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IN SILICO DRUG DESIGN AND EXTRACTION OF PIPERINE AN INHIBITOR FOR FERNESYLTRANSFERASE IN CRYPTOCOCCUS NEOFORMANS

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ABSTRACT

Piper nigrum (black pepper) extract contains several important alkaloids. These alkaloids are important in medicinal biology. In this present work we have attempted to extract an important component from black pepper by solvent extraction procedure. Extracted product was isolated, purified and identified by TLC,UV-VIS spectroscopy, FT-IR Spectroscopy and ^{1}H-NMR, ^{13}C-NMR spectroscopy. Finally, the isolated compound of piperine activity was checked and proved through the insilico analysis using HEX software. The synthesized compound piperine was used to stop the function of the protein farnesyltransferase available in the disease causing organism Cryptococcus neoformans and also compared with the existing drugs.

KEYWORDS: 1. Piper nigrum 2. Dichloromethane 3. Molecular docking 4. Cryptococcus neoformans.

INTRODUCTION

The fruits of Piper nigrum (black pepper) have been widely used in household spices and also in various systems of medicine since time immemorial [1-5]. It belongs to the family piperaceae Piper nigrum L; (fig.1). The South West part of India is well known for Pepper growing regions and there pepper is referred to as black gold [6-8]. It is used not only in human dietaries but also for other purposes such as medicinal, a preservative, in perfumery

and even as an insecticide [9-11]. Piperine is a naturally occurring compound present as the major pungent ingredient (1±9%) in various parts of plants from the family Piperaceae [12]. Piperine, an alkaloid (1-peperoyl piperidine), has been previously evaluated for its potential to enhance the serum levels of drugs and nutrients in animals and humans [13-20]. A thorough literature study on black pepper (piper nigrum) shows that studies on a tremendous of pharmacological, physiological activities and other applications. First extraction methods involve identifying the medicinal compound and then computational studies were done for the isolated compound.

Cryptococcus neoformans is encapsulated yeast that can live in both plants and animals. Its teleomorpha is Filobasidiellaneoformans, a filamentous fungus belonging to the class Tremellomycetes. It is often found in pigeon excrement. The most serious infections usually develop in patients with detective cell-mediated immunity. The global emergence of AIDS, the induce of Cryptococcusneoformans is increasing and now represent major life threatening fungal infection in those patients [21]. There are thirty different species of Cryptococcus. C. neoformans can be found in soil throughout the world. People at risk can become infected after inhaling microscopic, airborne fungal spores. Sometimes these spores cause symptoms of a lung infection, but other times there are no symptoms at all. In people with weakened immune systems, the fungus can spread to other parts of the body and causeserious disease [22]. Most infections with C. neoformans consist of a lung infection. However, fungal meningitis and encephalitis, especially as a secondary infection for AIDS patients, are often caused by C. neoformans making it a particularly dangerous fungus. Infections with this fungus are rare in those with fully functioning immune systems. For this reason, C. neoformans is sometimes referred to as an opportunistic fungus [23].

It is a facultative intracellular pathogen. Few antifungals can treat C. neoformans infections, and drug resistance is increasing. Protein farnesyltransferase (FTase) catalyzes post-translational lipidation of key signal transduction proteins and it is essential in C. neoformans. A multidisciplinary study validating C. Neoformans FTase (CnFTase) as a drug target, showing that several anticancer FTase inhibitors with disparate scaffolds can inhibit C. neoformans and suggesting structure-based strategies for further optimization of these leads [24-27].

In this paper we involved in wet lab process of extraction methods and characterization of spectroscopic techniques such as UV-VIS, FTIR, NMR spectral studies, and computational studies.

MATERIAL AND METHODS

Plant Material

The mature berries of piper nigrum obtained from a local market were used in the present study. The seeds used for the study were cleaned manually to free them from stones and other undesirable matter and then ground in an electrically operated grinder to fine particle size.

Chemicals

All chemicals were of analytical grade except petroleum ether (60-80 ^{0} C), Chloroform, Ethyl acetate, Acetone, Dichloromethane (CH_2 Cl_2), Silica gel for TLC, Silica gel for Column and ethanol is of laboratory grade. Except ethanol all other solvents were purchased from Merck chemicals private Ltd. (India).

