International Journal of Research in Pharmaceutical Sciences (IJRPS)

Volume No. 15

Issue No. 1

January - April 2024

ENRICHED PUBLICATIONS PVT. LTD

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PHONE: - + (91)-(11)-47026006

ISSN : 0975 - 7538

International Journal of Research in Pharmaceutical Sciences (IJRPS)

Aims and Scope

International Journal of Research in Pharmaceutical Sciences (IJRPS) is publishing online peer reviewed scientific journal sponsored by JK Welfare & Pharmascope Foundation. The journal publishes research articles, review articles, short communications, case studies and reports in Pharmaceutical Sciences.

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Coverage Area: Pharmaceutics, Novel Drug Delivery Systems, Pharmaceutical Chemistry and Drug Design, Pharmaceutical Analysis and Quality Assurance, Pharmaceutical Nanotechnology, Pharmacology and Toxicology, Hospital and Clinical Pharmacy, Clinical Research, Clinical Report, Case Studies, Pharmacognosy and Phytochemistry, Chemistry, Medical Biochemistry, Natural Products, Medicinal Chemistry, Molecular Biology, Microbiology and Biotechnology, Alternative Medicine, Polymer Science, Industrial Pharmacy, Drug Regulatory Affair, Intellectual Property Rights (IPR), Molecular Modeling, Herbal Medicines, Dental Medicines, Paramedical and Lifesciences.

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(Volume No. 15, Issue No.1, January - April 2024)

Contents

Development and Validation of a simple UV spectrophotometric method

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A B S T R A C T

Using a Shimadzu UV-2600, a quick, precise, easy, and affordable UV spectrophotometric approach has been created. Solvent made with methanol to assess the bulk Delamanid content. A wavelength of 320 nm was used for the detection process. The parameters linearity, accuracy, precision, ruggedness, robustness, LOD, and LOQ were taken into consideration during method validation in accordance with ICH Q2R1 criteria. It demonstrated linearity in the range of 60-360 (/mL) at a predetermined λmax of 320 nm, and it had a strong correlation coefficient (R2-0.996) and outstanding mean recovery (99.00-100.07%). Determination of Delamanid used this technique effectively. The method's linearity, accuracy, repeatability, and reproducibility were statisti cally and by recovery experiments confirmed. The outcomes demonstrated the method's applicability for both regular Delamanid bulk analysis and com mercial formulations.

Keywords:Delamanid,UV Spectrophotometricmethod,Process validation,ICH guidelines

INTRODUCTION

The drug delamanid is efficient in treating MDR TB.It is referred to by its trademark, Deltyba. 1. It is the first of a brand-new class of anti-TB drugs known as nitroimidazoles [1]. The chemical name for dela manid is (2R)-2-[(4-(trifluoromethoxy)phenoxy]. -1-piperidinyl]] -6-nitro -2-[(4-4-[4-(trifluoromethoxy)phenoxy]methyl].

Dihydroimidazo[2,1-b] [2, 3] oxazole as shown in Figure 1. Delamanid is a medication used to treat individuals with multidrug-resistant tuberculosis (TB) that affects the lungs. By preventing the pro duction of mycobacterial cell wall constituents such as methoxy mycolic acid and ketomycolic acid, it functions as a prodrug [1, 2]. For delamanid's analysis, no spectroscopic, HPLC, or HPTLC approach is available. thus Delamanid's analytical procedure needs to be developed. The current study's objective was to create a precise, repeatable, accurate UV approach for the analysis of delamanid. The. The developed method looked for linearity, accuracy, precision, robustness, ruggedness, LOD, and LOQ in accordance with ICH guidelines [4].

It is branded as Deltyba 50mg available in tablet form. Methanol, DMSO (Dimethyl Sulfoxide), and DMF are all solubilizing solvents for it. Keto mycolic acid and methoxy mycolic acid, two crucial mycolic acid constituents, have been demonstrated to be prevented by delamanid. Delamanid's inhibitory effect is restricted to mycobacteria since mycolic acids are only present in the cell walls of mycobacteria and are not present in the cell walls of other Gram positive or Gram-negative bacteria [3,

5]. Breaking the cell wall enables increased medication penetra tion and hence a shorter treatment schedule since these acids make it harder for pharmaceuticals to

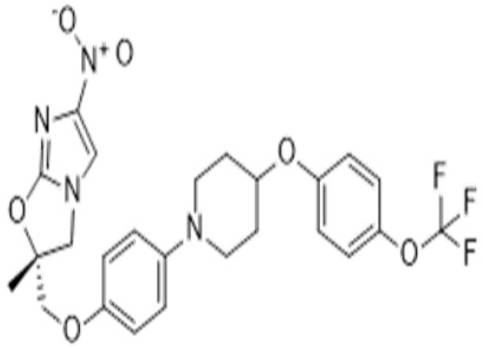

Figure 1: Delamanid

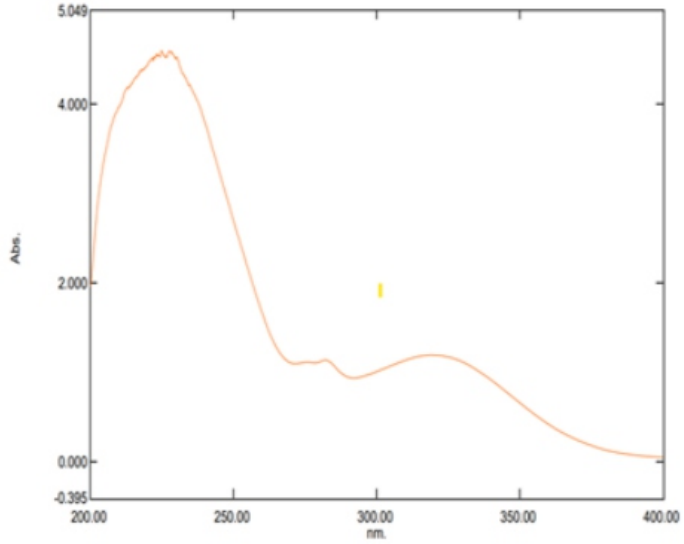

Figure 2: UV spectra showing Absorption Maxima (λ max) of Delamanid

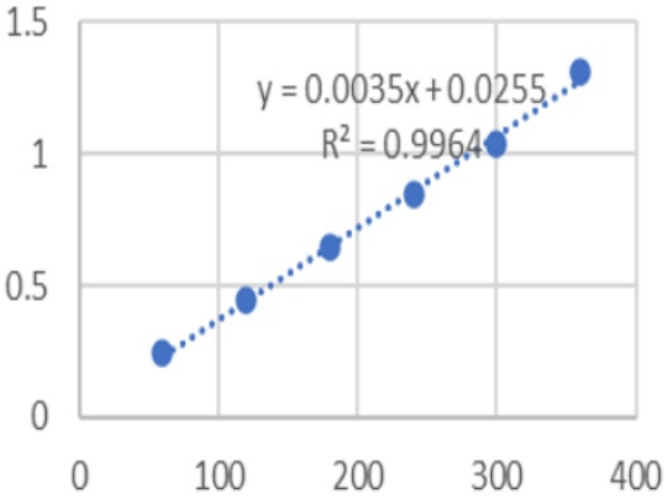

Figure 3: Calibration Curve of Delamanid

permeate the mycobacterium's cell wall [6, 7].

MATERIALS AND METHODS

Delamanid pure drug was received as a gift sam ple from Mylan, Hyderabad. Analytical grade Ethylacetate, methanol and n-hexane were acquired from Qualigen (India) Ltd., Mumbai, India. All other chemicals come from S.D. Fine Chemical Ltd. in Worli, India, and are of analytical quality and for all of the experimental work, volumetric glassware of class A grade was employed. On a SCHIMADZU 2600 Double Beam UV-Visible Spectrophotometer with two 10 mm matched quartz cells, the UV spec trophotometric technique was used.

Solvents such as methanol, ethanol, toluene,petroleum ether, acetonitrile, chloroform, n-hexane and acetone were tried for the estimation of Dela manid. Because of easy availability, better solubility and cost effectiveness. Methanol was chosen as the solvent for the Delamanid analysis

Table 1: Calibration Parameters

Sl.No.	Concentration (μg/mL)	Absorbance
1	60	0.247
2	120	0.447
3	180	0.646
4	240	0.845
5	300	1.038
6	360	1.314

Standard Stock Solution Preparation

Delamanid was carefully measured at 50 mg and added to the volumetric ẟlask at 50 ml. Prepared up to 50 ml with the same after being dissolved in a small amount of methanol to obtain a concentration of 1000 /ml(1000 ppm). 3.6 ml of the stock solution was transferred into a 10 ml volumetric flask and diluted with methanol. The dilution was observed to contain 360µg/ ml.

Determination of Wavelength of Maximum Absorption

Using methanol as a blank, the 360 g/ ml concentration solution was scanned between 200 and 400 nm. (Figure 2) λmax was determined to be 320 nm from the UV spectra and was chosen as the analyti cal wavelength. The UV spectrum of Delamanid was shown in Figure 2.

Preparation of Calibration Graph

In this method, 0.6-3.6 ml of the aliquots of stock solution of Delamanid containing 1000µg/ ml were transferred into six 10 ml volumetric flasks in a sequence. (60 - 360 µg/ml) and made up to the volume with methanol.

At 320 nm, the absorbance of solutions of various concentrations was measured in comparison to a

blank. By graphing concentration vs absorbance, the calibration curve was created.

At 320 nm, it was discovered that the sample was linear with a concentration range of 60 to 360 µg / ml.

Table 2: Recovery study

Drug	Initial amount Standard drug(100ppm) %	Amount added (200ppm)	Amount found	Recovery (%)	% RSD (n = 3)
Delamanid	80%(10ppm)	180ppm	181.2	101.2	0.07
	100%(10ppm)	200ppm	197.3	98.9	0.2
	120%(10ppm)	220ppm	218.6	99.3	0.3

Table 3: Precision

Drug		Intra – Day			Inter – Day		
Delamanid	Con. (μg/ml)	Absorbance measured Mean ± SD %	RSD	Average Potency %	Mean ± SD	RSD	Average Potency %
120	0.455± 0.005	1.1	104	0.462±0.002	0.6	103.4	
180	0.648± 0.006	1.1	100.1	0.66±0.003	0.5	100.7	
240	0.842± 0.004	0.45	100.8	0.851±0.006	0.14	98.2	
	Mean RSD	0.8			0.4		

Validation of Developed UV Spectrophotometric Method

Linearity

A calibration graph was created between concentration and absorbance. With a concentration range of 60–360 µg / ml at 320 nm, delamanid was linear.Additionally, the assay's limit of quantitation (LOQ) and limit of detection (LOD) were determined.

Precision

By conducting intra-day and inter-day variation trials using just three concentrations of Delamanid (120, 180, and 240 ppm) nine times, the suggested method's accuracy was evaluated. Intra-day studies and inter-day studies were determined by analysing three sample solutions of concentrations 120,180,240. The mean, standard deviation and % RSD were calculated.

Accuracy (Recovery Studies)

A blend of pure Delamanid and common excipients were used in this investigation. Calculations were made using the label claim and the typical final product weight.

The admixture was diluted using the same method as before to achieve three concentrations: 80%, 100%, and 120% of the reference solution. By incorporating a known quantity of the sample solution into the standard stock solution, the investigation was carried out.

LOD and LOQ

The set of six calibration curves used to assess the method's linearity was utilised to estimate LOD=3.3/S and LOQ=10/S Where σ is the standard deviation of the regression line's y-intercepts, The calibration curve's slope is denoted by S.

Ruggedness

The degree of repeatability of outcomes under various settings is known as ruggedness. Delamanid's decision to switch the analyst proved the robustness of the process.

The method's ruggedness was found by changing the analyst. The findings of the statistical analysis of the data are given as mean, standard deviation, and percent RSD.

Table 4: LOD and LOQ

Drug	LOD	LOQ
Delamanid	22.9ppm	69.54ppm

Robustness

For determination of the robustness of the method,wavelength change was applied as ±2 nm.

Following the statistical analysis of the data, the results are presented as mean, standard deviation, and % RSD

Table 5: Ruggedness

Drug Concentration (ppm)	Analyst-1			Analyst-2		
	Mean Absorbance	%Amount found	% RSD (n=3)	Mean Absorbance	%Amount found	% RSD (n=3)
120	0.447	102	0.8	0.440	98.7	0.3
180	0.646	100.6	0.4	0.651	97.7	0.5
240	0.845	101.5	0.7	0.858	99.10	0.3

Table 6: Robustness data of UV-Vis spectrophotometric method by taking absorbance at 320±2nm

Sl. No	Concentration (ppm)	318nm	320nm	322nm	%Amount	%RSD
1	120	0.436	0.438	0.437	97.9	0.2
2	180	0.660	0.661	0.665	101	0.3
3	240	0.868	0.860	0.865	99.9	0.4

RESULTS AND DISCUSSION

Method Development and Validation

Delamanid is easily soluble in organic solvents including methanol, DMF, and DMSO but practically insoluble in aqueous media. A few millilitres of methanol used as the diluent during the development phase produced a more favourable UV analysis result. The predetermined maximum absorption wavelength (λmax) was 320 nm.

Method Validation

Linearity

It was discovered that the calibration curve exhibits linearity in the range of 60-360µg/ml with a regression coefficient of 0.996 (Figure 3). With a correlation coefficient (r) of 0, the equation y = 0.0035+0.0255 was produced via linear regression of absorbance on concentration. 996. The maximal wavelength at 320 nm is visible for the detecting wavelength.

Accuracy (Recovery Studies)

RSD and the percentage recovery were computed.The present study work is correct in the technique creation of Delamanid since the mean percentage recovery and RSD were discovered to be within limits and less than 2. Calculations were made to determine the mean, standard deviation, and percentage relative standard deviation (%RSD). Table 2 presented the outcomes.

Precision

The repeatability (intraday) and intermediate precision (inter-day) of the assay were used to calculate its precision, which was reported as RSD%. For this,120 µg/mL, 180 µg/mL and 240µg/mL concentration solution were measured three times a day and RSD% was calculated. (Table 3). In terms of intra- and inter-day precision, Delamanid's% RSDwas discovered to be 0.6366 and 0.666, respectively.Table 3 displayed the results for intra-day and inter day. The devised technique's intra-day and inter-day precision study (Table 1), where all the RSDs were 2%, demonstrated appropriate sample stability and method dependability.

LOD and LOQ

Limit of Detection (LOD) and Limit of Quantitation (LOQ) estimates were used to determine the suggested method's sensitivity. Results are shown in Table 4.

Ruggedness

Table 5 displays the results for toughness. The method's robustness is demonstrated by the low percentage RSD value

Robustness

A slight planned modification in the analytical wavelength proved the method's robustness. The wavelength change applied as ±2 nm. At the selected variable wavelength, the amount of formulation was found. The % RSD was found to be 0.15, 0.36,0.10 for variables shown in Table 6.

CONCLUSION

The proposed UV-Vis Spectrophotometric approach was validated in accordance with ICH requirements and proved to be quick and accurate for estimating Delamanid. Therefore, without interfering with routinely used excipients, this method can be quickly and conveniently employed for routine analysis of Delamanid in quality control laboratories, whether in bulk or dosage formulations.

ACKNOWLEDGMENT

For giving a gift sample, the authors are grateful to Mylan Lab in Hyderabad, India. Also grateful for the facilities provided for doing this study work by the Principal of Mahatma Gandhi Vidyamandir's Pharmacy College Panchavati Nashik.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

[1] A Bahuguna and D S Rawat. An overview of new antitubercular drugs, drug candidates, and their targets. Medicinal Research Reviews, 40(1):263– 292, 2020.

[2] S K Field, D Fisher, J M Jarand, and R L Cowie. New treatment options for multidrug-resistant tuberculosis. Ther Adv Respir Dis, 6(5):255–268, 2012.

[3] M Matsumoto, H Hashizume, and T Tomishige. OPC-67683, a nitro-dihydro-imidazooxazole derivative with promising action against tuberculosis in vitro and mice. PLOS Med, 3(11):2131–2144, 2006.

[4] Validation of analytical procedure: text and methodology. International Conference on Harmonizations, pages 1–17, 2005.

[5] M S Glickman and W R Jacobs. Microbial pathogenesis of Mycobacterium tuberculosis: dawn of a discipline. Cell, 104(4):236–243, 2001.

[6] Stephen K Field. Safety and Efficacy of Dela manid in the Treatment of Multidrug-Resistant Tuberculosis. Clinical medicine insight: therapeutics, 5(5):137–149, 2013.

[7] J Nicola, Jin Han Ryan, and Lo. Delamanid: first global approval. Drugs, 74(9):1041–1045, 2014.

Diagnostic Aids and Techniques of Oral Cancer- An Updated

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A B S T R A C T

Oral cancer is the most prevalent and lethal type with a four in every 3000 incidence rate worldwide and a 3% survival rate. Oral squamous cell car cinoma (OSCC), which is multifactorial, is brought on by genetic and epigenetic instability. Oral potentially malignant disorders (OPMDs) are precursor lesions that often precede oral cancer, and their early diagnosis is advanta geous for patients since it may lengthen their productive longevity. Currently, oral cancer screening, early identi6ication, and its pre-invasive intraepithelial phases are still largely focused on visual inspection of the mouth. These methods are subjective and early lesions can be easily missed. Due to a lack of early identi6ication, oral cancer's 6ive-year survival rate is still low. Recently, the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) emphasised that by developing efficient cancer control and screening strategies, we can prevent a third of the 15 million cancer cases that are expected to occur in the future and better manage a second third. Oral cancer screening aids and techniques have witnessed a lot of advancements recently. This article reviews the current diagnostic methods and instruments for detecting oral cancer, which include the oral CDx brush, Velscope, Chemi luminescence, DNA ploidy, microarray technology, colposcopy, and oral scan. It also adds on the molecular (genetic and epigenetic alterations) & decoding of the oral carcinogenesis genomics data.

