

**INTERPRETATIVE *IN VITRO* PHYTOCHEMICAL, TLC, SYNTHESIS
OF SILVER NANOPARTICLES AND THEIR ANTIBACTERIAL
SCREENING OF AQUEOUS AND ETHANOLIC EXTRACTS OF
MORINDA CITRIFOLIA L. (NONI) FRUIT AND THEIR
COMPARATIVE STUDY**

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ABSTRACT

NONI is common name for *Morinda citrifolia* fruit. This belongs to Rubiaceae family. Noni's unique medicinal property is known to mankind since more than 2000 years. Noni helps in normalization of abnormally functioning cells by delivering the bio-chemical nutrients. It contains Proxeronine and more than 160 nutraceuticals. NONI is beneficial for the unique food supplement of which will be added to the probable list of drugs for cancer, heart diseases (hypertension), arthritis, allergy, menstrual irregularities, gastric ulcers, diabetes, immune system, eye inflammations and it is a most important nutritional booster etc.. The major aim of present study was to evaluate

extractability of the aqueous and ethanolic extracts were assessed by the ecofriendly method and also to screen the ethno medicinal use, the phytochemical screening of *Morinda citrifolia* L., (Noni fruit) excavate that most of the important constituents like alkaloids, glycosides, carbohydrates, flavonoids, terpenes, saponins, phenols, tannins, quinones, celluloses, steroids and gums which interprets its medicinal values. Biosynthesis of silver nanoparticles is an important in the field of nanotechnology, which has the economic and ecofriendly beneficial for chemical and biophysical characterization. The present study reveals the synthesis of silver nanoparticles using both the aqueous and ethanolic extracts of *Morinda citrifolia* fresh fruits were assessed by 1mM Silver nitrate solution. The synthesized nanoparticles were

characterized by UV-Visible Spectrophotometer, Transmission Electron Microscopy (TEM), Fourier Transform Infrared (FTIR), Atomic Force Microscopy (AFM), also thin layer chromatography (TLC) for chromatogram visualization (Rf values) of both the aqueous and ethanolic extracts of *Morinda citrifolia* fresh fruits and also antibacterial studies for the formed nanoparticles.

KEY WORDS: *Morinda citrifolia*, UV, TEM, FTIR, AFM.

INTRODUCTION

Morinda citrifolia L is commonly known as Indian NONI, also called *Indian Mulberry*, is an ever green small tree bearing flowers and fruits throughout the year. It belongs to family Rubiaceae which is shown in the Fig., 1. and Fresh fruit in Fig., 2.



Fig., 1 Noni twig with fruits.



Fig., 2 Noni Fresh Fruits.

Plant Profile

Botanical Name: *Morinda citrifolia* .L

Family : Rubiaceae

Kingdom : Plantae

Order : Gentianales
Genus : Morinda
Species : citrifolia

It is the biggest pharmaceutical unit in the universe because it has more than 160 nutraceuticals, several vitamins, minerals, micro and macro nutrients that help the body in various ways from cellular level to organ level. Noni contains chemicals like Proxeronine, Xeronine, Scopolitin, Anthraquinones, Damnacanthal and so many major nutrients for the human body as health booster.

The NONI fruit juice is an immense need as an alternative medicine for different kinds of diseased conditions such as arthritis, diabetes, high blood pressure, muscle aches and pains, menstrual difficulties, headaches, heart disease, AIDS, cancers, gastric ulcers, sprains, mental depression, senility, poor digestion, atherosclerosis, blood vessel problems, and drug addiction^[1, 2]. A vast variety of pharmacological actions have been reported from fresh fruit extracts of noni such as analgesic^[3], anti-inflammatory^[4], antioxidant^[5,6], immunomodulatory^[7], anti-tumor^[8], hepatoprotective^[9], blood pressure lowering and vasodilatory^[10,11], cardio protective^[12], antifungal^[13], phytoestrogenic^[14], wound healing^[15], insulinotrophic^[16] and anti-osteoporotic activity^[17]. Though, there have been only a few reports on the use of noni for CNS disorders such as anxiolytic and sedative^[18], nootropic^[19], antiepileptic^[20], neuroprotective effect against stress-induced cognitive impairment^[21] and some neuropharmacological effects^[22].

Nanotechnology is mainly concerned with synthesis of nanoparticles of volatile sizes, shapes, compositions and controlled distinctive nature of their future use for human benefits for the advanced modern technology (23). Bioinspired synthesis of silver nanoparticles implement growth over chemical and physical methods as it is a cost effective and eco-friendly (24). The products based on nanotechnology were estimated to be more than 800 and expected to raise more in the market within the next few years^[25,26]. A wide range of metal oxide like nanoparticles (ZnO, TiO₂, Al₂O₃, FeO, Fe₂O₃, etc.), fullerenes, carbon nanotubes, quantum dots, etc. have an increasing range of applications for different purposes^[27] and make their way easily in the environment^[28]. Hence, in the present investigation, green synthesis of silver nanoparticles using both aqueous and ethanolic extracts of *Morinda citrifolia* L. of fresh fruits and their antibacterial activities are presented and discussed.

MATERIAL AND METHODS

Collection of Noni Fruits

Fruits of *Morinda citrifolia*, L (Noni) were collected from Pentlavelly Village, Kollapur Mandal, Mahabubnagar District. The plant material was authenticated by Dr. Mandla Bichanna.

