ANTIOXIDANT&ANTI BACTERIAL ACTIVITIES OF SLIVER NANO PARTICLES BIOSYNTHESIZED FROM AQUEOUS LEAF EXTRACT OF HYMENODICTYON ORIXENSE

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ABSTRACT

An Aqueous leaf extract *HYMENODICTYON ORIXENSE* was employed to synthesize silver nano particles Commonly known as Bhorsal belongs to the family *Rubiaceae.* The plant shows the activity of microbial, Inflammation, oxidant, cancer, and bacterial. The present study deals with Anti-oxidant by using DPPH and Reducing power Assay method anti-bacterial activity by cup plate and cylindrical plate method. Preliminary phytochemical screening revealed the presence of flavonoids, tannins, etc.

SEM, TEM, UV-analysis, and XRD were performed to identify the compounds present in the aqueous plant extractof *hymenodictyon orixense.* In this study, we assessed for anti-oxidant activities with aqueous extract by in vitro method as a, the DPPH, Reducing power Assay method extract significantly reduced DPPH to 63.52 at the concentration of (100µg/ml) as compared to 76.41 with standard ascorbic acid (100µg/ ml)and also extract showed antibacterial activity by in vitro method as cup plate and cylindrical plate method.

Keywords: *HYMENODICTYON ORIXENSE*, silver nanoparticles phytochemical screening, anti-oxidant anti-bacterial.

Nanotechnology

Nanotechnology is a multidisciplinary field of study with a wide range of applications. It involves the manipulation of atoms, electrons, protons, and neutrons in a number of different ways to produce new knowledge about the development of materials that can be used to address a wide range of issues in engineering, agriculture, biology, chemistry, surface science, space exploration, ocean and marine science, geography, and geology.[1-6] Science uses nanotechnology to arrange matter at the molecular level. All of the following are included in what the US National Nanotechnology Initiative (NNI) refers to as nanotechnology: i) the development of bulk materials at the atomic, molecular, or macromolecular levels, in the range of around 1-100 nanometers (nm); ii) the development of structures, devices, and systems, and their application in such a way that their use results in novel features and functionalities as a result of their tiny and/or intermediate sizes. organise, stabilize, or change on a large scale (USEPA, 2007). A prominent field of contemporary research is nanotechnology, which is defined as the synthesis, characterization, exploration, and application of nanosized (1-150nm) materials for the development of science. In the recent years, the idiom[7-12]

PLANT INTRODUCTION:

Hymenodictyon is a genus of flowering plants in the family Rubiaceae. It has about 30 species. All are native to the Old World. Hymenodictyon orixense possesses a soft, limited-use wood that is mostly used for boxes. The type In an addition to William Roxburgh's Flora Indica that was published by Carey and Wallich after Roxburgh's passing, Nathaniel Wallich gave the plant its name in 1824. species for Hymenodictyon is Hymenodictyon orixense (synonym: Hymenodictyon excelsum). The generic name is derived from two Greek words, hymen,"membrane",and diktyon,"net". It refers to the wing that surrounds seach seed.[13] Molecular phylogenetic studies have shown that Hymenodictyon is paraphyletic over the Madagascan genus Paracorynante.Large deciduous glands known as collectors are present on the stipules of Hymenodictyon and Paracorynanthe. The corolla tube is small at the base and gets bigger as it gets closer to the tip. [(14-19)] It looks like a woody capsule. A common Thai medicinal plant, Hymenodictyonorixense (Roxb.) Mabb. (syn. H. Excelsum), is found in mixed forests and rainforests in northern, central, and southern Thailand. In Thai, it is referred to as "U Lok" and "Som K This plant is a deciduous tree that reaches heights of 9 to 12 meters. While the bark of this plant is mostly employed as febrifuge and astringent, the leaves have historically been used to treat inflammation, sore throat, tonsillitis, sialitis, and ulcers Previous studies on this genus led to the isolation of coumarins iridoids, anthaquinones, triglyceride, steroids and acetylenic fatty acids whereas chemical investigation of the bark of this plant provided β-sitosterol, stigmasterol and coumarins, hymexelsin, aesculin and scopoletin . A potential AChE inhibitor that restores neurotransmitter deficit in Alzheimer's disease is coumarins The MeOH extract of H. orixense bark demonstrated little inhibition of AChE at 500 mg by TLC bioautography test in a screening for AChE inhibitors A member of the Rubiaceae family, Hymenodictyon orixense (Roxb.) Mabb is widespread over most of India and is a native of tropical Asia and Africa.

H. orixense (Roxb.) Mabb., which grows wild from Bago Yoma to the hill areas of upper Myanmar, is utilized in indigenous medicine in Myanmar. It generates alkaloids and scopoletin, which are used as febrifuges, as a quinine alternative in traditional medicine, as probable regulators of tumor promotion, and as a remedy for sore throat and appetite. The system of traditional medicine in Myanmar has existed for many hundred years. Traditional and herbal treatments from Myanmar are becoming more and more well-liked and acknowledged. Ku-than is a less well-known plant constituent that is frequently utilized in

Myanmar, it has been noted. by those who practice traditional medicine. Although Ku-than was not a widely utilized substance like Cinchona, which was used to cure malaria, its usage as a substitute for or comparable to Cinchona should prompt further research into the plant's ingredients. As a result, the current study's goal is to learn more about and identify the chemical components of ku-than barks.[20-23]