Methods of Extraction

Place 25.0 g of pure ground pepper and 30 mL of CH_2Cl_2into a 100 mL 19/38 round bottom flask with a magnetic stir bar. Attach a water condenser to the top of the flask and allow water to run through it to condense the CH_2Cl_2 vapours while refluxing the solution for 20 min. After cooling the flask, use vacuum filtration with a Buchner funnel and filter paper to filter out the pepper grounds. Wash the grounds with 10 mL of CH_2Cl_2 and save two drops of the filtrate for TLC analysis.

Isolation and Purification

Transfer the filtrate to a 50 mL round bottom flask and using a sand bath or rotary evaporator, remove the CH_2Cl_2until dark brown oil is left. Cool the oil in an ice bath and add 6 mL of cold ether. After stirring for 5 min, remove the solvent again via sand bath heating or rotovap. Cool the oil in an ice bath and once again add 6 mL of cold ether. Allow the flask to sit for 15 min in an ice bath and do occasional stirring. Piperine should precipitate out; if not, repeat the above procedure. Using the Hirsch funnel, vacuum filter the yellow piperine crystals. Wash them with cold ether (2x4 mL).Thenrecrystallizedwith 3:2 acetone: hexane solution of pure piperine compound (fig.2).

Characterization Techniques

Melting points were recorded with melting point apparatus Macro Scientific Works (Delhi). Thin Layer Chromatography (TLC) was performed on glass plates coated with silica gel 60 (E.Merck, India Ltd.).UV-VISIBLE spectrum of the compound was recorded employing systronics double beam spectrophotometer: 2202FTIR spectrum was recorded employing Perkins Elmer FTIR spectrometer using the KBr pellet technique. The ^{1}H NMR and ^{13}C NMR spectra of the compound were recorded using the AMX 300 spectrometer with tetra methyl silane as the internal standard reference.In addition of the drug designwith Hex molecular modelling software.

RESULT AND DISCUSSIONS

Spectral analysis

In this present work we have attempted to extract an important component from black pepper by solvent extraction procedure. We have selected non-polar solvent, petroleum ether ($60\text{-}80^{0}C$) throughout the extraction process. The extraction was further done with pure Dichloromethane (CH_2Cl_2) using Magnetic stirrer bar extraction process. The TLC of the isolated compound showed a single spot in the non-polar solvent mixture that indicates that compound is pure and is not much polar. The UV absorption spectrum with a peak at **342nm** (fig.3) reflects that the compound may contain a highly conjugated aromatic ring. The FT IR spectrum of the compound is shown (fig.4) and the spectral data were given in Table.1 IR data revealed that the compound consisted of aromatic ring, conjugated double bond, carbonyl specially amide functional group.

^{1}H-NMR Spectrum was recorded in 300 MHz spectrometer (fig.5). The NMR results again confirmed the purity of the compounds. It also indicates nineteen protons within the molecule of which twelve are in aliphatic region. The ^{1}H-NMR Spectrum of the isolated compound showed characteristic signals (1H-NMR, CDCl3): δ 1.58-1.65 (m, 4H), 1.56-1.59 (m, 2H) 3.52-3.63 (m, 4H), 5.96 (s, 2H), 6.44 (d, 1H), 6.66 (m, 1H),6.67 (d, 1H), 6.74 (d, 1H), 6.97 (ddd, 1H),6.90 (m,1H),7.36-7.40 (ddd, 1H) and 7.26 (Solvent). This NMR data is shown in table 2 and it matches with the reference standard of piperine in the literature. But from this NMR data we cannot conclude whether extracted compound is E or Z isomer.

In the ^{13}C NMR spectrum, ($CDCl_3$): δ ppm the ranges of carbon atoms are absorbed between 24-165 ppm, suggesting only aromatic and unsaturated carbon atoms were present. The peak at 0-50 is due to a carbon-carbon single bond. The peak at 50-100 due to the carbon-oxygen

singly bonded atoms present in the molecule and the peak shows at 100-150 due to the carbon-carbon double bond. Finally the absorption peak at range of 165.29 ppm is the carbon due to carbon in a carbon-oxygen double bond. The ^{13}C-NMR spectrum of the compound is shown in (fig.6) and the spectral data are given in Table 3.

