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Synthesis, Crystallographic, Monoclinic, Inflexible, Antifungal Assessment of Schiff based resin

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Abstract: The present study investigates the preparation of a newly prepared Schiff base BHNMXD and Schiff based resin PMBHNMXD via polymerization. The condensation or polymerization procedure was used with formaldehyde (HCHO) at temperature 70 to 80 ˚C for 2-3 h. The prepared Schiff base BHNMXD and its respective resinPMBHNMXD were recrystallized from methanol to attain pure resin. After successful synthesis of Schiff base and resin was characterized by different instrumental techniques such as; CHN, elemental microanalysis, thermogravimetric analysis (TGA) studies of Schiff based resin, energy-dispersive x-ray spectroscopy EDS mass spectrometry, E.I-MS (electron impact ionization), ^{1}HNMR spectroscopy and MALDI-TOF mass spectrometry clearly investigated the molecular weight and structural information of synthesized compounds. The FT-IR spectra of resin PMBHNMXD showed stronger absorption band at 1632 cm^{-1} of azomethine >C=NH confirming the compound formation. The SEM analysis showed the morphology of Schiff base and resin showing, longitudinal spherical rough, inflexible and rigid structure. The structures and components were carefully investigated by X-ray diffraction .The crystallographic evaluation of Schiff base and its resin, aproved the monoclinic sharp crystalline structure. These active materials have shown high efficiency against the antifungal growth of *Aspergillus Flavus (G-ve), Candida albicans (G-ve) and Aspergillus Niger (G-ve),*by agar well diffusion method.

Keywords: Resins, Crystallographic, Monoclinic, Synthesis, Polymerization, Antifungal.

1. INTRODUCTION

Preparation of *bis*-imines via polycondensation of various amines and aromatic aldehyde results in the formation of derivatives of *bis* imines. Compounds containing an azomethine group (-CH=N-) are known as Schiff base (Sonnekar. *et al.*, 2013). Schiff base are *bi*- or tri-dentate ligands and very stable complexes with the transition metals (Arulmurugan.*et al.*,2010).Schiff base resin and their complexes retain motivating factors, like presence of catalyst, properties regarding thermal stability (Ismet, *et al.*,2007) Schiff base complexes have been strongly considered to be used as representative molecules for biological oxygen carrying system (Ahmed. *et al.*, 2015) (Hacer, *et al.*, 2009) Number of infections due to microorganisms has increased extensively due to resistance against antimicrobials. Schiff base resins have always concerned the researchers due to their infinite applicability in number of fields of industrial and biological sciences and in synthesis of drugs. They are appropriate in the field of biology, as anticancer, antioxidant, anti-inflammatory, antibacterial, antifungal, antiviral and antimalarial activities (Mughal. *et al.*, 2013) (Mughal, *et al.*, 2013) (Kumar. and Sheriff, (2015) (Kumar, *et al.*, 2014).The poly metal complexes have beneficial possessions such as thermal stability ion selectivity, conductivity and antimicrobial properties (Sahu. *et al.*, 2012) (Mughal, *et al.*, 2013). The structural morphology of the compound plays an essential role in formation of antibiotics and antifungal study relays on it, followed by the choice of solvent in the process and the bacterial or fungal strain used (Suha.*et al.*, 2016) (Othman. *et al.*, 2007) Schiff bases and their complexes are mostly studied because of their significant properties such as their capability to bind reversibly to oxygen (Farmanullah, *et al.*, 2015). The Scientists worldwide acknowledge that Schiff based compounds had been used as antimicrobials and such as anticancer and broad application in several fields (Monier. *et al.*, 2019) (Abdul. *et al.*, 2013) (Ravikumar, and Hussain, 2003).Schiff bases complexes have gained much significance due to their chelating ability (Xin. and Sun. 2010). Chelating resins are simply regenerated from the metalions (Khuhawar.*et al.*, 2007).Chelating polymer resins are established to be more selective by nature (Mohammad.*et al.*, 2004).Schiff base resins are mostly insoluble material and holds poor solubility in organic solvents and sharp melting point (Kumar.*et al.*, 2009) (Mohamed. *et al.*, 2011). Schiff base resins are also motivating because of thermal stability, semiconductor and some consumption as anti-fertility and enzymatic agents (Islam. *et al.*, 2013) (Nair, *et al.*, 2006). Schiff based compounds kept excellent characteristics, structural comparisons with natural biological substances and various structural characteristics, as chelating agents. Subsequently special attention has been focused by researchers on Schiff base complexes (Memon.*et al.*, 2014).

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Subsequently special attention has been focused by researchers on Schiff base complexes (Memon. *et al.*, 2014).

The present studies explore a novel synthetic method for preparation of Schiff base (BHNMXD) and its resins (PMBHNMXD). All reactions were monitored through TLC-UV visualization 254(nm). The synthesized compounds (BHNMXD) and (PMBHNMXD), was assessed via using different instrumental techniques. Such as CHN, elemental microanalysis, FTIR (Fourier Transform microscopy), Mass spectrometry EI-MS (Electron impact ionization), SEM (Scanning electron microscopy), XRD analysis and MALDI-TOF mass spectrometry,^{1}HNMR (Nuclear magnetic resonance spectroscopy). The calculated values (CHN, ^{1}HNMR, and Mass spectrometry) proposed the compound's structure and morphology and confirmed determination of its molecular weight. All compounds displayed highly active values against *Aspergillus Flavus* (G-ve), *Candida albicans (G-ve) and Aspergillus Niger* (G-ve).

