



ISOLATION AND PURIFICATION OF NIMBIDIN FROM *Azardichta indica* AND ITS PHARMOCOLOGICAL ACTIVITY

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ABSTRACT

Azardichta indica known to have wide range health benefits all over many nations in India. It has been determined that natives in Asian countries have three main bioactive compounds. Namely, Nimbin, Nimbidin, and Nimbiene. The compound was isolated and identified as nimbidin using chromatography. The leaves were extracted with ethanol and water as a solvent, and the procedure was change based upon the results because ethanol extraction shows better numbers in Phytochemical Analysis, and subjected to column chromatography. It was conducted and the extractions were collected. It is used in high performance liquid chromatography as a confirmation test for nimbidin. In the current study, antibacterial properties was studies in bacillus and pseudomonas bacteria were detected, and another study found anticancer activity in HepG-2 liver cancer cell lines.

KEY WORDS

Azardichta indica, nimbidn, phytochemical analysis, antibacterial activity, anticancer activity.

INTRODUCTION;

Azadirachta indica is known as neem or nim tree or Indian lilac and belongs to the mahogany family of Maliaceae neem a very similar tree with enormous characteristics neem is

an all-purpose medicine plant and its uses involve various biological activities the history of neem is inevitably connected to Indian civilization.

Modern research has uncovered of Neem, that the neem leaf has a strong anti-septic, anti-fungal, anti-oxidant, anti-inflammatory, anti-carcinogenic, anti-diabetic, anti-parasitic, anti-snake venom anti-HIV, anti-spasmodic, anti-pyretic, anti-diarrheal, anthelmintic and immunomodulation properties.

Azardichta indica trees can be found all over the world, including Africa, India, America. Neem serves as a major solution to the greater concern faced by humans in the environment. *Azardichta indica* does not have any harmful impact on living organisms.

The US environment protection agency has approved its use on food crops. Many countries use neem for pest control when establishing new lands, contains certain components for killing pathogens and ulcers that can help us. The important element of "margosic acid" is in the neem oil. Neem oil contains a high concentration of nimbidin, a bitter principle derived from neem oil.

Natural bioactive compounds are found in a variety of parts, such as fruit, flowers, stems, leaves, and roots. These phytochemical compounds are helpful in the production of new drugs.

Phytochemical Analysis is used for the identification and separation of bioactive compounds found in plants, like alkaloids, phenol, flavonoids, reducing sugar, tannins, saponins, carotenoids, etc. Several Phytochemical researchers discovered a variety of naturally occurring chemicals. Because leaves are used to and have antimicrobial properties, they should be able to control airborne bacterial contamination. Also used as a tumour and diagnosis process. The goal of this research is to isolate and purify nimbidin from neem leaves, as well as to test it in vitro. Antibacterial activity and anticancer activity (HepG-2 liver cancer cell line).

MATERIAL AND METHODS

COLLECTION OF RAW MATERIAL AND SOLVENT EXTRACTION

Healthy neem leaves were collected and washed under running tap water. It should be free of dust and foreign particles.

WATER EXTRACTION

10g of freshly picked leaves. Distilled water (100 ml) grained with a mortar and pestle. The extraction filtered it even further.

ETHANOL EXTRACTION

5g of leaves. 50ml of ethanol grained with a mortar and pestle. The extraction filtered it even further.

PHYTOCHEMICAL ANALYSIS

Phytochemical study was performed on the extracts by using the below the standard test.

Bioactive compounds	Water Extract Phytochemical analysis – As suspension based on the concentration of plant extract.	Ethanol Extract Phytochemical analysis- As suspension based on the concentration of plant extract.
Alkaloids	About 1 ml of plant extract add 200µl Wagner's reagent.	About 1ml of plant extract add 200µl Wagner's reagent.
Phenol- ferric chloride test	About 1ml of the extract add 200µl ferric chloride solution.	About 1ml of the extract add 200µl ferric chloride solution.
Saponins- Foam test	To 1 ml of the plant extract added 2ml of in water a test tube. The mixture was shaken vigorously.	To 1ml of the plant extract added 2ml of water in a test tube. The mixture was shaken vigorously.
Flavonoids	To about 1ml of plant extract, 200µl NaOH was added.	To about 1ml of plant extract, 200µl NaOH was added.
Phytosterol- Salkowski's test	1 ml of the plant extract was treated with 100µl H ₂ SO ₄	1 ml of the plant extract was treated with 100µl H ₂ SO ₄ .
Proteins	To a 1ml of plant extract added 200µl Ninhydrin reagent.	To a 1ml of plant extract added 200µl Ninhydrin reagent.
Steroids	About 100µl of chloroform and 100µl of Acetic hydride	About 100µl of chloroform and 100µl of Acetic hydride

	was added to 1ml of plant extract.	was added to 1ml of plant extract.
Tannins	To about 1ml of plant extract added few drops of dilute ferric chloride solution.	To about 1ml of plant extract added few drops of dilute ferric chloride solution. .
Glycosides	To 1ml of plant extract added 100µl chloroform and 100µl H ₂ SO ₄ .	To 1ml of plant extract added 100µl chloroform and 100µl H ₂ SO ₄ .
Reducing sugar	To 1ml plant extract added solution (A) and solution(B) 100µl respectively.	To 1ml plant extract added solution (A) and solution (B) 100µl respectively

