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# An Approach to Antifungal Efficacy through Well Diffusion Analysis and Molecular Interaction Profile of Polyherbal Formulation

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**In the current scenario, there is a thirst for research against emerging microorganisms, and it becomes challenging to introduce new drugs against organism virulence are pretty interesting. Herbal medicines are now gaining popularity as a treatment option for various diseases worldwide. The present study analyzes the antifungal effect of a polyherbal formulation through in vitro well diffusion method using fungal strains such as Candida albicans, Aspergillus niger, Aspergillus fumigatus, Cryptococcus neoformans, and Sporothrix schenckii. Molecular docking is done using the Auto dock vina tool to predict the mechanism of action of the phytomolecules present in the polyherbal formulation. The molecular interactions are visualized using molecular modelling (PyMOL) software. The antifungal effect was observed in a concentration-dependent manner with a significant zone of inhibition. Also, phytomolecules in polyherbal formulation showed potential inhibition on CYP450 Lanosterol 14 a-demethylase 1, 3 ß-Glucan synthase, and Thymidylate synthase from docking analysis.**

**Keywords:** Anti-Fungal; Binding energy; Molecular Docking; Polyherbal formulation; well diffusion; zone of inhibition.

Polyherbal combination due to its various phytochemical constituents found effective against various disorders in which it becomes an approach for developing the potential and promising traditional therapy [1]. Predominantly, polyherbal drugs are used in the Ayurveda system to treat numerous infections like Indukantha Ghritha (IG), a polyherbal formulation containing 17 different phytochemical components, is widely prescribed by ayurvedic physicians to treat a variety of ailments [2]. The relevance of antifungals in medical advances has grown significantly over last 30 years. Because of overwhelming amount of reality fungal diseases influence individuals with weakened immune systems, a rise in the number of people living with innate immunity circumstances or therapies can be connected to an increasing number of fungal infections.[3] Invasive Fungal infections in the population are currently posing a threat to treatment. New drug development for treating fungal infections has become more challenging, particularly with post-covid patients.[4] The fungal

cell wall is an essential structure which is absent in mammalian hosts that gives easy access to drug targets against fungi. The proteins anchoring to the plasma membrane act as a potential target for the drug having an antifungal activity.[5]

Currently available anti-fungal drugs like fluconazole, itraconazole, voriconazole, and posaconazole act by C14-Demethylase inhibition that blocks ergosterol synthesis.[6] The polyenes like amphotericin B inhibit ergosterol synthesis in the cell membrane.[7] The drugs that act on (1, 3)-6-D glucan synthase, which is responsible for cell wall synthesis, are echinocandins such as caspofungin, micafungin, and anidulafungin. The degradation of fungi can also be achieved by fluoropyrimidine i.e., 5-fluorocytosine acts by thymidylate synthase for nucleic acid synthesis.[8] The receptors involved in the pathogenesis of fungal infections can targeted to develop a new drug against fungal infections.[9] Microorganisms like *Candida albicans*, a polymorphic fungus, can cause infections ranging from superficial skin to life-threatening infections in the systemic circulation. Oral candidiasis is caused by *Candida albicans* which affects around 70 % of the population in which immune system is affected mainly oropharynx and esophagus.[10]

Aspergillus species mainly *Aspergillus fumigatus* and *Aspergillus niger* cause morbidity and mortality due to infection, specifically causing otomycosis, cutaneous infections, and pulmonary diseases. *Aspergillus niger* mainly causes Pulmonary aspergillosis that affects about 3.6% of Chronic obstructive pulmonary disease (COPD) patients.[11] Similarly, *Cryptococcus neoformans* is a human fungal pathogen that causes symptomatic infections highly in immune compromised patients with immunity defects.[12] And also, Sporotrichosis affects humans and animals mainly due to the hyphomycete genus Sporothrix, and among Sporothrix species, *Sporothrix schenckii* was found to have high genetic viability.[13] The polyherbal formulation consists of aqueous extracts of eleven herbs, namely *Aerva lanata* (L.) (Whole plant), *Boerhavia diffusa* L. (Whole plant), *Hemidesmus indicus* (L.) (Root), *Salacia reticulata* Wight (root), *Berberis aristata* DC. (Stem), *Gymnema sylvestre* (Retz.) (Leaves), *Tinospora cordifolia* (Willd.) (Stem and leaves),