Keywords:Delamanid,UV Spectrophotometricmethod,Process validation,ICH guidelines

INTRODUCTION

Due to its high rates of morbidity and mortality,oral cancer—a disease that affects everyone—has developed a reputation for being dif6icult to treat [1].

Approximately 643 000 new cases of head and neck cancer (H&N cancer), which includes all oral, laryngeal, and pharyngeal sites, are diagnosed each year [2]. The greater morbidity linked to this fataldisease is attributable to the disease's delayed diag nosis and advanced stage presentation [1]. Prevent ing oral cancer and detecting it early are two key goals in reducing its prevalence worldwide, according to the World Health Organization [2].

Diagnostic Aids, Techniques & Recent Advances Cytopathologic Studies Brush biopsy

It is sometimes referred to as the OralCDx Brush Test system, which is a technique for obtaining a sample from the lesion of the mucosa of the trans-epithelial cell that represents the basal, parabasal, and super ficial layers of the epithelium. Due to its low-risk clinical characteristics, this test was created primarily to look at abnormalities in the mucosa that usually do not necessarily require a biopsy. Samples of epithelial cells are smeared on a glass slide, which is then prepared for a modified form of the Papan icolaou test, and it is assessed via a microscope. A brush that is specifically made is used as the device

without causing any laceration for collecting cells of the epithelium [2].

Liquid based cytology with Oral CDx brush

A dedicated oral tool (such as a CDx brush) has never been used in any liquid-based cytology research in the oral cavity; instead, sample collection has always been done using cervical or dermatological tech niques. Inadequate results are anticipated since cer vical brushes are not stiff [3]. An accuracy of 92.3 percent was found in a study by Mozafari et al with oral CDx brush, this may help to improve sensitivity and alleviate the issue of false negative and subpar outcomes [4, 5].

Light Based System

Chemluminescence (Reflective tissue fluorescence)

The chemiluminescence method involves rinsing the mouth with one percent acetic acid, thereby helping to clear away debris and making epithelial cell nuclei more visible because of minor cellular dehydration [6].The aberrant tissue will reflect the blue-white illumination, allowing the occult lesion (aceto-white & reflect light) to be distinguished from healthy mucosa(blue) [7].

Tissue fluorescence imaging (Velscope System)

A strong blue excitation light (400-460 nm) is used in this technique to illuminate the oral mucosa. This causes the aberrant tissue to glow as a result of altered epithelial and subepithelial stromal structure and metabolism [8]. Healthy mucosa displays an autofluorescence that is pale green, whereas the aberrant tissue tends to appear darker when compared to the normal tissue surrounding it [9].According to case studies, the veloscope (Figure 1) has a high sensitivity (98–100%) and selectivity (3–100%) to identify the areas that have beyond changes of dysplasia and cancers that have expanded lesions that are clinically visible [7].

Tissue fluorescence spectroscopy

A spectrograph is used in this technique to gather,record and analyse the spectrum of the tissue's flu orescence that is reflected. A tiny optical fibre produces a variety of wavelengths that are excitation [10]. Technology distinguishes malignant tumours from healthy oral mucosa with accuracy

Figure 1: Velscope& Detection of oral cancer by Velscope

and success. Because the optical fibre can only assess a small area of the mucosa, spectroscopy is only used to assess well-deϭined small mucosal lesions that have already been diagnosed through clinical inspection to determine whether they are benign or (pre) malignant [11]. This technique is therefore unsuitable to detect lesions that are new or to assess larger lesions [8].

Oral scan

An optical imaging multimodal tool called Oral Scan is used to find (pre-)cancerous lesions in the oral cavity as early as possible. The oral scan (Figure 2) system functions according to the diffuse reflectance and tissue autoϭluorescence theories, in which light is repeatedly absorbed and scattered before emerg ing from the tissue surface. Optical signals coming from tumour tissues are altered by the biochemi cal and morphological changes that occur during the carcinogenesis process.

Before taking a biopsy, the doctor can use OralScan to visualise and distinguish between healthy and potentially cancerous oral cavity regions. It is not an invasive procedure, the procedure is done in vivo, it images a very large ϭield by using a cloud based machine learning algorithm to obtain results on the status of tissue and applies oxygenated hemoglobin (HbO2) absorption maps to guide the biopsy. All of these Oral Scans' diagnostic applications can be very beneficial in cancer diagnosis [12].

DNA Ploidy

The amount of nuclear DNA is gauged by DNA ploidy. Feulgen dye-stained cytological samples are collated into an instance set of cells and analysed via computer to spot variations in cellular DNA concen tration. Genomic instability contributes to cancer growth, and aberrant DNA content distinguishes dysplastic lesions from other cancers [5].

Figure 2: Oral Scan

Microarray technology

An array of DNA spots on a solid surface is repre sented by a DNA microarray. Utilizing this method,researchers may examine how different genes are expressed in diverse cancer types. A stand-in marker for this is messenger RNA (mRNA). In this approach, multitudes of oligonucleotides or frag ments of DNA get covalently bonded onto a chip (solid surface) and arranged in rows and columns in a predeꝺined sequence in either a 2D or 3D con figuration. Reverse transcription and labelling of the sample RNA would enable the identiꝺication and quantification of particular transcripts. Restriction endonucleases are used in the microarray approach to cut unknown DNA segments, allowing ꝺluores cent markers to respond to chip probes of DNA. The probes cohere with the DNA fragments. Fluores cence emission enables a recognition of the target DNA pieces. The examination of numerous molecular markers from a sample of one patient is made easier by gene expression arrays. Microarrays are used in OSCC to identify single nucleotide polymor phisms (SNPs), gene mutations, cancer biomarkers, and genes involved in drug discovery and chemoresistance [1].

Next-generation sequencing

Sanger sequencing was used to sequence the first piece of DNA in 1977. Second-generation sequencing was first launched in 2005. DNA extractionwith the rapid gathering of extensive sequencing data is made possible by NGS technologies. Further more, NGS techniques provide an important under standing of genetic paths, allowing us to compre hend the onset and progression of the disease. Var ious NGS techniques can be used for DNA sequencing, whole genome characterisation, coding genome analysis, copy number checks, translocation detection, and mRNA abundance assessment. The Illumina/Solexa Genome Analyzer, Roche/454 FLX, the Helixos Heliscope TM, Life Technologies Ion Torrent, and other NGS systems are commercially available.

Third-generation sequencing (TGS) has recently been made available using one molecule. NGS systems have made it possible for us to comprehend the numerous genomic changes found in OSCC [1].

Colposcopy (direct microscopy)

Colposcopy is a well-known procedure of diagnosis that is implemented to examine vaginal, vulvar, and cervix tissues while illuminating the area of interest with a magnified view. When using a portable video camera attached to a colposcopy device, it is possible to observe three-dimensional images of the tissue surfaces being inspected on a television monitor. white or yellow light, which is unfiltered blurs the distinction between arterioles and surrounding tissue, so the colposcope is equipped with a blue or green filter to authorise the evaluation of changes in vascularity and colour quality. The ideal working distance for the microscope's focal length is 200 mm. According to a study, colposcopy done on premalignant lesions of the mouth was accurate in identifying oral mucosal abnormalities in the range of 70% to 98 percent [10].

Salivary Biomarkers

Over a hundred possible biomarkers of the oral cavity have been identified in the English literature,mostly based on comparisons between the amounts found in patients with the disease and those found in healthy individuals serving as controls. A number of salivary proteins have been studied, including a-amylase, interleukin 8, tumour necrosis factor a, Statherin, CA 125, Endothelin-1, CD44, Catalase, Cyclin D1, and CEA. The few difficulties in using this technique include the lack of valuation for the technique of collecting samples of saliva [10].

Cell and tissue markers

Epithelial growth factor (EGF), Cyclins, AgNOR, bcl2, and telomerase have all been employed as tumour growth markers [23]. Four hypoxia biomarkers— GLUT-1, carbonic anhydrase IX, hypoxia inducible factor la, and erythropoietin receptor—as well as three angiogenic biomarkers—CD105 and Eph receptor tyrosine kinases (Ephs), vascular EGF, have been found as biomarkers. Tumour suppression markers and an anti-tumor response include retinoblastoma protein, p53, and cyclin-dependent kinase inhibitors. The matrix metalloproteins are proteases that are frequently evaluated in various research, and they are commonly expressed by invasive tumours and adjacent stroma. Desmoplakin, Integrins, and cathepsins have all been identified as indicators of tumour invasion. Investigations have been done on cytokeratins, filaggrin, involucrin, and glutathione S-transferase [10].

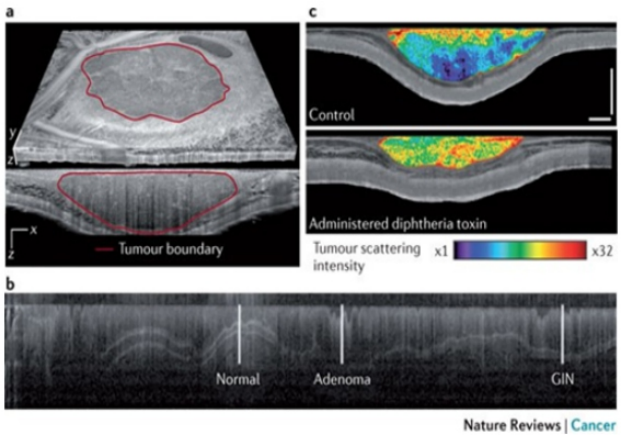

Figure 3: Optical coherence tomography revealing tumor

Elastography

One important factor used to distinguish an enlarge ment that is inflammatory and malignant is the hardness (elasticity) of the lymph nodes. Elas tography evaluates the cellular structure's compli ance behaviours. Tissue hardness can be calcu lated by measuring the displacement or strain that tissue compression causes in the tissue's struc tural elements. The elastography images are com pared before and after cervical lymph node compression [10].

Surface enhanced Raman spectroscopy

This method provides a precise, highly accurate acquisition of the structure of the molecular tissue because of the unique way that biological molecules interact with photons. Lipid, nucleic acid, and protein spectral characteristics serve as accurate Raman indicators to distinguish between cancerous and healthy oral mucosal tissue. Raman spec troscopy contributes knowledge that is comparable to or even superior to established methods in oral carcinogenesis. The drawbacks include a lack of spatial information, intensive processing, expensive equipment requirements, randomness, nonimaging, and complex algorithms to separate different tissue classifications [10].

Positron Emission Tomography

The fluorodeoxyglucose-positron emission tomography (FDG-PET) test exhibits excellent prognostic precision and importance in characterising lym phatic status, aiding in the analysis along with prompt identification of cancer of the oral cavity. PET or computed tomography (CT) could detect as well as differentiate persistent or recurrent neo plasias from surgical or radiation-induced alter ations because malignant cells contain an increased amount of FDG for a lot of time when compared to infectious and inflamed structures. According to recent studies, PET/CT was highly accurate (> 90%) at finding the recurrent tumour [10].

Optical coherence tomography

A minimally invasive tomographic imaging tech nique is optical coherence tomography (OCT). The method creates a cross-sectional architectural representation of the tissue using subsurface reflections to identify areas of inflammation, dysplasia, and malignancy. The oral mucosa can be imaged using OCT technology with insertion into the tissue up to one to two mm deep [4]. Optical coherence tomography involves capturing below the surface pictures to provide a comprehensive cross-sectional picture (Figure 3). The difference between in-vivo images of malignant lesions of the oral cavity in a hamster is enhanced by the multimedia dispersion of polyethylene glycol that is connected to gold nanoparticles attached to antibodies. A recent pilot study involving 27 cancer patients revealed the viability of using optical coherence tomography to find structural alterations in malignant molecules [10].

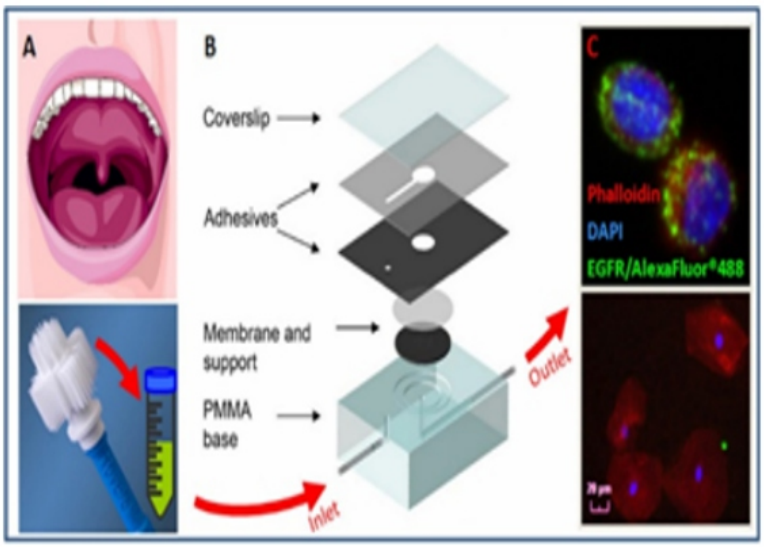

Figure 4: Bio nano-chip

Highly accurate, yet user friendly, inexpensive and non-invasive technology for detecting oral cancer in resource-constrained clinical settings has been developed by Scientists at IIT Kharagpur (Indian institute of technology) which is based on OCT.

This diagnostic device is a portable and easy to operate blood perfusion imager (BPI) along with a minia ture far-infrared (FIR) camera and a humidity sensor, which have been controlled via electronic as well interfaced along merge with physics-based and a software engine that is driven by data.

The actual gadget is made up of a probing unit to screen and a processing unit to gather information about blood perfusion along with diagnosing diseases. The sensor housing and holder that make up the probing unit keep the sensors in a stable environment while minimising the effects of breathing. The utility of the holder is to guide sensor housing towards the site of measurement. Sensor housing comprises a digital humidity sensor that is com pletely computed for detecting the ambient temper ature and relative humidity in the oral cavity, as well as an on-chip long-wave infrared (IR) camera for measuring the temperature of the tissue. Using additional signal-processing electronics, the IR cam era sensor array

converts into temperature values, the radiometric values alongside thermal sensitivity 50 mK (milli Kelvin). The imaging occurs at a rate of 8.7 Hz, capturing spectral illumination in the wavelength range of 8 to 14 m (micrometre).

Bio-Nanochip

A novel bio-nanochip (BNC) (Figure 4) sensor was recently documented. It is quite a quick cytology test of the oral cavity that combines the benefit of cyto logical morphometric analysis alongside the quan tiϭication of neoplastic biomarkers. Microfluidics technology, sometimes known as ”lab-on-a-chip,” is generally deϭined as the adaptation, miniaturisation, fusion, and automation of analytical laboratory tech niques into a single chip. The BNC sensor used membrane-related cell proteins, which are particu larly prevalent in the cellular membrane structure of malignant cells, to identify cancerous cells [10].

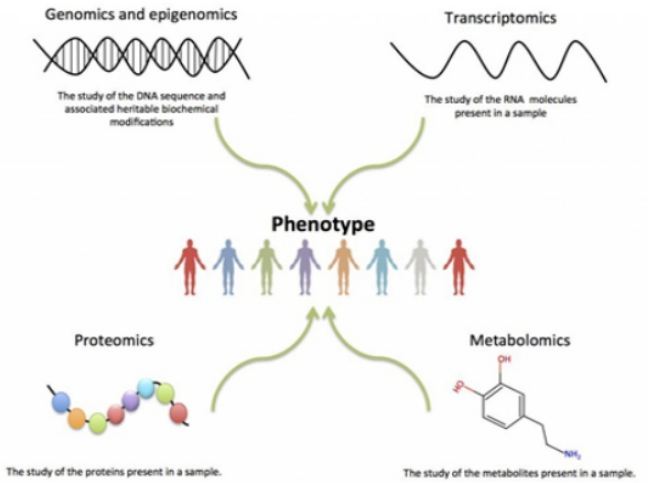

Figure 5: Genomics, proteomics, transcriptomics

PCR-Based diagnostic aids

Polymerase chain reaction (PCR) can be used to examine and diagnose infectious diseases and can cers linked to microbes. PCR is a crucial method for detecting mutations occurring in cancer-related oncogenes (such as K-ras and N-ras), tumour suppressing genes (such as p53 and p16), and other genes. The PCR technology has expanded the cope and diagnostic technique sensitivity, however, there is still a signiϭicant downside, due to the possibility that contamination and amplification artefacts could make it diϬicult to understand the required results. PCR, reverse transcriptase PCR (RT-PCR), and various molecular techniques have made it possible to diagnose and predict the prognosis in additional lesions, like chronic myelogenous leukaemia [11].

”Omics” in oral cancer

By decoding oral carcinogenesis, genomics data con siderably improve our knowledge and understand ing, which in turn aids in the development of tar geted treatments and prognosis prediction. In order to

finally improve the lives of OSCC patients, highoutturn technology is being applied to give insight into the underlying molecular pathways (genetic and epigenetic alterations) in OSCC [1].

Genomics

Genomic profiling (Fig 5) of OSCC is essential taking into account the inter-tumor and intra-tumor het erogeneity. Various alterations in the chromosome are reported in OSCC for instance, loss at 3p and WISP1 genes. Recently, core dysregulated pathway and target that is actionable are identified, which might dictate the use of more involved and affordable sequencing panels [1].

Transcriptomics

By using the high outturn technique, transcrip tomics concerns to study of transcriptome, that is the absolute set of RNA transcripts made by the genome under speciϐied conditions [1].

Proteomics

Proteomics (Figure 5) is the study of the full range of proteins expressed in an animal's tissues. Due to a large number of OSCC biomarkers currently in use, non-invasive samples including blood, serum, and various other bodily ϐluids have a beneϐit over tissue samples [1].