If we have better colour changes in ethanol extract, so it is treated as column chromatography techniques for the separation of extraction.

COLUMN CHROMATOGRAPHY;

10g of silica gel mixed in 50 ml of distilled water and filled the burette, adding 1000 µl of ethanol extraction. After a few minutes, the fraction will be collected carefully.

UV VISUALIZATION;

Then the extraction was transferred to an Eppendorf tube. Then placed as UV, visualised as a bioactive compound, named as like 2,3,4,5,9,10.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY;

The samples are moved to HPLC (high performance liquid chromatography). First selection of a chromatographic layer. The following samples are 2, 3, 4,5, 9, and 10. It is then prepared as standard (nimbidin) is prepared. I need special procedure requirements. Then detector selection and setting. To development of chromatography and detection of spots. Then the result (peaks both nimbidin and commercial nimbidin) will be observed. Then finally presence of nimbidin in sample 9th. Then, antibacterial activity and anticancer activity.

ANTIBACTERIAL ACTIVITY;

Antibacterial nimbidin tests against gram-positive bacteria namely bacillus, and gram-negative bacteria namely pseudomonas all sample were cultured in nutrient agar plates to be used the plots were in seven wells. In the well, a 50µl sample was injected plates were kept at 37°C for incubation 24 hours, the 9th sample nimbidin zone will be observed.

ANTICANCER ACTIVITY

Take T-flask subculture (liver cancer cell line) and take as an ELISA well and choose six wells, namely as control and various concentration of samples(6.25µg,12.5µg,25µg,50µg,100µg). After 48 hours of incubation, they added 100µl phosphate saline buffer to each well and incubated at 37°C at 4 hours. After incubation, added 100µl of DMSO (dimethyl sulfoxide) solution. A microplate reader used to measure absorbance at 570nm .To measure the viability of cells using microplate reader , then move on to SEM(scanning electron microscopy, where the results will be observed.

RESULTS

PHYTOCHEMICAL ANALYSIS;

The analysis used to screen different types of metabolites like alkaloids, phenol, flavonoids, reducing sugar etc.

TABLE 1-PHYTOCHEMICAL ANALYSIS

Bioactive compounds	Water extraction phytochemical analysis	Ethanol extraction phytochemical analysis
Alkaloids	++	++
Flavonoids	+++	+
Phenol	+++	+++
Protein	+	++
Tannins	++	+++
Glycosides	+	++
Phytosterol	++	++
Reducing sugar	+++	+++
Steroids	++	++
Saponins	+++	++

COLUMN CHROMATOGRAPHY AND ULTRA VIOLET VISUALIZATION

The technique is used to separate bioactive compounds using slicagel. The extraction will be collected. To visual the bioactive from extraction of column chromatography. As shown as fig.1



Fig:1 -UV visualization (Bioactive compounds)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The 10ul sample was injected into HPLC auto injectors. Acetone was used as a solvent. Segregation of nimbidin. The peak was detected at a wavelength. As shown as fig 2.