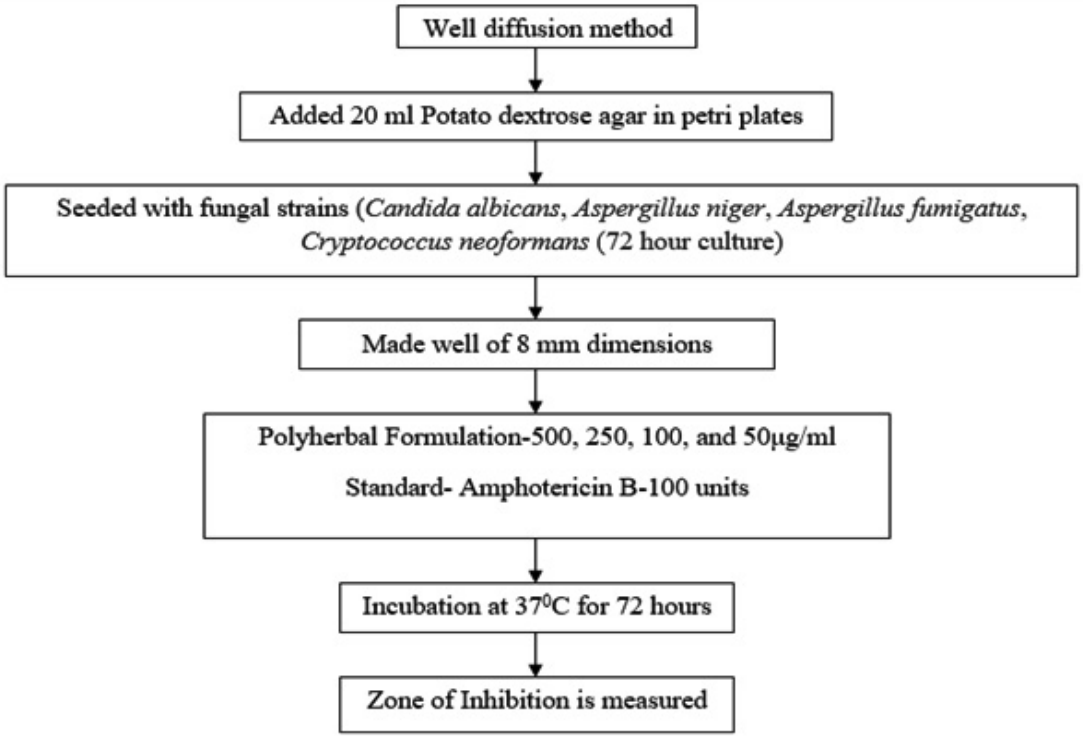


**Fig. 1.** Methodology for well diffusion method

**Table 1.** List of selection of phytocompounds and its biological activity

| Herbs | Parts used | Phytocompounds | Biological activity |
|---|---|---|---|
| *Aerva lanata* (L.) (whole plant) | Whole plant | Kaempferol-3- Rhamnoside[15] | Anti-Inflammatory[16] |
| *Boerhavia diffusa* L. (whole plant) | Whole plant | Punarnavine[17] | Immunomodulatory[18],Antiangiogenic[19] |
| *Hemidesmus indicus* (L.) (Root) | Root | 2-Hydroxy-4-Methoxy Benzaldehyde[20] | Antivenom [21] |
| *Salacia reticulata* Wight (root) | Root | Salacinol[22] | Alpha-glucosidase inhibitory activity [23] |
| | | Mangiferin[24] | Antifungal Antioxidant [25,26] |
| *Berberis aristata* DC. (stem) | Stem | Berberine[27] | Anti-inflammatory[28] ,Antifungal [29], Anti-convulsant [30] |
| *Gymnema sylvestre* (Retz.) (leaves) | Leaves | Gymnemic acid I [31] | Antiviral [32],Anti-sweet [33] |
| | | Quercetin[34] | Antioxidant, Anti-inflammatory[35], Anti mutagenic[36] |
| *Tinospora cordifolia* (Willd.) (stem and leaves) | Stem and leaves | Tinosporin A[37] | Antibacterial [38] |
| *Camellia sinensis* (L.) (leaves) | Leaves | Epigallocatechin-3-Gallate [39] | Anti-cancer [40] |
| *Vitis vinifera* L. (seed) | Seed | Gallic acid[41] | Anti-cancer [42] |
| | | Epicatechin[43] | Anti-oxidant [44] |
| *Curcuma longa* L. (rhizome) | Rhizome | Curcumin[45] | Anti-inflammatory[46], Immunomodulatory[47] , Antioxidant [48] |
| *Moringa oleifera* Lam. (leaves) | Leaves | *N*-á-L-Rhamnopyranosyl vincosamide[49] | Cardio protective [50] |

*Camellia sinensis* (L.) (Leaves), *Vitis vinifera* L. (seeds), *Curcuma longa* L. (rhizome), *Moringa oleifera* Lam. (leaves) and it was subjected to antifungal activity by well diffusion method against *Candida albicans*, *Aspergillus niger*, *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Sporothrix schenckii*. Auto dock vina has been used to predict the mechanism of action of the phytomolecules present in the polyherbal formulation.

## MATERIALS AND METHODS

### Polyherbal formulation

The polyherbal formulation is a proprietary preparation which consists of eleven different parts of medicinal herbs aqueous extract mixed in different ratios and it is coded as DNF11.

### Determination of antifungal activity by well diffusion method

### Fungal Strains, Chemicals, and Reagents

Fungal strains were purchased from MTCC, Candida albicans (MTCC 183), *Aspergillus niger* (MTCC 545), *Aspergillus fumigatus* (MTCC 2550) purchased from MTCC, *Cryptococcus neoformans* was purchased from Himedia, Cat No: 0291P, and *Sporothrix schenckii* isolated from the environment. Potato dextrose agar (HiMedia) and Amphotericin B (Zydus) were used to carry out the *in-vitro* antifungal activity.