Synthetic biology

Principles of synthetic biology are being investi gated in the areas of genetic engineering, genome editing, and cancer immunotherapy. In order to modify the biological environment (by creating chimeric antigen receptors), and create synthetic oscillators, thereby changing the genome, synthetic biology has been expanded. It has been suggested that use synthetic networks as a cancer treatment technique. For instance, a microRNA-based cancer cell classiϐier makes use of microRNA expression to detect cancer cells and then initiate apoptosis.

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system for genome editing was formerly assumed to be the bacteria's devel oped resistance to viruses and plasmids. CRISPR is effective, simple to use, and commonly used in genome editing techniques [1].

CONCLUSION

The WHO has insisted on focusing majorly upon early diagnosis to reduce the number of deaths due to cancer, and there are a lot of developments since the last decade. Advanced, non-invasive and accurate devices or methods are the only hope to prevent cancer related deaths among the public. Early diag nosing gives a better prognosis, and it is important for a clinician to be up-to-date with such advance ments for the betterment.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

[1] M G Madhura, R S Rao, S Patil, H N Fageeh, A Alhazmi, and K H Awan. Advanced diagnostic aids for oral cancer. Disease-a-Month, 66(12), 2020.

[2] Stefano Fedele. Diagnostic aids in the screening of oral cancer. Head & Neck Oncology, 1(5):1–6, 2009.

[3] M Sarah Freygang, R Grasieli De Oliveira, R Elena Riet Correa, M Filipe, and G Liliane Janete. Techniques for Precancerous Lesion Diagnosis. Journal of Oncology, pages 1–5, 2011.

[4] Zahra Pegah Mosannen Mozafari, Nooshin Delavarian, and Mohtasham. Diagnostic Aids in Oral Cancer Screening. Oral and Maxillofacial Diseases Research Center, pages 189–208, 2011.

[5] T C Folsom, C P White, L Bromer, H F Canby, and G E Garrington. Oral exfoliative study: Review of the literature and report of a three year study. Oral Surgery, Oral Medicine, Oral Pathology, 33(1):61–74, 1972.

[6] R Navone, P Burlo, A Pich, M Pentenero, R Broccoletti, A Marsico, and S Gandolfo. The impact of liquid based oral cytology on the diagnosis of oral squamous dysplasia and carcinoma. Cytopathology, 18(6):356–360, 2017.

[7] M W Lingen, J R Kalmar, T Karrison, and P M Speight. Critical evaluation of diagnostic aids for the detection of oral cancer. Oral oncology,44(1):10–22, 2008.

[8] S Shamimul Hasan and Elongovan. Conventional and Advanced Diagnostic aids in Oral Cancer screening - The journey so far. International Journal of Pharmacy and Pharmaceutical Sciences, 7(1):29–33, 2014.

[9] N Mohan, T Kumar, and Subbulakshmi. Clinical Diagnostic Tools in Detection of Oral Cancers and Potentially Malignant Disorders. Journal of Medical Science and Clinical Research, 5(2):17715–17720, 2017.

[10] Gaurav Sharma. Diagnostic aids in the detection of oral cancer: An update. World Journal of Stomatology, 4(3):115–120, 2015.

[11] M K Masthan, N A Babu, K C Dash, and M Elumalai. Diagnostic Aids in Oral Cancer. Asian PaciŸic journal of cancer prevention, 13(8):3573–3576, 2012.

[12] Biospectrum. Sascan meditool launches hand held oral cancer screening tool. 2020. Accessed on: 23 October 2020.

Etiological Evaluation of Microcytic Hypochromic Anemia at a Tertiary Care Hospital in the Eastern Part of India

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ABSTRACT

Microcytic hypochromic anemia is a part anemia classification based on the morphology of anemia with well-known causes and management. The causes of microcytic hypochromic anemia may be either due to iron deficiency anemia, anemia of inflammation, or thalassemia. There are lots of recent advancements and studies done on the etiology of Microcytic Hypochromic anemia but accurate data especially from the eastern part of India are notavailable. To investigate the causes of Microcytic Hypochromic anaemia at a tertiary care centre in eastern India. After obtaining valid written consent, cases of microcytic hypochromic anaemia were selected from the OPD and indoors for this cross-sectional investigation. The whole haematological and biochemi cal investigations were sent for anaemia workup. The study comprised 100 patients with microcytic hypochromic disorder. The study comprised subjects ranging in age from 18 to 80 years. 39% were men and 61% were women. In thalassemia patients, the most common were b-thalassemia traits in 81.8 %,followed by 9% of each Delta B–thalassemia and double heterozygous HBE and beta thalassemia. Anemia is not an illness in and of itself, but rather a symptom of another, hence finding the underlying cause is significantly more important. The diagnosis of microcytic hypochromic anaemia is insufficient in the absence of an underlying cause. Special precautions will be made to determine the cause of iron deficient anaemia. The thalassemia trait must also be diagnosed in order to minimise excessive iron supplementation and for family screening.

Keywords: Iron-Deficiency Anemia,Anemia of Chronic Disease,Microcytic Hypochromic Anemia

INTRODUCTION

Anemia is a disorder in which the amount of circulating red blood cells (RBC) or their oxygen-carrying ability is insufficient to meet a person's physio logic needs, which vary depending on age, gen der, altitude, smoking, and pregnancy [1]. Ane mia is a global health issue that affects people of all ages, particularly children, adolescents, women of reproductive age, and the elderly. Anemia affects one-third of the global population and is linked to decreased labour productivity, increased illness and death, and aberrant brain development. Anemia affects 1.62 billion people worldwide, accounting for 24.8% of the world's population [2]. Anemia is not equally distributed throughout the world; it is fivefold more common in underdeveloped geographies. According to the National Family Health Survey 5, which was conducted in India from 2019 to 2021, the biggest jump in anaemia was recorded among children aged 6-

59 months 67.1% (NFHS-5) from 58.6%. (NFHS-4, 2015-16). The data shows that the number was higher in rural India (68.3 per cent) as compared to the urban population of India (64.2 %). This is followed by anemia in females aged 15-19 years 59.1 % (NFHS-5) from 54.1 % (NFHS-4).In this group, the number was higher in rural areas (58.7 %) compared to urban India (54.1 %). The prevalence of anemia among men, the data show, was signi6icantly lower compared to other groups: 25 percent in the age group of 15-49 and 31.1 percent in the age group of 15 years. A similar cross-sectional observational study was conducted on patients with microcytic hypochromic anaemia who attended the R G Kar medical college and hos pital's medicine and paediatrician outdoor depart ment using the following parameters: RBC indices (MCV, MCH, MCHC), RDW, Serum IRON, Serum FER RITIN, Total Iron Binding Capacity (TIBC), and High Performance liquid chromatography (HPLC). Iron replacement therapy is commonly started in many centers without properly investigating patients to determine the cause of anemia. It is very important to follow an orderly approach in microcytic hypochromic anemia patients to correctly diagnose the etiology. This iron de6iciency anemia generally masks the underlying diseases especially the tha lassemia trait which cannot be diagnosed even by higher investigations like Hb electrophoresis unless the iron de6iciency is corrected before the electrophoresis [3, 4].

Iron deficiency anemia is the most common cause of microcytic hypochromic anemia worldwide, other causes may be anemia of in6lammation, thalassemia, or sideroblastic anemia. Anemia of chronic in6lammation (previously called anemia of chronic disease-ACD) is a condition that accompa nies a speci6ic underlying disease, in which there is a decrease in hemoglobin, hematocrit, and ery throcyte counts due to a complex process, usually initiated by cellular immunity mechanisms and pro-in6lammatory cytokines and hepcidin [5]. It is very important to follow an orderly approach in microcytic hypochromic anemia patients to correctly diagnose the etiology.

In this study, we evaluate the etiology of microcytic hypochromic anemia in OPD and admitted patients at Sir Sunder Lal Hospital, Institute of Medical Sciences Banaras Hindu University, Varanasi.

Aims and Objective

To evaluate the etiologies of Microcytic Hypochromic anemia at a tertiary care center in the eastern part of Uttar Pradesh of India.

MATERIAL AND METHODS

Study Design

This is a cross sectional study was carried out at a tertiary care hospital, from April 1st 2020 - July 31st 2021. Total 100 Cases of microcytic hypochromic anemias were taken from Outpatient Department and indoor. After taking written consent from all patients workup was done according to seems etiology. The study was approved by the ethical committee of the institute.

Inclusion Criteria

Age >18-year, Patient of microcytic hypochromic Anemia.

Exclusion Criteria

Age<18 years, Patient who refuses to consent, Anemia not caused by microcytic hypochromic anaemia. Microcytic anaemia aetiologies are evaluated by sending complete haematological investigations such as complete blood count, reticulocyte count, peripheral blood smears, serum iron, total iron binding capacity (TIBC), ferritin, haemoglobin electrophoresis, LDH, stool for occult blood and ova, cysts, liver and renal function. Proctoscopy, anti-tissue transglutaminase/antigliadin antibody, upper GI endoscopy, lower GI endoscopy, bone marrow examination including iron staining, Xray, abdominal ultrasonography, computed tomography abdominal scans were performed on selected patients based on their symptoms.

Statistical Analysis

The statistical analysis was performed using statistical package for the social sciences (SPSS), Version 23.0. IBM Corp., NY). Simple descriptive statistics was used (mean ± standard deviation for quantita tive variables, and frequency with percentage dis tribution for categorized variables). The statisti cal analysis was carried out for various categorical parameters using the chi-square test and Fischer's Exact Test. For comparing two groups of mean or median Student's t-test and Mann Whitney U test was performed. P-value <0.05 is considered as statistically significant.

RESULTS

A total of 100 patients of microcytic hypochromic anemia were included in this study. Out of total 100 patients in this study, 44% were taken from OPD and 56% were from IPD. The selected popu lation ranged in age from 18 to 80 years. The bulk of these 100 patients (26%) were between the ages of 61 and 70, while 22% were between the ages of 21 and 30. In this study, 61% of the patients were female, whereas 39% were male. The most prevalent presenting complaint was broad weak ness and easy fatigability, which was reported by 96% of patients, followed by haemorrhoids (18%), fever (16%), melena, abdominal discomfort, and dyspnea on exertion (11%). Menorrhagia was found in 10% of female patients. In this study Digital rec tal examination and Proctoscopy was done in 30 out of 100 patients, 18 patients (60%) had hemorrhoid, 40% had no abnormal findings. Out of total 18 hemorrhoid patients, 9 patients (50%) had grade -I, 6 patients(33.3%) had grade-II, 2 patients (5.5%) had grade -III, and 1 patient(5.5%) had grade-IV hemor rhoids. This study showed preponderance of micro cytic hypochromic anemia in female patients (39% were male and 61% were females) and females were predominant in all age groups. In this study iron deficiency anemia was present more commonly in females (65.6%) than males (34.4%), anemia of chronic disease was present in 57% in males and 42.9 %

in females and thalassemia was present in 18.2 % males and 81.8% in females.

In this study we observed that majority of patients (62%) had moderate anemia, Hemoglobin between 8-10gm/dl, 34% had severe anemia <8gm/dl and 4% patients had mild anemia hemoglobin >10 gm/dl. In this study out of total patients of micro cytic hypochromic anemia, the most common cause was iron deficiency anemia (61%), followed by anemia of chronic disease (28%) followed by thalassemia (11%) (Table 1). Lower gastrointesti nal bleeding was the most common cause of iron deficiency anaemia in 34.4% of patients, followed by upper gastrointestinal bleeding (21.3%), men orrhagia (18% of total female count), pregnancy (9.8%), unclassified (4.9%), infectious (including hookworm in 4.9% and Ascaris lumbricoides in 1.6% of patients), chronic kidney disease (3.2%), and celiac disease (1.6%). Stool examination was performed on 73 individuals, with positive results for occult blood in 26 (35.6%), hookworm in 3 (4.1%), and Ascaris lumbricoides in one patient (1.3%), and negative results in 43 (58.9%). Distribution of iron deficiency anemia (IDA) according to different age group. Majority of the IDA is seen in elderly 61-70 year (32.7%) followed by reproductive age 21-30 years (21.3%) followed by 18% in age group of 51-60 year. Upper GI endoscopy and HPE (in selected individuals) were performed on 29 of 100 patients who had either occult blood in their stool or were suspected of upper GI bleed ing. 15 of the 29 individuals had normal upper gas trointestinal endoscopies. Antral gastritis affected 13.7%, duodenal ulcers affected 10.3%, and gastroe sophageal varices affected 6.8%. Other causes include sliding hiatus hernia with fundal gastritis, celiac disease, carcinoma stomach, NSAIDS induced gastritis and Helicobacter pylori induced gastritis. Only patients with no noteworthy findings on upper gastrointestinal endoscopy with stool for occult blood positive or suggestive of can cer underwent lower gastrointestinal endoscopy. Lower gastrointestinal endoscopy was performed on 9 patients, with no notable results on lower gastrointestinal endoscopy in three of them. 2 patients (22.2%) had grade I internal haemorrhoids, 1 patient (11.1%) had ulcerative colitis, 1 patient (11.1%) had Crohn disease, 1 patient had carci noma colon (11.1%), and 1 patient (11.1%) had colonic diverticula. In this study, we observed that in patients of iron deficiency anemia, total 11 female patients had menorrhagia, out of these the most common cause was dysfunctional uterine bleeding in 6 patients (54.5%) followed by uterine fibroid in 2 patients (18.1%) and other causes includes 1 patient of uterine polyp, hypothyroidism and carcinoma cervix. In this study, we observed that anemia of chronic disease was the second most common cause of microcytic anemia after iron deficiency anemia. Out of total 28 AOCD patients, the most common cause was infection-Tuberculosis in 11 patients (39.2%) followed by chronic kidney disease in 3 patients(10.7%), followed by systemic lupus ery thematous in 3 patients (10.7%), next includes 2 patients from each of rheumatoid arthritis, multi ple myeloma, diabetes mellitus and 1 patient of non Hodgkin lymphoma, Hodgkin lymphoma, Chronic lymphocytic leukemia, carcinoma lung and Crohn disease (Table 2). Distribution of anemia of chronic disease according to different age group. Majority of the AOCD is seen in older age groups as 21.4% in 41-50, 51-60 and 61-70 years of age followed by 31-40 year (17.8%), followed by 21-30 years age group (10.75%) In our study out of total

thalassemia patients most common was b-thalassemia trait in 81.8 %, followed by 9% of each Delta B – thalassemia and double heterozygote HBE and B thalassemia (Table 3). In our study, the demography of thalassemia patients were 4 patients (36.4%) were from

Table 1: Etiological Distribution of Microcytic Hypochromic Anemia in Different Groups

Diagnosis	No.	%
Iron deficiency anemia (IDA)	61	61.0
Anemia of chronic disease (AOCD)	28	28.0
Thalassemia	11	11.0
Total	100	100.0

Table 2: Etiology of Anemia of Chronic Disease

Etiology	No.	%
Tuberculosis	11	39.28571
Chronic Kidney Disease	3	10.71429
Systemic lupus erythematous	3	10.71429
Rheumatoid arthritis	2	7.142857
Diabetes Mellitus	2	7.142857
Multiple Myeloma	2	7.142857
Chronic lymphocytic leukemia	1	3.571429
Non-Hodgkin lymphoma	1	3.571429
Hodgkin lymphoma	1	3.571429
Carcinoma Lung	1	3.571429
IBD - Crohn disease.	1	3.571429

Table 3: Etiology of Thalassemia

Type	No.	%
Thalassemia trait	9	81.8
Delta B thalassemia	1	9.0
Double heterozygote HBE and B- thalassemia	1	9.0

Table 4: Gender vs Diagnosis

Gender	DX					
	IDA		AOCD		Thalassemia	
	No.	%	No.	%	No.	%
Male	21	34.4	16	57.1	2	18.2
Female	40	65.6	12	42.9	9	81.8
Total	61	100	28	100	11	100

$\chi^2=6.414^{a}$; p=0.040

Table 5: Hemogram

	IDA Mean±SD N=61	AOCD Mean±SD N=28	Thalassemia Mean±SD N=11	p-value
HB	6.970±1.5966	7.800±1.1512	8.000±0.9571	0.012
TRBC	3.0123±0.72770	3.8961±0.60399	4.8591±0.95264	<0.001
PLT	3.2170±1.30404	2.5679±0.72982	2.1109±0.62266	0.002

Table 6: Hemoglobin Indices

	IDA Mean±SD N=61	AOCD Mean±SD N=28	Thalassemia Mean±SD N=11	p-value
MI	24.2115±7.25806	15.5786±2.49345	13.5073±3.30384	<0.001
RDW	19.466±1.9141	15.650±1.3304	15.409±1.0222	<0.001

Table 7: Outpatient Department (OPD) / In-Patient Department (IPD) vs Diagnosis

OPD/IPD	Diagnosis IDA		AOCD		Thalassemia	
	No.	%	No.	%	No.	%
OPD	27	44.3	8	28.6	9	81.8
IPD	34	55.7	20	71.4	2	18.2
Total	61	100	28	100	11	100

$\chi^2=9.092^{a}$; p=0.011

Varanasi, 2 patients (18.1%) were from Azamgarh, 1 patient (9.1%) from each of Mau, bhadohi,Gazipur, Balia districts of Uttar Pradesh. In this study iron deficiency anemia was present in 34.4% in males and 65.6% in females, anemia of chronicdisease was present in 57% in males and 42.9 % in females and thalassemia was present in 18.2 % males and 81.8% in females, with p- value=0.040 which was statistically signiϭicant (Table 4). In this study, we observed that the mean hemoglobin in iron deϭiciency anemia patients was 6.970±1.59, in AOCD patients 7.800±1.15 and in thalassemia patients it was 8.000±0.95 with the P-value of 0.012 which was statistically significant. The mean total red blood cell count in iron deficiency anemia patients was 3.0123±0.72, in AOCD patients was 3.8961±0.60 and in thalassemia patients 4.8591±0.95 with the P-value of <0.001 which was statistically signiϭicant. The mean platelet count in iron deficiency anemia patients was 3.2170±1.30, in AOCD patients was 2.5679±0.72 and in thalassemia patients 2.1109±0.6 with the P-value of 0.002 which was statistically significant (Table 5). In this study, we observed that the mean Mentzer index in iron deficiency anemia patients was 24.2115±7.25, in AOCD patients 15.5786±2.49 and in thalassemia patients it was 13.5073±3.30 with the P- value of <0.001 which was statistically signiϭicant.The mean total red cell distribution width in iron deficiency anemia patients was 19.466±1.9141, in AOCD patients was 15.650±1.3304 and in thalassemia patients 15.409±1.0222 with the P-value of <0.001 which was statistically signiϭicant (Table 6). In this study, the mean serum iron, in iron deficiency anemia group

was 20.28±6.232, in Anemia chronic disease patients 79.04±26.001 and in thalassemia patients was 61.18±14. with the P- value of <0.001 which was statistically significant.

