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Synthesis and molecular docking of terephthalic dihydrazide from poly(ethylene terepthalate) for antimicrobial activity and biochemical changes

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ABSTRACT

The increasing use and generation of solid plastic wastes have imposed challenges of their disposal for the society. Thereby, the recycling and treatment of solid plastic waste to ecofriendly compound may help in sustaining and integrating the wastes for useful purpose. Herein, this paper the aminolysis of poly(ethylene terphthalate) (PET) is being carried out in presence of excess of hydrazine monohydrate to form terphthalic dihydrazide (TDH). The characterization and structural confirmation was done by FTIR, NMR, UV and thermal analysis. The derivatives of amide are related with broad spectrum biological activities including antimicrobial activities. This aromatic amide was further analysed for antifungal property by well diffusion method viz., MIC (minimum inhibitory concentration), zone of inhibition and biochemical changes. The synthesized aromatic amide showed broad spectrum antifungal property. The amide derivative was proposed as an inhibitor of Cytochrome P450- 14DM14, a-demethylase from Aspergillus niger and S12 protein of ribosomal subunit from Escherichia coli. Drug-likeness and hidden potential of compound and descriptors related to ADMET were deliberated to foresee pharmacokinetic properties of the molecule. Thereby molecular docking data study helped in evaluating probable mode of action of molecules in active site of receptor.

Keywords:Antifungal agent, Aromatic amides, *Aspergillus niger*, PET (Polyethylene terephthalate), TDH (terephthalic dihydrazide), minimum inhibitory concentration (MIC), Molecular docking, Receptor

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INTRODUCTION

The first commercial and industrial scale synthetic polymers were produced in early 1940s, ever since the consumption and generation of solid waste from plastic has increased exponentially in daily household, packaging, industries etc. Now the researchers all over the world are working in regulating the environmental issues generated by plastic wastes [1]. The high cost of disposal of plastic solid waste and the considerable decrease in space for landfills have forced to look for alternatives for disposal of plastic solid wastes [2]. Several methods and chemical routes have been proposed for recycling of plastic wastes [3]. PET is a commonly used as a thermoplastic polymer, used in sheets, fibres and bottles. PET wastes can be recycled chemically and mechanically both. The mechanically recycled PET wastes generate low grade products like fibre [4,5,6]. Chemical recycling is carried out by depolymerization processes like hydrolysis, methanolysis and glycolysis and also by the use of the organocatalytic depolymerization of PET with excess amount of ethylene [7]. Another little investigated chemical degradation technique of PET waste is aminolysis. Soni and Singh [8] reported the degradation of PET by ammonia and methylamine in the presence ammonium salt as a catalyst at room temperature. Thus various efforts have been done in the study of degradation of PET waste with different amines by solvolysis. The product obtained by solvolyis of PET waste may be used for further application such as terephthalic dihydrazide was reported for the use in PVC

compounding as secondary plasticizers [9]. In addition of this application amide derivatives are also associated with another use as an antimicrobial agent having biological and pharmacological activities [10]. Aromatic amides may be used as fungicides by affecting cell wall production through inhibition of ergosterol production [11]. Amides derivatives show broad spectrum of biological activities which includes antituberculosis, anticonvulsant, analgesic, anti-inflammatory, insecticidal, antimicrobial, and antitumor properties. These amides play a pivotal role in molecular recognition and also an important component in supramolecular chemical anion sensor technology. These amides show good thermostability [12] can sustain higher temperature which positively affects its use as an antimicrobial agent. In today's scenario there is continual need to keep exploring new classes of antimicrobial agent because wide use of antibiotics has developed multidrug resistance in microbial pathogens [13]. In this way, synthesis of amide and amides derivative may be used as good approach for antimicrobial agent.

In this work, an effort was made to synthesize a biologically useful compound from the PET waste by aminolysis method of chemical degradation. PET was aminolysed to aromatic amide TDH (Terephthalic dihydrazide) by the reaction of PET waste with hydrazine monohydrate and screened for antifungal activity on *Aspergillus niger*. *Aspergillus* species cause the group of diseases such as aspergillosis, otomycosis etc. [14]. These are secondary invader of diseases of sinuses, lungs, ear, eye, CNS, urinary tract and dissemination in case of immunosupression. The amide derivative acted as an inhibitor of Cytochrome P450- 14DM14a-demethylase from *Aspergillus niger* and ribosomal subunit of S12 protein from *Escherichia coli.* The drug-likeness and hidden potential of compound and ADMET-related descriptors were considered to expect pharmacokinetic properties of the molecule. Molecular docking studies helped to evaluate possible mode of action of molecules in active site of receptor.