2. RESULTS AND DISCUSSION

Chemistry;

The synthesis of resin included two steps, the first step comprises of a Schiff base BHNMXD followed by the preparation of its resin PMBHNMXD by common method. The synthetic compounds was then characterized by instrumental techniques such as EI-MS,^{1}H-NMR, FT-IR, MALDI-TOF mass spectrometry, CHN elemental microanalysis, XRD, and SEM analysis. Scheme **1** and Scheme **2**represents the formation of Schiff base and its resin respectively. The Schiff base (BHNMXD) and Schiff based resin (PMBHNMXD) were used for in vitro antifungal activities, against *Aspergillus Flavus* (G-ve), *Candida albicans* (G-ve) and *Aspergillus Niger*(G-ve).

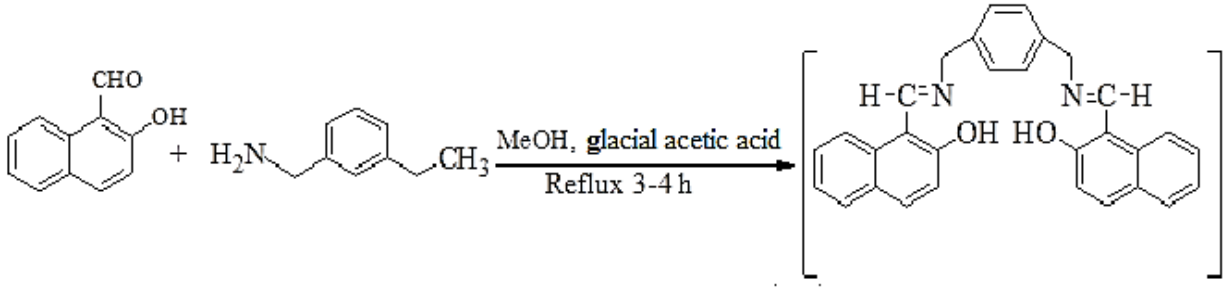

Fig. 1:-Reaction Scheme of Schiff Base BHNMXD

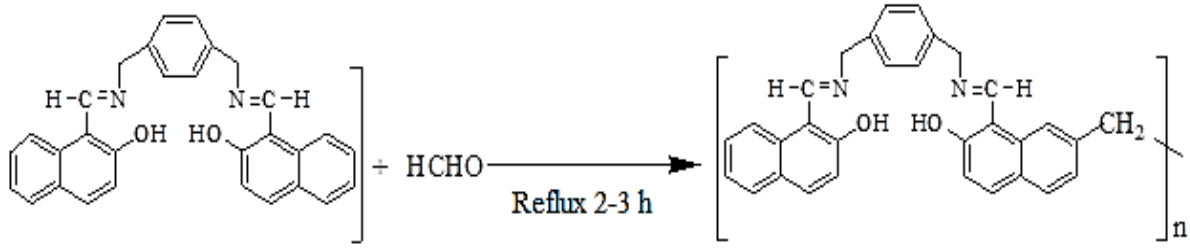

Fig. 2:-Reaction Scheme of Schiff Based Resin PMBHNMXD

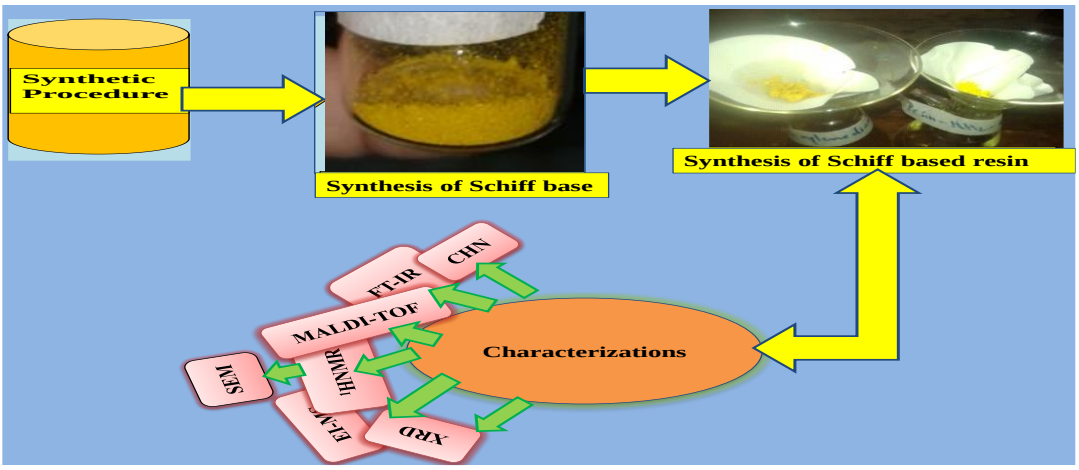

Fig.-3: Graphical Abstract of Synthesized Compounds

Synthesis

General procedure for the synthesis of Schiff base :

In a reaction flask 2-hydroxy-1-napthalehyde (0.344g, 2mmol) was dissolved in methanol,few drops of glacial acetic acid were added in the reaction mixture, m-xylenediamine (0.03ml, 1mmol) was then added andreaction mixture was then kept on refluxing for 2-3 hours. The progression of the reaction was monitored with TLC.The presence of yellow precipitates indicated the completion of reaction,the precipitates were then filtered washed with n-hexane and dried to get pure product.(Vasant. *et al.*, 2012).