MATERIALS AND METHODS

3.1 Materials

Fig:2 Hymenodictyon orixense

taxonomical study of Hymenodictyon orixense

- Kingdom: Plantae
- Phylum: Tracheophyta
- Class : Mangoliopsida
- Order: Gentianales
- Family: Rubiaceae
- Genus: Hymenodictyon
- Species: Hymenodictyonorixense(Roxb)Mabb

Plant collection and authentication :

The fresh leaves and dried bark of Hymenodictyon Orixense were collected during the months of December-January from the Horsley Hills. The plant material was taxonomically identified and authenticated by Dr. Madhava Cheety, Department of Botany, Sri Venkateswara University, Tirupati. The flower voucher number-0417. The fresh leaves and dried bark were collected and kept shaded dry for 15 days. The dried bark and leaves were taken and milled into coarse powder by a mechanical grinder and stored in a airtight container

MATERIALS / CHEMICALS USED

The chemicals used in the experiment they are methanol, NAOH, and sulphuric acid, Phenolphthalein, dilute HCL, $Fecl_3$, DPPH,by SDFCL Laboratories from Mumbai.

Methods:

Preparation of leaf extracts of H. orixense

Fresh A. spinosus leaf were washed with deionized H2O and chopped into small pieces. 10 g of leaf transferred into Erlenmeyer flask containing 50 mL Milli-Q H2O and maintained at 50 °C for 45 min. The supernatant was filtered through the Whatmann No. 1 paper to get the leaf extract and was stored at 4 °C for until further use. The obtained extract used as a reducing agent as well as a stabilizer for NPs synthesis. The antioxidant capacities of leaf extract were evaluated.

Fig: 2 Preparation of leaf extracts

Synthesis of FeO NPs using H. orixense leaf extract:

The synthesis of FeO NPs using 40 mL of leaf extract and 50 mL of 0.5 M ferric chloride (FeCl3) were taken as burette and beaker solution respectively. The leaf extract (pH 6) was added to FeCl3 solution and continuously stirring with magnetic stirrer maintaining at 37 ± 1 °C for 90 min. The solutions pH was adjusted using 0.1N HCl and 0.1N NaOH. The formation of NPs was confirmed by colour changes from brown to colourless solution with the black precipitate. The precipitate of B FeNPs was collected and washed wit absolute ethanol for completely removal of H2O. The BFeNPs were dried in oven at 60 °C for 180 min. These Fe O NPs samples were stored in sealed bottles under dry conditions prior to use.

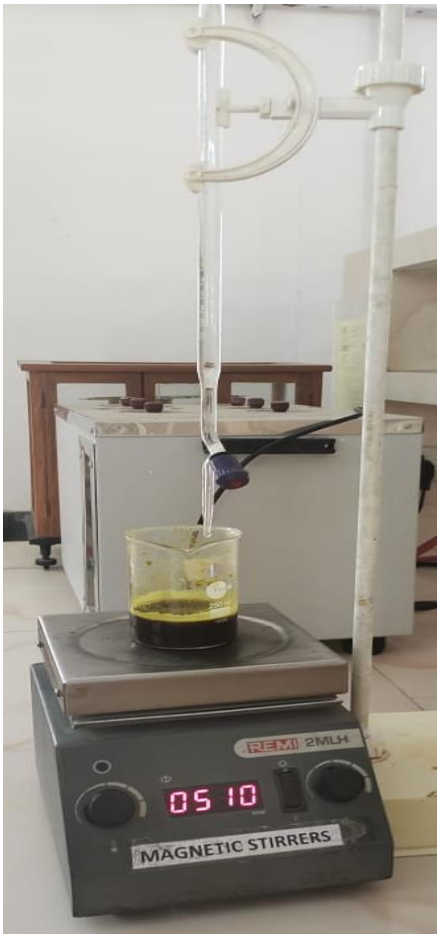


FIG :3 Synthesis of FeONPspercentage of yield

Sample	Weight of sample in gms	Weight of the extract	% yield
Ho aqueous plant extract	25 gm	20 gm	80 %

Calculation:

$$\text{Percentage yield} = \frac{\text{Weight of the extract}}{\text{Weight of sample}} \times 100$$

$$= \frac{20}{25} \times 100$$

$$= 80\ \%$$

***IN-VITRO-ANTI*- OXIDANT ASSAY**

DPPH Radical Scavenging Assay.

Both hydrogen atom transfer (HAT) and electron transfer (SET) processes are the foundation of the DPPH test. Since DPPH is a stable radical, this assay takes into account not only the concentration of the tested sample but also the reaction time and the temperature; both of which, when carefully controlled, enable this assay to be highly reproducible. One benefit of the DPPH assay is that it is an easy, economical, and quick method to evaluate the radical scavenging activity of non-enzymatic antioxidants. However, this assay has limitations when used to assess the antioxidant activity of brewed coffee. These limitations are connected to the color of the EO, which may interfere with the DPPH absorption. Furthermore, the current hydrophilic components have limited accessibility to the lipophilic radical DPPH. Alcohol must therefore be present in the reaction mixture to guarantee maximal solubility in brewed EO. When standards are created for measurement purposes, the background antioxidant activity caused by the presence of methanol must be taken into account. if there are proteins The DPPH assay's major drawback is that it doesn't target specific free radicals with physiological significance. [33-36]

Procedure:

The DPPH method was used to determine the amount of radical scavenging activity. One mL of various EO or extract concentrations was combined with one mL of a 90 M DPPH solution in methanol, and the final amount was increased to four mL using methanol. After thoroughly shaking, the combinations were stored at 25°C in the dark for one hour. At 517 nm, the absorbance was measured. Using the following equation, the radical scavenging activity (RSA) was estimated as a percentage of DPPH discoloration:

$$\%\text{RSA} = \left[\frac{(A\,0 - A\,s)}{A\,0}\right] \times 100,$$

where As is the absorbance of the test compound and A0 is the absorbance of the control, which contains all of the reagents but not the test compound. Plotting inhibition percent against oil concentration allowed us to determine the oil concentration that provides 50% inhibition (IC50) from the graph. BHT served as the standard.