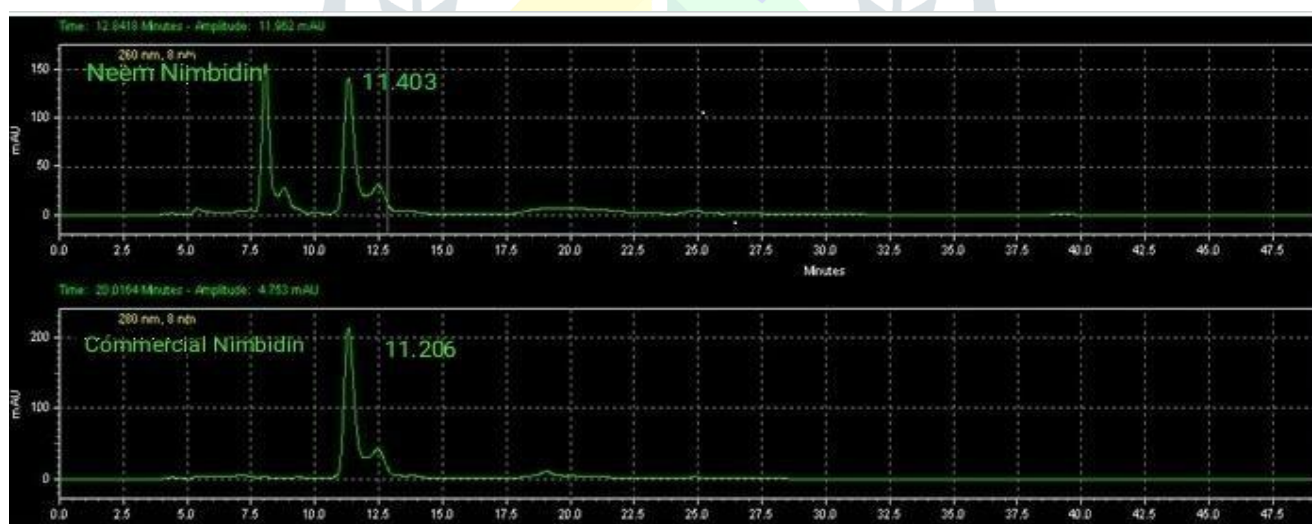


Fig 2-HPLC analysis

TABLE 2- CALCULATION

Neem Nimbidin;

Peak	Retention time	Area (%)	Height (%)
1.	10.250	6.272	9.643
2.	11.403	5.068	4.290

Commercial Nimbidin:

Peak	Retention time	Area (%)	Height (%)
1.	11.20	5.008	4.374

ANTIBACTERIAL ACTIVITY

To determine the antibacterial activity of neem extraction in culture against *Bacillus* and *pseudomonas*. As shown as fig 3,4

Fig3 *Pseudomonas* and fig 4 *Bacillus* -(9th compound nimbidin)**TABLE 3- ZONE OF INHIBITION**

BACTERIA	2 nd Sample	3 rd sample	4 th sample	5 th sample	9 th sample (Nimbidin)	10 th sample
<i>Bacillus</i>	1.8 cm	1.5cm	1cm	1.3cm	1cm	1.6cm
<i>pseudomonas</i>	2.7cm	2cm	2.5cm	2.3cm	2.4cm	2.8cm

ANTICANCER ACTIVITY

It is the cancer activity of an analysed sample of various concentration to be used.