Preparation of Aqueous Extract

Noni fruits were subjected to aqueous extract. Fresh fruits of *Morinda citrifolia*, L (Noni) were used to make the aqueous extract. 25 gms of fresh fruits were thoroughly washed thrice with distilled water followed by double distilled water to remove the dust particles and other contaminants. Then the crushed fruit material was taken in a clean 250 ml Erlenmeyer conical flask and 100 ml of Millipore water was added and incubated on a sand bath for 20 mins to facilitate the formation of aqueous fruit extract. The extract was then filtered using Whatman No. 1 filter paper. The aqueous fruit extract is used for further phytochemical analysis, silver nanoparticle synthesis, TLC and antibacterial studies.

Preparation of Ethanolic Extract

Ethanolic Extraction of *Morinda citrifolia*, L was done by using ecofriendly method. Fresh fruits of *Morinda citrifolia*, L (Noni) were used to make the ethanolic extract. 25 gms of fresh fruits were thoroughly washed thrice with distilled water followed by double distilled water to remove the dust particles and other contaminants. Then the crushed fruit material was taken in a clean 250 ml Erlenmeyer conical flask and 100 ml of 90% ethanol was added followed by closing it with cotton plug and incubated on shaker incubator for 4 days to promote the formation of ethanolic fruit extract by filtering the solution with Whatman No. 1 filter paper. The ethanolic fruit extract is used for further phytochemical analysis silver nanoparticle synthesis, TLC and antibacterial studies by storing it in refrigerator by closing it with cotton plug in order to evaporate the extract.

Preparation of 1mM AgNO₃ Solution

Systematic concentration of 1mM AgNO₃ (Merck India Ltd) was prepared by dissolving 0.169gms of AgNO₃ in 1000 ml of Millipore water and stored in Amber colored bottle to avoid auto oxidation of Silver.

Preliminary Qualitative Phytochemical screening

The Phytochemical screening of aqueous and ethanolic extracts of *Morinda citrifolia*, L(Noni) were analysed by the standard methods and shown the presence of various phytochemical constituents such as alkaloids, glycosides, carbohydrates, flavonoids, terpenoids, saponins, phenols, tannins, quinones, cellulose, steroids and gum.

Test for Alkaloids

To reveal the presence of alkaloids, few drops of Mayer's reagent (potassium mercuric iodide) reagent were added to the extract, cream colour precipitate visualises the presence alkaloids. (Siddiqui and Ali, 1997).

Test for Glycosides

To 2ml of extract, add 1ml of glacial acetic acid, few drops of 5% FeCl₃ and Conc.H₂SO₄ were added reddish brown colour at the junction of two layers and upper layers appears bluish green visualises the presence of glycosides (Trease and Evans;1989).

Test for Carbohydrates

To 2.3ml of extract, few drops of Molisch reagent (α -naphthol) was added, shaken well and Conc. H₂SO₄ was added from the sides of the test tube, violet ring formation at the junction of two layers visualises the presence of carbohydrates (Krishnaveni et al;1984).

Test for Flavonoids

To the 1 ml of extract few drops of 10% Conc. H₂SO₄ was added and followed by adding 1ml of ammonia, formation of greenish yellow precipitate visualises the presence of flavonoids(Siddiqui and Ali;1997).

Test for Terpenes/Terpenoids

To 2ml of extract, 5ml of chloroform and 2ml of Conc. H₂SO₄ was added.Reddish brown colourations of interface visualises the presence of terpenes(Harborne;1973).

Test for Saponins

To 2ml of extract add water and shaken vigorously for frothing presence visualises saponins(Siddiqui and Ali ;1997).

Test for Phenols

To 1ml of extract add alcohol and few drops of ferric chloride solution is added for the formation of greenish yellow visualises the presence of phenols(Mukherjee PK;2002).

Test for Tannins

To 1ml of extract, 1ml of 5% FeCl_3 was added which visualises by the presence of greenish black precipitate (Mukherjee PK; 2002).

Test for Quinones

To 2ml of extract add Conc.Hcl by formation of green colour visualises the presence of quinones.

Test for Celluloses

To 1ml of extract add iodine, followed by addition of Conc. H_2SO_4 by formation of brown visualises the presence of celluloses.

Test for Steroids

To 2ml of extract, 1ml of chloroform and drop of glacial acetic acid was added, followed by heating and add Conc. H_2SO_4 which visualises by the presence of orange colour (Liebermann Burchard test, Salkowski test and Liebermann's reaction).

Test for Gums

To 1ml of extract add 3ml of Dil.Hcl, Fehling's solution is added drop by drop till red colouration visualises the presence of gums.

T L C Analysis Of Fruit Extracts Of *Morinda Citrifolia*. L(Noni)

Both the aqueous and ethanolic noni fruit extracts were checked for Thin Layer Chromatography. For Ethanolic Extract and aqueous extracts Hexane: Ethyl acetate (8:2) was found to be the best solvent system for separation.

Biosynthesis of Silver Nanoparticles and their Characterization

In this ecofriendly biosynthesis of silver nanoparticles, the 90ml of prepared 1mM AgNO_3 solution and also 10ml of both the aqueous and ethanolic extracts were taken individually and incubated on sand bath at 60°C for 20mins for aqueous extract and 30mins for ethanolic extract for reduction of Ag^+ ions which resulted in the colour change of the extracts.

UV-visible Spectra Analysis

Synthesized silver nanoparticles of both the extracts were initially characterized by taking small aliquot into UV-Visible Spectrophotometer absorption spectra at 300-700nm using

Elico SL-159 UV Spectrophotometer against both the extracts are used for the baseline scan correction.

FTIR Analysis

Fourier-Transform Infrared Spectroscopy Thermo Nicolet Nexus-670 Spectrophotometer was used for analysis of reduced silver for both the extracts. The spectrum was recorded in mid-IR region of 400-4000 cm^{-1} in KBr pellets with diffuse reflectance mode.