MATERIALS AND METHODS

Chemicals and Methods: The chemicals hydrazine hydrate was procured from SRL, India. PEG was procured from SRL. Fluconazole and Media was purchased from Himedia, India. A fungus species *Aspergillus niger* was cultured and maintained in our laboratory in Sabouraud Dextrose media (SDA) and streptomycin antibiotics. UV-Visible spectroscopy was carried out by Systronic double beam spectrophotometer 2203 SMART. The FTIR spectra of compounds were recorded on Cary 630 FTIR, Agilent Technologies. DSC was done by Lab: Mattler STAR^e SW 8.10. ^{1}H NMR spectra were recorded on Bruker 300 MHz. The chemical shifts measured are relative to TMS which is used as an internal standard and are expressed in ppm.

Experimental Section

Recycling of PET wastes: The waste flakes from PET were collected from industry and washed with methanol several times. Then it placed into a water bath at 70-100°C for separation of PET flakes from impurities. This process shrinked PET flakes which separated other impurities leaving behind high density polyethylene cups and polypropylene cups [8]. PET waste was reacted with Hydrazine monohydrate in the ratio 1:10 (w/v). The sealed reaction flask was used to carry out the reaction with continuous stirring at ambient temperature and pressure. The aminolysed product was separated after 24 hours and washed with distilled water. The precipitate was dried in oven [12]. Characterization of reaction product was done by UV-Visible spectroscopy, FTIR spectroscopy, Nuclear magnetic resonance spectroscopy and thermal analysis for the identification of molecular structure.

Antifungal activity: Determination of antifungal activity of TDH was done by measurement of zone of inhibition by well diffusion method. The petridish was casted with media and well was created in petridish with the help of sterile cork borer (8 mm in size). The test compound dissolved in DMSO was filled in wells. The antifungal activity of compound was compared with Fluconazole which is used as a standard antifungal agent [15]. Fungal strain *Aspergillus niger* was exposed to different concentration of synthesized amide in DMSO to determine zone of inhibition.

Preparation of an ointment and antifungal activity study: Ointment was prepared by fusion method followed by mixing PEG 400 and PEG 4000 in adequate ratio to prepare a cream base then different concentration of amides in DMSO were added to it. The ointment with different concentrations of amide was examined for *Aspergillus niger* fungal growth.

Biochemical Studies: Biochemical analysis of the fungus was studied in the presence of ointment. It involved the estimation of carbohydrate, protein, lipids and nucleic acid. Biochemical changes in fungal cells were studied during growth, development of fungus which was measured by dry weight. Fluconazole was used as a positive control and

DMSO as a blank. Total carbohydrate was estimated by Anthrone method. Protein estimated was done by Lowry's method. Nucleic acid as DNA and RNA were measured by diphenylamine and Orcinol method, respectively. All parameters was calculated by extrapolation on standard curve.

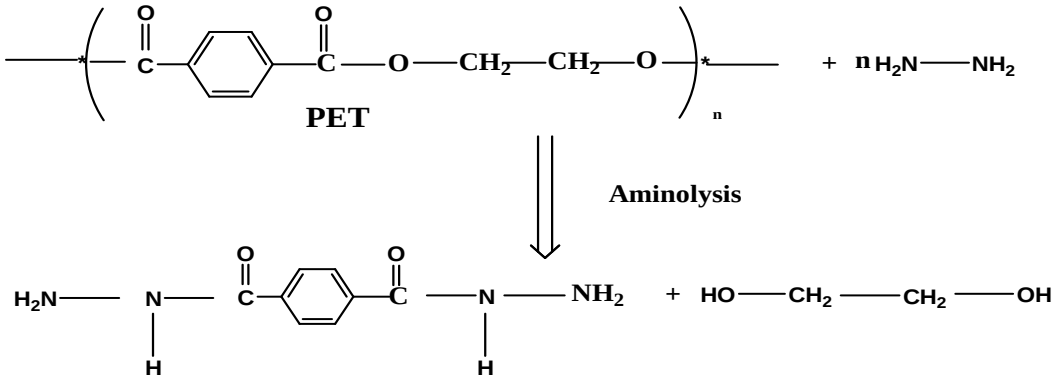
Molecular Docking: The 3D structure of Cytochrome P450-14DM from *C. albicans* have been constructed through homology Modeling. Modeller9v9 package was used to generate homology model of Cytochrome P450-14DM [16]. The template chosen for study was human Cytochrome P450-14DM protein that displayed resemblance with protein sequence used here. The low value of Modeller objective function or DOPE and assessment score of GA341 helped in choosing five best models among 100 models. Procheck analysis assisted in choosing the best model [17] which showed the occurrence of just one residue in excluded region analogous to the template.