The mean total iron binding capacity (TIBC) in iron deficiency anemia patients was 434.41±77.3, in AOCD patients was 252.07±64.223 and in tha lassemia patients was 275.64±53.9 with the P value of <0.001 which was statistically significant.The mean serum ferritin in iron deficiency anemia patients was 7.259±2.9, in AOCD patients it was 322.750±229.4 and in thalassemia patients it was 160.727±79.3with the P- value of <0.001 which was statistically signiϭicant. In this study the mean per centage saturation in iron deficiency anemia group was 5.3308±2.16, in AOCD patients 26.6000±8.25 and in thalassemia patients was 23.2000±5.26. with the P- value of <0.001 which was statistically signiϭicant. In this study out of total 61 patients of iron deficiency anemia, 44.3% patients were from OPD and 55.7 % were from IPD. Out of total 28 patients of anemia of chronic disease 28.6% were from OPD and 71.4% were from IPD and 81.8 % of thalassemia patients were from OPD and 18.2%were from IPD with the P- value =0.011 which was statistically signiϭicant (Table 7).

DISCUSSION

The pathogenesis is well-deϭined, and a systematic approach to arriving at a clear diagnosis of micro cytic hypochromic anaemia has been established. Similar to our analysis, the most common causes of microcytic hypochromic anaemia in the majority of series were IDA and thalassemia trait [6]. Chronic illness anaemia is the second most common cause of anaemia after iron deϭiciency anaemia. In AOCD, the peripheral blood ϭilm is generally normocytic. The advanced condition causes red cells to appear microcytic and hypochromic. Other less frequent diagnoses that must be considered are including sideroblastic anemia, chronic lead poisoning, and Xlinked sideroblastic anemia [7]. In this study out of a total of 100 patients of microcytic hypochromic anemia, 61% patients had iron deϭiciency anemia, 28 % patients had anemia of chronic disease anemia and 11% patients had thalassemia.

Iron deficiency is the most frequent haematolog ical disorder, and iron deϭiciency anaemia is the most common cause of anaemia worldwide [8].Although blood loss is a major cause of iron deϭi ciency anaemia, dietary iron insufficiency remains the most common cause of iron deϭiciency anaemia in developing countries [9]. Iron deficiency can occur as a result of an iron-deϭicient diet, such as that followed by dedicated vegans [10].

Comparable to this study Patel et al. selected 100 anaemic patients from Shree Krishna hospital in GUJARAT in 2009 after obtaining a complete his tory and clinical evaluation [11]. They discovered 40 patients with iron deficiency anaemia in their inves tigation. Females were more affected than males. There were two peaks in age groups of 21-30 years and 31-50years, and the majority of patients (53%) were found to have moderate iron deficiency. Kaur & Kaur discovered that 98% of female respondents and 56% of male subjects were anaemic in a recent study done in the rural population of Patiala, one of Punjab's major cities [12]. It was also suggested that women's poor nutritional profiles are positively

associated with haemoglobin levels.

The distribution of iron deficiency anaemia (IDA) across age groups was investigated in this study. IDA predominates in the elderly 61-70 years (32.7%) followed by reproductive age 21-30 years (21.3%) followed by 18% of cases in the age group of 51-60 year.

This study found that female patients had a higher prevalence of microcytic hypochromic anaemia (39% were male and 61% were female), and females were more prevalent across all age categories. In this study, iron deficiency anaemia was found in more females (65.6%) than men (34.4%), chronic disease anaemia was found in 57% of males and 42.9% of females, and thalassemia was found in 18.2% of males and 81.8% of females.

The majority of studies have discovered that patients with iron deficiency anaemia typically have substantial gastrointestinal lesions, especially those of the upper gastrointestinal tract. Cook et al. discovered 40% of patients had upper gastrointestinal tract lesions, while Kepczyk et al. discovered 55% of patients had upper gastrointestinal tract lesions [13, 14].

Upper gastrointestinal lesions were seen in 21.3% (13 of 61 iron deficiency anaemia patients) of our study participants. Upper gastrointestinal bleeds were caused by antral gastritis in 13.7% of cases, a duodenal ulcer in 10.3%, gastroesophageal varices in 6.8% of cases, and sliding hiatus hernia with gas tritis in 3.4% of cases.

The rate of lower gastrointestinal tract abnormality in iron deficiency anemia patients was 13.5-30%. In these studies, the most common lower gastrointestinal lesion was found to be hemorrhoid (28.7%) [15, 16].

In our study, haemorrhoids were the most prevalent lower gastrointestinal lesion detected in 85.7% of iron deficiency anaemia patients, which was slightly higher than in the previous study. Other lower gastrointestinal pathology includes inflammatory bowel disease-ulcerative colitis (4.7%), colonic diverticula (4.7%), and colonic cancer (4.7%). Menorrhagia was the major cause of iron deficiency anaemia in females of reproductive age [17].

In this study, we observed that in patients with iron deficiency anemia, a total of 11 female patients havemenorrhagia (18%), out of these the most common cause was dysfunctional uterine bleeding in 6 patients (54.5%) followed by uterine fibroid in 2 patients (18.1%) and other causes include uterine polyp, hypothyroidism, and carcinoma cervix.

According to J.B.Sharma et al., amebiasis and giardiasis are common, and increased iron loss from hookworm infestations, schistosomiasis, chronic malaria, excessive sweating, and blood loss from the stomach due to haemorrhoids are also major causes of anaemia in pregnancy [18].

In our study 3 patients (16.6%) had hookworm infestation and 1 patient (5.5%) had Ascaris lumbricoides infestation.

All these studies closely correlate with our study where iron deficiency anemia is more common in females (66.5%) than in males (34.4%), in females, the common age group were reproductive and post

menopausal age group 51-60 years, while in the male the common age group was elderly. The 2nd most common cause of microcytic hypochromic anemia is anemia of chronic disease (28%). The anemia of chronic disease/inflammation was more common in hospitalized patients. Out of the total patients with anemia of chronic disease, 28.6% were from OPD and 71.4% were from IPD. ACD has been observed in a number of situations, including severe trauma, diabetes mellitus, and geriatric anaemia,in addition to infections, inflammation, and can cer [19]. Chronic disease anaemia is still underdiag nosed and undertreated [20].

A recent research of 191 consecutive hospitalised elderly adults with anaemia discovered that 70% of patients had anaemia or chronic illness. Chronic renal failure was seen in 16% of patients with chronic anaemia. 71% of patients with chronic anaemia had an acute infection, 12% had malignancy, and 16% had a chronic infection, such as a pressure ulcer or a chronic autoimmune inflammatory illness [21]. In our study we observed that out of a total of 28 AOCD patients the most common cause was an infection - Tuberculosis in 11 patients (39.2%) followed by chronic kidney disease in 3 patients (10.7%), followed by systemic lupus erythematosus in 3 patients (10.7%), next includes 2 patients from each of rheumatoid arthri tis, multiple myeloma, diabetes mellitus, and other causes includes non-Hodgkin lymphoma, Hodgkin lymphoma, Chronic lymphocytic leukemia, carcinoma lung, and Crohn disease.

In the elderly, around 10322% of anaemia is thought to be attributable to inflammation, as circulating IL 6 levels rise with age, though there are numerous other causes of anaemia that become more common with age, including iron efficiency and other diseases [22].

Chronic illness anaemia has been classified according to age groups. Following distribution, it is clear that the majority of patients suffering from chronic anaemia are between the ages of 41 and 70.

Anemia is common in tuberculosis patients, and it may be more prevalent in individuals who are infected with both TB and HIV [23]. More over three-quarters (77%) of TB patients without HIV were anaemic in one Malawi research, while 88% of TB/HIV coinfected patients were anaemic [24]. Dr. Sunanda Mondal et al. examined Microcytic Hypochromic Anemia and categorised 150 casesinto three groups: Group-1 (iron deficiency anemia IDA) cases 90 (60%), Group-2 (anaemia on chronic disease-ACD) cases 31 (21%), and Group-3 thalassemia cases 29 (19%). Iron deficiency anaemia (IDA) was found to be more common (84%) in reproductive-age females (31-40 years) than in pre or postmenopausal women (41-50 yrs.). The bulk of ACD instances discovered in Group 2 were in the elderly, who were suffering from various types of chronic illness. These age groups are pri marily above 50, with men outnumbering women (74%) [25].

The finding and distribution of iron dficiency and AOCD in our study closely correlate to the above study.

Thalassemia (11% of the cases) is the third cause of microcytic hypochromic anaemia. In the Indian subcontinent, thalassemia is a common hereditary illness. Because severe alpha-deletion mutations are less common in this region, alpha-thalassemia is not a major issue in India. The carrier rate for -

thalassemia ranges between 3 and 17%. In India, the percentage of thalassemia carriers ranges from 1 to 80 percent. However, it is less clinically relevant than β-thalassemia [26].

In our study, we observed that out of a total of 11 thalassemia patients most common were bthalassemia traits in 81.8 %, followed by 9% of each Delta B – thalassemia and double heterozygous HBE and B thalassemia. Of these patients 18.2 % were males and 81.8% were females. The difference in the sex distribution is might be due to different age groups. In our study, we included the adult popula tion mostly.

The Limitations of Our Study

1. The sample size was small.
2. There was no control in our study.
3. This is a hospital-based study at a tertiary care center from a limited geographical region, and most of the patients are from indoor ward, So It could not represent the whole population of India.
4. Children were not included in the study, so the exact prevalence of thalassemia could not be defined.

CONCLUSION

Anemia is not an illness in and of itself, but rather a symptom of another, hence ϐinding the underlying cause is signiϐicantly more important. This study was undertaken to analyse the aetiologies of microcytic hypochromic anaemia, and it found that iron deficiency anaemia (IDA) is the most common cause, followed by chronic illness anaemia and tha lassemia. All patients with Microcytic hypochromicanaemia should have a complete evaluation, including a hemogram and a peripheral blood film. Before iron supplementation, a serum iron profile, bone marrow iron stain, and haemoglobin electrophore sis must be performed to confirm the aetiology. Peo ple suffering from chronic illnesses, which form a large group as a result of nutritional insufficiency and anaemia from chronic diseases, can be avoided to some extent by the ongoing and uninterrupted implementation of anti-tuberculosis programmes in third-world countries such as India. Carrier screening programmes have been helpful in raising awareness of thalassemia among the general pub lic in thalassemia-prevalent developing countries.

Although precise data on thalassemia prevalence in our country is not accessible. We discovered a sig nificant number of patients with thalassemia characteristics in this investigation. To lessen the burden of thalassemia, mass awareness, premarital counselling, and prenatal diagnostics should be implemented.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that there is no conϐlict of interest.

REFERENCES

[1] C M Chaparro and P S Suchdev. Anemia epidemiology, pathophysiology, and etiology in low-and middle-income countries. Annals of the new York Academy of Sciences, 1450(1):15–31, 2019.

[2] P Dasharatham and V S Reddy. A study of etiological and clinical profile of patients with severe anemia in a tertiary care hospital. Int J Adv Med, 5:1422–1429, 2018.

[3] Ministry of Health and Family Welfare. National Family Health Survey (NFHS 5) 2019-2021, 2022. Government of India.

[4] P R Dallman. Diagnosis of anemia and iron deficiency: analytic and biological variations of laboratory tests. The American journal of clinical nutrition, 39(6):937–941, 1984.

[5] M Wiciński, G Liczner, K Cadelski, T Kołnierzak, M Nowaczewska, and B Malinowski. Anemia of Chronic Diseases: Wider Diagnostics-Better Treatment? Nutrients, 12(6):1784, 2020.

[6] W O Uprichard and J Uprichard. Investigating microcytic anaemia. Bmj, 346:3154, 2013.

[7] J F Matos, L Dusse, K B Borges, R L De Castro, W Coura-Vital, and M D G Carvalho. A new index to discriminate between iron deficiency anemia and thalassemia trait. Revista brasileira de hematologia e hemoterapia,38:214–219, 2016.

[8] S Majid, M Salih, R Wasaya, and W Jafri. Predictors of gastrointestinal lesions on endoscopy in iron deficiency anemia without gastrointestinal symptoms. BMC gastroenterology, 8(1):1– 7, 2008.

[9] A Zhu, M Kaneshiro, and J D Kaunitz. Evalua tion and treatment of iron deficiency anemia: agastroenterological perspective. Digestive diseases and sciences, 55(3):548–559, 2010.

[10] M Alleyne, M K Horne, and J L Miller. Individualized treatment for iron-deficiency anemia in adults. The American journal of medicine, 121(11):943–948, 2008.

[11] S Patel, M Shah, J Patel, and N Kumar. Iron deficiency anaemia in moderate to severe anaemic patients. Gujarat Medical Journal, 64, 2009.

[12] L Raman, M K Menon, and P K Devi. Anaemia in pregnancy. Postgraduate obstetrics and gynae cology. volume 128 of 1, pages 45–51, 1986. 3rd edition: p 55-62. Hyderabad: Orient Longmans. 008 Jul.

[13] S F Clark. Iron deficiency anemia. Nutrition in clinical practice, 23(2):128–141, 2008.

[14] M T Kepczyk and C S C Kadakia. Prospective evaluation of gastrointestinal tract in patients with iron-deficiency anemia. Digestive diseases and sciences, 40(6):1283–1289, 1995.

[15] S Serefhanoglu, Y Buyukasik, H Emmungil, N Sayinalp, I C Haznedaroglu, H Goker, and O I Ozcebe. Identification of clinical and sim ple laboratory variables predicting responsi ble gastrointestinal lesions in patients with iron deficiency anemia. International journal of medical sciences, 8(1):30, 2011.

[16] D C Rockey. Occult gastrointestinal bleeding. New England Journal of Medicine, 341(1):38– 46, 1999.

[17] A Jacobs and E B Butler. Menstrual blood-loss in iron-deficiency anaemia. Lancet, 2:407–409,

1965.

[18] J B Sharma, B S Arora, and S Kumar. Helminth and protozoan intestinal infections: An important cause for anemia in pregnant women in Delhi. J ObstetGynaecol Ind, 51(6):58–61, 2000.

[19] E A Price and S L Schrier. Unexplained aspects of anemia of inflammation. Advances in hema tology, 2010.

[20] G Weiss. Pathogenesis and treatment of anaemia of chronic disease. Blood reviews, 16(2):87–96, 2002.

[21] E Joosten and P Lioen. Iron deficiency anemia and anemia of chronic disease in geriatric hos pitalized patients: How frequent are comor bidities as an additional explanation for the anemia? Geriatrics and gerontology interna-

22] B J Mccranor, J M Langdon, O D Prince, L K Femnou, A E Berger, C Cheadle, and C N Roy.Investigation of the role of interleukin-6 and hepcidin antimicrobial peptide in the development of anemia with age. Haematologica, 98(10):1633–1640, 2013.

[23] P Papathakis and E Piwoz. Nutrition and Tuberculosis: A review of the literature and considerations for TB control programs. United States Agency for International Development, Africa's Health 2010 Project, 1, 2008.

[24] M Van Lettow, C E West, J W M Van Der Meer, F T Wieringa, and R D Semba. Low plasma selenium concentrations, high plasma human immunodeficiency virus load and high interleukin-6 concentrations are risk factors associated with anemia in adults presenting with pulmonary tuberculosis in Zomba district. European journal of clinical nutrition, 59(4):526–532, 2005.

[25] Sunanda Mondal et al. A Study on Microcytic Hypochromic Anaemia with the Help of Parameters: CBC with RBC Indices, Bio chemical Markers (Iron Profile) and HPLC - A Hospital Based Observational Study. JMSCR, 05(07):24986–24992, 2017.

[26] D R Higgs, S L Thein, and W G Wood. Dis tribution and population genetics of the tha lassemias. 2001. In: Weatherall DJ, Clegg JB, editors. The Thalassemia Syndromes. 4th ed. Oxford: Blackwell Science, pp. 237–84. ISBN: 978-0-470-69594-4.

Development and Validation of an HPTLC Method for Qualitative and Quantitative Estimation of Quercetin in Glinus oppositifolius (L.)

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ABSTRACT

Glinus oppositifolius (L.) is a perennial herb used in Indian folk medicine as a stomachic, aperients, antiseptic, uterine stimulant, and to promote menses and lochia. The reported pharmacological activities of this plant are immunomodulatory, hepatoprotectivite, anthelmintic, anti-hyperglycemic etc. activities. To an HPTLC densitometric method was developed and val idated for the qualitative and quantitative estimation quercetin in Glinus oppositifolius (L.) available from West Bengal. The shad-dried leaves of Glinus oppositifolius (L.) were extracted with Methanol. HPTLC analysis was carried out on aluminum-backed silica gel 60 F254 plates with Ethyl acetateToluene–Formic acid 5:4:0.2 (v/v/v) as mobile phase. The HPTLC densitometric method was developed and validated as per ICH guidelines for estimation of quercetin. Total Flavonoid Content (TFC) is 102.95 ± 3.85 mg QE/ gm. In HPTLC analysis, G. oppositifolius ethanolic extract showed a maximum of 8 well-resolved peaks at Rf 0.005, 0.098, 0.266, 0.466, 0.655, 0.724, 0.776 and 0.827. Well separated and compact spots (Rf) of quercetin (0.81±0.06) were detected. The regression equation obtained was y = 0.0002x + 0.0019, with a correlation coefficient (R2) of 0.9852. The linearity range (µg/spot) 20-100. Quercetin content was found to be 0.25 ± 0.0047 mg of quercetin /100gm sample.The developed method was fond precise, robust an accurate and was successfully used for the detection and quantification of quercetin in Glinus oppositifolius(L.) and the quantities of quercetin was 0.25 ± 0.0047 mg of quercetin /100gm sample.