Reducing Power Assay.

The previously mentioned approach was determined by the reducing power of the EO/extract. We combined 2.5 mL of potassium ferricyanide [K3Fe(CN)6] (1%), 2.5 mL of 0.2 M phosphate buffer (pH 6.6), and various amounts of EO/extract. For 20 minutes, the mixture was incubated at 50°C. 10% trichloroacetic acid was added to the mixture in aliquots (2.5 mL). The previously combined was then centrifuged at 1036 g for 10 minutes. 2.5 mL of the solution's upper layer was combined with 2.5 mL of distilled water and 2.5 mL of a solution containing 1% ferric chloride. Through the use of a double-beam UVVIS spectrophotometer, the absorbance was measured at 700 nm. The reaction mixture's enhanced absorbance was a sign of its improved reducing power. The concentration of the essential oil/fraction that provided 0.5 of absorbance (EC50) was estimated based on a graph of the absorbance at 700 nm against the concentration of the EO/extract and compared to other common antioxidants.

INVITRO-ANTI-BACTERIA ASSAY CUP PLATE METHOD OR CYLINDER PLATE METHOD

PRINCIPLE

This approach relies on the diffusion of an antibiotic from a vertical cavity or cylinder. By placing a layer of solidified agar in a petri dish, the test microorganisms' growth is completely prevented in a circle or zone surrounding the cavity or cylinder carrying the antibiotic solution.

Requirements and materials

Gram Positive bacteria: Pneumococci Gram Negative bacteria: Klebsiella

Petri plate, Conical flask, Glass rod, Burner, pH paper, Inoculum loop, Heating mantle, Volumetric flask,hotair oven,autoclave,weighing balance.

Chemicals:

Agar-Agar, Beef extract, Peptone, Sodium chloride (NaCl), Hydrochloride (HCl), Sodium hydroxide(NaOH),distilled water.

Procedure:

A general-purpose nutrition medium called nutrient agar is used to cultivate microorganisms that support the growth of a variety of non-fastidious organisms. Because it can support a wide range of bacterial and fungal growth and is rich in the nutrients required for bacterial development, nutritional agar is widely used.

Composition of nutrient

0.5%peptone	It is an enzymatic digest of animal protein. Peptone is the principal source of organicnitrogen for the growing bacteria.
0.3% beef extract/yeast extract	It is the water-soluble substances that aid in bacterial growth, such as vitamins,carbohydrates, organic nitrogen compoundsand salts.
1.5%agar	It is the solidifying agent.

0.5%NaCl	The presence of sodium chloride in nutrient agar maintains a salt concentration in the medium that is similar to the cytoplasm of the microorganisms.
Distilled water	Water is essential for the growth of and reproduction of micro-organisms and also provides the medium through which various nutrients can be transported.
pH(7.4)	Stable pH is an important requirement for optimum microbial growth inculture media.

Preparation of Nutrient Agar

1. Suspend 28 g of nutrient agar powder in 1 litre of distilled water.

2. Heat this mixture while stirring to fully dissolve all components.

3. Autoclave the dissolved mixture at 121 degrees Celsius for 15 minutes.

4. Once the nutrient agar has been autoclaved, allow it to cool but not solidify.

5. Pour nutrient agar into each plate and leave plates on the sterile surface. until the agar has solidified

6..Replace the lid of each Petri dish and store the plates in a refrigerator.

Nutrient Broth

Widespread for the cultivation of undemanding microorganisms is the nutrient broth. Many standardized ways of analyzing meals, dairy products, water, and other products advocate it. The only difference between Nutrient Broth and Nutrient Agar is the absence of agar, which causes the sodium to crystallize at ambient temperature. The combination of tryptone and meat extract used in its production aids in the proliferation of bacteria. The purpose of sodium chloride is to maintain osmotic pressure.[24]

Procedure

Add 13gto 15g of nutritious broth powder in 1Lofdistilled water.

Mix and dissolve completely

Sterilize by auto craving at 121° Cfor15minutes.

Microbial culture by streak plate method:

1. Sterilize the inoculating loop in the Bunsen burner by holding it up to the flame until it is red-hot. Give it time to cool.

2Select an isolated colony from the agar plate culture, and either spread it evenly across the first quadrant (about 1/4 of the plate) using closely spaced parallel streaks, or insert your loop into the tube/culture bottle and take some inoculum out of it. There's no need for a significant portion.[25-30]

3. Immediately, lightly spread the inoculating loop across a fourth of the plate in a back-and-forth motion.

4. Continue to flame the loop and let it to cool. Return to the edge of area 1 where you streaked previously and continue the streaks into the second quarter of the plate (area 2).