**Table 2.** List of phytoconstituents that are selected as ligands for molecular docking

| Name of the Herb | Phytocompounds | Pubchem ID | Molecular formula | Molecular weight |
|---|---|---|---|---|
| *Aerva lanata* (L.) (whole plant) | Kaempferol-3- Rhamnoside | 5835713 | $C_{21}H_{20}O_{10}$ | 432.4 |
| *Boerhavia diffusa* L. (whole plant) | Punarnavine | 442922 | $C_{18}H_{15}NO_4$ | 469.31 |
| *Hemidesmus indicus* (L.) (Root) | 2-Hydroxy -4-Methoxybenzaldehyde | 358341 | $C_8H_8O_3$ | 328.4 |
| *Salacia reticulata* Wight (root) | Salacinol | 6451151 | $C_9H_{18}O_9S_2$ | 333.4 |
| | Mangiferin | 5281647 | $C_{19}H_{18}O_{11}$ | 422.3 |
| *Berberis aristata* DC. (stem) | Berberine | 2353 | $C_{20}H_{18}NO^{4+}$ | 336.4 |
| *Gymnema sylvestre* (Retz.) (leaves) | Gymnemic acid I | 11953919 | $C_{43}H_{66}O_{14}$ | 807 |
| | Quercetin | 5280343 | $C_{15}H_{10}O_7$ | 302.23 |
| *Tinospora cordifolia* (Willd.) (stem and leaves) | Tinosporin A | 122206355 | $C_{21}H_{26}O_8$ | 406.4 |
| *Camellia sinensis* (L.) (leaves) | Epigallocatechin-3-Gallate | 65064 | $C_{22}H_{18}O_{11}$ | 458.4 |
| *Vitis vinifera* L. (seed) | Gallic acid | 370 | $C_7H_6O_5$ | 170.12 |
| | Epicatechin | 72276 | $C_{15}H_{14}O_6$ | 290.27 |
| *Curcuma longa* L. (rhizome) | Curcumin | 969516 | $C_{21}H_{20}O_6$ | 368.4 |
| *Moringa oleifera* Lam. (leaves) | *N*-á-L-Rhamnopyran-osylvincosamide | 71717770 | $C_{32}H_{40}N_2O_{13}$ | 660.66 |

**Table 3.** Zone of inhibition against *Candida albicans, Aspergillus fumigatus, Aspergillus niger, Cryptococcus neoformans and Sporothrixschenckii*

| S. No | Name of the test organism | Zone of inhibition (mm) 500 µg/ml | 250 µg/ml | 100 µg/ml | 50 µg/ml | Positive Control |
|---|---|---|---|---|---|---|
| 1. | *Candida albicans* | 11±1.41 | 8.45±0.63 | 0 | 0 | 22±1.41 |
| 2. | *Aspergillus fumigatus* | 11.5±0.7 | 7.75±0.35 | 0 | 0 | 12.5±0.7 |
| 3. | *Aspergillus niger* | 12.25±1.76 | 8.25±0.35 | 6.2±0.28 | 0 | 16.5±0.7 |
| 4. | *Cryptococcus neoformans* | 13.5±0.7 | 7.25±0.35 | 0 | 0 | 26±1.41 |
| 5. | *Sporothrixschenckii.* | 10.5±0.7 | 8.35±0.49 | 6.25±0.35 | 0 | 29±1.41 |

SD ± Mean, SD – Standard Deviation.

**Preparation of culture media**

The 3.9 g potato dextrose agar medium was dissolved in 100 ml of distilled water and autoclaved at 15 lbs. pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100 mm Petri plates (25-30 ml/ plate) while still molten.

**Measurement of Zone of inhibition**

The well diffusion method was the standard method for carrying out the antimicrobial analysis using 100 µl of a suspension containing 106 spores/ml of fungal organisms which spread on Potato dextrose agar medium.[14] Petri plates containing 20 ml potato dextrose agar medium were seeded with a 72 hr. culture of a different fungal strain. The wells were made at the dimension of 8mm and different concentrations of test sample polyherbal formulation (500, 250, 100, and 50ìg/ml) were added to their respective wells. Amphotericin B 100 units were used as a positive control. The experiment was carried out in triplicates and the plates were incubated by inverting at 37°C for 72 hours. The antifungal effect was assessed by measuring the diameter of the inhibition zone formed around the wells and mean and SD were calculated using Graph Pad Prism 6.0 software (USA). Figure 1 represents the methodology of well diffusion method.

**In silico Molecular docking**

**Selection and Preparation of the target protein**

The targets of antifungal agents were selected based on the literature survey and the 3D structure of CYP450 Lanosterol 14 á-demethylase (PDB ID: 1EQ1), 1,3-glucan synthase ((PDB ID: 1EQP), and Thymidylate synthase (PDB ID: 1HZW) was retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein data bank and saved in program database (PDB) format for docking elucidation. Then, with the exception of metals, all water molecules, and hetero groups are removed and converted into PDBQT format.