TEM analysis

Transmission Electron Microscopy(TEM) analysis was carried out by using Philips model CM 200 instrument at an accelerated voltage 200 KV to determine the size and shape of the synthesized nanoparticles of both the extracts by maintaining the sample preparation with 10mg of ethanol thin films on carbon coated copper grids.

AFM Analysis

Atomic Force Microscopy (AFM) analysis was carried out by thin film of the sample prepared on a glass slide by dropping a 100 μl of sample on the slide which was allowed to dry for 5mins and slides were scanned with AFM using Veeco Nanoscope IV by non-contact mode.

Analysis of Antibacterial Activity

The antibacterial activity of both the aqueous and ethanolic extracts of *Morinda citrifolia*. *L* (*Noni*) fresh fruit and green synthesized silver nanoparticles were tested by the disc diffusion method against an antibiotic ampicillin(1 $\mu\text{g}/\mu\text{l}$) as standard drug. For the determination of antibacterial activity, the antibiotic resistant bacteria namely *Staphylococcus aureus*, *Pseudomonas putida*, *Bacillus subtilus*, *Escherichia coli*, *Klebsiella pneumonia* were used and plates were incubated at 37 $^{\circ}\text{C}$ overnight to know the zone of inhibition.

RESULTS AND DISCUSSION

Phytochemical screening

The aqueous and ethanolic extracts of *Morinda citrifolia*.,*L*(*Noni*) fruits were subjected to qualitative phytochemical screening of phytoconstituents like alkaloids, glycosides, carbohydrates, flavonoids, terpenoids, saponins, phenols, tannins, quinines, cellulose, steroids and gums. The results revealed the presence of alkaloids, glycosides,carbohydrates, flavonoids, terpenes, saponins, phenols, tannins,quinones and cellulose (Table. 1) and Fig.,3

Table 1: Results of phytochemical screening of aqueous and ethanolic extracts of *Morinda citrifolia*,L(Noni),(+) indicates the presence of the constituents and (-) indicates the absence of constituents.

S.No	Primary and Secondary metabolites	Aqueous Extract	Ethanolic Extract
1.	Alkaloids	-	+
2.	Glycosides	+	+
3.	Carbohydrates	+	+
4.	Flavonoids	-	+
5.	Terpenes/Terpenoids	+	+
6.	Saponins	+	+
7.	Phenols	+	+
8.	Tannins	-	+
9.	Quinones	+	+
10.	Cellulose	+	+
11.	Steroids	-	-
12.	Gums	-	-

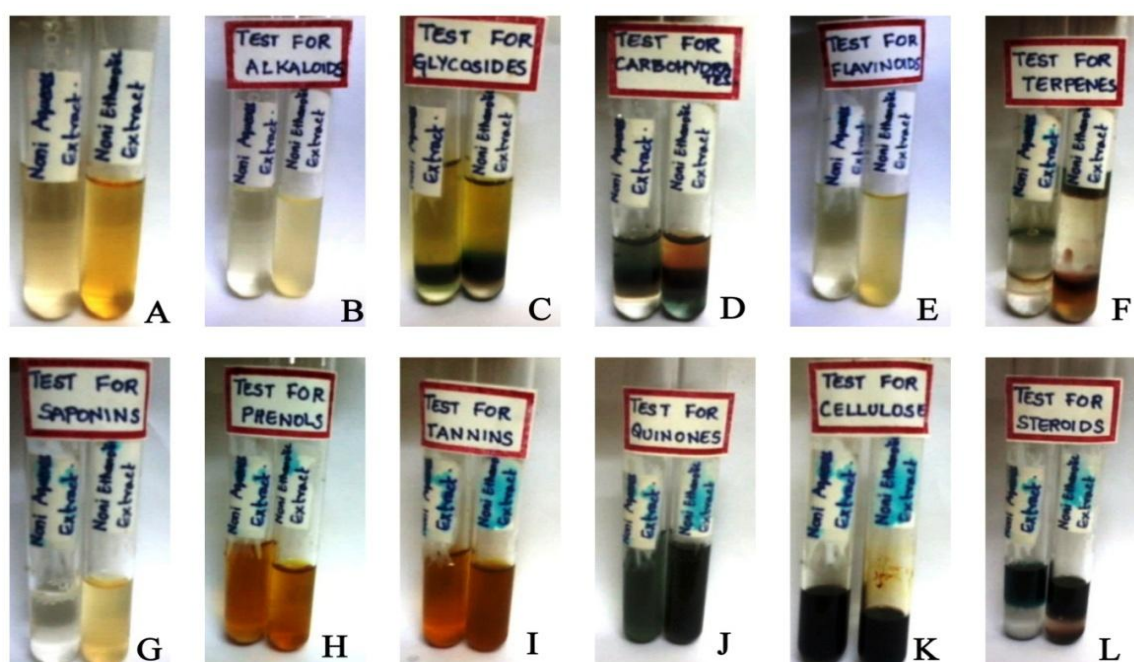


Fig.3: Results showing the phytochemical screening of both the aqueous and ethanolic extracts.

T L C Analysis Of Fruit Extracts Of *Morinda Citrifolia*,L(Noni):

Under UV illuminator 2 spots were observed in the aqueous and 3 spots were observed in ethanolic extracts of *Morinda citrifolia*,L(Noni) fruits. After proliferating TLC of both the extracts, R_f values were calculated for the spots visualized under UV illuminator. Table. 2 and Fig., 4a & 4b respectively.

Table 2: Results of Retardation factor (R_f) for both aqueous and ethanolic extracts of *Morinda citrifolia*,L(Noni).