5. Continue to flame the loop and let it to cool. Return to area 2 where you previously streaked and continue your streaks into the third quarter of the plate (area 3).

6. Continue to flame the loop and let it to cool. Return to the region you just streaked (area 3) and extend the streaks into the plate's center fourth (area 4).

7. Burn your loop once more.

Standard Solution:

Ingredients	gram/litre
Peptones	10g
Beef extract	1g
Yeast extract	2g
Sodium chloride	5g
pH Final	6.8±0.2at25°C

To make a stock solution, dissolve a sufficient amount of the USP reference standard of the supplied antibiotic, or, if necessary, the whole contents of a vial of the standard, in the solvents. After dilution to the desired concentration, store at 2–8* and use within the time frame mentioned .Prepare five or more test dilutions from the stock solution on the day of the

assay, with each succeeding solution's concentration increasing stepwise in a ratio of typically 1:1.25.Use the final diluent specified such that the concentration is at the median.

Sample drug derivatives Dilutions:

1. To prepare the stock solutions of the drug derivatives (vanillin, salicylic benzaldehyde, para-di methyl amino benzaldehyde, para- chloro benzaldehyde, cinnamaldehyde, and benzaldehyde), weigh each accurately at 20 mg and then transfer to the appropriate solvent (20 ml each of ethanol, chloroform, and acetone).

2. Take roughly 1ml, 2ml, 3ml, and 4ml from each stock solution and dissolve each in 20ml of distilled water, accordingly (10g/ml, 20g/ml, 30g/ml, and 40g/ml).

General procedure:

Sterilize all glass items using a hot air oven at 170° C about 30 minutes beforehand. Prepare the necessary quantity of agar-agar media and sterilize in an autoclave at 121 degrees. Next, take an inoculum from a pure culture of gram positive and gram negative bacteria, scatter it over agar-coated plates, and incubate the mixture for 48 hours. The cultured bacteria were put into nutrient broth for two to three hours, where they were allowed to proliferate until turbidity was visible. Before it begins to firm, prepare another freshly made agar-agar medium and add nutritional broth to it. Transfer the full contents of the petri plates, allow them to solidify, and then construct cylinder-shaped cups. Next, fill each petri plate with various drug derivative dilution concentrations, and set it in the incubator for around 24 hours. A zone of antibiotic action can be visible with respect to the individual conccentations after 24 hours Calculate the diameter by dividing it by the two inhibition zones, R/2, and record the results.[31-,32)]

Note: The absence of turbidity denotes negative control, whereas the appearance of turbidity denotes positive control.

Statistical analysis :

The resulting experimental data were statistically analyzed using Graph Pad Prism. San Diego Trail (Prism Graph Pad Version 8.2.3(263, Graph Pad Software.Inc La Jolla CA.U

RESULTS & DISCUSSION

PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF HYMENODICTYON ORIXENSE

Table :3.1 Phytochemical screening results

S. No	Test for	methanolic extract of *Hymenodictyon Orixense*
1	Carbohydrates	-
2	Steroids	-
3	Cardiac glycosides	-
4	Steroid glycosides	-
5	Coumarins	-
6	Flavonoids	+
7	Alkaloids	-
8	Tannins	-
9	Terpenoids	-
\10	Volatile oils	-

FT- IR results

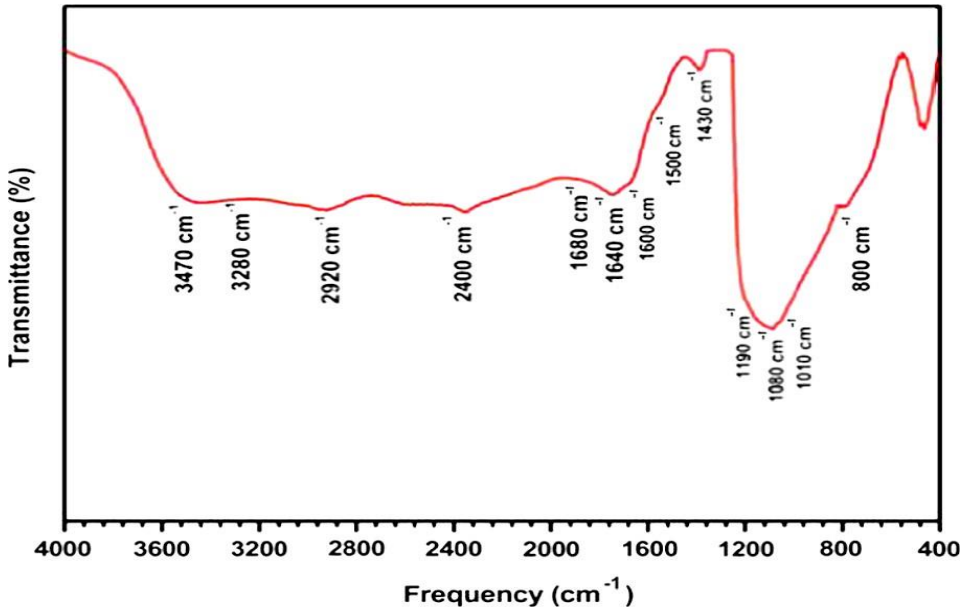


Fig. 4 FTIR spectra of the silver nanoparticles synthesized by the reduction of silver nitrate with the *Hymenodictyon Orixense* leaf extract.