**Selection and preparation of ligand molecules**

The polyherbal preparation constitutes aqueous extracts of eleven herbs, phytocompounds have been selected random from each herb based

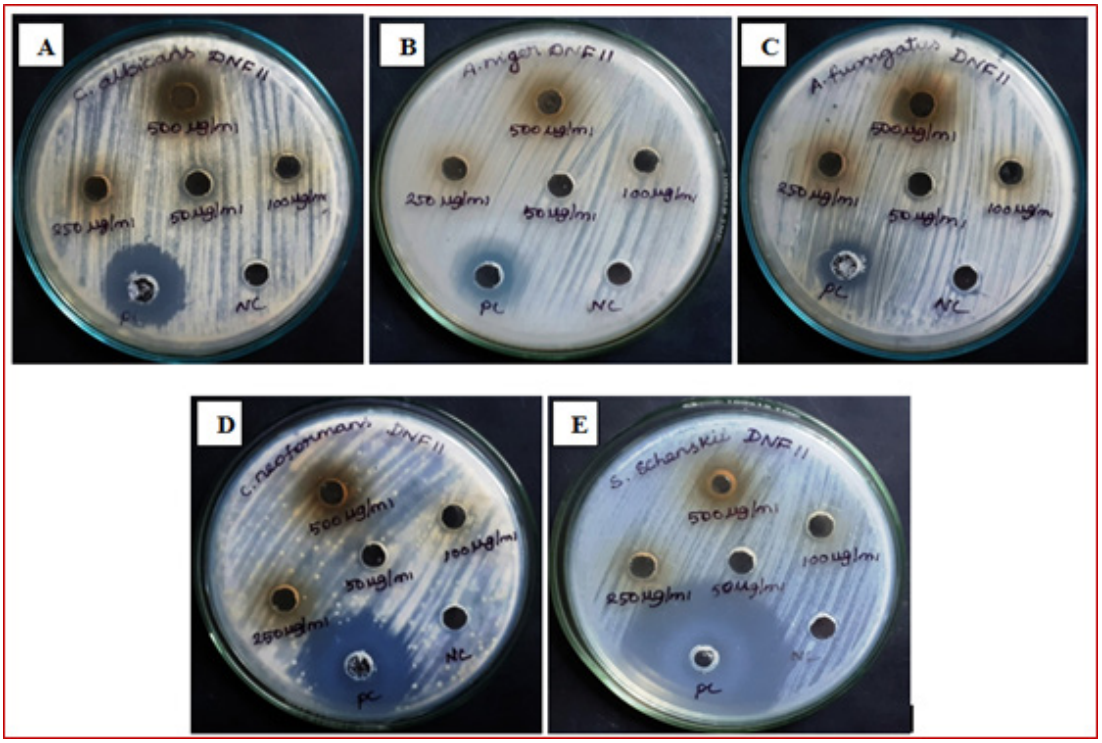


**Fig. 2.** Antifungal efficacy of polyherbal formulation and the positive control in various fungal species. A. *Candida albicans,* B. *Aspergillusniger,* C.*Aspergillus fumigates,* D. *Cryptococcus neoformans and Sporothrix schenckii*

on its importance from previous individual herb literature with its biological activity given in table 1 and its molecular formula in table 2. To compare the affinity and interacting residues, standard antifungal drugs such as isavuconazole (Triazole), caspofungin (echinocandins) and 5-fluorocytosine (anti-metabolite) were docked against their respective receptor based on their mechanism of action. The canonical smiles are obtained from the Pubchem database and converted into PDB (Program database) format or Protein Data Bank, Partial Charge (Q), & Atom Type (T) (PDBQT) format using appropriate tools.

**Prediction of molecular docking interactions**

The docking experiments between the ligands and the target were carried out with the AutoDock Vina 4.2.6 programme (The Scripps Research Institute), which has been used in medicinal chemistry. Based on the Lamarckian Genetic Algorithm, which combines energy evaluation with affinity potential grids to discover the best binding location for a ligand on a certain protein target. [51] The software was used to anticipate protein-ligand interactions, and it is known for its speed and flexibility in performing docking operations to demonstrate that the ligand binds to the target protein. The docking process begins with the ligand and receptor to identify potential binding sites on the target protein in order to anticipate the ligand-binding mode. Polar hydrogen atoms were introduced to the protein targets as per the usual technique, and Kollman unified atomic charges were computed. Hydrogen atoms were added to the ligands before the Gastiger partial charges were applied. The bond orders were examined after the current crystal ligand removal. To cover the entire protein, the target's grid map was generated and set with proper grid spacing. The target molecule's grid box was properly adjusted to cover the active residues, and the typical docking