Docking results of the drug

The Chemicalmolecules and their activities against biological assays can be obtained using Pub Chem database. The PubChem ID: 3Q73 is Cryptococcus neoformans (fig.7) protein farnesyltransferase (fig.8) [28].It reveals strategies for developing inhibitors that target fungal pathogens. A drug is a substance which may have medicinal, intoxicating, performance enhancing or other effects when taken or put into a human body or the body of another animal and is not considered a food or exclusively a food.

Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria: Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms) [29]. Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms). A molecular mass must be less than 500 Daltons. An octanol-water partition coefficient [log P] should not be greater than-2 to 5. To evaluate drug likeness better results of isolated compound piperine, shown in Table 4.

Hex is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules[30]. Hex can also calculate protein-ligand docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes. The synthesized compound piperine was used to stop the function of the protein farnesyltransferase available in the disease causing organism Cryptococcus neoformansand also compared with the existing drugs Amphotericin B, Sertaconazole, Itraconazole, Anidulafungin, Butenafine and Flucytosine. All the parameters (E-total, E-shape, E force, E air, V shape, V clash and Energy range) were showed under the suggested values and proved the efficiency of the drug. Energy ranges were confirmed between the acceptable values and the docking time also very less. Comparatively the existing drugs and selected lead compound had identical scores. Hence, the overall conclusion proved the pepper also has the ability to work as a fungicide an inhibitor for Fernesyltransferase in Cryptococcus Neoformans. Finally docking results of isolated compound was shown in table 5 and fig. 9.

Table 1: FT-IR Spectral frequencies of the compound

Characteristics Bond Stretching	Wave Number in (cm^{-1})
N-H asymmetric stretching vibration	3300
Aromatic C-H asymmetric stretching vibration	2919
Asymmetric stretching of conjugated diene	1844
stretching vibration =C=O Kenotic group	1610
Asymmetric stretching of =C-O-C	1226 & 1132
C-H Bending of Trans -CH=CH-	1000 & 928
C-H out-of plane bending vibration	831 & 717

Table 2: The ^{1}H-NMR Spectral data of the compound

Chemical shift values(δ in ppm)	Multiplicity	Number of Protons (^{1}H)	Assignment
1.58-1.65	m	4	11-H
1.56-1.59	m	2	10-H
3.52-3.63	m	4	9-H
5.96	s	2	8-H
6.44	d	1	7-H
6.66	m	1	6-H
6.67	m	1	5-H
6.74	d	1	4-H
6.97	dd	1	3-H
6.90	m	1	2-H
7.36-7.40	ddd	1	1-H
7.26			Solvent

Table 3: The ^{13}C-NMR Spectral data of the compound

S.No.	δ (ppm)	Assignment
1	24.57	C-10
2	25.52	C-11
3	26.64	C-11
4	43.12	C-9
5	46.81	C-9
6	101.17	C-8
7	105.55	C-2
8	108.37	C-4
9	119.98	C-7
10	125.26	C-3
11	138.08	C-5
12	130.91	C-13
13	122.39	C-6
14	142.35	C-1
15	148.01	C-14
16	148.09	C-15
17	165.29	C-12
18	76.5-77.5	Solvent

Table .4 Lipinski's Rule of five.

Parameters	Known Drugs						NewLead molecule
	AmphotericinB	Sertacanazole	Itraconazole	Anidulafungin	Butenafine	Flucytosine	Piperine from black pepper
Molecular weight [g/mol]	924.O7902	437.7699	705.63342	1140.23692	317.46718	129.0092463	285.33766
Molecular Formula	$C_{47}H_{73}NO_{17}$	$C_{20}H_{15}Cl_3N_2OS$	$C_{35}H_{38}Cl_2N_8O_4$	$C_{58}H_{73}N_7O_{17}$	$C_{23}H_{27}N$	$C_4H_4FN_3O$	$C_{17}H_{19}NO_3$
XLogp3-Values	0	6.3	5.7	2.3	6.3	-0.9	3.5
Hydrogen Bond Donor	12	0	0	14	0	2	0
Hydrogen Bond Acceptor	18	3	9	17	1	3	3