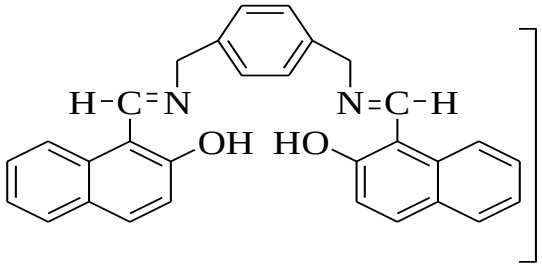

Fig.4: Structural Formula of Schiff Base BHNMXD.

General procedure for the synthesis of Schiff based resin;

The synthesized Schiff based reinPMBHNMXD (1g) was dissolved in25 mL distilled water. The solution was kept on stirring for 10 to 15 minutes. 15 drops of 2M NaOH was then added and the reaction mixture was refluxed at temperature 50˚ to 60˚C for 10 minutes, distilled formaldehyde 37% HCHO was then added (1:3 molar ratio).The reaction mixture was refluxed on oil bath at 120˚C for 2 hours. The precipitates obtained were filtered washed with water and diethyl ether. The recovered precipitates were then oven dried for 1-2 hours at 80 ˚C.(Sylwia. *et al.*, 2012).

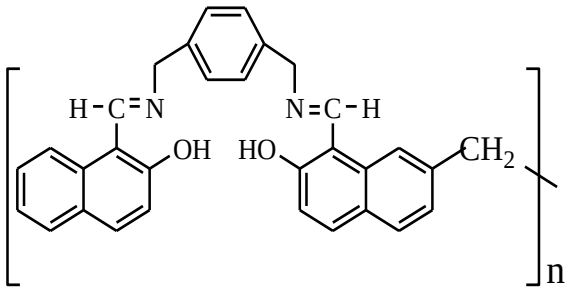

Fig.-5:Structural Formula of Schiff Based Resin PMBHNMXD.

Spectral, Characterization of Schiff Base and Schiff Based Resin;

^{1}H-NMR Spectroscopy of Compound BHNMXD

^{1}H NMR signals was recorded in deuteratedDMSO on 400 MHz instrument. Sharp multiples for OH appeared at δH 14.36 the most downfield signal of the spectrum. The doublet appeared at δH9.30 presented the presence of C=NH. H-8′ and H-8″ appeared as doublet at δH 8.09 having coupling constant $J_{8'}, _{7'/8''}, _{7''}$ 8.4 Hz, while H-4′ and H-4″, appeared at δH 7.44 as doublet having coupling constant $J_{4'}, _{3'/4''}, _{3''}$ = 7.6 Hz. Another doublet of H-5′ and H-5″ appeared at δH 7.65 having coupling constant $J_{5'}, _{6'/5''}, _{6''}$ = 8.0 Hz. While, H-7′ and H-7″ resonated at δH 7.46 as multiplet Another multiplet of H-6′ and H-6″ appeared at δH 7.40. 5A triplet peak at H-6′, H-6″, appear at δH 7.22 having coupling constant $J_{6'},_{5'/6'},_{7'/6''},''_{6''},_{7''}$ =7.4 Hz. while doublet peak of H-3′, H-3″ appeared at δH 6.73 having coupling constant $J_{3'},_{4'/3''},_{4''}$ = 9.6Hz. Another doublet peak of CH_2, CH_2 appeared at δH 4.89 having coupling constant J = 4.4 Hz compound.

^{1}HNMR (400 MHz, DMSO-d6): δ 14.36 (m, 2H, OH), 9.30 (d, 2H, J = 9.6 Hz, C=NH), 8.09 (d, 2H, $J_{8'},'/_{8''},_{7''}$ =8.4 Hz, H-8′, H-8″), 7.44 (d, 2H, $J_{4''},_{3''}/_{4'},_{3'}$ = 7.6 Hz,

H-4″, H-4′), 7.65 (d, 2H, $J_{5'6'/5''6''}$ =8.0 Hz, H-5′, H-5″), 7.46 (m, 2H, H-7′, H-7″), 7.40 (m, 4H, H-2, H-4, H-5, H-6), 7.22 (t, 2H, $J_{6'5'/6'7'/6''5''/6''7''}$ 7.4 Hz, H-6′, H-6″), 6.73 (d, 2H, $J_{3'4'/3''4''}$ = 9.6 Hz, H-3′, H-3″). 4.89 (d, 4H, J = 4.4 Hz, CH_2, CH_2)

EI-MS: Spectral Studies of Schiff base BHNMXD

The EI-MS spectra of compound 3 showed the M+ at m/z 444.1 for molecular formula $C_{30}H_{24}N_2O_2$.The significant fragment appears at m/z 170 showed that the molecule is cleaved into two equal halves with the formation of two 1-(iminomethyl) naphthalen-2-ol fragments, however, another fragment appeared at m/z 288 which showed the loss of an OH radical ion from the parent molecule, this fragment upon further loss of an 1-(iminomethyl) naphthalen-2-ol appeared at m/z 273.