Table: IR spectra standard values

S.No	Functional group	Standard value	Observed
1.	C-H	3200-2800	2920
2.	O-H	2400	2400
3.	C-O	2000-1600	1680
4.	C=C	1449-1618	1600 1500

UV-Vis spectral analysis

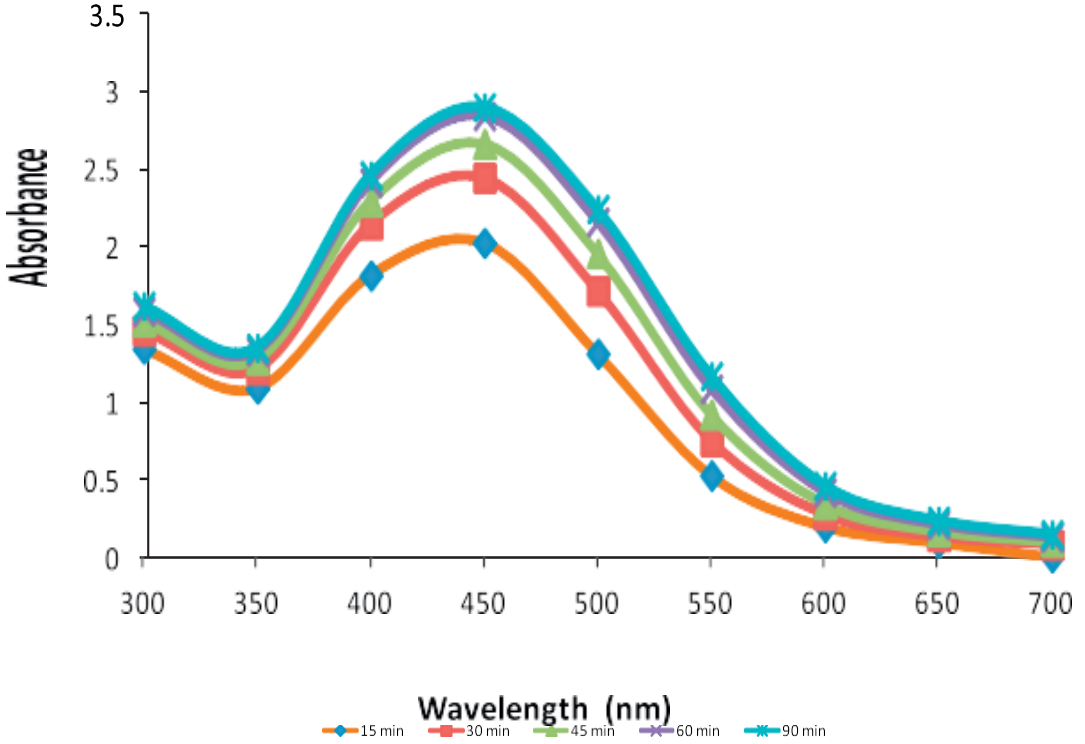


Fig. 5 UV/Vis spectra recorded from the reaction mixture that shows the production of A

TEM results:

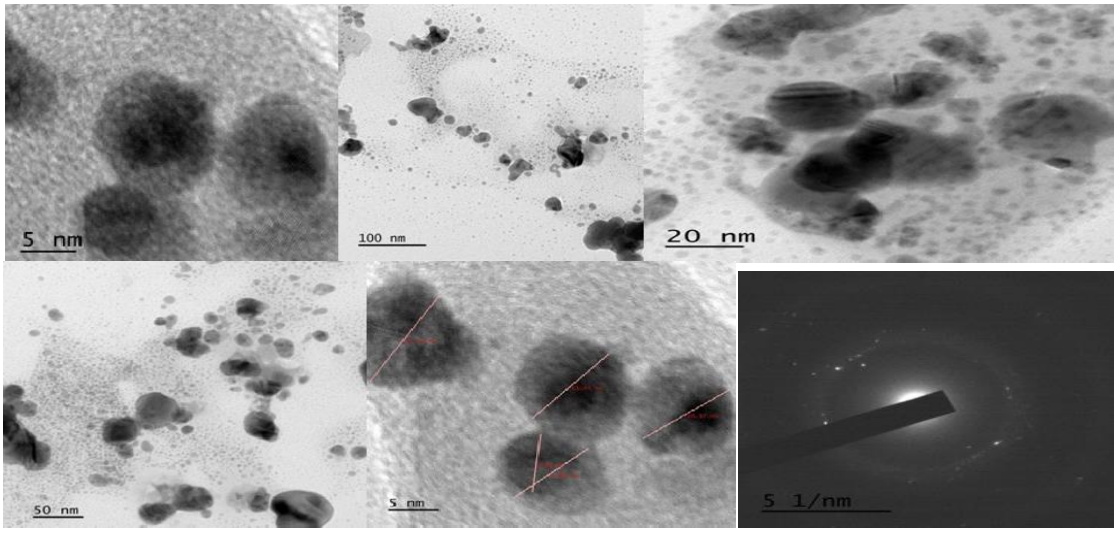


Fig: 6 HRTEM micrograph of silver nanoparticles synthesized from 1×10^{-3} M $AgNO_3$ solution and *Hymenodictyon Orixense* extract.