Plant Extract	Solvent System	Rf Values
Aqueous Extract	Hexane : Ethyl acetate (8:2)	0.08315cm,0.06559cm
Ethanolic Extract	Hexane : Ethyl acetate (8:2)	0.08595cm,0.06804cm,0.02204cm

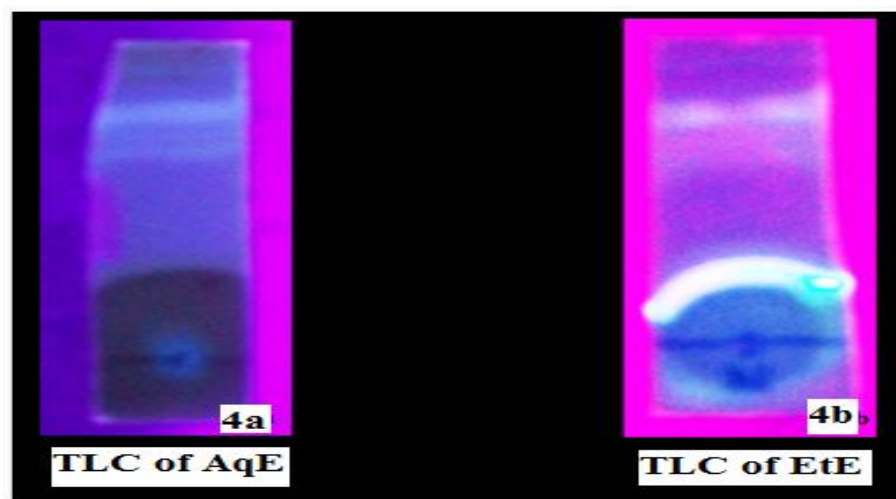


Fig.4a & 4b: Results showing the Solvent Front and position of the compound of both the aqueous and ethanolic extracts.

Characterization of Silver Nanoparticles

Firstly the present study reports that both the aqueous and ethanolic extracts of *Morinda citrifolia*,L(Noni) fruits may act as reducing and capping agents for the synthesis of silver nanoparticles. As both the extracts were mixed with the aqueous solution of silver ion complex which results in the color change of extracts to orange reddish colored solution due to excitation of the surface plasma vibrations indicating the silver nanoparticles synthesis were shown in the Fig.,5a and 5b.

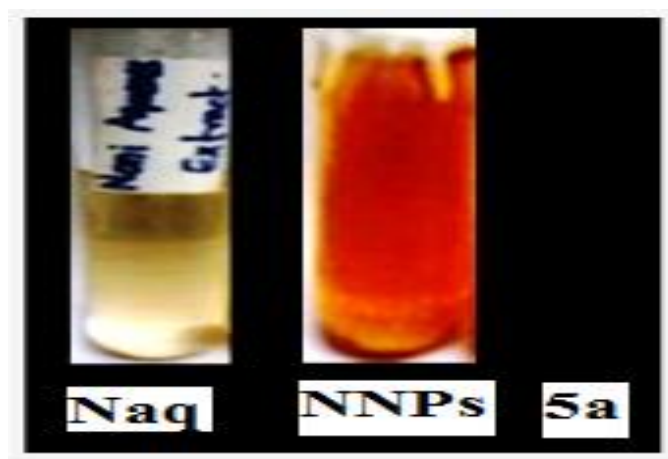


Fig.5a Noni Aqueous NPs

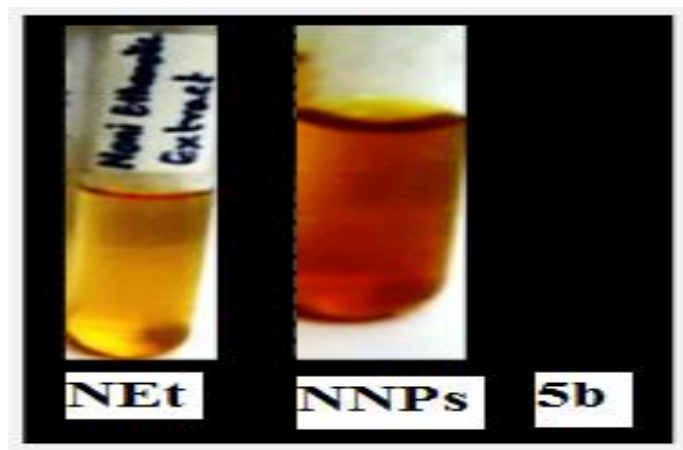


Fig.5b Noni ethanolic NPs

UV-Visible Spectrophotometer analysis

Synthesis of colloidal Silver Nanoparticles was performed by UV-Spectra which showed maximum chromatogram at 420-430nm in noni aqueous extract nanoparticles(NA-NPs) and 430nm in noni ethanolic extract nanoparticles(NE-NPs), indicating the presence of silver nanoparticles and UV Visible spectra is reported in Fig.,6a and 6b respectively.