Further, the protein and grid preparation required crystal structure of 30S Ribosomal subunit S12 (PDB Id:1FJG, L Chain) obtained from protein data bank. After the modeling of Cytochrome P450-14DM, the best model is selected. Schrodinger protein wizard is used for protein preparation that include hydrogen atom addition, followed by optimization by OPLS force field [18]. Ligand for docking was set in Schrodinger ligprep wizard and addition of hydrogens was followed by optimization in OPLS_2005 force field, generating 10 conformations for ligand. After the preparation of ligand library, proteins and the protein grid docking was carried out. The software Glide search and pose ligand flexibility using Systematic and Simulation method.

Post docking analysis: Ligplot program is used for analysis of docked protein-ligand complex interaction and to ensure hydrophobic and polar interactions between the ligand and receptor [19]. PyMOL software helped in generation of figures screening protein-ligand complex and ligand-protein interactions [20].

RESULTS AND DISCUSSION

Chemistry: The PET wastes were degraded using amines, such as primary amines (aliphatic, aromatic, click functionalized, tertiary functionalized), secondary amines, and asymmetric amines and the process was known as aminolysis. Masayoshi reported solid-phase polycondensation or melt polycondensation to produce copoly(ethyleneterephthalate/ethylene terephthalamide) using ethylene diamine [21]. The aminolysis reaction of PET with excess amount of hydrazine mono hydrate can be proposed as the chemical reaction in Scheme 1.



Scheme 1: Terephthalic dihydrazide (TDH) obtained by the aminolysis of PET waste

Characterization: The aminolysis product of PET waste with was characterized with several spectroscopic techniques (UV-Visible, FTIR and ^{1}H-NMR). The UV-Visible spectra of compounds were recorded using Systronic double beam spectrophotometer 2203 SMART at medium scan speed and range between 200 to 400 nm **(Figure 1)**. The sample was prepared by dissolving in DMSO. The UV spectrum showed absorption at 232.0 nm indicating the presence of carbonyl group.

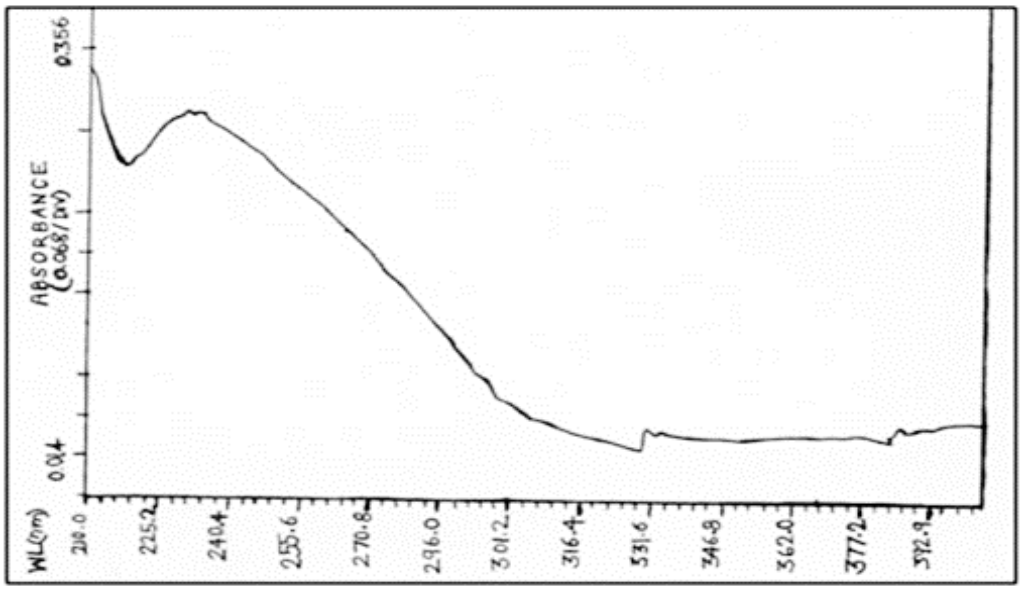


Figure 1: UV-Visible spectra of terephthalic dihydrazide in DMSO (200-400 nm)