XRD results:

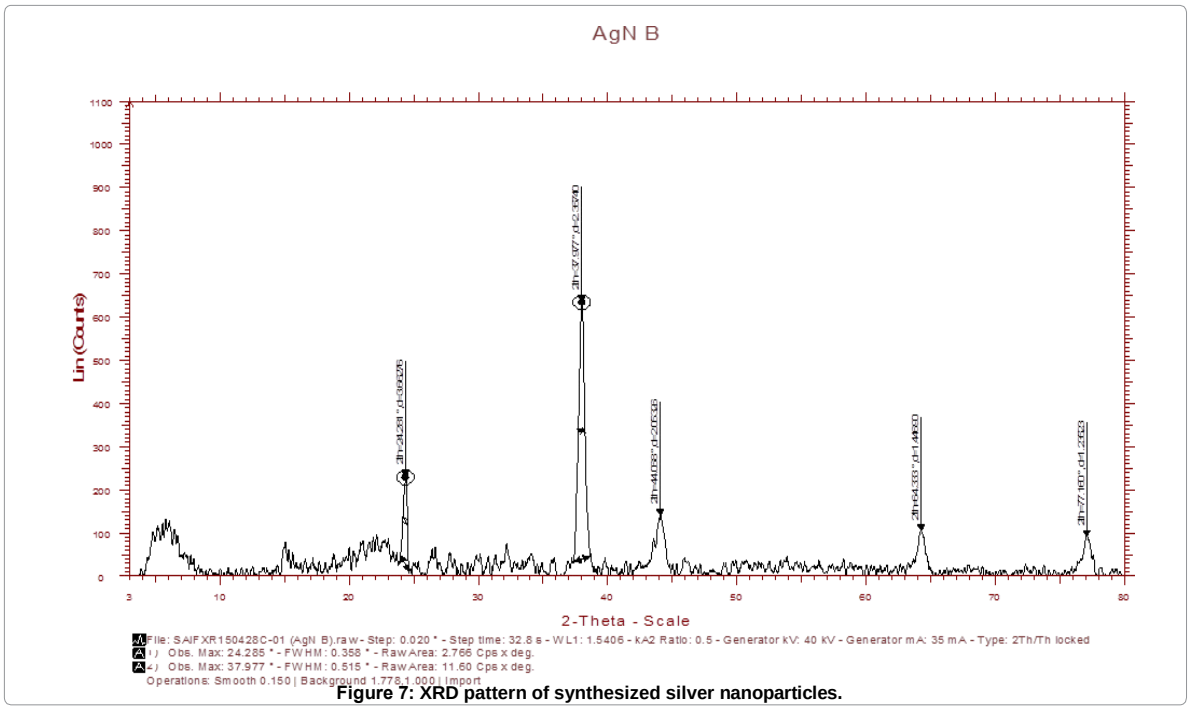


Figure 7: XRD pattern of synthesized silver nanoparticles.