Table 5.Results of the isolated Compound

Compound	E-total	E-shape	E-Force	E- air	V- shape	V-clash	Docking time	Energy Range	
								E min	E max
Piperine (from black pepper Nigrum)	-246.40	-246.40	0.00	0.00	0.00	0.00	7 min 29 sec	-236.48	-65.97
AmphotericinB	-337.48	-337.48	0.00	0.00	0.00	0.00	15 min 37 sec	-337.48	-124.86
Sertaconazole	-283.69	-283.69	0.00	0.00	0.00	0.00	13 min 14 sec	-283.69	-103.98
Itraconazole	-325.50	-325.50	0.00	0.00	0.00	0.00	14 min 14 sec	-325.50	-113.91
Anidulafungin	-395.16	-395.16	0.00	0.00	0.00	0.00	14 min 46 sec	-395.16	-137.52
Butenafine	-234.40	-234.40	0.00	0.00	0.00	0.00	13 min 32 sec	-234.40	-88.19
Flucytosine	-148.23	-148.23	0.00	0.00	0.00	0.00	12 min 34 sec	-148.23	-43.83

Fig.1: piper nigrum (Black pepper)

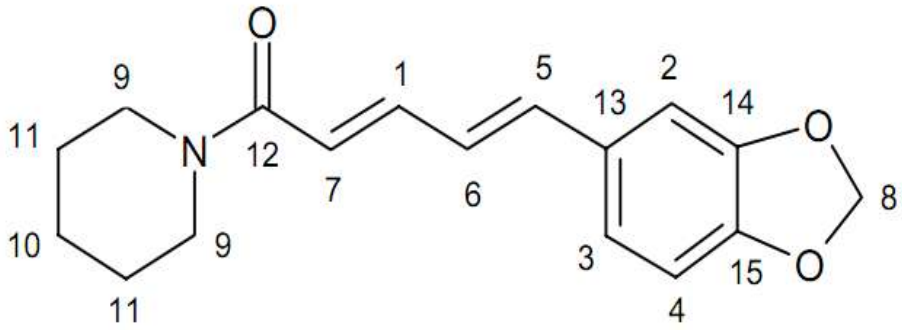


Fig.2. Structure of the piperine

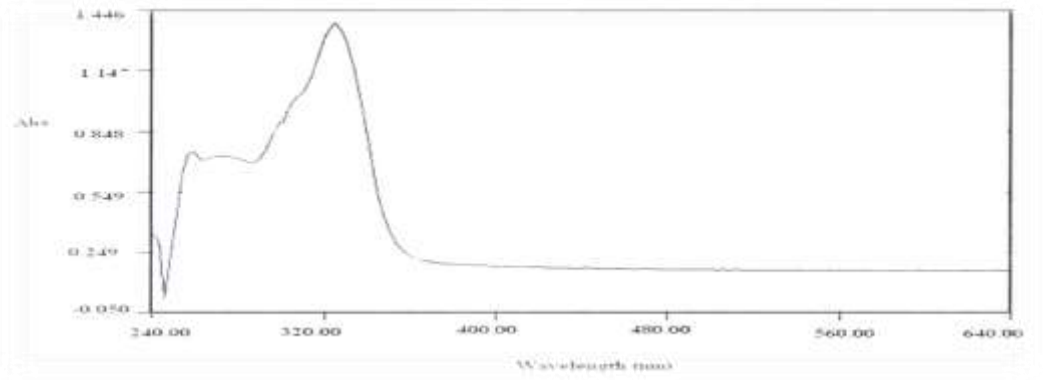


Fig. 3. UV-VIS Spectrum of isolated compound

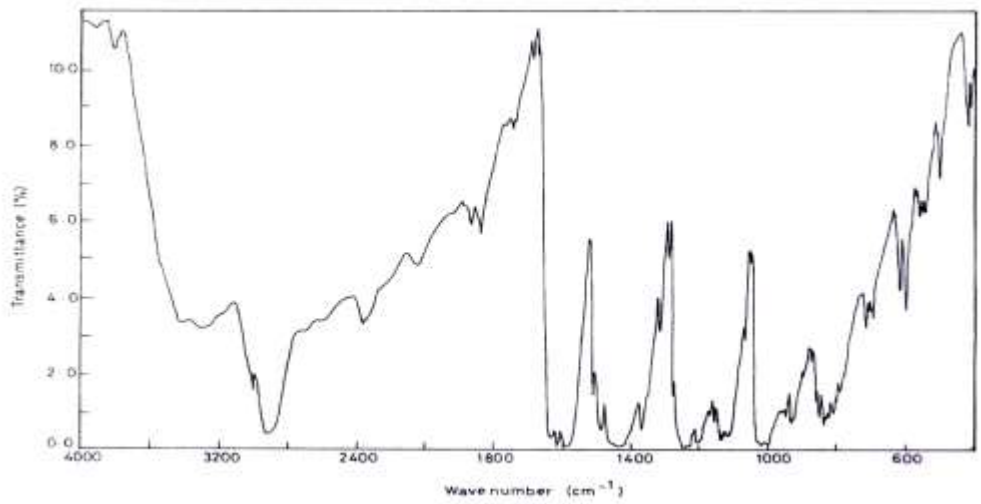


Fig.4. FIIR Spectrum of isolated compound

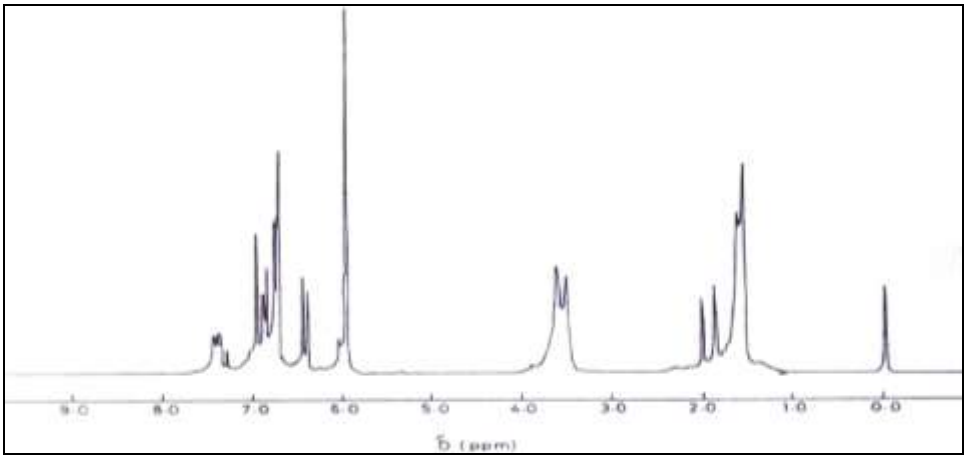


Fig.5. ^{1}H NMR Spectrum of isolated compound

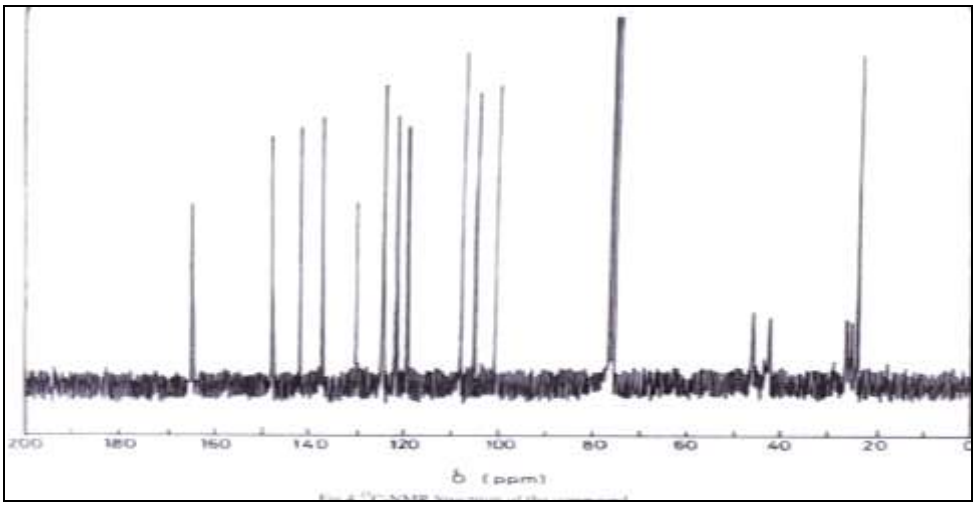


Fig.6. ^{13}C NMR Spectrum of isolated compound

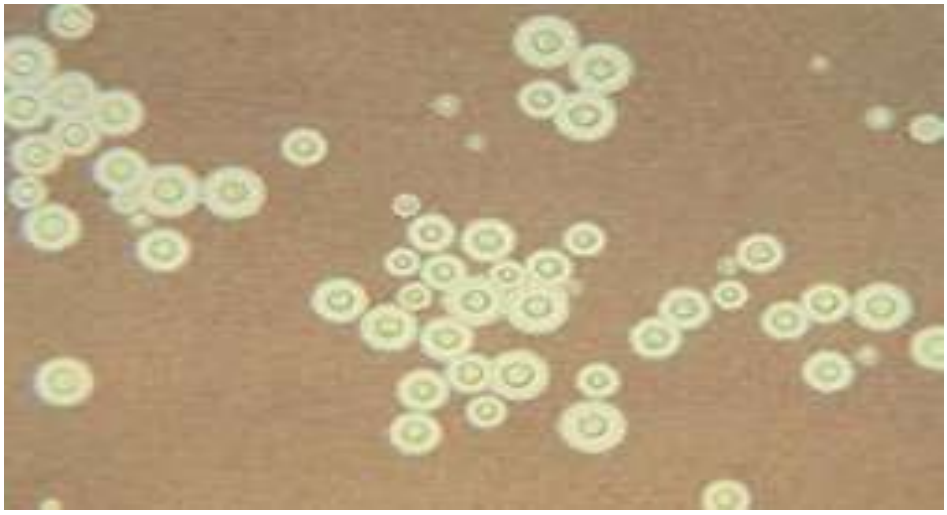


Fig: 7. Cryptococcus neoformans.