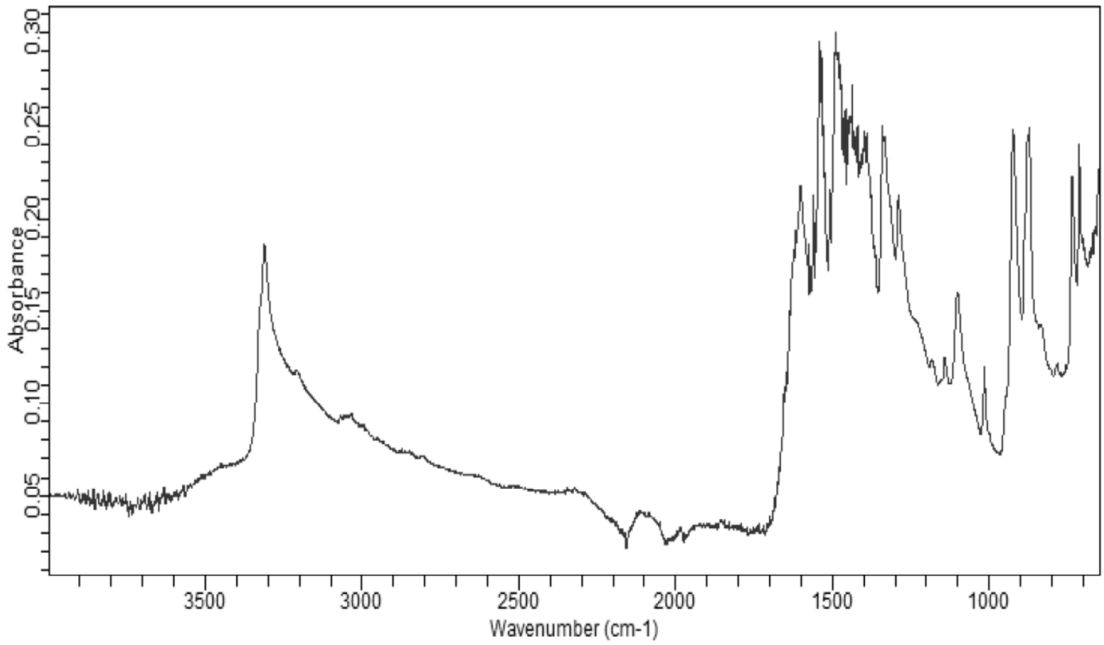


Figure 2: FTIR spectrum of terephthalic dihydrazide

The FTIR spectra of compounds were recorded on Cary 630 FTIR, Agilent Technologies. The FTIR spectra revealed absorption bands at 3313 and 1605 attributed to N-H stretching and C=O stretching, respectively. A bifurcate medium peak was obtained at 3320 and 3315 cm^{-1} which showed coupled N-H stretching and indicated the presence of primary amide. Peaks obtained at 1337, 1299, 1234, 1104 cm^{-1} showed C-O stretching. Two medium peaks were observed at 1544 and 1493 cm^{-1} that showed C=C stretching and indicated the presence of an aromatic ring (**Figure 2**). These spectral values of synthesized TDH are in good agreement with those reported in the literature [22, 23]. It confirmed the reaction product obtained was TDH.

The ^{1}H proton NMR spectrum shows three absorption peaks **(Figure 3)**. A sharp singlet at δ 7.86 was associated with four aromatic protons (C-H), peak at δ 9.88 was linked with CO-NH protons and a doublet at δ 4.537 accounted for NH protons.