Scanning electron micrograph (*SEM)studies*

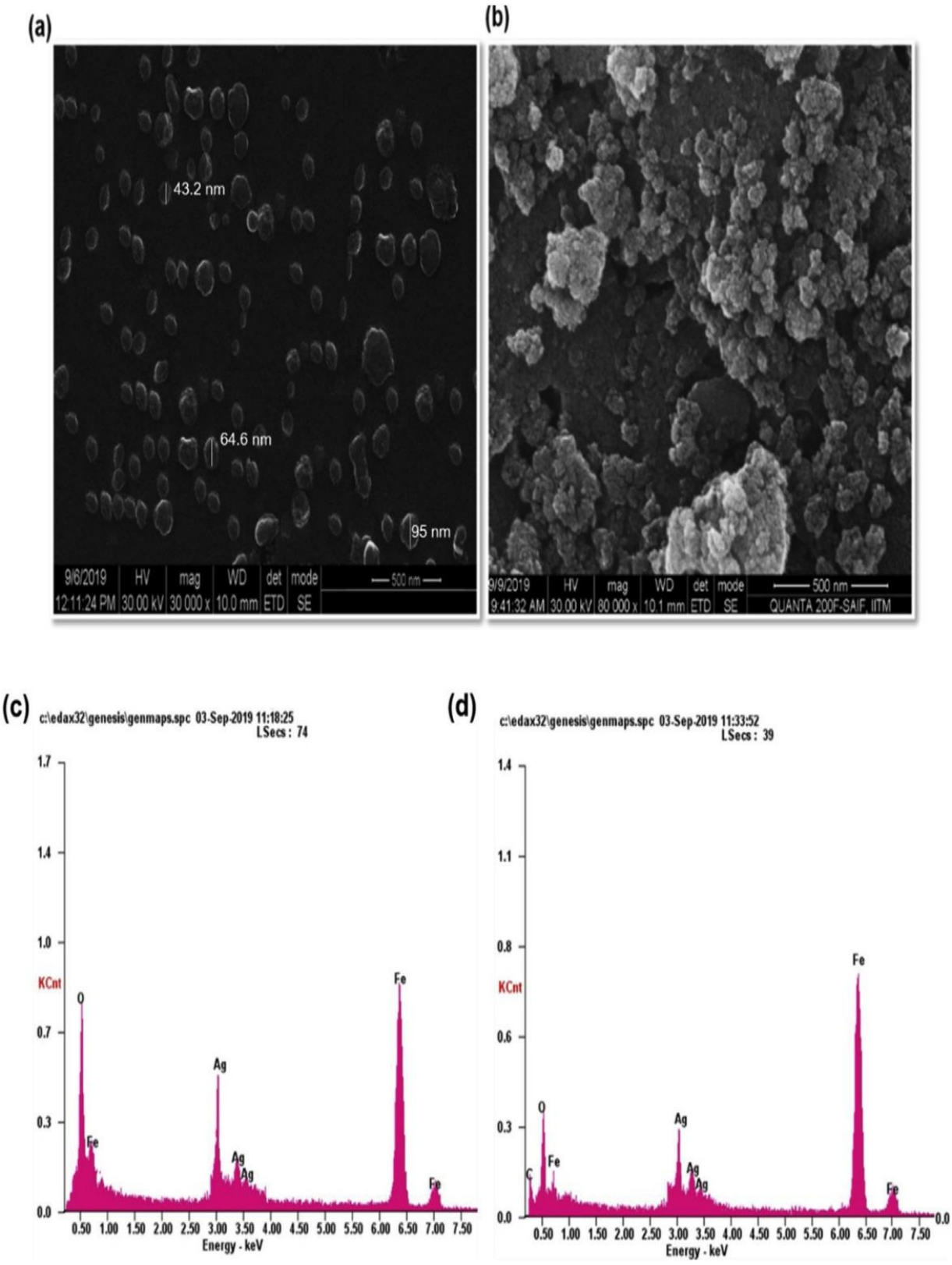


Fig. 8 Scanning electron micrograph of synthesized NPs SEM operating at a voltage of 30 keV corresponding energy dispersive X-ray spectra

Evaluation of *In-vitro* Anti-Bacterial Activity:

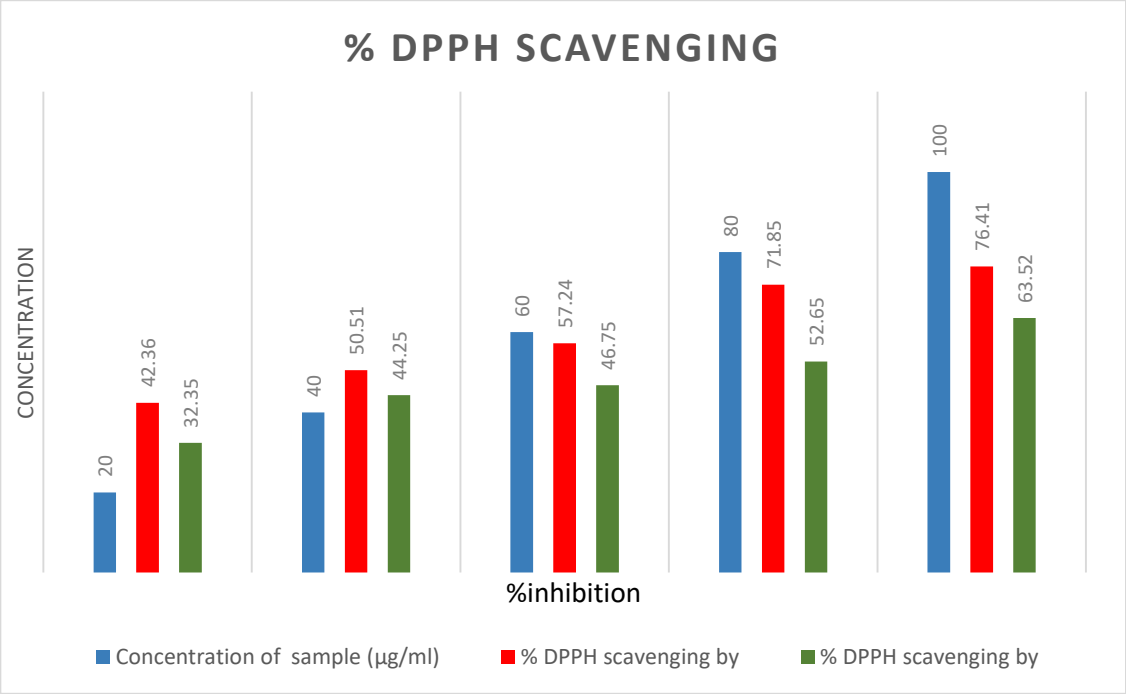
Table: evaluation of *in-vitro* anti-bacterial activity

S.No	Compound code	ZONE OF INHIBITION (diameter = R/2)								Positive control		Negative control
		Gram +ve				Gram -ve				+ve	- ve	Turbidity not observed
		10ug	20ug	30ug	40ug	10ug	20ug	30ug	40ug			
1.	PE HO	2.01	1.7	1.5	1.0	1.89	1.6	1.1	0.8			
2.	Gentamicin	1.7	1.5	1.0	0.7	1.6	1.4	1.2	0.8			

Table:DPPH scavenging assay by aqueous plant extact & Ascorbic acid :

S. No.	Concentration of sample (µg/ml)	% DPPH scavenging by ASCORBIC ACID	% DPPH scavenging by Plant extract
1	20	42.36±0.45	32.35±3.21
2	40	50.51 ±0.32	44.25± 2.35
3	60	57.24±1.23	46.75± 2.56
4	80	71.85 ±2.21	52.65±5.43
5	100	76.41±3.25	63.52±2.34