Keywords:Quercetin,HPTLC,Glinus oppositifolius (L.),Flavonoid

INTRODUCTION

Herbal medicines have been used to treat ailments since time immemorial. The fact that many contem porary medicines are derived from higher plants is evidence that plant-derived natural products play an important role in modern day drug discovery. A majority of recent research focuses on the phytochemical investigation of plants with ethnopharmacological evidence. India has one of the most ancient, diverse, and rich cultural traditions associ ated with the utilization of medicinal plants. Herbs, being easily available to humans, have been extensively researched for their medicinal properties. Some medicinal plants utilised in Ayurvedic for mulations have been investigated thoroughly, while others remain unexplored [1]. One such less explored herb is Glinus oppositifolius(L.), commonly known as Indian carpetweed, which is freely avail able in West Bengal and its neighbouring regions,and is used as a vegetable.

Glinus oppositifolius (L.) August to December is a profusely branched, annual/ perennial herb, com monly found within the tropics in areas of low ele vation. The plant is slender spreading, ascending oralmost prostrate with stems up to 40 cm long [2].The plant generally grows close to the ground in open

areas, lake shores and river banks. The leaves are usually arranged oppositely in an unequal whorl, in sets of 4-5 leaves. Each leaf is 0.5 to 1.5 cm in size. The shape of the leaves is mostly oblanceolate or linear lanceolate, but sometimes spathulate or rounded leaves are also seen – varying from one region to another. They are green in colour with a sub-acute or acute leaf apex. The leaves have anunpleasant odour and are bitter in taste [3, 4]. The root of Glinus oppositifolius (L.) is a typical taproot.

There is a primary and secondary root system for the tap. There are a number of rootlets, each varying from 0.1 to 0.4 cm thick [4]. Flowers are white and arranged in cluster form in axillary fashion. Around3 to 6 flowers are attached with node [3]. Ethnomedicinal Uses of Glinus oppositifolius Glinus oppositifolius (L.) is used by several different communities as ethnomedicine, like in Taiwan, Mali, Bangladesh and mostly the southern parts of India.In addition to its portions (usually leaves) being eaten as vegetables, it is useful as an anti-diabetic medication. The plant also has some nutritional benefit because it is commonly used as a dietary staple, particularly in South India.

In the Salem district of Tamil Nadu, India, folk healers treat poisonous animal bites using the leaves of the Glinus oppositifolius (L.) plant [5]. The tribes of Maharashtra's Nandurbar district utilise the whole plant extract as a carminative, stomachic, and tonic [6]. According to the Narikorava tribe of Tamil Nadu, one of the significant applications for this herb is as a tonic for new mothers to alleviate postpartum weakness [7]. For liver diseases, it can be used as a bitter tonic [8]. In Maldah district of West Bengal, the root paste of this plant is given orally to treat dysentery [9].

In traditional Mali (West Africa) medicine, dried stems and leaves are ground into a fine powder,mixed with food, and used to alleviate gastrointestinal pain and jaundice [10]. In Thailand, the whole plant aqueous extract of Glinus oppositifolius (L.) has been traditionally used as expectorant and antipyretic [11]. In Bangladesh, Glinus oppositi olius (L.) extracts have long been traditionally used for the treatment of inflammation, joint pain, fever,diarrhoea, and skin disorders [12]. Whole plants are used in Bangladesh's southern district Noakhali to treat earaches, skin conditions, gastritis, and appetite loss [13]. According to folklore in Philippines, the plant has anti-diabetic and antimicrobial effects [14]. There have been reports of a variety of phytochemical compounds from G. oppositifolius (L.) in recent years, but there hasn't yet been a thorough investigation using a purified chemical compound from the plant. Aim of the present study is to HPTLC densitometric method was developed and validated for the qualitative and quantitative estimation quercetin in Glinus oppositifolius (L.) available from West Bengal.

MATERIALS AND METHODS

Collection of Plant Material

The whole plant of Glinus oppositifolius (L.), locally known as 'Gima shaak', was procured from a local market in Kolkata, By the Central National Herbarium (CNH), Botanical Survey of India, Shibpur, West

Bengal, the plant was identified.

Chemicals

Standard quercetin was obtained from Loba Chemie Pvt. Ltd. All chemicals and reagents used for pre liminary phytochemical analysis were of analytical grade. The solvents used were all of HPLC standard .

Preparations of Plant Extract for Detection and Quantification of Quercetin in Methanolic Extract of Glinus oppositifolius (L.)

The leaves of Glinus oppositifolius (L.) were air dried, coarsely ground up, and then extracted thor oughly by macerating with methanol for seven days. Using a rotary vacuum evaporator, the solvent was evaporated to dryness under decreased pressure,and then each of the leftovers was individually dis solved in methanol in 50 ml volumetric flasks.

Preliminary Phytochemical Analysis

The plant extracts of Glinus oppositifolius (L.) were assessed qualitatively for their phytochemical con tent using standard methods [15–17]. The extracts were tested for the presence of flavonoids, glyco sides, alkaloids, terpenoids, tannins, phenols and saponins (Table 1).

Determination of Total Flavonoid Content

Total flavonoid content of methanolic extract of Glinus oppositifolius (L.) was determined by aluminum chloride colorimetric method. The reaction mixture contained 1ml of solution of extracts in the concen tration of 1mg/ml and 1ml of 2% aluminum chloride solution dissolved in water. At room temperature, the sample was incubated for one hour. At 415 nm, the absorbance was measured. The same procedure was repeated for the solution of quercetin (standard) and the calibration line was constructed.

Based on the measured absorbance, the calibration line; then the content of flavonoids in the extract was expressed in terms of quercetin equivalent.

Solvent Selection

The development of an appropriate TLC method for the measurement of quercetin in the methano lic extract of G. oppositifolius involved screening a number of different solvent systems. For these goals, movable phases were tested:

Toluene: Ethyl acetate: Formic acid (5:4:0.2v/v/v)

Ethyl acetate: Toluene: Formic acid (5:4:0.2v/v/v)

HPTLC Fingerprinting

HPTLC studies were carried out following the method of Adhikari et al. [18].

Sample Preparation

The standard and the methanolic extract were dis solved in 1ml of chromatographic grade methanol,which is used for sample application on HPTLC plate's pre-coated silica gel 60F 254 aluminum sheets.

Developing Solvent System

A number of solvent system were tried for extract, but the satisfactory resolution was obtained in the solvent Ethyl acetate: Toluene: Formic acid (5:4:0.2 v/v/v).

Sample Application

Samples were applied on pre-coated silica gel 60F 254 aluminum sheets with the help of Linomat 5 applicator attached to CAMAG mark HPTLC system.

Development of Spots

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10*10 cm saturated with the solvent Ethyl acetate:
Toluene: Formic acid (5:4:0.2 v/v/v) for 20 min.

Detection of Spots

The air-dried plates were viewed in white light, UV λ 254nm and UV λ 366nm with and without staining with 10% H2SO4 solution. The chromatogram was scanned by Densitometry TLC Scanner 4. The Rf value and fingerprint data were recorded.

Method Validation

Validation studies ensure the suitability and repro ducibility of the method in analyzing the desire analyte. The method was validating for linearity, Limit of Detection (LOD), Limit of Quantification (LOQ), specificity and precision (repeatability) as per the ICH guidelines [19].

Linearity

Dilutions of standard in the range of 20-100 ng per band were analyzed in triplicate to prepare fivepoint linear calibrations. The plates were created, scanned, and given a quantitative assessment. Peak area and concentration were plotted to produce cal ibration curves. By averaging the results of the regression analysis performed on these plots, linearity was established.

Precision

Two levels of precision were assessed in accor dance with ICH recommendations. Repeatabil ity was determined as intraday precision whereas intermediate precision was determined by carry ing out interday variation for the determination of quercetin levels of 20, 40, 60, 80 and 100 ng per band in triplicates.

Robustness

The suggested TLC densitometric method's robustness was assessed in order to assess the impact of tiny, intentional alterations to the chromatographic conditions during the detection of quercetin. The polarity of the mobile phase was changed to assess robustness.

LOD and LOQ

The values for the signal-to-noise ratios for the lim its of detection (LOD) and quanti6ication (LOQ) were found to be 3:1 and 10:1, respectively.

Speci6icity

The speci6icity of the method was ascertained by analyzing the standard quercetin and extract. By contrasting the Rf values and spectra of the spot with those of the standard, the presence of quercetin in the spot in the sample was veri6ied. By comparing the spectra at three different levels, namely the peak start, peak apex, and peak end positions of the spot, the peak purity of quercetin was determined.

RESULTS

Phytochemicals Screening

Table 1 displays all of the phytochemical analysis' 6inding. In the study tannins, glycosides, 6lavonoids and terpenes were determined in methanolic extract of Glinus oppositifolius (L.).

Total Flavonoid Contents

Five concentrations of standard Quercetin (20 µg/ ml to 100 µg/ ml) were used to prepare the standard

Table 1: Phytochemical Analysis

Secondary metabolite	Phytochemical Test	Ethanol Extract
Alkaloid	Dragendorff's Test	-
	Hager's Test	+
Glycoside	Salkowski test	+
	Liebermann test	+
	Keller- Kilani test	+
Flavonoid	Alkaline reagent test	+
	Shinoda test	+
Tannin	Lead acetate test	-
	Ferric Chloride test	+
Terpenoid	Chloroform test	+
Saponin	Frothing Test	+

Table 2: Linear Regression Data for the Calibration Curve of Quercetin

Linear Regression Parameter	Data
Linearity range (μg/ spot)	20-100
Regression equation	y = 0.0002x + 0.0019
Correlation coefficient	0.9852
Slope	0.00019005
Intercept	0.001859
SE of intercept	0.00089
SD of intercept	0.00199

Table 3: Recovery Study for Proposed Method (n=3)

Excess drug added to analyte (%)	Concentration found (μg±SD)	% recovery	%RSD
50	50.37±0.03	100.75	0.06
100	91.29±1.8	91.29	1.97
150	154.59±1.89	103.06	1.22

Table 4: Precision of the Proposed Method (n=3)

	Repeatability (Intra-day precision)			Repeatability (Intra-day precision)		
Conc.(μg/ml)	Area±SD	Std. error	%RSD	Area±SD	Std. error	%RSD
100	0.025±0.00025	0.00014	0.98	0.037±0.00034	0.0002	0.92
100	0.021±0.00028	0.00016	1.33	0.038±0.00034	0.0002	0.88
100	0.024±0.00032	0.00018	1.31	0.039±0.00040	0.00023	1.01

Table 5: Robustness of the Propose HPTLC Method (n=3)

Con (μg/ml)	Original Mobile Phase	Used Mobile Phase		Area±SD	%RSD	R_f
100	5:4:0.2	5:4:0.3	+0.1	0.03727±0.00048	1.30	0.85
		5:4:0.2	0	0.03916±0.0028	0.90	0.81
		5:4:0.1	-0.1	0.06089±0.00066	1.08	0.82

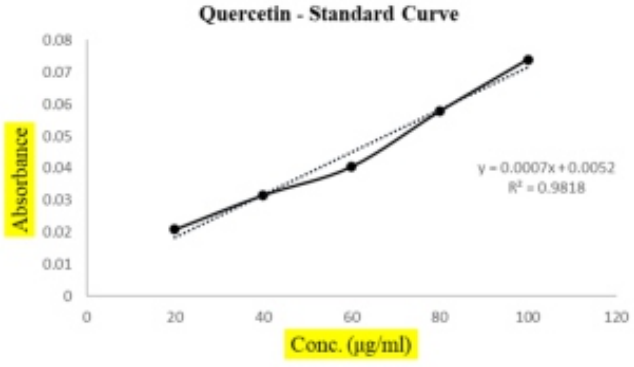

Figure 1: Standard Curve of Quercetin

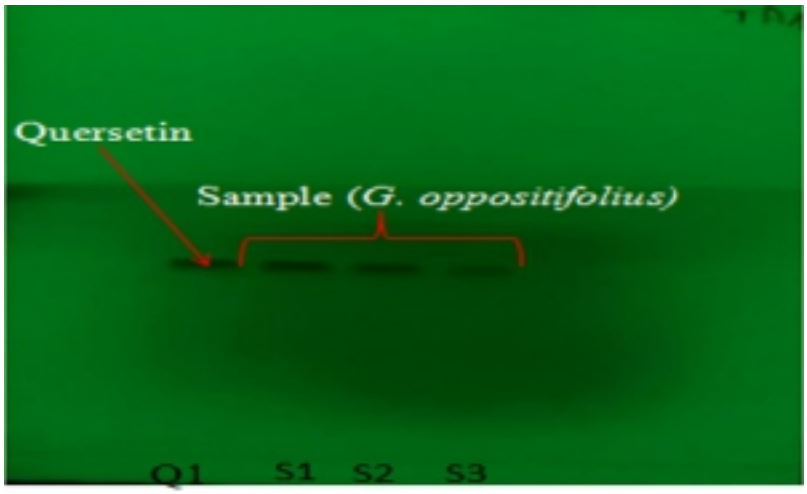

Figure 2: HPTLC Plate Showing Bands of Standard Quercetin (Q1)and Sample (S1-S3) at 254nm

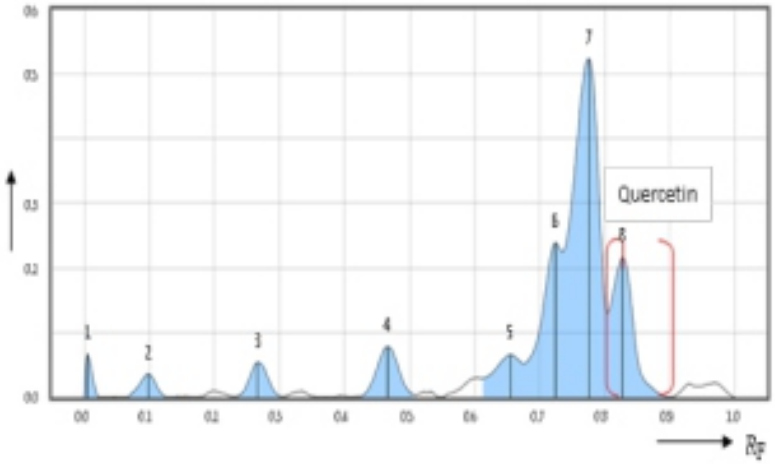

Figure 3: HPTLC Chromatogram of Glinus oppositifolius (L.)

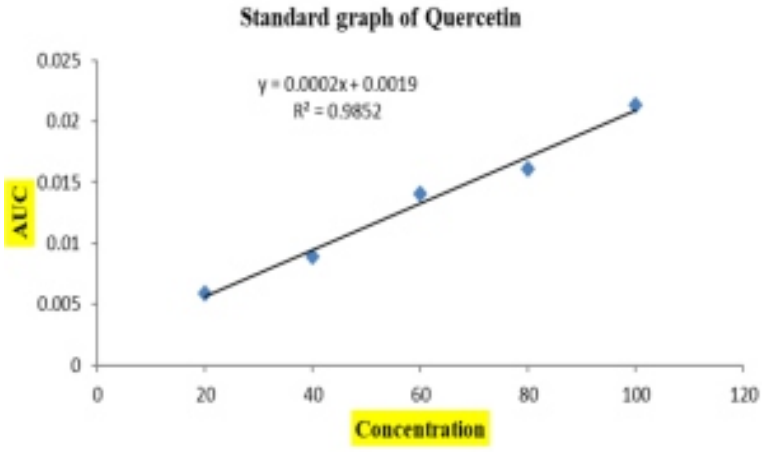

Figure 4: Regression Curve of Standard Quercetin

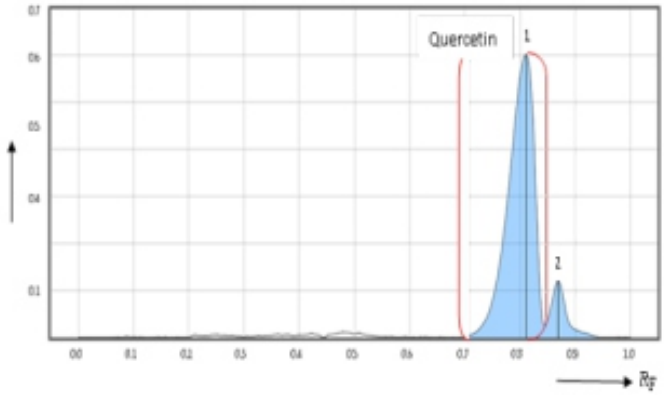

Figure 5: HPTLC Chromatogram of Standard Quercetin

curve - y = 0.0007x + 0.0052; R2 = 0.9818 (Figure 1)used for the determination of total flavonoid content in the sample extracts, as shown in the below.

Total flavonoid content represented as Quercetin equivalent (QE) per gram of sample was tested in the methanol extract. The absorbance for each extract was measured in triplicate and results were calculated as their mean. Total flavonoid content in the methanol extract of Glinus oppositifolius (L.) 102.95 ± 3.85 mg QE/ gm.

Solvent Selection

A clear, compact, and well-resolved band for quercetin was obtained via ascending development using ethyl acetate-toluene-formic acid (5:4:0.2v/v/v). This band's Rf value was 0.81±0.06. Figure 2 shows band of standard quercetin (Q1) and different volume of sample solution (S1-S3) at 254nm.