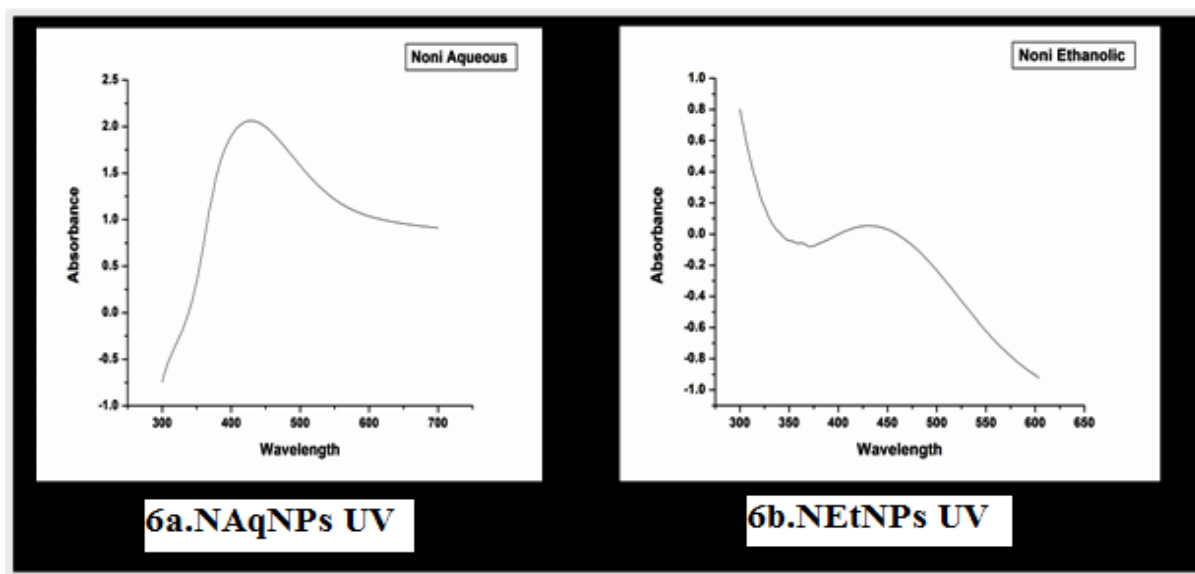


Fig., 6a Noni Aqueous NPs Spectra

Fig., 6b Noni ethanolic NPs Spectra

FTIR analysis

FTIR analysis was carried out to identify the possible biomolecules formed by the reduction of Ag^+ ions and capping of the bioreduced nanoparticles synthesized by both the aqueous and ethanolic extracts of Noni fruits. The FTIR spectrum is presented in Fig., 7a and 7b. The representative spectra of aqueous nanoparticles(NA-NPs) obtained manifests absorption peaks located at about 3451cm^{-1} (-OH groups of alcohols and phenols), 2928.94cm^{-1} (-CH of

alkanes), 1633.72cm^{-1} ($\text{C}=\text{O}$ of ketones), 1521.43cm^{-1} ($\text{C}=\text{C}$ of aromatic stretch), 1385.33cm^{-1} (CH of alkanes), 1083.46cm^{-1} (NH amines), 754cm^{-1} (CH of aromatic alkanes) and of ethanolic nanoparticles (NE-NPs) manifests absorption peaks at 3453.60cm^{-1} (OH of alcohols and phenols), 2925.65cm^{-1} , 2854.80cm^{-1} (CH of alkanes), 1638.61cm^{-1} ($\text{C}=\text{O}$ of ketones), 1463.18cm^{-1} (NO of nitro compounds), 1254.13cm^{-1} (CN of aromatic compounds), 1019.23cm^{-1} (CO of alcohols, carboxylic acids, esters).