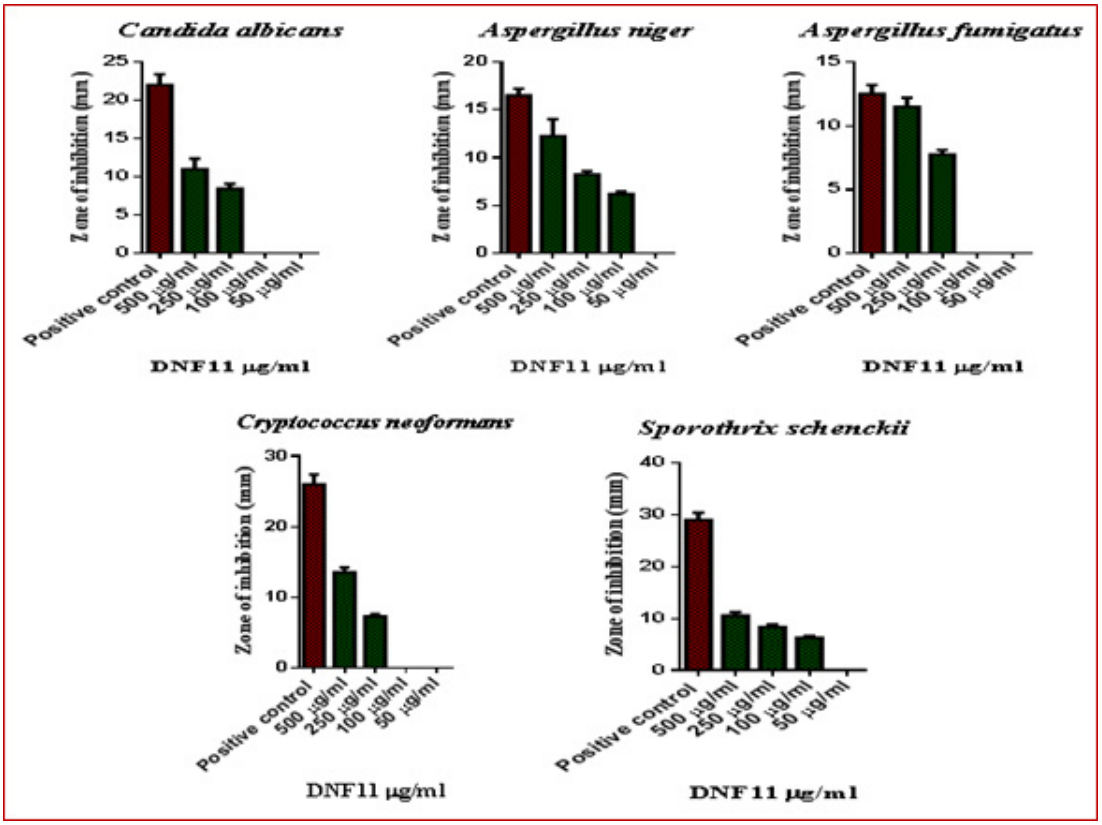


**Fig. 3.** Zone of inhibition of polyherbal formulation and the positive control in various fungal species at different concentrations

**Table 4.** Interaction between Phytomolecules and Standard against Cyp450 Lanosterol 14 á-demethylase (PDB ID: 1EA1)

| S. No | Phytomolecules | Binding energy (Kcal/mol) | Interacting residues in the target protein | Number of Hydrogen bonds |
|---|---|---|---|---|
| 1 | Standard - Isavuconazole | -9.8 | His253 | 1HB |
| 2 | *N*-á-L-Rhamnopyranosylvincosamide | -10.6 | Asp151, Tyr153, Gly143, Arg309(3), Tyr317 | 7HB |
| 3 | Epigallocatechin-3-Gallate | -10.5 | Arg309, Asp145, Asp145, Asn146, Tyr29, Tyr255 | 6HB |
| 4 | Kaempferol-3- Rhamnoside | -10.4 | Glu192(2), Tyr317, Asn305, Arg309, Leu304 | 6HB |
| 5 | Mangiferin | -10.1 | Glu27, Tyr29, Glu292, Tyr255, Arg309, Tyr317(2), Arg312(2) | 9HB |
| 6 | Punarnavine | -9.9 | Arg309, Tyr29, Asn146 | 3HB |
| 7 | Berberine | -9.8 | Asn146 | 1HB |
| 8 | Quercetin | -9.5 | Glu292, Tyr255, Asn146, Asp145, Arg309, Arg312 | 6HB |
| 9 | Epicatechin | -9.0 | Tyr255, Glu292, Glu27, Asn305, Arg305, Tyr29 | 6HB |
| 10 | Gymnemic Acid | -9.0 | Tyr317, Arg309, Asn305, Trp27(2),Tpr255(3) | 7HB |
| 11 | Tinosporin A | -8.4 | Asn146, Asp145, Asp145, Leu304, Tyr317 | 5HB |
| 12 | Curcumin | -8.2 | Glu27, Tyr317, Arg309 | 3HB |
| 13 | Salacinol | -7.0 | Tyr255, Glu292(2), Asn146(2), Asp145, Tyr29, Glu27, Arg312, Asn305 | 10HB |
| 14 | Gallic Acid | -6.5 | Glu192, Glu292, Tyr255, Tyr29, Glu27, Asn146, Asp145 | 7HB |
| 15 | 2-Hydroxy -4-Methoxybenzaldehyde | -5.5 | Glu192, Asn146(2), Asp145 | 4HB |

process was followed. [52] Finally, independent docking runs were carried out for each ligand, and results were retrieved as binding energies. The poses that showed high free energy values and less RMSD were tabulated and the molecular interactions are visualized using PyMOL 1.7.4.5.

## RESULTS AND DISCUSSION

### Antifungal activity of polyherbal formulation

Previously, polyherbal formulation which contains five herbs has been studied for antifungal efficacy like against *Candida albicans, Trichophyton rubrum, Microsporum gypseum, Epidermophyton floccosum* showed significant effect in a dose-dependent manner.[53] From the existing literature, some of the individual phytochemicals like quercetin, epicatechin, epigallocatechin has been proved for their antifungal effect against *Cryptococcus neoformans*, dermatophytes, and *Candida species*.[54-57] Similarly, mangiferin showed potential inhibition against *Aspergillus flavus* and *Aspergillus fumigatus.* [58] and berberine, which is an isoquinoline alkaloid proven to have potential antifungal effect against fluconazole resistant *Candida*, *Cryptococcus neoformans,* and other *Candida species*. Likewise, Curcumin was also studied for its antifungal activity in the form of silver nanoparticles. [59] From the results figure 2 and 3, Anti-fungal activity of polyherbal formulation assessed by a well diffusion method against different fungal strains comparing with standard drug Amphotericin B through zone of inhibition was found as concentration-dependent antifungal effect.