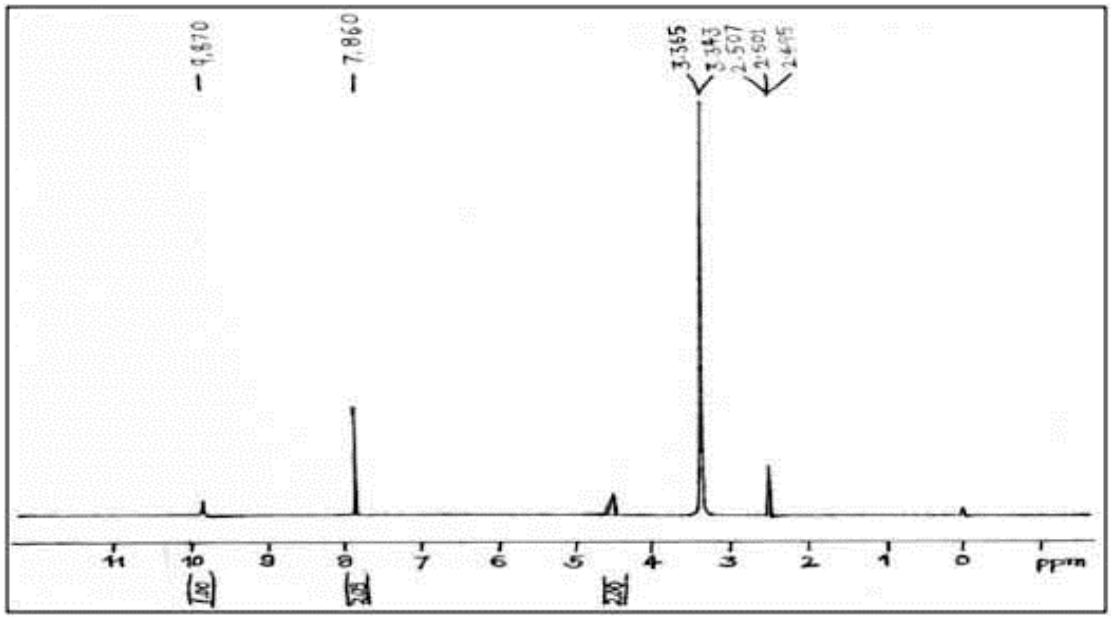


Figure 3: ^{1}H NMR of of terephthalic dihydrazide (TDH)

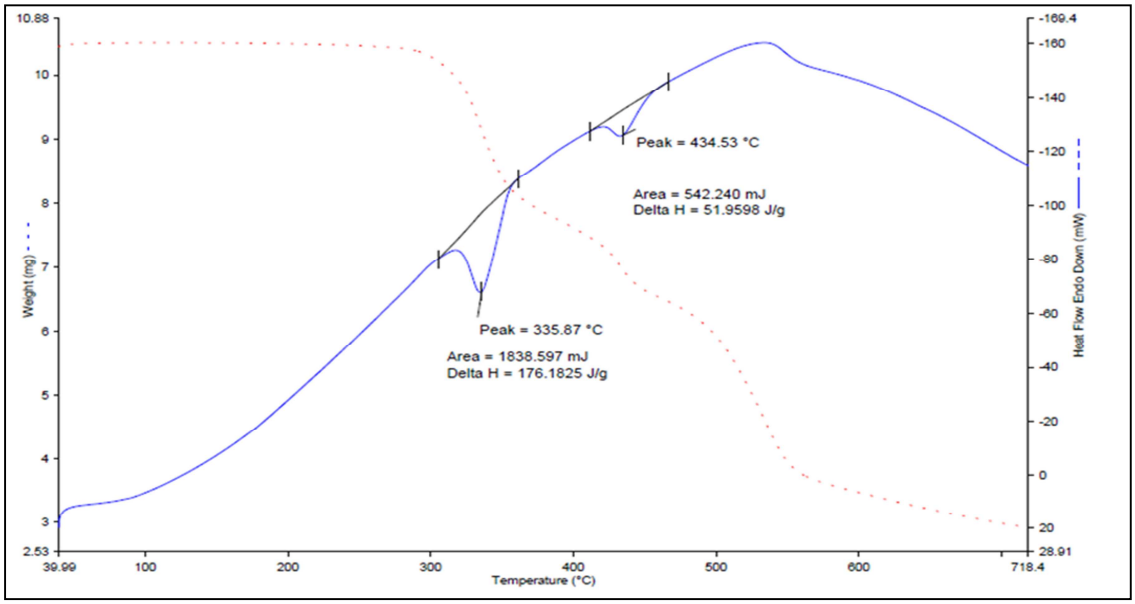


Figure 4: TGA/DTA of terephthalic dihydrazide (TDH)

Thermogravimetric analysis and Differential Thermal Analysis (TGA/DTA) of TDH was recorded from 0-700 ^{o}C. The loss in weight of the sample was observed after 300^{o}C, hence TDH was thermally stable till 300^{o}C (**Figure 4**). The DSC thermogram **(Figure 5)** was recorded from -50^{o}C to 500^{o}C at a heating rate of 10^{o}C per minute under air atmosphere for the recycled product obtained as the result of aminolysis The thermogram showed a large endothermic peak at 326.89^{o}C which indicated formation of single compound.