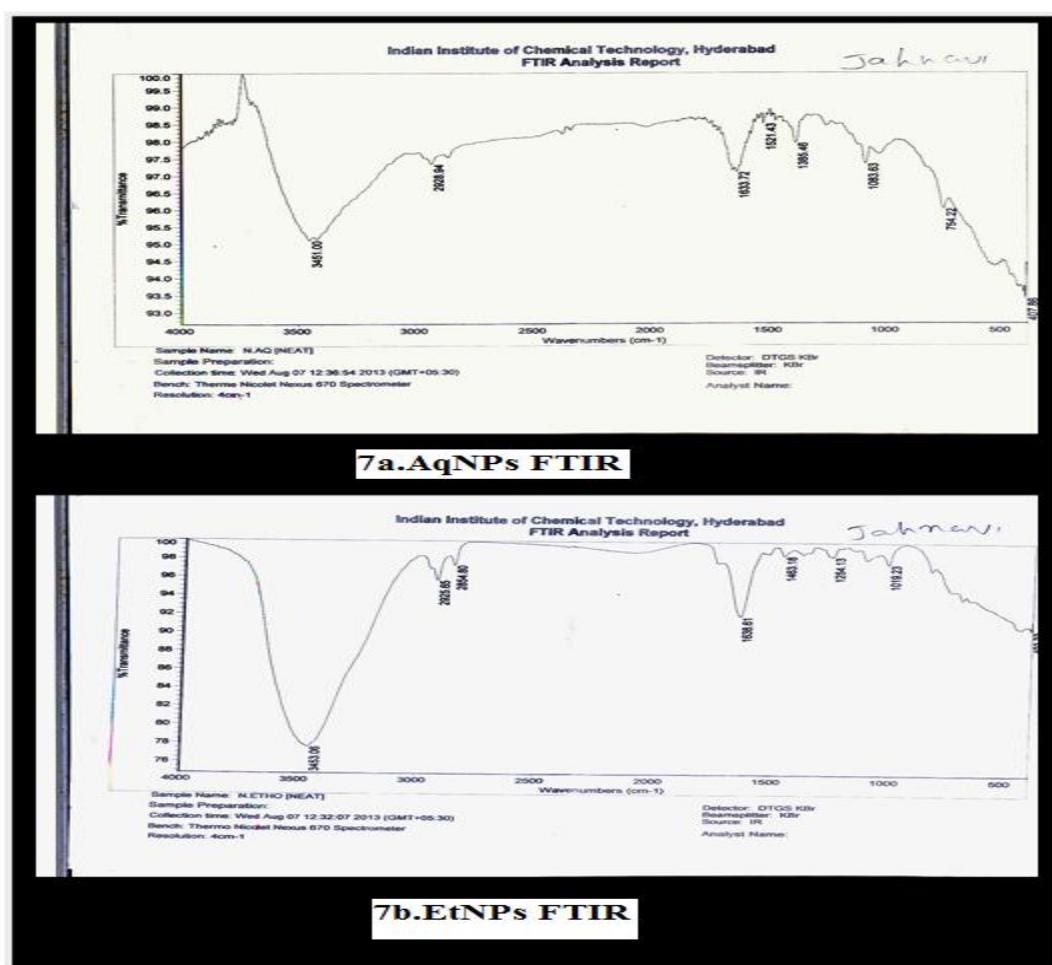


Fig., 7a Noni Aqueous & 7b Noni Ethanolic NPs FTIR Spectra

Atomic Force Microscopic Analysis

The Silver nanoparticles were characterized by AFM on mica sheet thin film for its detail size, morphology and agglomeration of Silver. AFM images were taken with showing spherical shape and size of 1-10nm and height 1-15nm, in non contact mode for both the aqueous and ethanolic extracts. The topographical images of irregular silver nanoparticles of both extracts is reported in Fig., **8a and 8b**. The particles size of the aqueous silver nanoparticles ranges in size from 1-96nm and for ethanolic size ranges from 1-68nm.

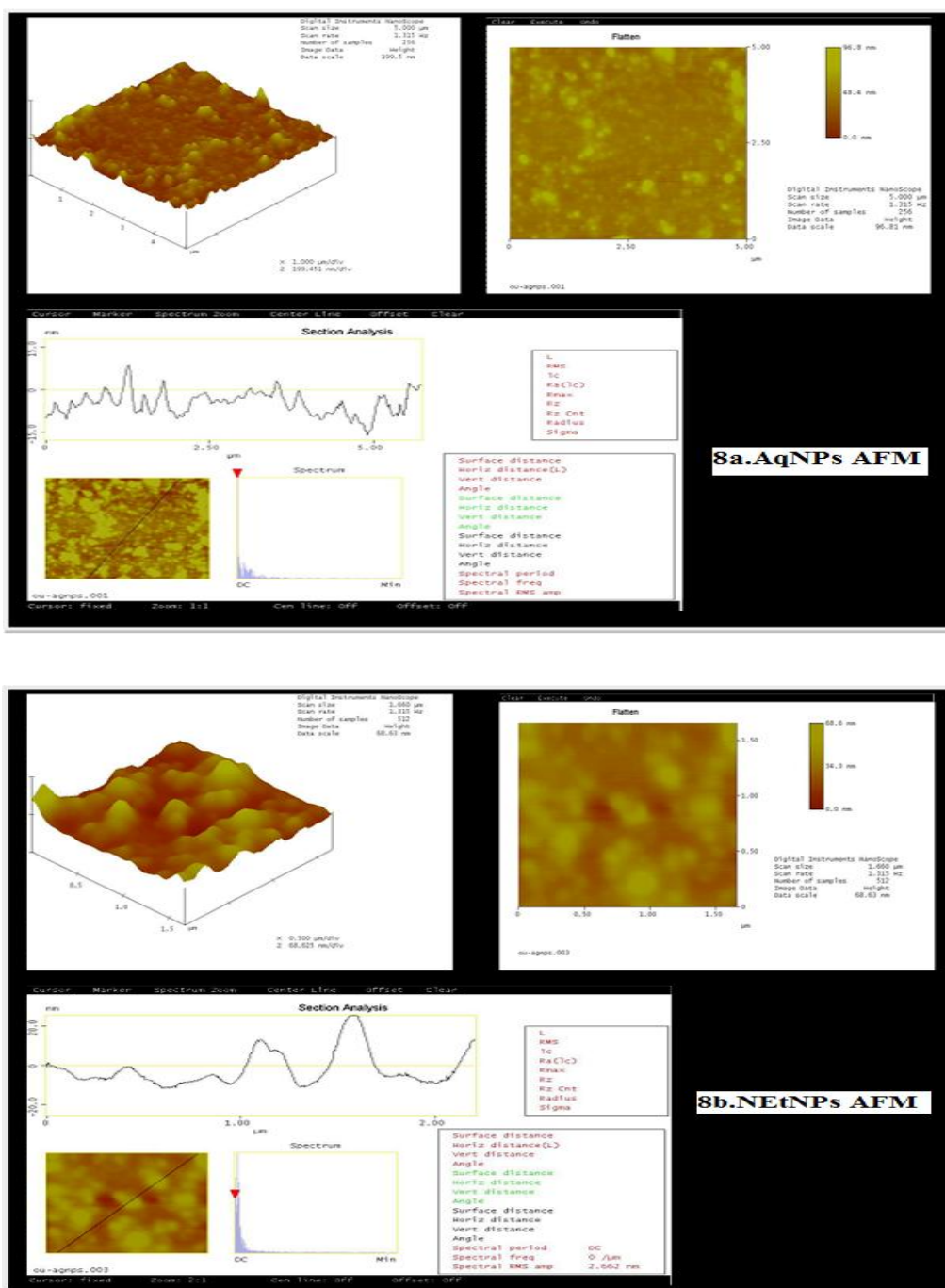


Fig., 8a Noni Aqueous & 8b Noni Ethanolic NPs AFM.

TEM Analysis

TEM analysis reveals that the synthesized silver nanoparticles of both the aqueous and ethanolic extracts were spherical in shape and majority of the particles 1-5nm for the aqueous and ethanolic extracts. The TEM images were represented in Fig., 9a and 9b respectively.