Among the fungal organisms, The zone of inhibition at various concentration (500-50 µg/ml) as given in table 3 the *Cryptococcus neoformans* showed 13.50 ± 0.70 mm zone of inhibition at the concentration of 500 µg/ml followed by

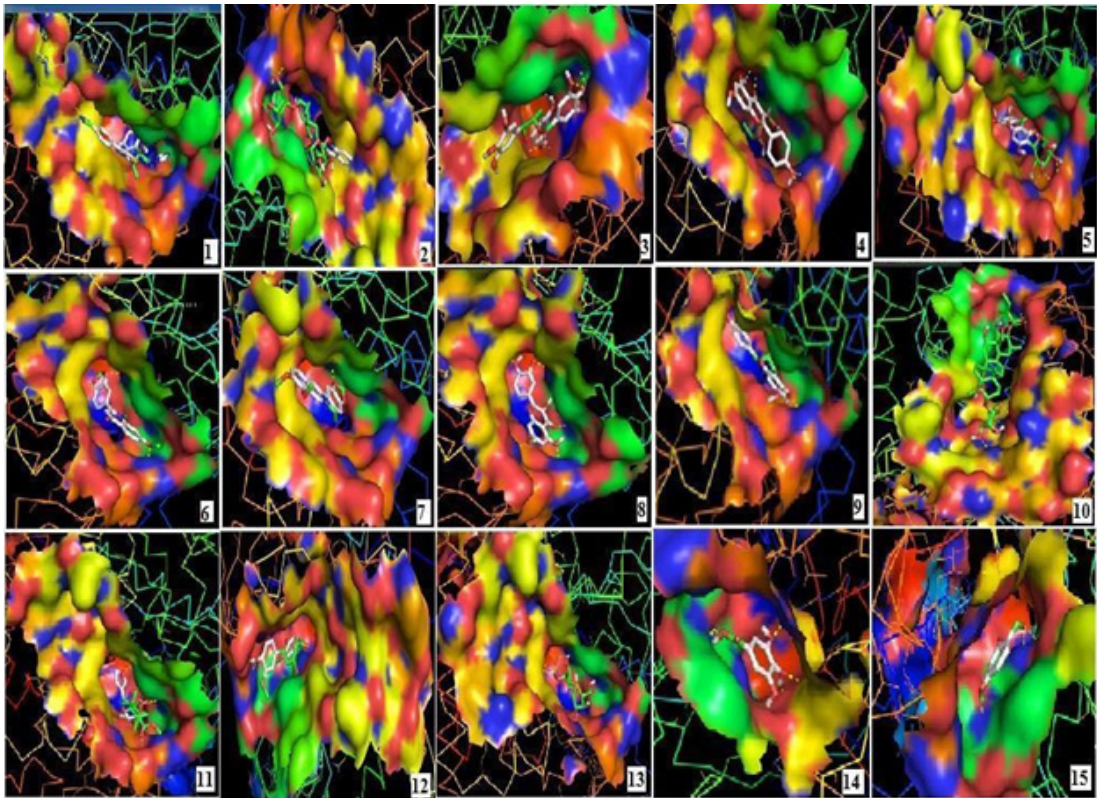


**Fig. 4.** Ligands binding sites on target receptor protein Cyp450 Lanosterol 14 demethylase (PDB ID: 1EA1). 1) Standard –Isavuconazole, 2)*N*-á-L-Rhamnopyranosylvincosamide, 3)Epigallocatechin-3-Gallate, 4) Kaempferol-3- Rhamnoside, 5)Mangiferin, 6)Punarnavine, 7)Berberine, 8)Quercetin, 9)Epicatechin, 10) Gymnemic Acid, 11)Tinosporin A, 12)Curcumin, 13)Salacinol, 14)Gallic Acid, 15)2-Hydroxy -4-Methoxy benzaldehyde