Values are expressed as mean±SEM



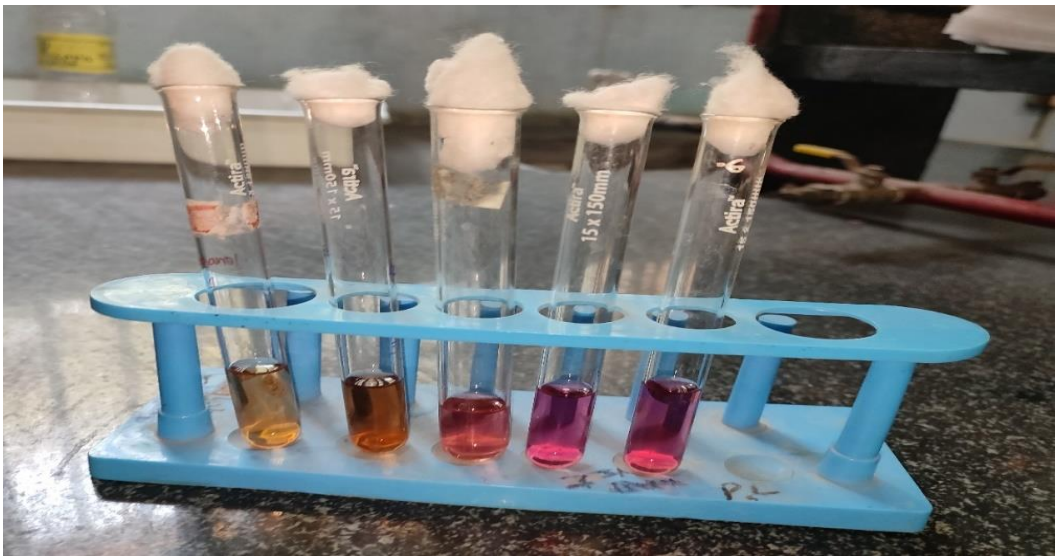
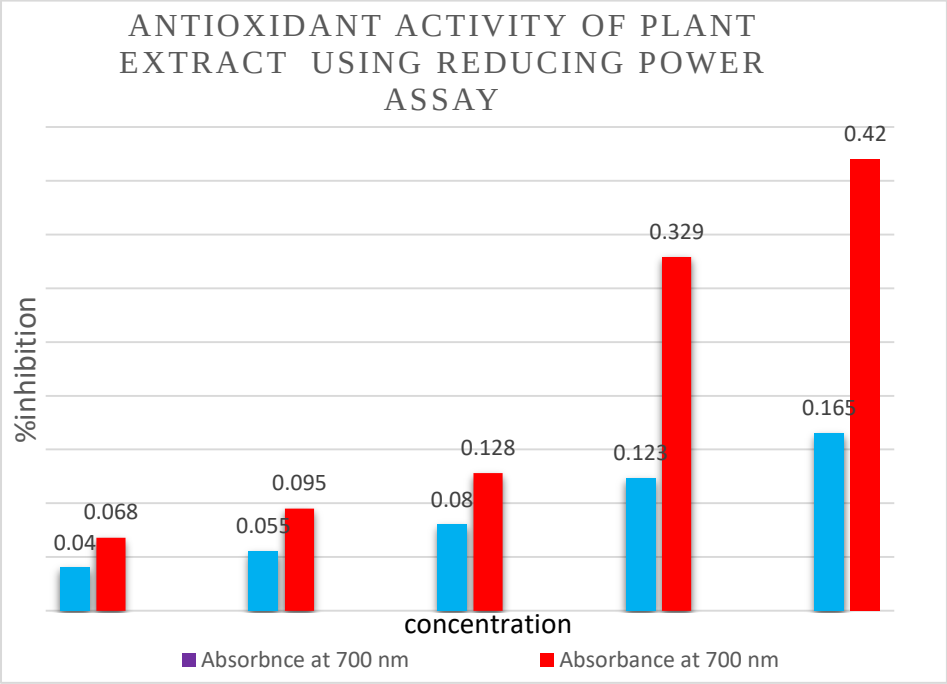


Fig :9.Evaluation of *In-vitro* Reducing power Assay by plant extract & Ascorbic Acid

S. No	Concentration of Test substance((µg/ml)	Absorbance at 700 nm (PLANT EXTRACT)	Absorbance at 700 nm (ASCORBIC ACID)
1	20	23.450	32.723
2	40	38.645	43.657
3	60	47523	58.123
4	80	53.641	65.73
5	100	62.725	74.16

Values are expressed as mean±SEM



CONCLUSION

A medicinal plant called Hymenodictyon Orixense was subjected to pharmacological research, anti-oxidant and anti-bacterial analysis, and phytochemical analysis. The results are summarized in this chapter. In the beginning, plant powder was extracted from methanol and ethanol extracts. Preliminary phytochemical study revealed that H. orixense plant extracts synthesized utilizing nanotechnology contained tannins, flavonoids, saponins, and resin components. The isolated compounds were examined for the presence of tannins, flavonoids, resin, and saponin using their FT-IR, SEM, TEM, and XRD properties. In this study, we evaluated the anti-oxidant activities of aqueous extract using in vitro methods such as DPPH and reducing assay method. The extract significantly decreased the value of the DPPH method through to 63.52 at the concentration of 100 g/ml as compared to 76.41 with standard ascorbic acid (100 g/ml), and it also demonstrated reduced assay activity. Plant extract's antibacterial activity was tested in vitro using the cup plate and cylinder plate methods on both gram+ve and gram-ve both microorganisms.

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