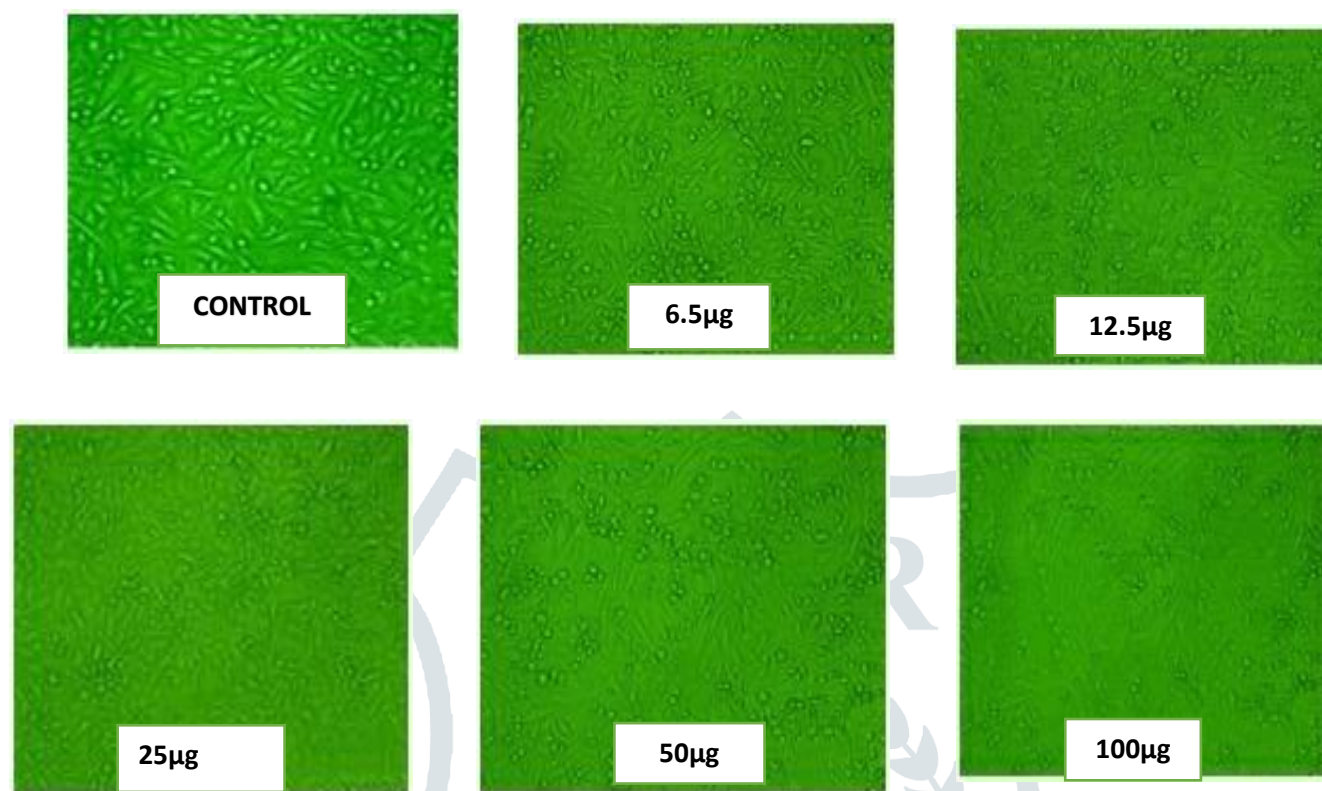


Fig 5- liver cancer (HePG 2 -cell lines)

TABLE 4-CALCULATION

Conc	6.25µg	12.5 µg	25µg	50µg	100µg	Cont
ABS	0.013	0.046	0.103	0.165	0.221	0.405
	0.011	0.045	0.103	0.164	0.233	0.402
	0.013	0.047	0.103	0.167	0.223	0.404
Avg	0.01233	0.046	0.102333	0.16533	0.222333	0.40366
Conc(µg/µl)	% cell inhibition					
6.25	3.055532				IC50	77.67µg/ml
12.5	11.3955				R ²	0.99
25	25.3509					
50	40.9578					
100	55.0784					

GRAPH

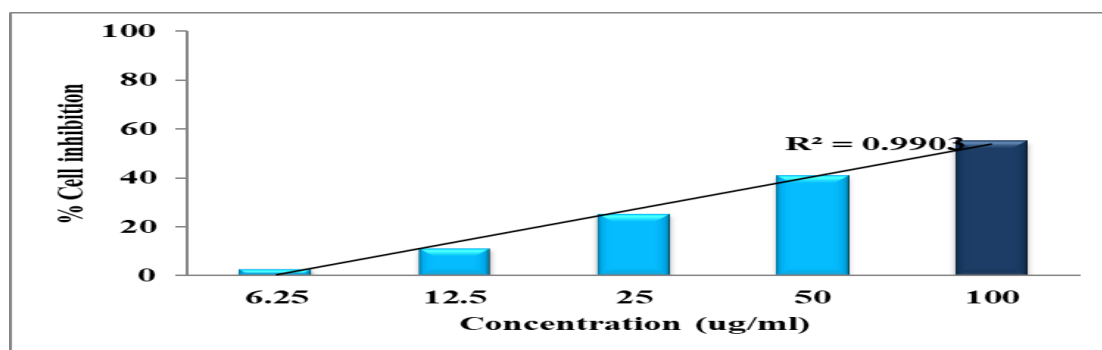


Fig 5- Graph of % of cell inhibition and concentration

DISCUSSION

For more than a century, the extract has been used for food preservation and medicinal purposes. The extract is a possible source of antimicrobial properties.

Kubmarawa et al., 2008¹⁰ reported nimbidn was one of the compounds with antimicrobial, antioxidant, and anti-diabetic properties. Grirish K and Bhatt S 2008¹⁴, biologically active compound, nimbidin extract from neem leaf. Cstiane p 2009¹⁵ described extracts produced by various chromatography methods. High performance liquid chromatography has been reported by Vebek et al.,1979¹⁶; Schneider. Jessinta et al., 2013²⁰ found that neem oil possesses antimicrobial properties similar to neem extract. Prochazkova et al., 2011²³ discovered that percentage inhibition was more when a higher concentration of plan extract used. Ross R 1993²⁷ described that the generation of nitric oxide take place in biological tissue by a process called (NOSS) Kumar, Suresh, et al., has been reported anticancer effects in presence of ethanolic neem leaf extract on prostate cancer cell line. Agrawal, Shikha et al., has been reported anticancer effect in *Azardichta indica* in oral cancer.

CONCLUSION

The study reveals that the extract contains bioactive compounds and effective drug-resistant microorganisms the biological active compounds are chemically assorted further research into primary metabolites of plant extract has been completed with advanced activities as a result *azardichta indica* will be a significant source of medicinal use in the pharmaceutical industry.

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