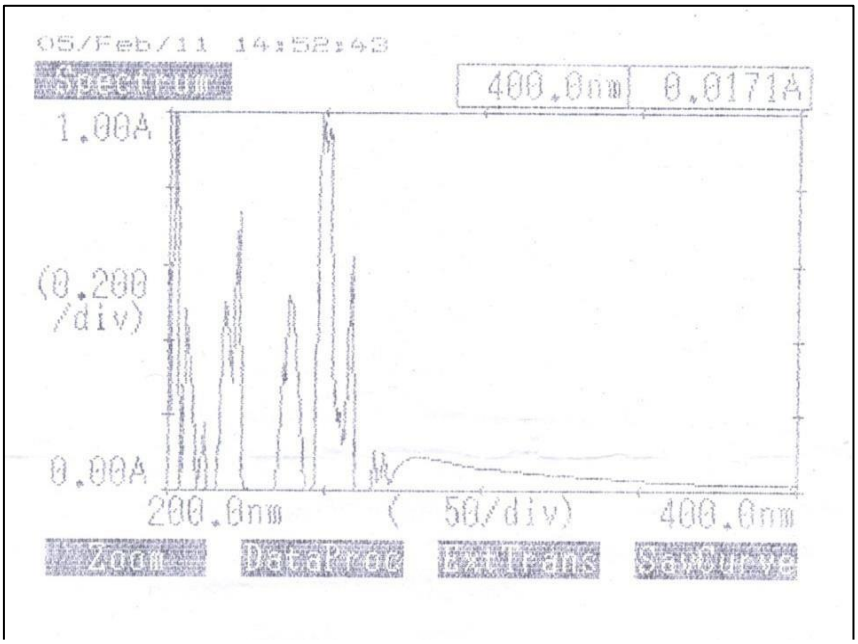
UV lamda maximum (λmax) of neem bark extract in methanol at 278.5nm.



UV absorbance of neem bark extract at 279nm in same concentration in methanol as UV lamda maximum (λmax) of extract previously found at 278.5nm.



UV lamda maximum (λmax) 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.

UV analysis for determining percentage purity

Concentration in µg/ml	Concentration in %w/v	Observed absorbance at 279 nm	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

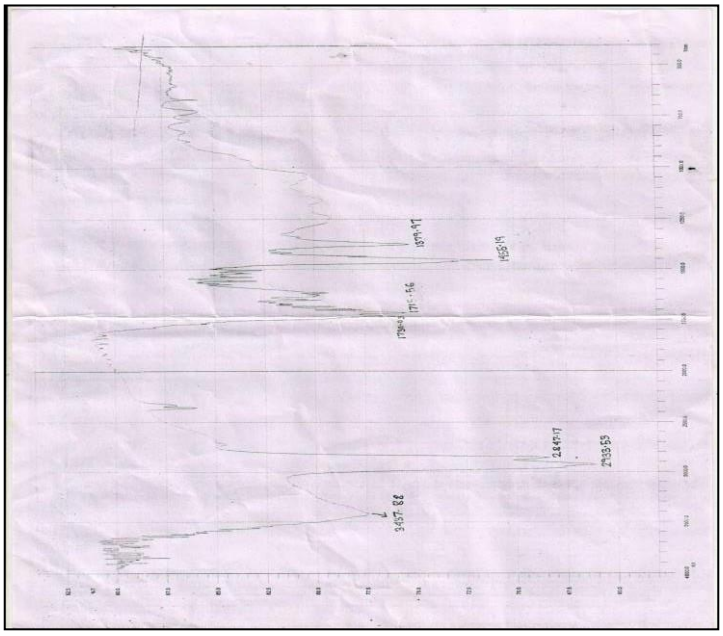
=0.005%

Structural elucidation by FTIR study IR Spectroscopy - The IR Spectroscopy was used as to confirm the structure of extract with respect to reference FTIR data of extract. The study reveals that as identified by IR spectrum and the main peaks was found at 3437.88, 2847.17, 2933.53, 1730.03, 1715.56, 1455.19, 1379.97 cm−1 of sample which is identical to reference spectra data.

FTIR spectra of prepared neem bark extract -

Compound	Stretching/Bending	Wave number (cm−1)
Prepared neem bark extract.	N-H stretching()	3437.88
	C-H stretching(alkane)	2847.17
	C-H stretching(alkane)	2933.53
	C=O stretching(aldehyde)	1730.03
	C=O stretching(acid)	1715.56
	C=C stretching(aromatic)	1455.19
	C-O stretching(phenols)	1379.97

FTIR spectra of prepared neem bark –



RESULTS AND DISCUSSION

The neem bark extract was prepared using methanol as a solvent. The obtained extract appeared as a dark brown crystalline solid and was readily soluble in water, methanol, and ethanol. This solubility may be attributed to intermolecular hydrogen bonding interactions between the extract constituents and polar solvents.

The antibacterial activity of the extract was evaluated against Staphylococcus aureus (ATCC 29737, IPRS) and Escherichia coli (ATCC 25992). A zone of inhibition was observed only against Staphylococcus aureus, measuring 12 mm, indicating that the extract effectively suppressed microbial growth around the well in the nutrient agar medium.

The maximum absorption wavelength (λmax) and optical density (absorbance) were measured at varying concentrations of the extract solution under optimized conditions. The λmax in methanol was found to be 278.5 nm with an absorbance of 0.1688, whereas in ethanol, the λmax was 282 nm with an absorbance of 0.0881. The absorbance at 279 nm for a specific concentration of the extract solution was 0.1685, which was used to estimate the percentage purity.

The purity of the neem bark extract at 0.005% w/v concentration was calculated to be 30.63% w/w, using a standard reference value at 279 nm.

The FTIR (Fourier Transform Infrared) spectroscopic analysis was carried out to verify the structural characteristics of the neem bark extract by comparison with reference IR spectral data. The characteristic absorption peaks were observed at 3437.88, 2933.53, 2847.17, 1730.03, 1715.56, 1455.19, and 1379.97 cm^{-1}, which were consistent with the standard reference spectrum.

CONCLUSION

Upon successful completion of the study, the neem bark extract exhibited antibacterial activity against Staphylococcus aureus (ATCC 29737). The maximum absorption wavelength (λmax) of the extract was observed to be 278.5 nm in methanol and 282 nm in ethanol. The percentage purity of the neem bark extract at a concentration of 0.005% w/v was determined to be 30.63% w/w, based on the reference value at 279 nm, using the absorbance measured at this wavelength.

Furthermore, FTIR (Fourier Transform Infrared) spectroscopic analysis was performed to verify the structural characteristics of the neem bark extract by comparison with standard IR spectral data. The characteristic absorption peaks were recorded at 3437.88, 2933.53, 2847.17,

1730.03, 1715.56, 1455.19, and 1379.97 cm^{-1}, which were found to be in agreement with the reference spectrum.

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