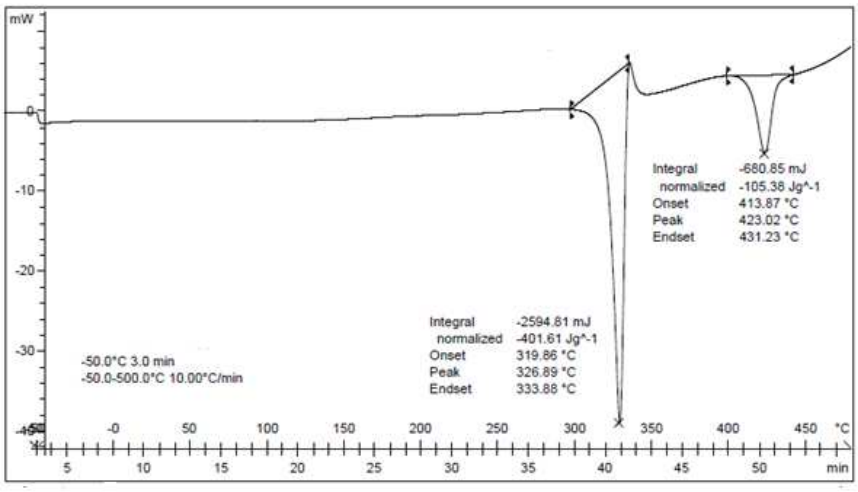


Figure 5: DSC thermogram of terephthalic dihydrazide (TDH) recorded from -50°C to 500°C at a heating rate of 10°C per minute

Antifungal activity of compound: The recycled compound (TDH) from PET wastes showed broad spectrum antifungal activity. Different concentration of compound viz., 20 mg/mL, 25 mg/mL, 30 mg/mL, 40 mg/mL and 45 mg/mL was made in DMSO. The DMSO was supposed to be blank and different concentration of fluconazole viz., 20 mg/mL, 25 mg/mL, 30 mg/mL, 40 mg/mL and 45 mg/mL were observed as positive control. *Aspergillus niger* growth inhibition was observed at the concentration of 40 mg/mL and 45 mg/mL. In the presence of DMSO, no zone of growth inhibition was seen. The measured zones of growth inhibition is showed in **Table 1.0.** The results showed that TDH respond to antifungal activity when compared to control fluconazole. Minimum inhibitory concentration (MIC) was determined by microdilution method. MIC is the minimum amount of compound that inhibits microorganism growth. MIC for *Aspergillus niger* in presence of TDH compound was 30mg/mL of DMSO and on the other hand, MIC in presence of Fluconazole was 10mg/mL. Further, to enhance and support the antifungal activity data, the ointment of TDH was prepared and its antifungal activity was analysed. Ointment was prepared by dissolving different concentration of TDH compound with polyethylene glycol by fusion method. The different concentration of TDH in the form of ointment inhibited fungal growth. The high melting point of TDH as suggested by DSC data shows heat stability of compound and favours it to be an antifungal ointment which can be stored at unfavourable temperature condition.

Table 1: Comparison of zone of inhibition of TDH in presence of fluconazole at different concentrations

S.No.	Concentration of TDH compound (mg/ml DMSO)	Zone of inhibition (mm) in presence of TDH	Concentration.of fluconazole (mg/ml)	Zone of inhibition (mm) in presence of fluconazole
1.	20	20	20	20
2.	25	20	25	22
3.	30	20	30	20
4.	40	22	40	20
5.	45	22	45	22

Different biochemical parameters as DNA, RNA, protein, carbohydrate and nitrogen content were analysed in fungal suspension. The spores and mycelium grown in the presence of TDH ointment and fluconazole showed different concentrations of biological parameters in comparison to blank **Table 2.0** . This results suggested that there is no significant difference of biological paramteres concentrations between TDH and fluconazole.