The key fragments are represented in Fig. 6 .Atm/z 288displayed the loss of an OH radical ion from the parent molecule, this fragment upon further loss of an 1-(iminomethyl) naphthalen-2-ol appeared at m/z273.

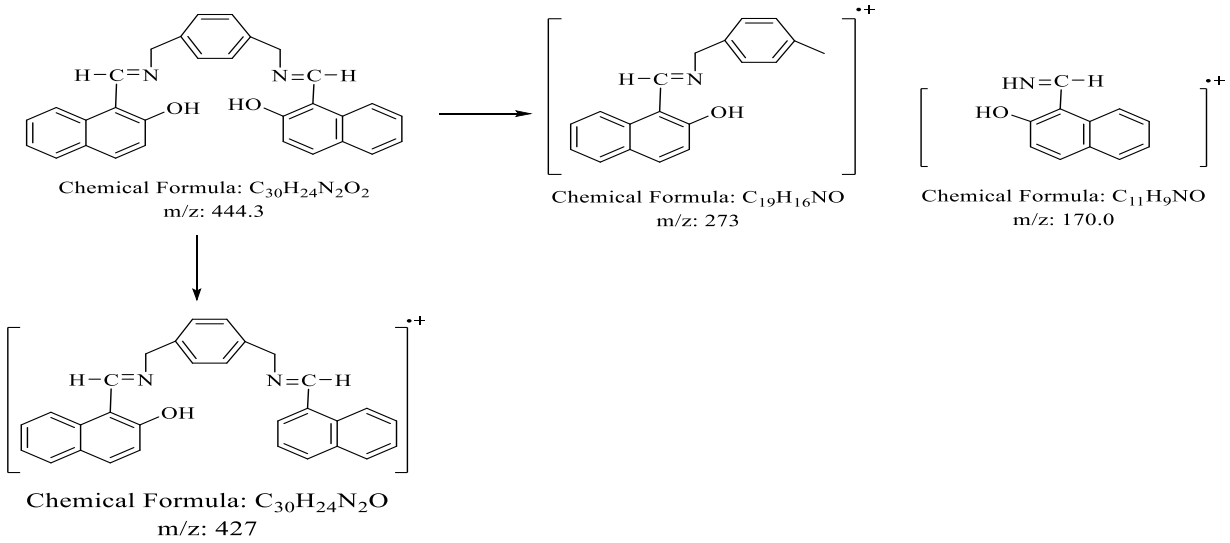

Fig.-6: Representative Fragmentation Pattern of Schiff Base BHNMXD

MALDI-TOF Mass Spectrometry results of Schiff Based Resin PMBNMXD:

MALDI + ve: MK+ =743.4,(M+, 220.0).

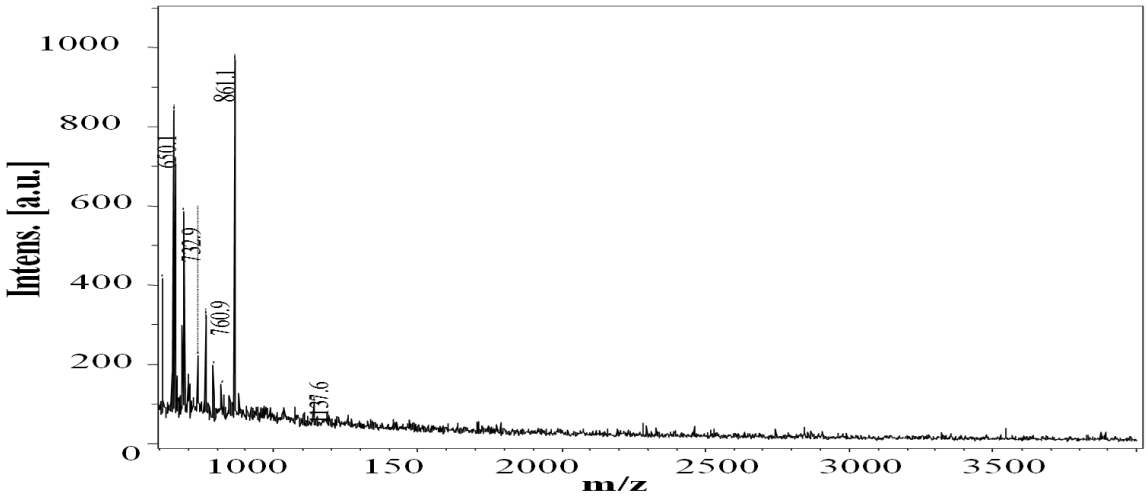

Fig-7: MALDI-TOF Spectra of Schiff Based Resin PMBHNMXD.

CHN Elemental Microanalysis of Synthesized Compounds:

The Schiff base and Schiff based resin were synthesized by a general method. TheCHN elemental microanalysis results were in good agreement to the calculated values as shown in (**Table-1).**

Table-1: CHN analysis of Schiff Base BHNMXD and Resin PMBHNMXD

Compounds	Chemical Formula	Melting Point	Calculated % (Found %)		
			C	H	N
1	$C_{30}H_{24}N_2O_2$	80°C	55.23 (53.2)	5.59 (6.06)	6.95 (7.02)
2	$C_{31}H_{26}N_2O_2$	85°C	69.3 (70.2)	6.87 (6.42)	6.44 (6.23)

Table-2: Solubility of different solvents of Schiff Base and Schiff Based Resin

	Compounds	H_2O	CH_3OH	C_2H_5OH	$CHCl_3$	Acetone	n-Hexane	Ether	DMSO
1	BHNMXD	--	+	±	_	_	_	_	+
2	PMBHNMXD	--	+	±	_	_	_	_	+

a:0.05gCompounds in 10 ml solvents at 30°C**. b:(+)**Soluble,(±)Partially soluble**,(-)** Insoluble.