HPTLC Fingerprinting

The results of the HPTLC fingerprinting of methanolextract of Glinus oppositifolius (L.) at 254 and 366nm are given in Figure 3. The methanol extract showed a maximum of 8 well resolved peaks, at 2µL sample volume, with Rf values of 0.005, 0.098, 0.266, 0.466, 0.655, 0.724, 0.776 and 0.827.

Among these peaks, the peak at Rf value 0.827 showed the highest peak area of 16.22%. The peak corresponding to Rf value 0.827 showed a sharp peak and significant area of 16.22%, was identified as quercetin.

Method Validation

According to the calibration plot in Figure 4, the response is a linear function of quercetin concentrations between 20-100µg/ml.

The slope, intercept, and correlation coefficient were 0.00019, 0.001859 and 0.9852 respectively. Table 2 displays the linear regression information for the quercetin calibration curve.

Recovery Study

Results from recovery studies, which are listed in Table 3, showed good accuracy and fell within acceptable ranges (91.29 to 103.06%).

Precision

Table 4 displays the findings from the determi nation of repeatability and intermediate precision, expressed as SD (%). For repeatability, RSD fell between 0.98 and 1.33, and for intermediate precision, it fell between 0.88 and 1.01. These small values demonstrated the method's accuracy.

Robustness

Results of robustness are shown in Table 5. Low values of %RSD (0.09- 1.30) were obtained after intro ducing small deliberate change into the densitometric TLC procedure proved the robustness of the pro pose HPTLC method.

Limit of Detection and Limit of Quantification

The suggested approach's LOD and LOQ for quercetin were determined to be 34.62 and 104.91 µg/spot, respectively, demonstrating that the method may be utilised successfully for quercetin detection and quantification in a range of conditions.

Method Development

The mobile phase composition was optimised with the goal of creating a reliable and accurate densit ometric HPTLC technique for the measurement of quercetin. The mobile phase Ethyl acetate-Toluene Formic acid 5:4:0.2 (%v/v/v) exhibited a crisp, sym metrical, and well-resolved peak at Rf value of (0.81±0.06) (Figure 5).

Quantification of Quercetin in the Methanolic Extract of Glinus oppositifolius (L.)

By contrasting the single spot at Rf = 0.81±0.06 (Figure 5) of the quercetin peaks from the methano lic extracts of the leafy vegetable Glinus oppositi folius(L.) with those obtained by chromatography of the standard under the same circumstances. Using the standard concentration and AUC, the quercetin content in methanolic extracts of Glinus oppositi folius (L.) is 0.258± 0.0047 mg of quercetin /100g sample.

DISCUSSION

Plant medicines have been used to treat illnesses for as long a time in the history of mankind. Many con temporary medications have their origins in higher plants. Ayurveda, the traditional medical system of India, has made a variety of therapeutic claims regarding these plant-based medicines. However, it is crucial to support the varied medical bene6its of the herbs with scienti6ic evidence.

For a variety of reasons, the stage of ignorance for plant medicine is fast changing. First, issues with side effects of contemporary medications have rekindled interest in plants as a signi6icant source of novel drug candidates. Second, since the majority of the already known lead structures have already been used, pharmaceutical scientists are in search for newer lead compounds from plants [20]. Traditional wisdom of the indigenous people can provide new sources for discovering leads. Third, herbal remedies have achieved remarkable success in the recent years.

The present study was undertaken to prepare the HPTLC Fingerprint pro6ile of the plant Glinus opposi tifolius(L.) (also known as Gima shaak) for the iden ti6ication of compounds present in its extracts, as well as to quantify the identi6ied 6lavonoid from it. Literature survey was conducted to 6ind out the eth nomedicinal uses of the plant in several tribes of India, as well as in other countries like Taiwan, Mali and Bangladesh.

The methanol crude extract of G. oppositifolius (L.) was used for the identi6ication of the plant sec ondary metabolites or phytochemicals present by the standard phytochemical screening tests. The methanol extract tested positive for most of the phytochemical tests, indicating presence of alka loids, glycosides, 6lavonoids, terpenoids, tannins and saponins (Table 1).

The total 6lavonoid content of the plant extracts was estimated using the Aluminium chloride method, using quercetin as the standard. The principle behind this assay procedure is that aluminium chlo ride forms acid-stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxyl groups of 6lavones and 6lavonols present in the sample. Additionally, it combines with the ortho-dihydroxyl groups on the A or B ring of 6lavonoids to produce complexes that are acid labile [21]. The concentration of 6lavonoids present in the reaction media linearly affects the intensity of the light absorbed, thus useful in estimating the 6lavonoid content in the sample. In this study, higher amount of TFC was obtained in the ethanolic extract (102.95 ± 3.85 mg QE/ gm sample).

Following development, 10% H2SO4 solution spray ing reagents are applied to the TLC plates. After being sprayed with a 10% H2SO4 solution, quercetin turned up as a red colour spot in both the sample

and the reference. Under chromatographic conditions, the Rf value of quercetin extracted from Glinus ppositifolius (L.) was almost identical to that of the reference standard. One of the solvent systems was discovered to be suitable for running the sample after all 2 were analyzed. Therefore the Ethyl acetate: Toluene: Formic acid (5: 4: 0.2 v/v/v) can be considered as good solvent system (Figure 3).High Performance Thin Layer Chromatography (HPTLC) is a very useful technique for the analytical validation of novel natural product forms. HPTLC is currently becoming one of the best methods for ensuring product quality, purity, stability, as well as for identification, or, validation of an herbal product's complex composition. In this study, HPTLC was used to develop the chromatographic fingerprint profile of the methanolic extract of Glinus oppositifolius (L.) in the optimized mobile phase Ethyl acetate: Toluene: Formic acid (5: 4: 0.2 v/v/v). The pattern of the fingerprint profile obtained from this study can be used for the quality control of the plant sample. The identified compound from the chromatogram of this plant is Quercetin, having Rf value 0.82. Quantification of quercetin was also carried out to estimate the amount of quercetin content per gram of plant sample. Quercetin content was found to be 0.258 ± 0.0047 mg of quercetin /100gm sample.

CONCLUSION

The presence of flavonoids in the methanolic extract of the plant could be responsible for its anti inflammatory, anti-viral and immunomodulatory activity. HPTLC Fingerprint profile of the methanolic extract was obtained, which could be used for authentication and quality control of the herbal plant or other herbal formulations containing this plant. The flavonoid identified from the plant is quercetin, at Rf value 0.82, which is being reported for the first time from this plant Glinus oppositifolius (L.). The flavonoid was quantified by comparing the area under the curve with standard quercetin and the quantity of quercetin is 0.258 ± 0.0047 mg of quercetin /100g sample.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] N Hari and V P Nair. Preliminary Phytochemical Evaluation and HPTLC Fingerprint Profile of Jasminum azoricum L. Int J Sci Res Sci Eng

2] C Y Ragasa, D L Espineli, E H Mandia, M J Don, and C C Shen. A new triterpene from Gli nus oppositifolius. Chinese Journal of Natural Medicines, 10(4):284–286, 2012.

[3] S T Ramaseshan, P Pitchaiah, V Bharti, K K Ramakrishna, V Gaddam, D Tewari, and D K Singh. Pharmacognostical, Phytochemical and Nutritional Evaluation of Glinus oppositifolius (L.) Aug. DC. Pharmacognosy Journal, 8(1), 2016.

[4] R K Dewangan, S Dubey, and A K Dixit. Pharmacognostical and Qualitative Phytochemical Study of Glinus oppositifolius (Linn.) Aug. DC An Important Ethnobotanical Plant. GIS Sci J, 8:2105–2119, 2021.

[5] T Thirunarayanan. Ethnobotanical survey on Folk Medicine in the management of animal bite poisons in the forest tract of Salem region of Tamil Nadu, India. International Journal of Pharmacology and Clinical Sciences, 2(2), 2013.

[6] N Dongarwar, Uma Thakur, and S Dongarwar. Ethnomedicines Among Some Tribes Of Nandurbar District Of Maharashtra (India). Life Sci LeaŸl, 4:48–53, 2012.

[7] G Siromoney, L D Giles, and C Livingstone. Herbal medicines of the Narikoravas. Folklore, 14(10):363–369, 1973.

[8] C P Khare. Indian medicinal plants: an illustrated dictionary. 2008. Springer Science and Business Media.

[9] M Chowdhury and A P Das. Inventory of some ethno-medicinal plants in wetlands areas in Maldah district of West Bengal. Pleione, 3(1):83–88, 2009.

[10] K T Inngjerdingen, S C Debes, M Inngjerdin gen, S Hokputsa, S E Harding, B Rolstad, and B S Paulsen. Bioactive pectic polysaccharides from Glinus oppositifolius (L.) Aug. DC., a Malian medicinal plant, isolation and partial characterization. Journal of ethnopharmacology, 101(1-3):204–214, 2005.

[11] P Sahakitpichan, W Disadee, S Ruchirawat, and T Kanchanapoom. L-(−)-(N-trans Cinnamoyl)-arginine, an Acylamino Acid from Glinus oppositifolius (L.) Aug. DC. Molecules, 15(9):6186–6192, 2010.

[12] N Hoque, M Z Imam, S Akter, M E H Mazumder, S R Hasan, J Ahmed, and M S Rana. Antioxidant and antihyperglycemic activities of methanolic extract of Glinus oppositifolius leaves. Journal of Applied Pharmaceutical Science, pages 50– 53, 2011.

[13] R Bhowmik, M R Saha, M A Rahman, and M A U Islam. Ethnomedicinal survey of plants in the Southern District Noakhali. Bangladesh Pharmaceutical Journal, 17(2):205–214, 2014.

[14] C Y Ragasa, E C Cabrera, O B Torres, A I Buluran, D L Espineli, D D Raga, and C C Shen. Chemical constituents and bioactivities of Glinus oppositifolius. Pharmacognosy Research, 7(2):138, 2015.

[15] O O Odebiyi and E A Sofowora. Phytochemical screening of Nigerian medicinal plants II. Lloydia, 41(3):234–246, 1978.

[16] G E Trease and W C Evans. Phenols and pheno lic glycosides. In Textbook of Pharmacognosy, volume 12, pages 343–383, 1989. Tindall and Co Publishers, London, UK.

[17] B N Shah and A K Seth. Textbook of Pharmacognosy and Phytochemistry. 2010.

[18] T Adhikari and P Saha. Quantitative Estimation of Immunomodulatory Flavonoid Quercetin by HPTLC in Different Leafy Vegetables Available in West Bengal. Pharmacognosy Research, 14(4), 2022.

[19] R C Guy. International Conference on Harmonisation. Encycl Toxicol Third Ed, 2:1070–1072, 2014.

[20] B Patwardhan. Ayurveda: The designer medicine. Indian drugs, 37(5):213–227, 2000.

[21] G C Bag, P G Devi, and T H Bhaigyabati. Assess ment of total flavonoid content and antiox idant activity of methanolic rhizome extract of three Hedychium species of Manipur val ley. International Journal of Pharmaceutical Sciences Review and Research, 30(1):154–159, 2015

Madhuca longifolia leaf extract mediated synthesis of ZnO nanoparticles and their Antibacterial, Antioxidant, and Photocatalytic activity

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ABSTRACT

The biogenic synthesis of ZnO NPs is a promising substitute for the standard method of NP synthesis. In the current study, ZnO nanoparticles were pro duced biologically. Leaf extract from the Madhuca longifolia (M-ZnO Nps) plant was used to create ZnO NPs, which were then examined using UV-vis, XRD, FTIR, SEM, and TEM. SEM and TEM examination and XRD validated the size and crystalline nature of Zinc oxide nanoparticles, respectively. Func tional groups involved in the production of ZnO NPs were visible in the FTIR spectra. By scavenging DPPH free radicals at various concentrations, the antioxidant activity of green ZnO NPs was determined. By using the agar well diffusion method, ZnO NPs were tested for their bactericidal potential against Gram-negative bacteria E. coli and Gram-positive bacteria Staphylococcus aureus bacterial strains. A 96% photodegradation of MB dye and 91% degra dation of textile wastewater was observed in green-produced ZnO NPs when exposed to sunshine. The recent work proved that ZnO NPs have substan tial antioxidant, antibacterial, and photocatalytic activity. Therefore, the study offers a straightforward, practical, economical, and ecologically secure green synthesis technique for the biofabrication of multifunctional ZnO nanoparticles.

Keywords:Antibacterial activity,Antioxidant activity,Green synthesis,Madhuca longifolia,Photocatalytic activity,ZnO nanoparticles

INTRODUCTION

Nanotechnology is a rapidly growing and widely accepted research 6ield in modern material science with the purpose of synthesis of new materials at the nanoscale level. Many researchers reported that nanomaterials have various functions and huge efficiency that are not noticed in their bulk phase and nano-dimension particles are extensively studied due to having electronic, magnetic, catalytic, optical, antimicrobial, wound healing, and antiin6lammatory properties [1].

Zinc oxide is a well-known semiconductor (n-type) with low-coat, nontoxic, and photocatalytic proper ties. ZnO has a wide bandgap semiconductor with 3.2 eV and photocatalytic activity is a commonly investigated function of semiconductors [1]. Zinc oxide nanoparticles are well known for their wide range of applications in diverse 6ields. Due to the extremely low toxicity of ZnO nanoparticles, recent research works have paid high attention to their function and employment, especially in the biomedical 6ield property. The demand and production of green ZnO NPs increased gradually because a better eco-

friendly alternative is required to avoid the use of high-energy inputs and toxic chemicals.

Looking at the harmful and toxic effects of nanomaterials on the environment, current research focused on a low-toxic, cost-effective, and ecofriendly green approach. The biological process of NPs synthesis using plant extracts, microorganisms, algae, and enzymes is considered a healthier substitute for existing processes of NPs synthesis because of their hazards to the environment [2, 3].

Nanotechnology has offered a ray of hope in the biomedical field as an antimicrobial and drug delivery agent for various diseases. However, the mechanisms of the bactericidal potential of ZnO NPs are still unknown, but Rajani et al.(2022) [4]; explain some mechanisms such as membrane dis ruption, generation of reactive oxygen species (ROS), disruption of the cell wall, leakage of nuclear and cytoplasmic material by the action of nanomaterials.

Textile wastewater contains a huge amount of non biodegradable hazardous dyes which causes some serious health and environmental issues. It is an immediate need to introduce a promising technology for dye removal with high efficiency and low tox icity. Nanotechnology has emerged as one of the prime technology with great potential for the degradation of textile dyes from wastewater more efficiently and effectively than previous methods. Current methods involve in the treatment of wastew ater and water purification, are disgraced due to their insufficiency in water purification and high cost demands. Nanotechnology can help to con quer this issue by removing organic dyes, heavy metal pollutants, pesticides, and other chemical pollutants from water that are related to a serious hazard to the ecosystem because of their toxicity to water inhabitants and every living organism, including humans [5].

ZnO NPs are semiconductors that have photocatalytic properties so ZnO NPs can photodegrade tex tile dyes efficiently. ZnO NPs are widely employed as photocatalysts in the photodegradation of organic pollutants in air and water [6]. Vasantharaj et al. (2021) [7]; was successfully reported the degradation of synthetic textile dyes by green synthesize ZnO NPs using Ruellia tuberosa plant extract.

In this study, Madhuca longifolia plant leaf extract was applied for the facile green synthesis of ZnO nanoparticles. The phytochemistry study of M. longifolia plant shows that it is rich in protein, sugar, alkaloids, vitamin, phenolic compounds, sapogenins, triterpenoids, steroids, saponins, flavonoids, glycosides, etc. [8, 9]. ZnO nanoparticle synthesis using M. longifolia leaf extract is an environmentally benign synthetic method. M. longifolia has higher constituents of phenolic compounds which are amenable to the ZnO NPs synthesis.

These bio-compounds have reducing properties and acted as capping agents in the nanoparticle synthesis process. The bio-synthesized M-ZnO NPs were investigated for their antioxidant, antibacterial, and photocatalytic activities. The photodegradation performances of methylene blue (MB) dye by the green synthesized ZnO nanoparticles under sunlight irradiation were investigated.

EXPERIMENTS

Materials and Methods

Leaves of Madhuca longifolia were taken from the local garden of Jaipur, India. Plant leaves were cleaned with distilled water and dry to grind to make their powder. 5 g of M. longifolia plant leaves pow der boiled in 100 ml distilled water for 20 min utes. After cooling, leaf extract was filtered using Whatman's filter paper and stored in the refrig erator for further use. Zinc nitrate hexahydrate (Zn(NO3)2.6H2O) was used for ZnO NPs synthesis. MB dye was purchased from Thermo Fisher Sci entific company, India. Textile dye samples were collected from the textile industry area, Sanganer, Jaipur.

Biosynthesis of ZnO Nps

8 gm zinc nitrate was added into 25 ml distilled water and heated at 60oC. 50 ml M. longifolia plant leaf extract was added drop by drop into the heated zinc nitrate solution under the continuous stirrer. The solution was continuously stirred until a pale yellow colour paste was obtained. The paste wasdried in the furnace at 400oC for 2 hours. The ZnO NPs were collected and stored for further characterization and applications.

Characterization

The bio-synthesized ZnO NPs were sonicated before characterization and every application for uniform dispersion and obtaining higher efficiency. The ZnO NPs synthesis was confirmed by the UV-Vis spec troscopy of the compounds. The UV-Vis absorption peak of ZnO NPs centred at 376 nm. The optical analysis was carried out by the UV-Vis spectrophotometer (GENESIS 180). The morphology and size of ZnO NPs were analyzed by SEM images (ModelZESIS), at a working distance of 10 mm and a voltage of 20 kV. Transmission electron microscopy was performed using a TALOS HR-TEM apparatus oper ating at 80 kV. The crystalline structure and grain size were confirmed by XRD using Rigaku (Model SMART LAB) diffractometer. The functional groups and chemical composition of ZnO NPs were studied by using an FTIR spectrometer (Bruker ALPHA).