**Table 5.** Interaction between Phytomolecules and Standard against 1,3â-Glucan Synthase (PDB ID: 1EQP).

| S. No | Phytomolecules | Binding energy (Kcal/mol) | Interacting residues in target protein | Number of Hydrogen bonds |
|---|---|---|---|---|
| 1 | Standard-Caspofungin | -7.8 | Ser259, Tyr255, Trp277, Phe229(2) | 5HB |
| 2 | *N*-á-L Rhamnopyranosyl vincosamide | -10.6 | Tyr317, Arg309(3), Asp151, Tyr153 | 6HB |
| 3 | Epigallocatechin-3-Gallate | -10.5 | Asn146, Asp145, Leu304, Asp145, Tyr255, Tyr29 | 6HB |
| 4 | Kaempferol-3- Rhamnoside | -10.3 | Tyr317, Arg309, Glu192, Leu304, Asp145 | 5HB |
| 5 | Mangiferin | -10.1 | Arg309,Tyr317(2), Arg312(2), Glu292, Tyr255, Tyr29, Glu27 | 9HB |
| 6 | Berberine | -10.0 | Asp146(2), Tyr317, Arg312 | 4HB |
| 7 | Punarnavine | -9.9 | Arg309, Asn145, Tyr29 | 3HB |
| 8 | Quercetin | -9.5 | Arg312, Arg309, Asp145, Tyr255, Glu292, Asn146 | 7HB |
| 9 | Epicatechin | -9.0 | Arg309, Asn305,Tyr255, Tyr29 | 4HB |
| 10 | Gymnemic Acid | -9.0 | Trp277, His254, Tyr255(2), Arg309, His253, Tyr317, Asn305, Asp318 | 9HB |
| 11 | Tinosporin A | -8.4 | Tyr317, Asn146, Asp145(2) | 4HB |
| 12 | Curcumin | -8.2 | Tyr255, Asn146(2) | 3HB |
| 13 | Salacinol | -7.1 | Tyr29(2), Tyr255, Glu292, Glu27, Asn146(2), Asn305, Arg309 | 9HB |
| 14 | Gallic Acid | -6.5 | Glu27, Tyr29, Tyr255, Glu192, Glu192, Asp145, Asn146 | 7HB |
| 15 | 2-Hydroxy -4-Methoxy benzaldehyde | -5.5 | Asp145, Glu192, Asn146(2) | 5HB |

*Aspergillus niger* showed 12.50 ± 0.70 mm, *Aspergillus fumigates* showed 11.50 ± 0.70 mm, *Candida albicans* showed 11.00 ± 1.41 mm and *Sporothrix schenckii* showed 10.50 ± 0.70 mm zone of inhibition. Compared to other organisms, the highest zone of inhibition was found in Candida albicans (8.45± 0.63 mm) with a concentration of 250 µg/ml. *Sporothrix schenckii* showed 6.25 ± 0.35 mm and *Aspergillus niger* showed 6.20 ± 0.28 mm of the zone of inhibition at 100 µg/ml whereas the remaining organisms did not respond. At the concentration of 50 µg/ml, no inhibition was observed in all five organisms. Positive control, Amphotericin B showed an effective zone of inhibition in all the fungal organisms. Among all the fungal strains, the polyherbal formulation has effectively inhibited the growth of *Cryptococcus neoformans*, *Aspergillus niger,* and *Aspergillus fumigatus* at higher concentrations. This antifungal potency can be better to take into a therapeutic advantage against fungal infections due to phytoconstituents present in the polyherbal formulation.

**In silico molecular docking prediction**

The phytoconstitutents present in each herb of polyherbal formulation selected based on marker estimation and solubility from existing literature has been studied for the molecular interaction to predict the pathway behind the mechanism of that particular phytomolecule. The fungal metabolic enzymes were considered as antifungal targets and the results obtained from the study are given as follows.

**CYP450 Lanosterol 14 á-demethylase**

The fungal species like *Candida albicans* contain cytochrome p450 that converts *N*-alkanes to alkanols and grows with *N*-alkanes as its carbon source. Cytochrome p450 comes under the class of protoheme proteins showing Soret absorption band at 450 nm in reduction co-complex. This is due