Thermo gravimetric analysis (TGA) Studies of Schiff Based Resin (PMBHNMXD)

The thermal analysis of Schiff based resin PMBHNMXD is shown figure the. Initial weight loss of about 2 % in the region of 50-100 ^{0}C is due to the water in the sample between 200 and 355^{0}C. The material suffered rapid weight loss. The loss rate is 0.39% at 160 ^{0}C which is maximum rate of weight loss (T**max**).

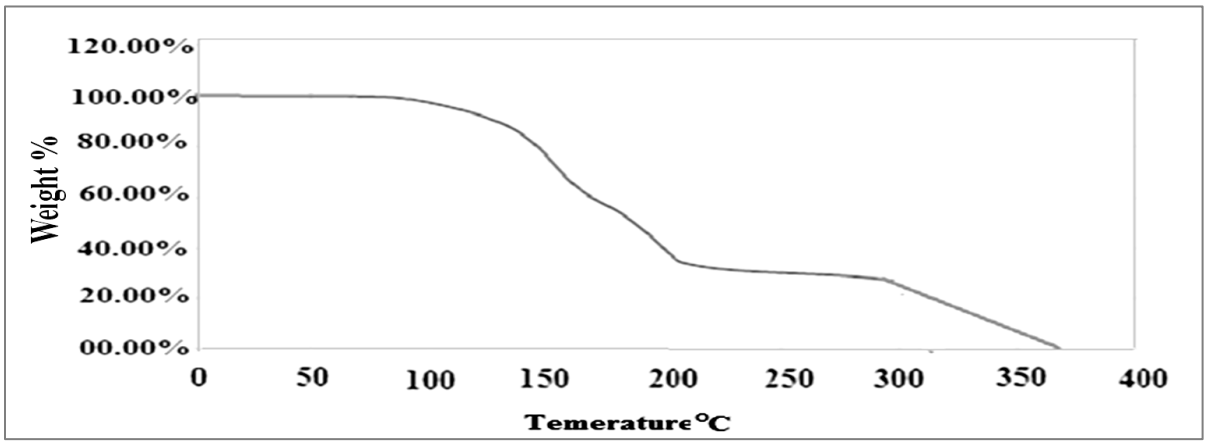

Fig-8: TGA of Schiff based Resin PMBHNMD

Infrared Spectral Studies of Schiff Base BHNMXD and Schiff Based Resin PMBHNMXD

The results of FT-IR (Fourier Transform Infrared) Spectra of Schiff base and resin were presented in figure-9 and figure-10respectively.The spectra of compound Schiff base clearly displayed presence of >HC=N azomethine sharp peak around 1630 cm^1, whereas the FT-IR spectra of compound Schiff based resin PBHNMX D indicated shift in the peak at 1632 cm^{-1}.The 2cm shift of band in resin towards higher frequency as compared to Schiff base, confirmed the formation of resin as it differentiated a Schiff base from resin. The higher intensities absorption bands confirm the formation of polymerization resin

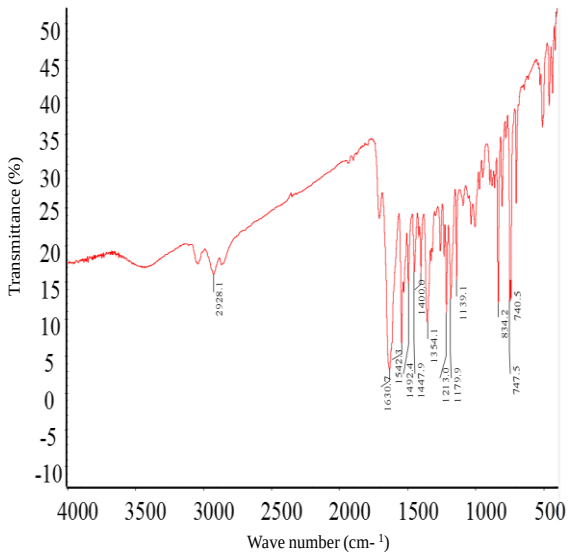

Fig-9: FT-IR spectra of Schiff base BHNMXD.

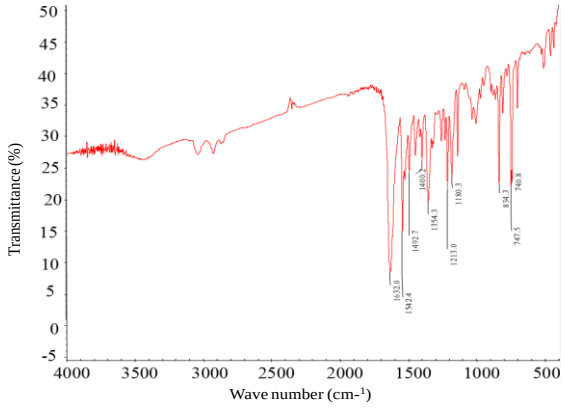

Fig-10: FT-IRSpectra of Schiff base resin PMBHNMXD.