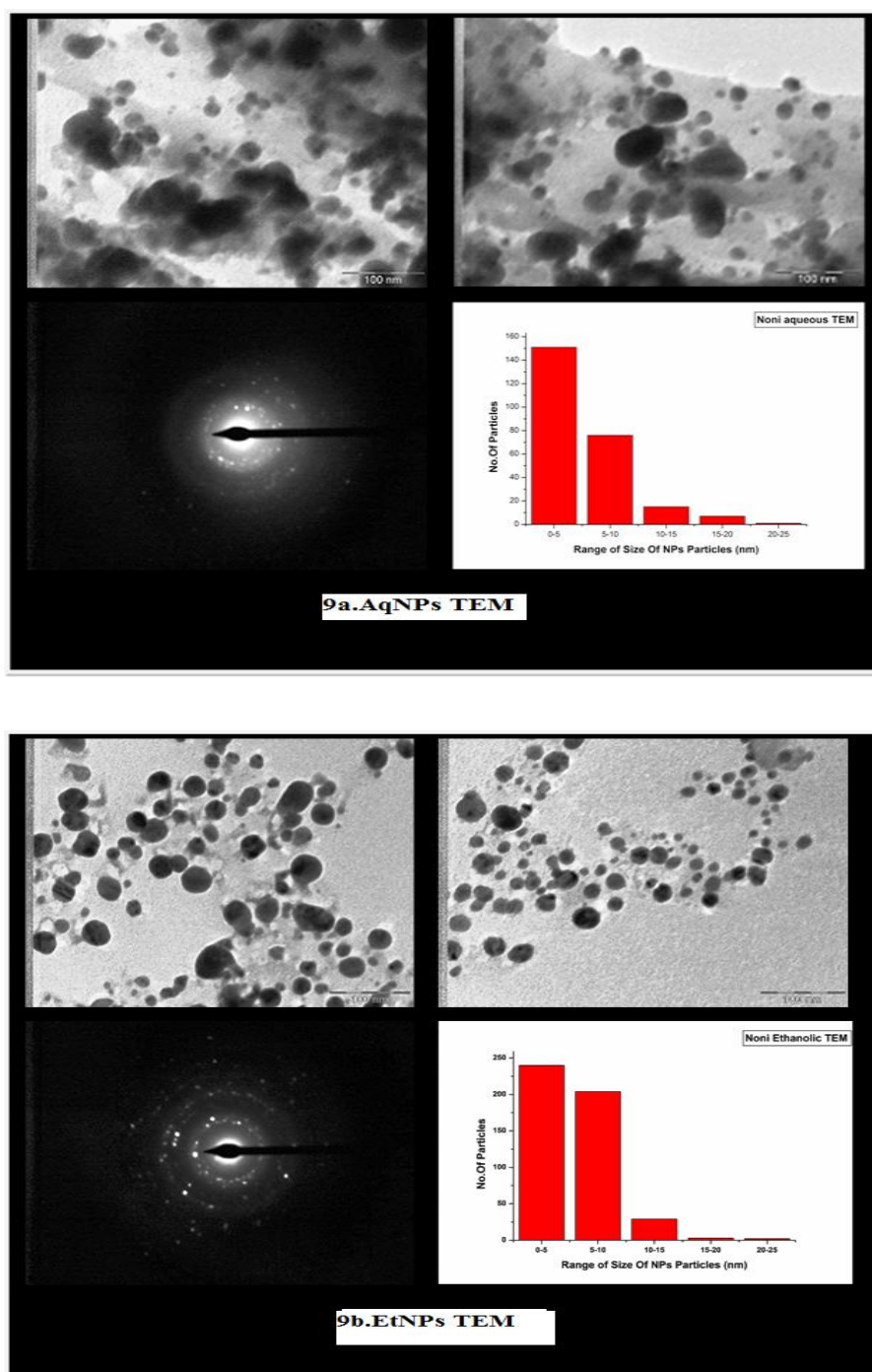


Fig., 9a Noni Aqueous & 9b Noni Ethanolic NPs TEM.

Antibacterial Activity of both Aqueous and Ethanolic Extracts & NPs

Antibacterial analysis reveals that the synthesized silver nanoparticles possess mild potential antibacterial activity against *Staphylococcus aureus*, *Pseudomonas putida*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* (Fig., 10a & 10b and Table 3a & 3b). This shows the maximum zone of inhibition in *B. subtilis*, *E. coli* and also *K. pneumoniae* in ethanolic extracts and in *B. subtilis* of aqueous extract shows maximum zone of inhibition.