Antioxidant activity

The free radical scavenging capacity of green synthesized ZnO NPs was evaluated against DPPH radi cal. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) provides an easy method of determining antioxidant activity. DPPH was offered as a free radical source and ZnO NPs were applied as a radical scavenger. In the presence of ZnO NPs, the DPPH solution's deep violet colour gradually turns pale yellow. The absorbance at 517 nm gradually decreases as ZnO NP concentration increases. The ZnO nanoparticles' ability to scavenge free radicals is assessed by the reduced absorbance in the DPPH solution. M-ZnO NPs were treated in ten different doses to test the antioxidant potential of ZnO

nanoparticles.

Antibacterial assessment

M-ZnO NPs were tested for their bactericidal activity against S. aureus and E. coli bacterial strains using an agar well diffusion assay. It is a relatively quick and effective test to determine antimicrobial activity. Different concentrations of M-ZnO NPs were pipetted into the well of agar plates. After that, the plates were incubated at 37 0C in the bacterial incubator for 24 hours. The bactericidal activity of MZnO NPs was in the form of a diameter of the Inhibi tion Zone (IZ). After 24 hours, the inhibition zones were observed.

Photocatalytic activity of ZnO Nps

The prepared green ZnO NPs examined for the pho tocatalytic degradation of textile dyes. The pho tocatalytic activities of M-ZnO NPs was assessed for dye methylene blue and dye-containing textile wastewater. The degradation of dye was observed in three different conditions-1.) Dye degradation in direct sunlight with M-ZnO NPs, 2.) Dye degradation in sunlight without NPs, 3.) Dye degradation in dark (without sunlight) with M-ZnO Nps.

After certain time intervals, degradation of pollutants was observed and absorbance of dye pollutants at their respective wavelength was used to measure the residual dye amount measured using a UV-vis spectrophotometer

RESULT AND DISCUSSION

Characterization of ZnO nanoparticles UV visible studies

UV-vis absorption spectra are used to evaluate the optical properties of nanoparticles. UV- vis absorption spectra of green ZnO NPs shown in Figure 1 UV-vis absorption spectra reveal the mono dispersion of zinc oxide nanoparticles. The wavelength of absorption peaks of ZnO nanoparticles at 376 nm can be allied with ZnO intrinsic band-gap absorp tion. This happened because of the bounce of e− from the valence to the conduction band [10].

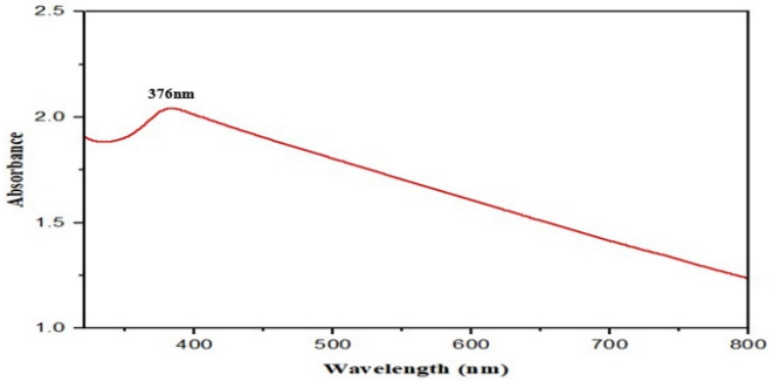

Figure 1: Uv-vis absorption spectra of M-ZnO Nps

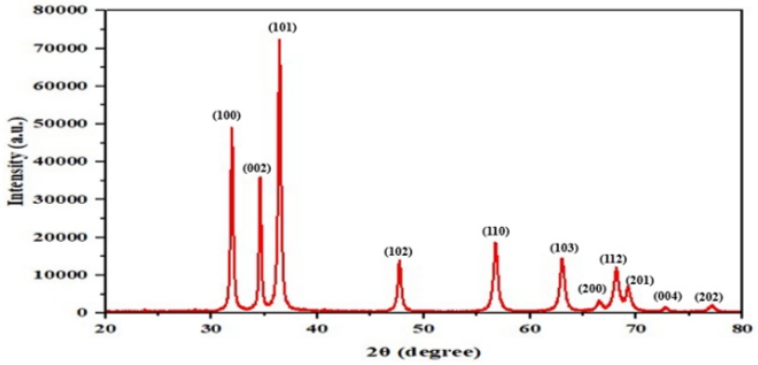

Figure 2: XRD pattern of M-ZnO nanoparticles

XRD analysis

X-ray diffraction is used to determine the phase and crystallographic structure of a material. The green synthesized ZnO nanoparticles were characterized using powder XRD to conϭirm the nanoparticles as zinc and to examine the structural information and crystalline behaviour. The XRD proϭile of the opti mized green ZnO NPs is reported in Figure 2. The prominent XRD peaks were obtained at 2θ values of 31.79◦ , 34.43◦, 36.28◦, 47.53◦, 56.5◦, 62.79◦, 67.83◦, and 68.67◦ Bragg peaks corresponding entirely to(100), (002), (101), (102), (110), (103), (112) and (201) indicating crystalline wurtzite structure ofZnO NPs and this data matched with JCPDS No. 36- 1451 [11, 12]

SEM analysis

SEM images are used to predict the structural morphology of particles. SEM image of ZnO NPs (Figure 3) observed that the shape of most of the particles is spherical and the average size is between 70-120 nm. The SEM analysis of ZnO NPs coupled with the EDX spectrum conϭirms the zinc oxide particles and thepresence of other elements.

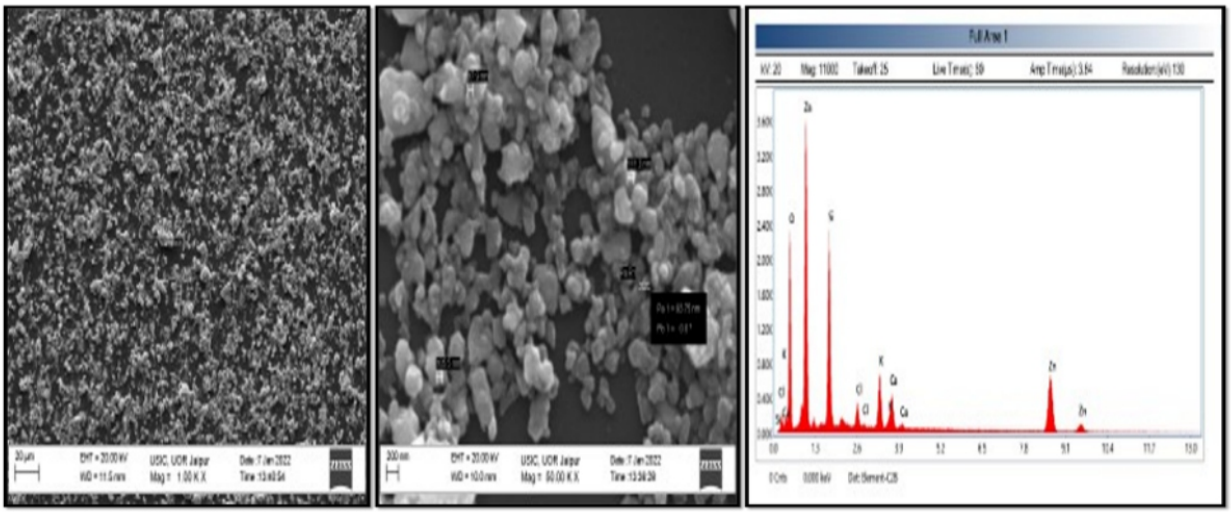

Figure 3: SEM image of M-ZnO nanoparticles at different magniϭications

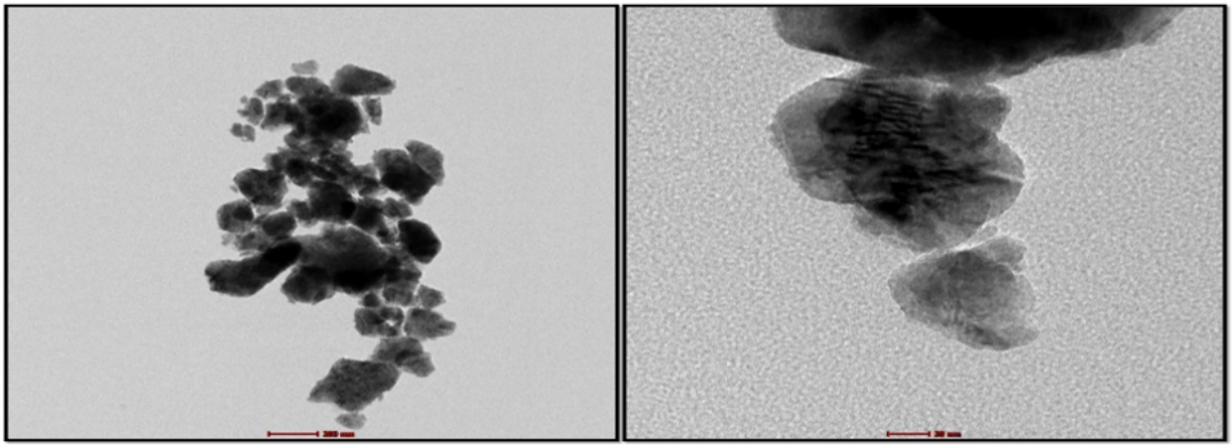

Figure 4: HR-TEM micrograph of M-ZnO NPs at different magnifications

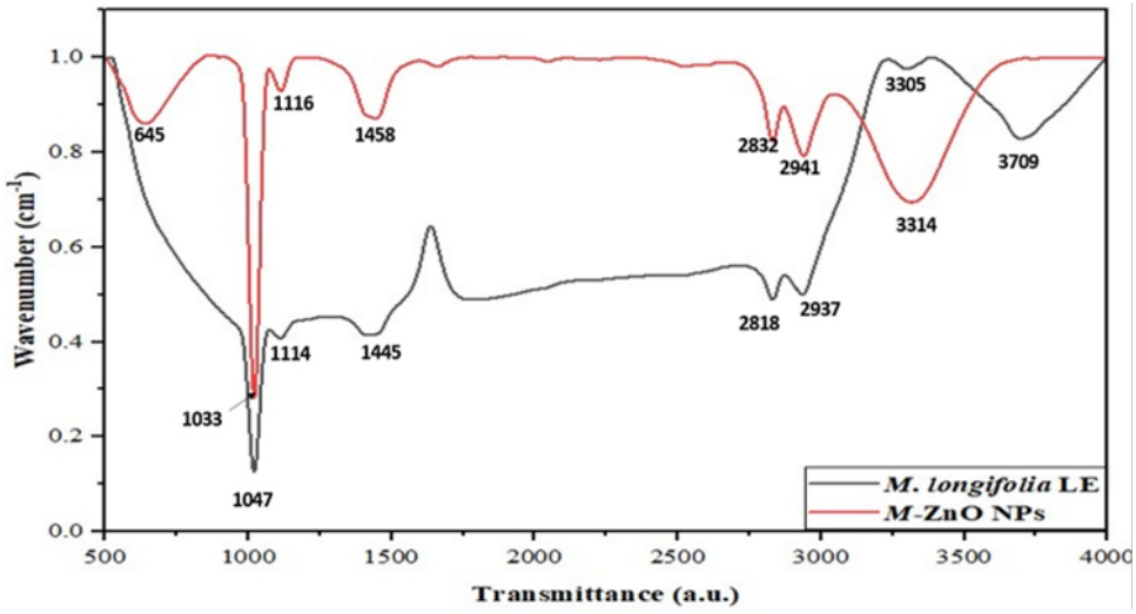

Figure 5: FTIR spectra of M. longifolia leaf extract and M-ZnO Nps

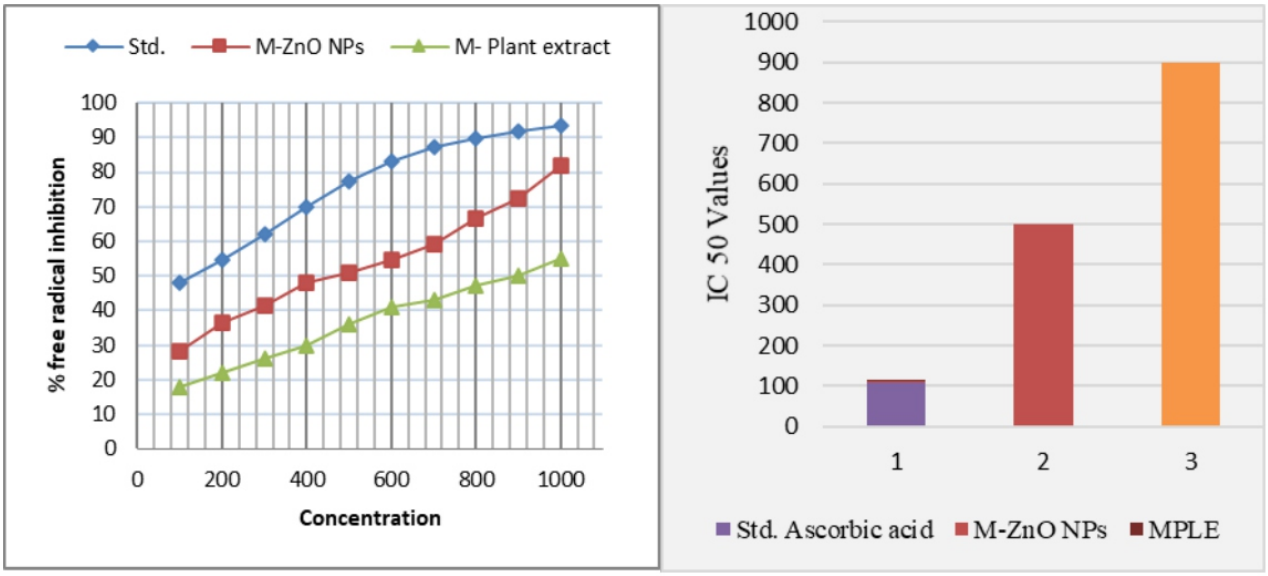

Figure 6: Free radicals inhibition percentage of M. longifolia leaf extract and M-ZnONPs and

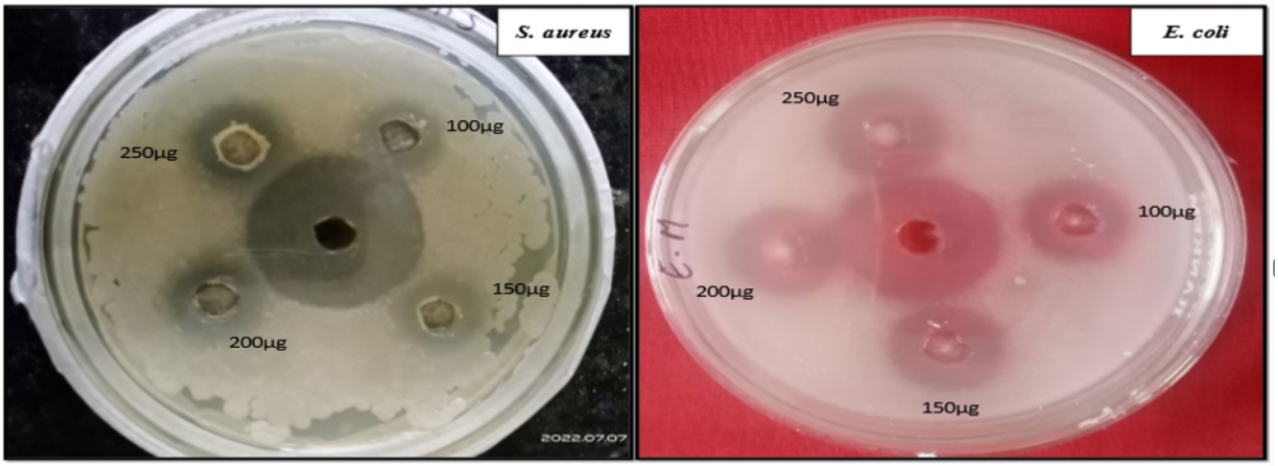

Figure 7: Inhibition zone of M-ZnO NPs against S. aureus and E. coli bacteria at different concentrations

Table 1: Antibacterial activity of M. longifolia leaf extract mediated synthesized ZnO nanoparticles

Bacteria	Inhibition Zone (mm)				
	Standard	100μg/ml	150μg/ml	200μg/ml	250μg/ml
S. aureus	32	16	17	19	19
Escherichia coli	31	19	20	22	23

The elemental inspection by the EDX spectrum shows that zinc is the major element that is present in the highest amount. Oxygen is also observed in the EDX spectrum which is related to the formation of ZnO NPs. Other elements observed in the EDX pro6ile, are correlated with plant extracts that were used for ZnO NPs synthesis. Silica impurities were observed due to the glass slide used for thin 6ilm formation.

TEM analysis

High resolution of the TEM was used to analyze the shape, size, and density of nanoparticles. The TEM image at different magi6ications (20 and 200 nm) are hown in Figure 4. ZnO are quite monodisperse and nearly spherical in shape. However, some larger aggregates were also observed in the sample image because the aggregation is the result of the high surface energy of ZnO nanoparticles.