EDS ANALYSIS OF SCHIFF BASE (BHMNMXD) AND SCHIFF BASED RESIN (PMBHMNMXD)

EDS spectrum and elemental composition demonstrates the schiif base BHMNMXD and schiff based resin PMBHMNMXD and identified the material is mainly composed of carbon and oxygen as a esential material along with N as a constitute in the sample.

EDS analysis shows a composition of BHMNMXD 51.51 % C, 17.49% O, and 30.99% N (wt.%) and Schiff based resin PMBHMNMXD composition of 80.92% C,19.0 % O, and 34.98% N(wt.%) as evidence of successful inter-diffusion and the formation of compound.

SEM analysis Schiff Base BHNMXD and Schiff Based Resin PMBHNMXD

The synthesized compounds schiff base and schiff based resin were subjected to SEM analysis. The two images present the morphological alteration before and after the polycondensation reactions. The topogrphic

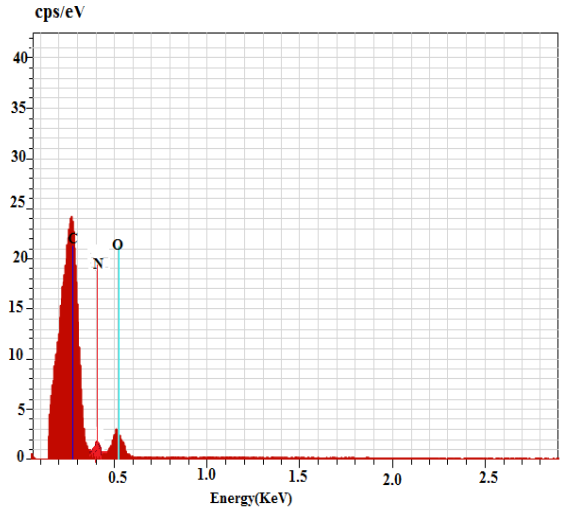

Fig-11: EDS Spectrum of Schiff Base BHMNMXD

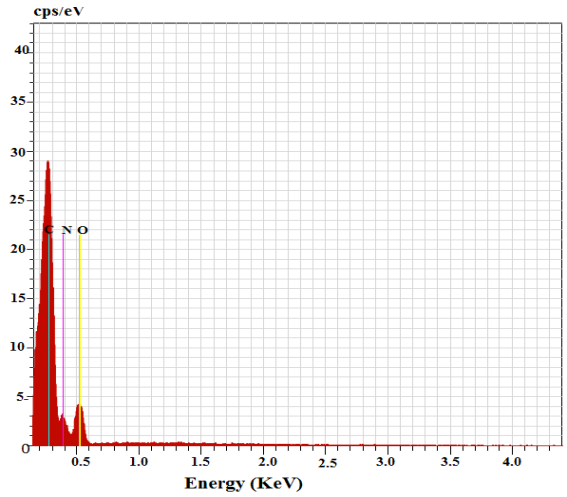

Fig- 12: EDS Spectrum of Schiff Based Resin PMBMHNMXD

results of Schiff base particles indicated observable variation from crystalline,irregular,longitudional to submerged some what porous particlesof resin, confirming formation of resin.

In **(Fig. 13)** represents the images of Schiff base BHNMXD, at a magnification×1,000 to 500 indicating the, sphericalsurface, with longitudinal particles and size distribution approves a sharpcrystalline structure. The diameter of Schiff baseparticles, is in the range of 10 micron, and **(Fig.14)** showed the SEM images of resin PMBHNMXD, at a magnification × 500 to1,000 indicating the strong binded merged particles ranged between 10-50 micron.The SEM image of schiff based resinshowed most of its particles spherical, displaying uniform average size and close-packed surface. The images could directly reflect the effect of polymerization,thus resulting in increase homogenous distribution**.**

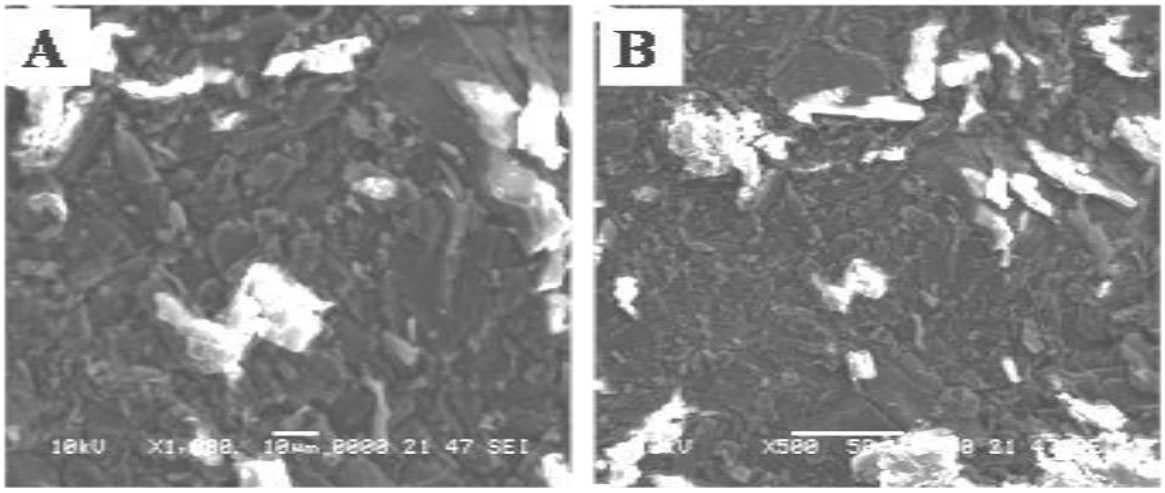

Fig-13:SEM images of Schiff Base BHNMXD.