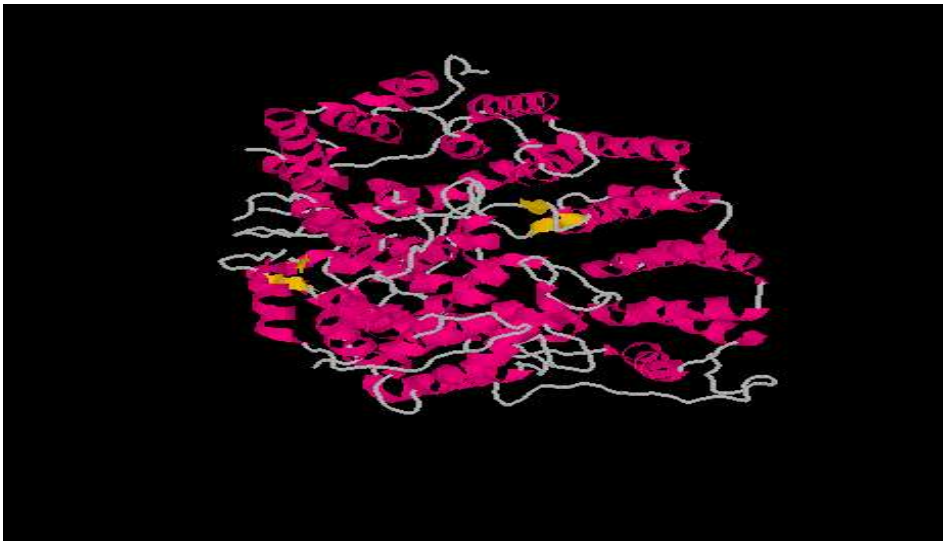
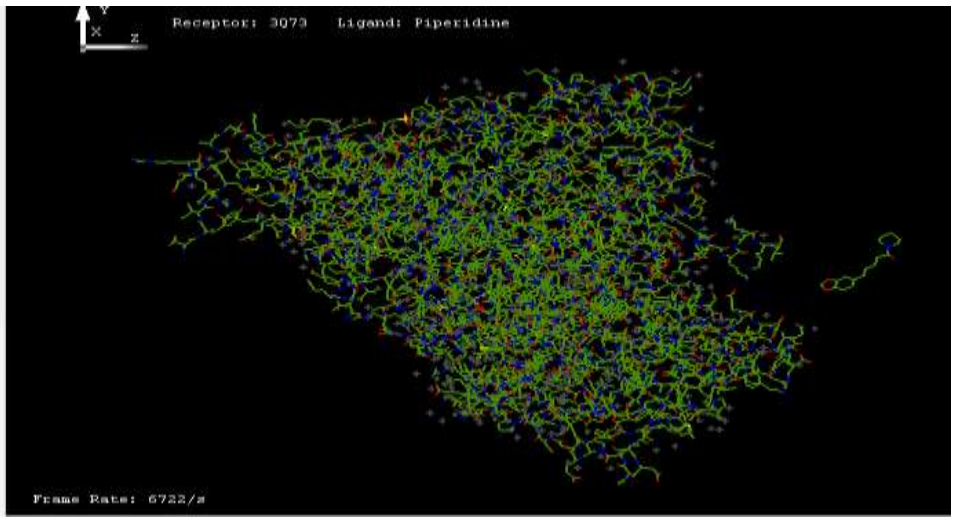
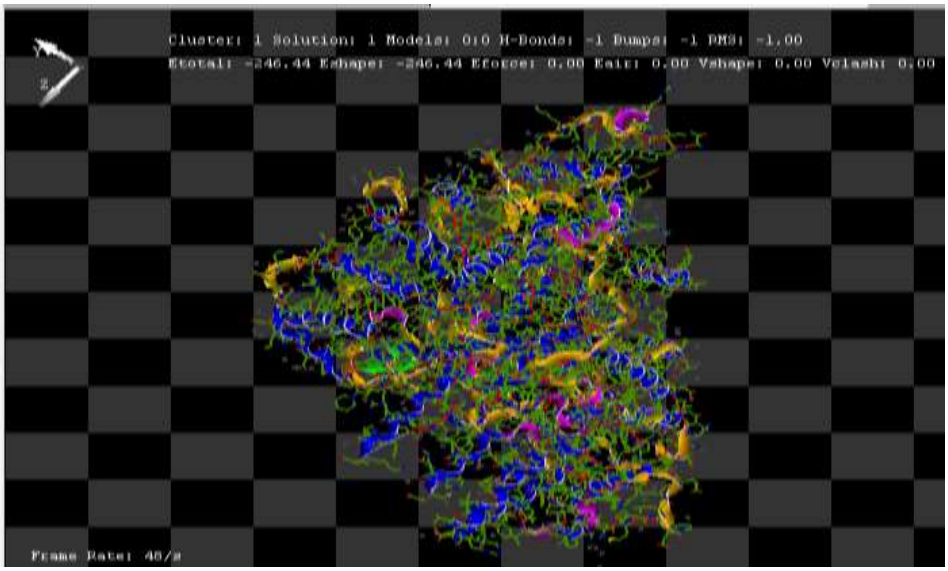


Fig. 8 Structure of fernestyltranferase protein



Protein and ligand molecule (before docking)



After docking (Protein with Ligand)

Fig. 9. Docking Result Of Drug

CONCLUSION

In conclusion, this study is a complementary survey to phytochemical and spectral studies carried out upon piperine. This work involves extraction of the piper nigrum with petroleum ether from which a pure component has been identified by crystallization with dichloromethane. Thus, our method, reported here is found to give moderate yield of a compound likely to be piperine of fairly pure quality. TLC report also gives the indication of a pure component. Melting point determination, UV-VIS Spectrum, FTIR Spectrum, ^{1}H-NMR and ^{13}C-NMRspectral techniques identify the purity of the compound and also the spectral data indicate that the isolated compound ispiperine. Finally, the chemical compound piperine activity was checked and proved through the in silico analysisof molecular docking (Lipinski's rule, ADMET). In silico drug design of piperine states, it was an inhibitor for Fernesyltransferasein Cryptococcus Neoformans.

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