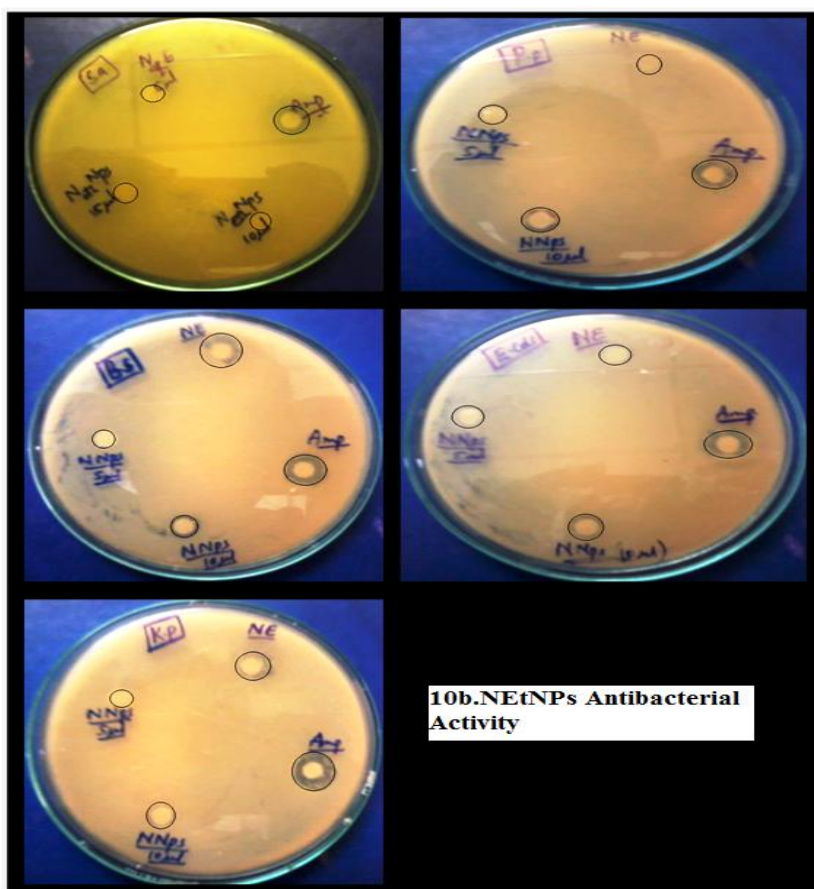
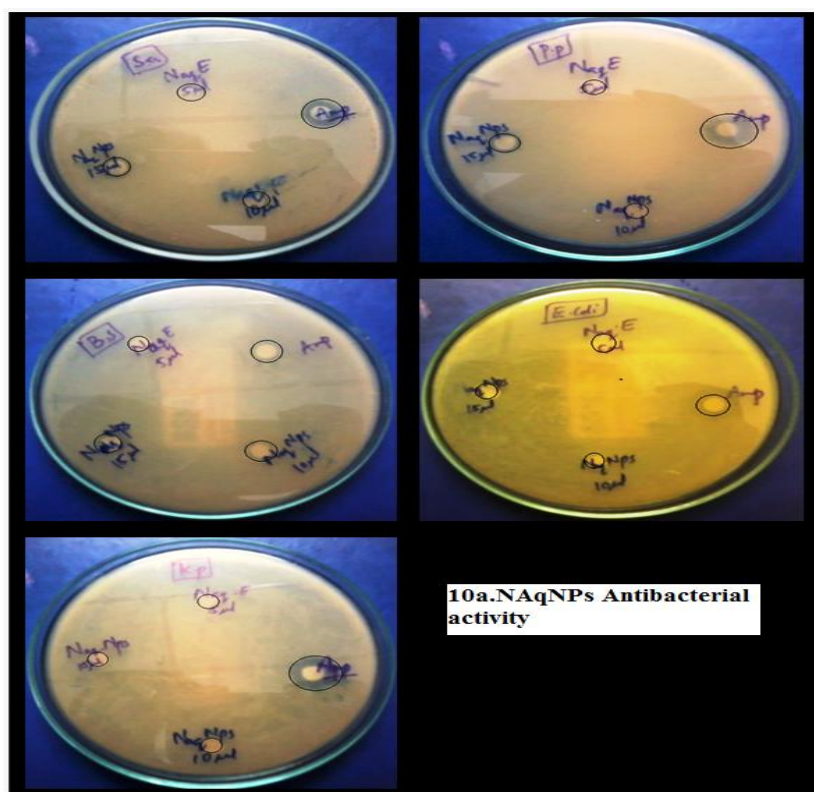


Fig., 10a & 10b Antibacterial Activity of both aqueous and ethanolic extracts & NPs against the reference drug Ampicillin.

Table 3a: Antibacterial Activity of both aqueous NPs against the reference drug Ampicillin.

S.No	Name of the Organism	Diameter of Zone of Inhibition (mm)			
		Aqueous Ex (5µl)	Aqueous NPs (10µl)	Aqueous NPs (15µl)	Ampicillin (5µl)
1.	<i>Staphylococcus aureus</i>	-	1	1	4
2.	<i>Pseudomonas putida</i>	-	1	-	5
3.	<i>Bacillus subtilis</i>	2	-	2	3
4.	<i>Escherichia coli</i>	-	-	-	3
5.	<i>Klebsiella pneumoniae</i>	-	-	-	6

Table 3a: Antibacterial Activity of both aqueous NPs against the reference drug Ampicillin.

S.No	Name of the Organism	Diameter of Zone of Inhibition (mm)			
		Ethanollic Ex (5µl)	Ethanollic NPs (10µl)	Ethanollic NPs (15µl)	Ampicillin (5µl)
1.	<i>Staphylococcus aureus</i>	-	1	1	4
2.	<i>Pseudomonas putida</i>	1	2	2	4
3.	<i>Bacillus subtilis</i>	3	1	2	5
4.	<i>Escherichia coli</i>	1	2	3	5
5.	<i>Klebsiella pneumoniae</i>	2	-	2	6

CONCLUSION

The present study concludes the preliminary phytoconstituents of both the Aqueous and ethanolic extracts of the fresh fruits of *Morinda citrifolia*, TLC profile studies reveals authentication for quality control. The biologically established ecofriendly and rapid synthesis of silver nanoparticles using *Morinda citrifolia* L of both aqueous and ethanolic extracts were shown to produce particles of fairly uniform size and shape providing easy, cost effective and proficient way for synthesis. The development of Silver nanoparticles was observed by appearance of color change in the solutions and by UV spectrophotometry. The FTIR analysis was meant to identify the functional groups present in the fruit extract which are responsible for reduction. The synthesized particles ranged in size from 1-5nm and were spherical in shape, and confirmed by the TEM and AFM analysis. Finally synthesized silver nanoparticles were emphasized the plant mediated synthesis with mild antibacterial effect of both the aqueous and ethanolic extracts. It is however anticipated that the usage of nanotechnology will reinforce the future aspects to raise our knowledge with forceful decline in cost of nano-based food and medicines. Thus, this present studies are significant for the present day scenario to explain the safety and potency of *Morinda citrifolia* for its boosting nature of productivity to overcome its scarcity for their constituents in clinical environment to

treat inevitable diseases like cancer and AIDS.

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