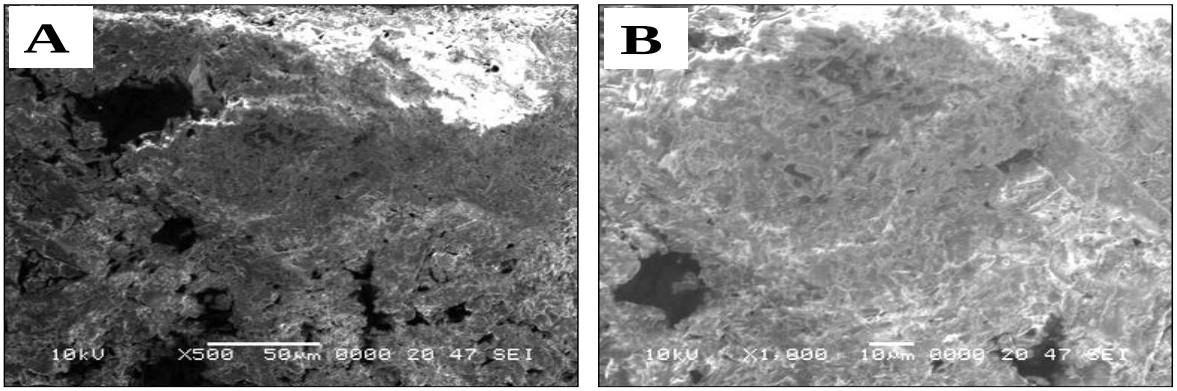

Fig.-14: SEM images of Schiff based Resin PMBHNMXD

The X-ray Diffraction Crystallographic Evaluation of Schiff base BHNMXD and its Resin PMBHNMXD

The XRD diffraction pattern of Schiff base and Schiff based resin is indicated by black and red lines respectively. The X-ray diffraction pattern of compound BHNMXD and PMBHNMXD are planes with peaks at 2θ, the peak 2θ = 20.2 and 20.5, are associated with the (111), (002)planes,(as in figure-12).The crystallographic evaluation of Schiff base, and Schiff based resin display black line in diffractrogram represents Schiff base BHNMXD about 2θ angle around12.5, 19.9, 20.1, 24.1 and 28.3, 31.5.

While red one represents the resin PMBHNMXD about 2θ angle around 12.2, 19.9, 21.9, 23.9, 27.9,29.9and 32.4,.The red line in diffractrogram showing of Schiff based resin, very sharp and high intense peaks compared to the Schiff base .The Schiff based resin showed 2θ angle around 12.2, 19.9, 21.9, 23.9, 27.9,29.9and 32.4, these peaks represents that the sample are crystalline in nature, and are associated with the (200), (-111), (202),(-112),(311),(440) and (531) planes, respectively. These reflection planes were approved and found in agreement with JCPDS card no: 5970-45-6 (The powder diffraction file (PDF) for Monoclinic structure.

The result of Schiff based resin as compared to Schiff base was observed, displaying extra 1 peak 32.4, at 2θ. A highest intensity peaks 12.2, 19.9 and 21.1at 2θ was also observed.

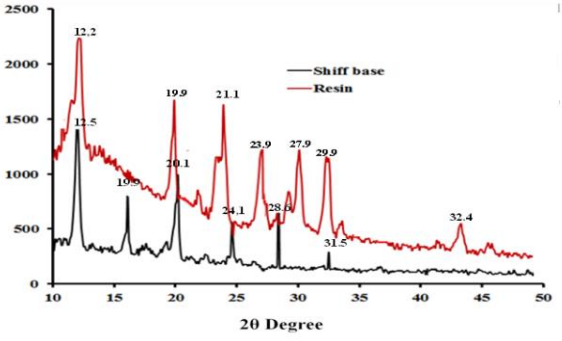

Fig.-15: XRD Diffractogram of Synthesized Compounds

Biological Studies

Antifungal activity of Schiff base BHNMXD and Schiff based Resin PMBHNMXD.

The infections due to fungus are not usually limited to the apparent part of an organism possessing cell aggregation; a substantial enhancement life intimidating infections due to fungus has been reported.

The major cause for this is the additive number of patients endangered, especially those aging above 65 to 70 years, battling surgeries, under the influence of drugs that lowers body's immune response, Anticancer treatments, and transplantation of hematogenic system of cells. The search and development of more effective antifungal agents are obligatory and some Schiff bases are recognized to possess positive antifungal mediators.

The antifungal studies of Schiff base and resin were examined by using ager well diffusion method on Potato dextrose agar (PDA), which is a commonly used medium for growing fungus by a composition, water, potatoes,(silica washed) used the fungal strains were grown on'' potato dextrose agar (PDA) and were kept for incubation at the temperature of 24±˚C for five days.