FTIR analysis

The functional groups found in plant extracts that were utilised in the production of ZnO NPs were con6irmed by FTIR spectroscopic analysis. For bioreduced ZnO NPs, phytochemicals function as cap ping agents. The bio-reduction of ZnO NPs is carried

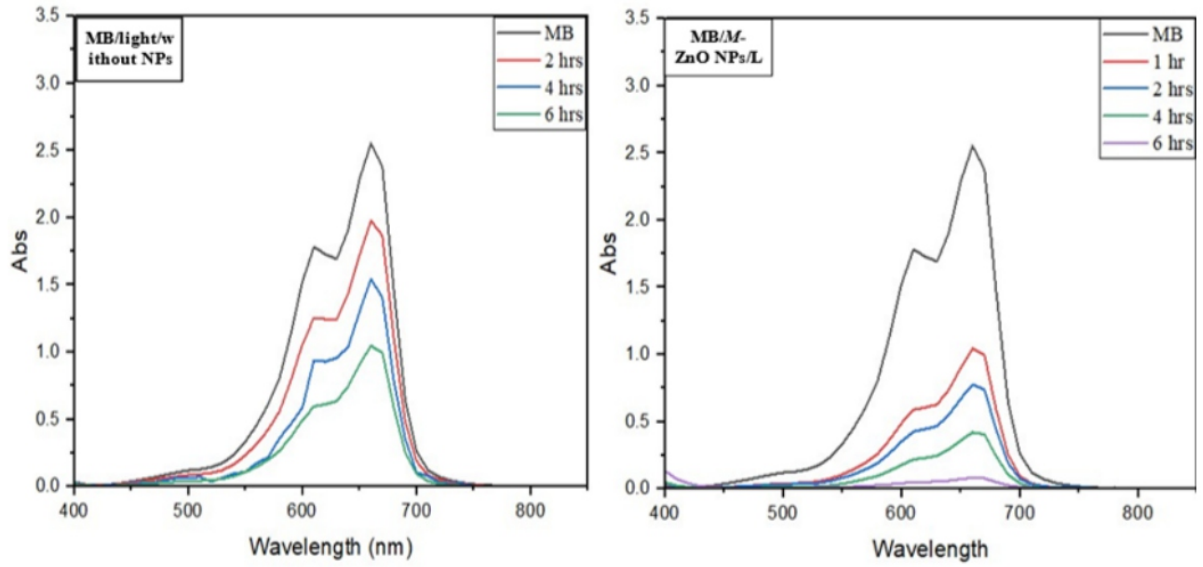

Figure 8: MB dye degradation in the absence and presence of M-ZnO NPs under sunlight illumination

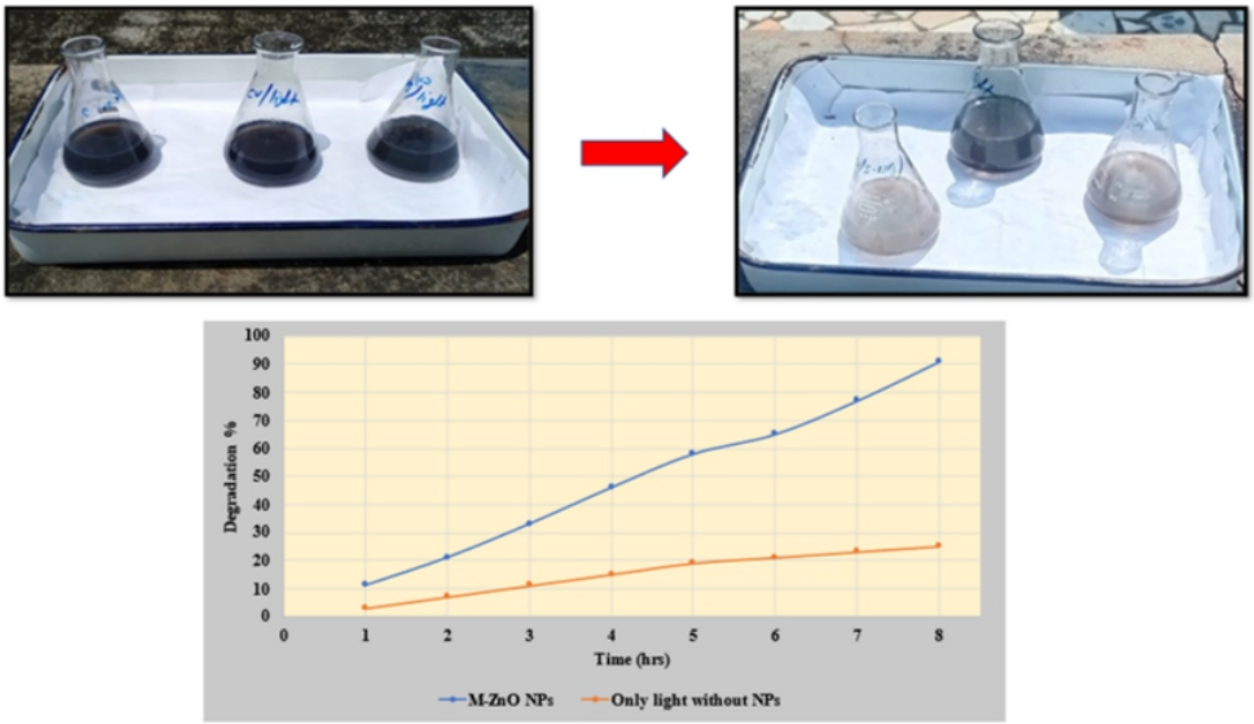

Figure 9: Dye degradation percentage of textile wastewater in the presence and absence of ZnO Nps

out using predetermined wave numbers to identify speciFic bond vibration peaks in the FTIR method. Figure 5 displays the FT-IR spectra of M. longifolia leaf extract and M-ZnO NPs. Because ZnO NPs span between 400 and 800 cm-1, the band at 645 is a sign of their presence. The absorption bands at 1047, 1114, 1445, 2818, 2937, 3305, and 3709 cm−1 in M. longifolia leaf extract wound be shifted to 645,1033, 1116, 1458, 2832, 2941, 3314 cm−1 in synthesized ZnO NPs. The absorption between 3200-3400 (peak band at 3314, 3305) indicates the O-H stretch of the phenol group [13]. The peak at 2818,

2937 for M. longifolia leaf extract and 2832, 2941 for M-ZnO NPs is because of C-H stretching vibrations in the aromatic compound. Further, the peak at 1033 and 1047 indicate the starching between C-O of polyphe nol. The absorption region at 942–1714 cm−1 is due to C=N, C=O, NH, and C=C stretching vibrations of aromatic compounds [14].

Antioxidant activity of green ZnO nanoparticles

The antioxidant potential of green synthesized ZnO NPs was evaluated against DPPH radical. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) provides an easy method of determining antioxidant activity. DPPH was used as the free radical source and ZnO NPs were used as a radical scavenger. In the presence of ZnO NPs, the deep violet colour of the DPPH solution gradually changed into pale yellow colour. With the increase of ZnO NPs concentration, absorbance at 517 nm gradually decreases. The decrease in absorbance gives evidence of the radical scavenging ability of ZnO Nps.

DPPH solution was prepared by dissolving 0.004gm DPPH in 100 mL of 80% methanol. In the presence of a free radical scavenger (ZnO NPs) that can change in absorbance at 517 nm which was determined spectrophotometrically. 80% of methanol was prepared by dilution of methanol with distilled water. In brief, a series of with 10 various concentrations values from 100µl/ml to 1000µl/ml of ZnO NPs solution was added and mixed with 3ml DPPH solution. ZnO NPs solution was made by dispersing 1mg NPs in 10ml distilled water. The mixture was left in dark for 30 minutes. The absorbance against a blank (80% methanol), and a control sample (DPPH+ methanol) without the addition of a test sample and a series of different concentrations of the test samples (ZnO NPs) was recorded at 517 nm. The absorbance difference between control and test samples is considered as actual absorbance.

$$Antioxidant\ \ activity\ \ Index = \frac{IC_{50}\ of\ the\ standard\ Ascorbic\ acid\ (\mu l/ml)}{IC_{50}\ of\ Sample\ (\mu l/ml)}$$

The IC50 value for M. longifolia leaf extract is 900 µl/ml and green ZnO NPs are 500 µg/ml (Figure 6). results indicate that the antioxidant activity of M. longifolia leaf extract-mediated ZnO NPs is much higher than M. longifolia leaf extract.

Bactericidal activity of green ZnO nanoparticles

Green synthesized ZnO nanoparticles were tested for their bactericidal activity against S. aureus and E. coli bacteria by agar well diffusion assay. Inhibition zone values were determined for theM-ZnO Nps at different concentrations (100 µg/ml, 150 µg/ml, 200 µg/ml, and 250 µg/ml). ZnO NPs showed an excellent inhibitory effect against both S. aureus and E. coli bacteria due to their surface activity. In Fig ure 7, the diameter (mm) of inhibition zones of ZnO NPs solution around each well is shown. The result shows that inhibition zone diameter increase with the increasing concentration of ZnO NPs. M. longi folia leaf extract-mediated synthesized ZnO NPs are found the highest bactericidal activity against E.

coli (23 mm) at 250 µg concentration (Table 1). But Pachaiappan et al., 2021 [15]; reported that green synthesized ZnO NPs using Justicia adhatoda leaves extract showed higher bactericidal activity against S. aureus bacteria than E. coli. The result indi cates that the bactericidal activity of green ZnO Nps depends on the concentration of NPs. Asha et al.(2022) [16]; reported that the antimicrobial potential of zinc oxide nanoparticles depends upon the size, smaller size NPs has better bactericidal effect than a larger one.

Photodegradation of Textile dyes

In the sunlight irradiation but absence of M-ZnO, little degradation of MB dye was observed. The effect of both ZnO NPs on the photocatalytic degra dation of MB was investigated and shown in Uv vis spectroscopy graphs. However, no photocat alytic degradation of MB dye was observed in dark conditions. The absorbance of dye-polluted water decreases with the increasing illumination time of sunlight irradiation. It is clearly seen in Uv-visible spectroscopy data that the relative concentration of dye reduced with sunlight illumination time. In the photocatalytic degradation process, the photogen eration of electron-hole pairs is generally responsi ble for the elimination of dye pollutants [17]. The degradation percentage of MB dye in the presence of ZnO NPs was calculated using the equation [18].

$$Degradation\ \% = [A_o - \frac{A_t}{A_o}] \times 100$$

where A0 is the absorbance value at the initial stage and the absorbance at time "t" is At . After 6 hours, 96.08% degradation of MB dye was observed in the presence of M-ZnO NPs (Figure 8). Only 48% degra dation was observed in the absence of nanoparti cles. Anbuvannan et al. 2015 [1]; also reported the efficient photocatalytic property of green ZnO Nps against MB dye in sunlight illumination.

As we can see in Figure 9, textile industrycontaminated water becomes transparent in the presence of M-ZnO NPs after 8 hours of sunlight illumination. After treatment of M-ZnO Nps, 91% of dye degradation was observed while only 25% degradation was observed in the absence of nanoparticles.

ZnO NPs have been shown to exhibit inhibitory and antibacterial properties, antioxidant properties and photocatalytic activity. It was also proven in previ ous Findings that green synthesis of nanoparticles improves their properties and reduced their hazards [19–21].

CONCLUSION

In this study, Zinc oxide nanoparticles were synthe sized by a simple, cost-effective, and eco-friendly approach using Madhuca longifolia plant leaves extract. The phytochemicals of M. longifolia leaf extract effectively work as reducing agents in ZnO NPs synthesis. The biogenic ZnO NPs were found to have 376 nm absorption spectra with a hexagonal wurtzite structure. Powdered XRD results confirm the

the crystalline structure of NPs. SEM and TEM images validated the formation of nanoparticles with 90 nm of average size. FTIR result showed the presence and role of phytochemicals in the synthesis of ZnO NPs. Results revealed that ZnO NPs showed significant antioxidant and antimicro bial activity. ZnO nanoparticles effectively photode grade methylene blue dye under sunlight illumina tion. The objective of this study is to investigate the properties of bio-synthesized ZnO NPs which can help to reduce the hazards of chemically syn thesized nanoparticles and enhance the efficiency of ZnO NPs. This study successfully facile green synthesized multifunctional ZnO nanoparticles using plant parts.

Abbreviations

ZnO- zinc oxide, NPs- nanoparticles, M-ZnO NPsMadhuca longifolia leaf extract mediated zinc oxide nanoparticles, XRD- X-ray Diffraction, SEM- Scanning Electron Microscope, EDX- Energy Dispersive X-ray spectroscopy, TEM- Transmission Elec tron Microscope, FTIR- Fourier Transform Infrared spectroscopy, Uv-vis spectroscopy- Uv-visible spectroscopy.

ACKNOWLEDGEMENT

This research is supported by JOINT CSIR-UGC and RUSA 2.0 MHRD grants.

Authors Contributions

Rajani: conceptualization, formal analysis, investigation, writing-original draft;
Rishi Kesh Meena: investigations, writing-review,and supervision;
Preeti Mishra: conceptualization, writing-review,and editing.

Declarations of Competing Interest

The authors declare that they have no competing interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

[1] M Ramesh, M Anbuvannan, and G Viruthagiri. Green synthesis of ZnO nanoparticles using Solanum nigrum leaf extract and their antibacterial activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 136(Pt B):864–870, 2015.

[2] P C Nethravathi, G S Shruthi, D Suresh, H Nagabhushana, and S C Sharma. Garcinia xanthochymus mediated green synthesis of ZnO nanoparticles: photoluminescence, pho tocatalytic and antioxidant activity studies. Ceramics International, 41(7):8680–8687, 2015.

[3] A Muthuvel, M Jothibas, and C Manoha ran. Effect of chemically synthesis compared to biosynthesized ZnO-NPs using Solanum nigrum leaf extract and their photocatalytic, antibacterial and

in-vitro antioxidant activity. Journal of Environmental Chemical Engineer ing, 8(2):103705–103705, 2020.

[4] P M Rajani, S Kumari, P Saini, and R K Meena. Role of nanotechnology in management of plant viral diseases. Materials today: Proceedings, 69(1):1–10, 2022.

[5] H Sadiq, F Sher, S Sehar, E C Lima, S Zhang, H M Iqbal, and M Nuhanović. Green synthesis of ZnO nanoparticles from Syzygium Cumini leaves extract with robust photocatalysis applications. Journal of Molecular Liquids, 335, 2021. Article ID 116567.

[6] F Davar, A Majedi, and A Mirzaei. Green synthesis of ZnO nanoparticles and its application in the degradation of some dyes. Journal of the American Ceramic Society, 98(6):1739–1746, 2015.

[7] S Vasantharaj, S Sathiyavimal, P Senthilku mar, V N Kalpana, G Rajalakshmi, M Alsehli, and A Pugazhendhi. Enhanced photocat alytic degradation of water pollutants using bio-green synthesis of zinc oxide nanoparticles (ZnO Nps). Journal of Environmental Chemical Engineering, 9(4), 2021. Article ID 105772.

[8] P Yadav, D Singh, A Mallik, and S Nayak. Mad huca longifolia (Sapotaceae), a review of its traditional uses, phytochemistry and pharma cology. International Journal of Biomedical Research, 3(7):291–305, 2012.

[9] J Sinha, V Singh, J Singh, and A K Rai. Phy tochemistry, ethnomedical uses and future prospects of Mahua (Madhuca longifolia) as a food: a review. J. Nutr. Food Sci, 7(1), 2017. Article ID 1000573.

[10] D Suresh, P C Nethravathi, H Rajanaika, H Nagabhushana, and S C Sharma. Green synthesis of multifunctional zinc oxide (ZnO) nanoparticles using Cassia fistula plant extract and their photodegradative, antioxidant and antibacterial activities. Materials Science in Semiconductor Processing, 31:446–454, 2015.

[11] F T Thema, E Manikandan, M S Dhlamini, and M J M L Maaza. Green synthesis of ZnO nanoparticles via Agathosma betulina natural extract. Materials Letters, 161:124–127, 2015.

[12] M Zare, K Namratha, K Byrappa, D M Surendra, S Yallappa, and B Hungund. Surfactant assisted solvothermal synthesis of ZnO nanoparticlesand study of their antimicrobial and antioxidant properties. Journal of materials science & technology, 34(6):1035–1043, 2018.

[13] K J Rao and S Paria. Green synthesis of sil ver nanoparticles from aqueous Aegle marme los leaf extract. Materials Research Bulletin, 48(2):628–634, 2013.

[14] N Matinise, X G Fuku, K Kaviyarasu, N Mayedwa, and M Maaza. ZnO nanoparticles via Moringa oleifera green synthesis: Physical properties & mechanism of forma tion. Applied Surface Science, 406:339–347, 2017.

[15] R Pachaiappan, S Rajendran, G Ramalingam, N Dai-Viet Vo, P Mohana Priya, and M Soto Moscoso. Green synthesis of zinc oxide nanoparticles by Justicia adhatoda leaves and their

antimicrobial activity. Chemical Engineering & Technology, 44(3):551–558, 2021.

[16] S Asha, T C Bessy, J J Sherin, C V Kumar, M R Bindhu, S Sureshkumar, and A A Hatamleh. Efficient photocatalytic degradation of industrial contaminants by Piper longum mediated ZnO nanoparticles. Environmental Research, 208, 2022. Article ID 112686.

[17] I Kazeminezhad and A Sadollahkhani. Photocatalytic degradation of Eriochrome black-T dye using ZnO nanoparticles. Materials Letters, 120:267–270, 2014.

[18] C H Kwon, H Shin, J H Kim, W S Choi, and K H Yoon. Degradation of methylene blue via photocatalysis of titanium dioxide. Materials Chemistry and Physics, 86(1):78–82, 2004.

[19] H Agarwal, S V Kumar, and S Rajeshkumar. A review on green synthesis of zinc oxide nanoparticles-An eco-friendly approach. Resource-EfŸicient Technologies, 3(4):406–413, 2017.

[20] Y Y Chan, Y L Pang, S Lim, andW C Chong. Facile green synthesis of ZnO nanoparticles using natural-based materials: Properties, mecha nism, surface modiőication and application. Journal of Environmental Chemical Engineering, 9(4), 2021. Article ID 105417.

[21] V N Kalpana and V Devi Rajeswari. A review on green synthesis, biomedical applications, and toxicity studies of ZnO NPs. Bioinorganic chemistry and applications, 2018. Article ID 3569758.

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