Table 2.0: Estimation of different Biochemical parameters in presence of Fluconazole and TDH

S.No.	Biological parameter	Blank	In presence of TDH	In presence of fluconazole
1.	DNA	1.4mg/100ml	1.0mg/100ml	0.9mg/100ml
2.	RNA	2.8mg/100ml	2.4mg/100ml	2.45mg/100ml
3.	Protein	4mg/100ml	3.4mg/100ml	3.5mg/100ml
4.	Carbohydrate	4.8mg/100ml	3.8mg/100ml	3.5mg/100ml
5.	Nitrogen content	0.64mg/100ml	0.54mg/100ml	0.52mg/100ml

Absorption, Distribution, Metabolism, Excretion (ADME) and toxicity prediction: A 2D ADME-Plot is calculated using ADMET_PSA_2D and ADMET_A logp98 properties. The graph was plotted against ADMET_PSA_2D vs. ADMET_A logp98 (**Figure 6**). In blood brain barrier (BBB) plot, TDH was falling outside 99% ellipse suggesting it to be inactive compound for central nervous system and blood brain barrier. As a result, central nervous system toxicity is low. The bioavailability of the drug is an impotant property that is decided by aqueous solubility. TDH was found to have similar aqueous solubility score as that of standard compound (Fluconozole) as referred in (**Table 3**). TDH does not show liver toxicity and with respect to CYP2D6 liver the compound is non-inhibitors of CYP2D6 (**Table 3**). These data suggest that TDH is metabolized well in Phase-I metabolism.

Table 3 : Computer aided ADME screening of TDH and Fluconozole (standard)

Compd	ADMET BBB_Level	ADMET_Absorption_Level	ADMET_Solubility_Level	ADMET_Hepatotoxicity	ADMET_CYP2D6
3365 (Fluconozole)	3	0	4	1	0
67294 (TDH)	4	1	4	1	0

BBB: Blood Brain Barrier, CYP2D6: Cytochrome P450-14DM enzyme inhibition using 2D chemical structure as input.

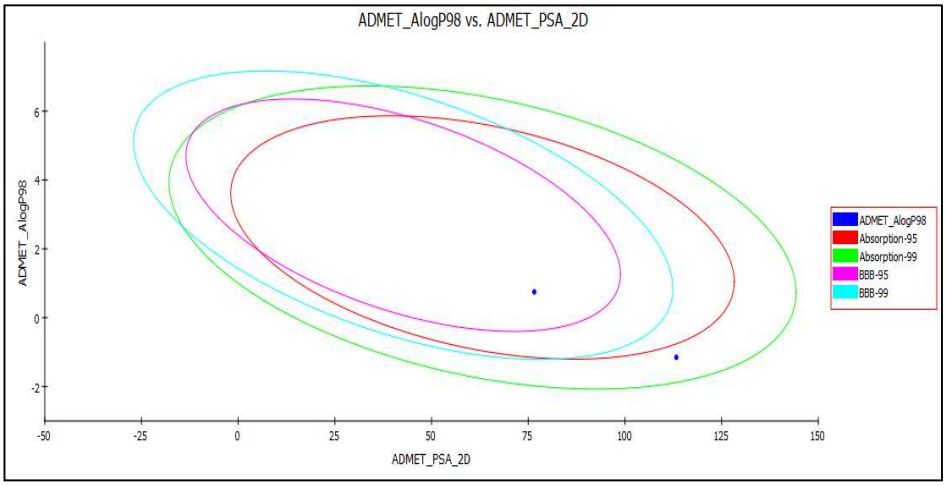


Figure 6: Drug absorption for the synthesized compound TDH for anti-microbial activity. Discovery studio 2.5 ADMET Descriptors, 2D polar surface area (PSA 2D) in _A2 for compound is plotted against their corresponding calculated atom-type partition coefficient (ALogP98)

Table 4.0 shows the ligand molecule with different types and number of molecular interactions and binding strength. The binding sites on the protein of hydrophobic nature are concerned with drug disposition leading to the significance of lipophilicity as referred in **Figure 7**.