The fungal slants were added with two to three milliliters of distilled water and after incubation at the said temperature, the cultures were filtered through ordinary whatman filter paper. Miconazole an antifungal was used as a standard drug.10 mg/ml of the test samples of (BHNMXD) and (PMBHNMXD) were used for recording the activity. The activity was observed by measuring colony diameter in millimeters of all the zones formed. The Schiff base and Schiff based resin showed inhibitory effects against cultures. The found results conveyed that the resin indicated enhanced activity as compare to its Schiff base against *Aspergillus Flavus* (G-ve), *Candida albicans* (G-ve) and *Aspergillus Niger* (G-ve). The Schiff base showed(**30**, **20**, **15**) zone of inhibition, whereas the resin exhibited **(35, 25, 15)** zone of inhibition. The test compounds showed a good sensitivity against all antifungal strains.

Table - 3: Antifungal screening results of synthesized Compounds Schiff base and Schiff based resin

Standard drug –ve control antifungal active

	Zone of Inhibition (mm) 50 µg/ml-1			
	Compound	**A flavus**	**Calbicans**	**A. Niger**
1	BHNMXD	+ + +	+ + +	+ +
2	PMBHNMXD	++ +++	+++++	++
3	Miconazole	+++	++	++

- = inactive **5** mm
+ = weakly active 8 – **10** mm
++ = moderately active 11 – **15** mm
+++ = highly active **16** – **20** mm
++++ = most active **21** – **25** mm

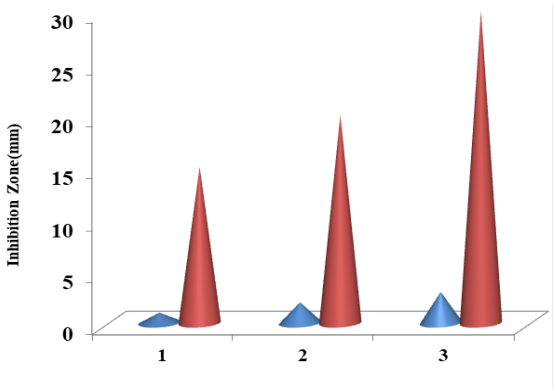

Fig.-16: Antifungal activities of Schiff base against *A flovus, C.allican, A. Nige,* fungal isolate

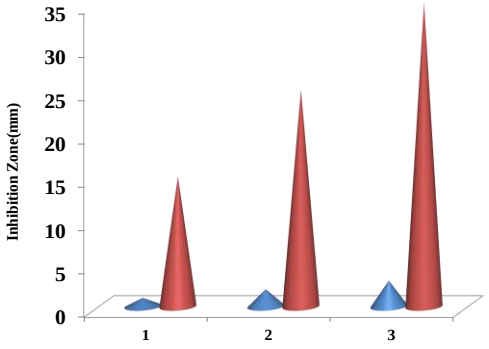

Fig.17: Antifungal activities of Schiff based resin against *A flovus, C.albicans, A. Nige,* fungal isolate,

Chemicals and spectral measurements:

^{1}H NMR spectra were verified on Bruker 300 MHz spectrometers.Mass experiments were carried out on a Finnigan MAT-311A (Germany) mass spectrometer. Thin-layer chromatography (TLC) was examined on pre-coated silica gel aluminium plates (Kieselgel 60, 254, E. Merck, Germany). Visualization of TLC chromatograms was performed at wavelengths of 254 and 365 nm. SEM analysis were recorded on model JSM-6380 lv.XRD analysis were recorded D8-Bruker. FT-IR spectra were recorded on Thermo Nicolet Corporation,SF-AV 400 USA. The CHN analysis (Carbon-Hydrogen-Nitrogen) was performed atNational center of excellence in analytical Chemistry, Jamshoro. 2-Hydroxy-1-naphthaldehyde was purchased from alfa aesar, Germany, m-xylenediamine, acetic acid, formaldehyde, methanol, ethyl acetate, n-hexane, NaOH were obtained from E Merck, Germany.

3. CONCLUSION

The compounds were synthesized followed by characterization^{1}H-NMR, EI-MS, MALDI-TOF mass spectrometry, X-ray diffraction powder analysis, SEM analysis, FT-IR, and CHN elemental microanalysis successfully. The FT-IR spectra of resin PMBHNMXD indicated stronger sharp peak at 1632 cm-^{1} of >C=NH

approving polymerization and confirming the structure of the newly prepared resin. The found CHN results match remarkably to the calculated values assuring the forming of Schiff bases and resin. Scanning electron microscopy analysis showed the morphology of Schiff base BHNMXD and resin PMBHNMXD showing, fibrous, longitudinal spherical rough, inflexible and rigid structure. The melting point enhancement was observed after recrystallization, which improved the purity of compounds. TGA showed that heat stability of synthesized resin.EDS showed C, O_2 and N essential element present in synthesized Schiff base and Schiff based resin. The X-ray diffraction crystallographic evaluation of Schiff base and its resin proved that monoclinic sharp crystalline structure. The Schiff base and resin presented highly active antifungal activities in all fungal strains such as, *Aspergillus Flavus* (G-ve), *Candida albicans* (G-ve) and *Aspergillus Niger* (G-ve).

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