Table 4: Ligand molecules with types and number of molecular interactions and binding strength

Compound	Gold Fitness Score	No. of Hydrogen Bonding Interactions	No. of Lipophilic Interactions	No. of Non-Bonded Interactions
Fluconazole	57.52	NIL	6; Tyr76, Arg96, Ala256, Thr260, Leu321, Hem450	30
TDH	43.61	5; Arg96, Phe255, Ala256, Thr260, Hem450	1; Leu321	21

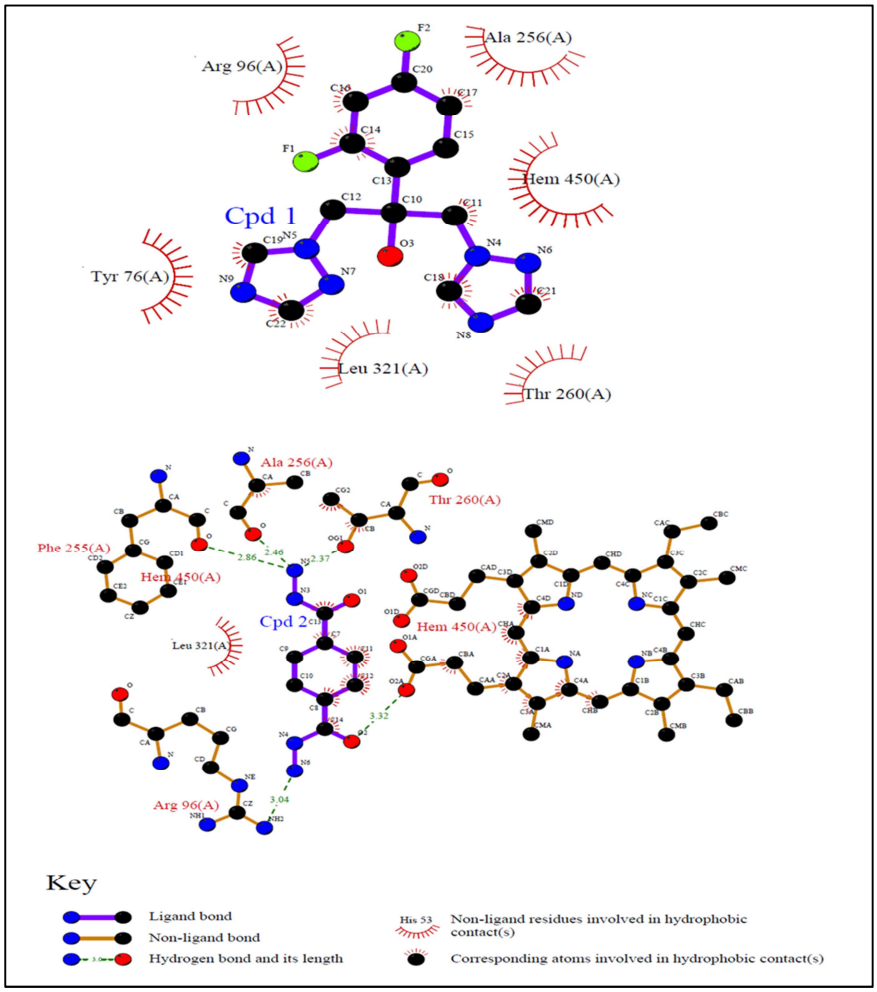


Figure 7: The interaction plot (Ligplot) of Flucanozole (a) and TDH (b) with the binding site residues of Cytochrome P450-14DM of *C. albicans*. The hydrophobic interactions and hydrogen bonds are holding the ligand within the binding site

CONCLUSION

This work deals with the formation of amide (terephthalic dihydrazide) through degradation of PET with hydrazine monohydrate (aminolysis) at room temperature. The ultimate degraded product was analysed and characterized with spectral data (UV-Visible, FTIR, ^{1}H NMR) and thermal data (DSC, TGA-DTA). This TDH was analysed for the fungal growth inhibition. Further, ointment was prepared by compound TDH which inhibited growth of fungus *Aspergillus niger* and decreased the content of carbohydrate, protein, DNA and RNA of fungus as compared to blank media. It suggested that synthesized compound showed antifungal activity and reduced the biological contents of the fungi species. The results are significantly comparable with fluconozole which is used as an standard antifungal agent. The amide derivative was designed as inhibitors of Cytochrome P450- 14DM14, a-demethylase from *Aspergillus niger* and ribosomal subunit of S12 protein from *Escherichia coli.* The drug-likeness and hidden potential of compound and ADMET-related descriptors were calculated to predict pharmacokinetic properties of the molecule. Molecular docking studies helped in evaluating possible mode of action of molecule in active site of receptor. Herein, an attempt is made to recycle the solid polymer waste in development of antifungal agent. Further, this type of docking and antipathogenic screening studies would help in targeting microbial protein synthesis.

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