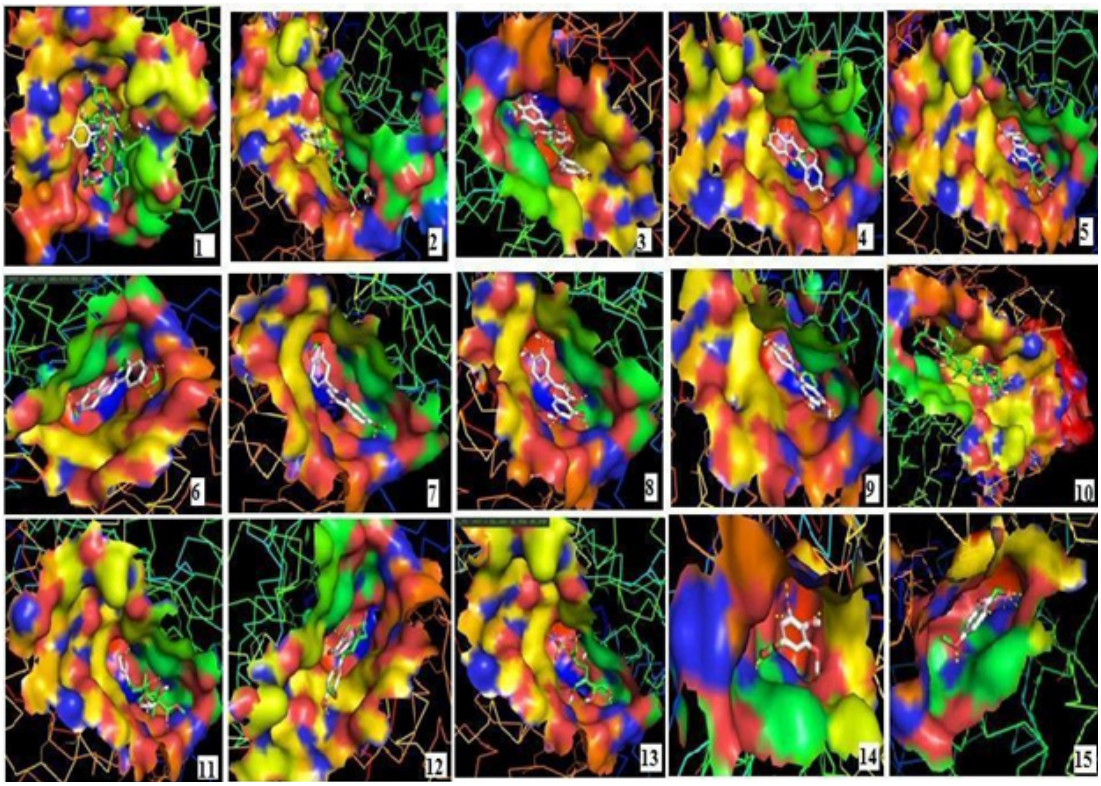


**Fig. 5.** Ligands binding sites on target receptor protein 1,3â-Glucan Synthase (PDB ID: 1EQP). 1) Standard –Isavuconazole, 2)*N*-á-L-Rhamnopyranosyl vincosamide, 3)Epigallocatechin-3-Gallate, 4)Kaempferol-3-Rhamnoside, 5)Mangiferin, 6)Punarnavine, 7)Berberine, 8)Quercetin, 9)Epicatechin, 10)Gymnemic Acid, 11) Tinosporin A, 12)Curcumin, 13)Salacinol, 14)Gallic Acid, 15)2-Hydroxy -4-Methoxy benzaldehyde

**Table 6.** Interaction between Phytomolecules and Standard against Thymidylate synthase (PDB ID: 1HZW)

| S. NO | Phytomolecules | Binding energy (Kcal/mol) | Interacting residues in the target protein | Number of Hydrogen bonds |
|---|---|---|---|---|
| 1 | Standard 5-Fluorocytosine | -4.9 | Met149, Ser151, Ser154, His141, Tyr153 | 5HB |
| 2 | *N*-á-L-Rhamnopyranosyl vincosamide | -9.0 | Ile108, Tyr258, Arg215, Asn226(2), Ser216, Arg215 | 7HB |
| 3 | Kaempferol-3- Rhamnoside | -8.7 | Tyr258, His256, Gln214, His196, Asn226, Asp218, Tyr135, Ile108, Asn226 | 11HB |
| 4 | Epigallocatechin-3-Gallate | -8.6 | Ser216, Asp218, Gln214, Leu221, His196 | 5HB |
| 5 | Punarnavine | -8.5 | His196,Glu87(2) | 3HB |
| 6 | Tinosporin A | -8.3 | Leu221, His196, Asn226 | 9HB |
| 7 | Gymnemic Acid | -8.2 | Arg215, Leu251 | 2HB |
| 8 | Mangiferin | -7.9 | Lys77, Phe80, His196, Glu87 | 4HB |
| 9 | Berberine | -7.7 | Phe80 | 1HB |
| 10 | Epicatechin | -7.6 | Ala293, Arg140, Ile92 | 3HB |
| 11 | Quercetin | -7.2 | Phe80, Asn226 | 2HB |
| 12 | Curcumin | -6.8 | Tyr135, His196, Asn226 | 3HB |
| 13 | Gallic Acid | -5.8 | Glu100, Ser95, Thr96(3), His141 | 8HB |
| 14 | Salacinol | -5.6 | Ser216, Asp218, His196(2), Tyr135, Glu87(2), Asn226(2) | 9HB |
| 15 | 2-Hydroxy -4-Methoxy benzaldehyde | -5.0 | Asn226(2), His126 | 3HB |

to thiolate anion coordination in cysteine residue present in apoprotein to heme protein. It has thiolate-ligated iron protoporphyrin IX as its prosthetic group [60]. Among 14 phytomolecules present in polyherbal formulation, *N* á-l-rhamnopyranosyl vincosamide which is mainly present in *Moringa oleifera* has a higher binding energy of -9.8 Kcal/mol with seven hydrogen bonding interacting with Asp 151, Tyr 153, Gly 143, Arg 309, Tyr 317 amino acids. It forms a good affinity with Arg 309 with 3 hydrogen bonds. Salacinol present in *Salacia reticulata* interacted with the target enzymes, formed 10 hydrogen bonds with the binding energy of -7.0, Kcal/mol. Since this is one of the major ingredients in the polyherbal formulation, this could be the reason for the inhibition of fungal growth. Other phytoconstituents binding interactions and their energy values are given in table 4 and the evidence for the interactions was given in figure 4.

**1,3 â-Glucan synthase**

â (1,3)-D-glucan is a polysaccharide component present in the cell wall of fungi that plays a major role in cell wall synthesis. The enzyme 1,3 â-Glucan synthase was suspected to be a target for many natural products like Aculeacin A, B, and Paulacandin. The inhibition of â (1,3)-D-glucan in the organisms such as *Candida albicans, Aspergillus fumigatus,* and *Cryptococcus neoformans* appeals to have a potential broad-spectrum fungal specific target that brings interest in new drug development. [61] Among all phytomolecules against 1,3 â-Glucan synthase enzyme, *N*-á-L Rhamnopyranosyl vincosamide showed higher binding energy of -10.6 Kcal/mol with 5 hydrogen bonds followed by Epigallocatechin-3-Gallate -10.5 Kcal/mol with 6 hydrogen bonds and Kaempferol -10.3 Kcal/mol with 6 hydrogen bonds respectively. Caspofungin,

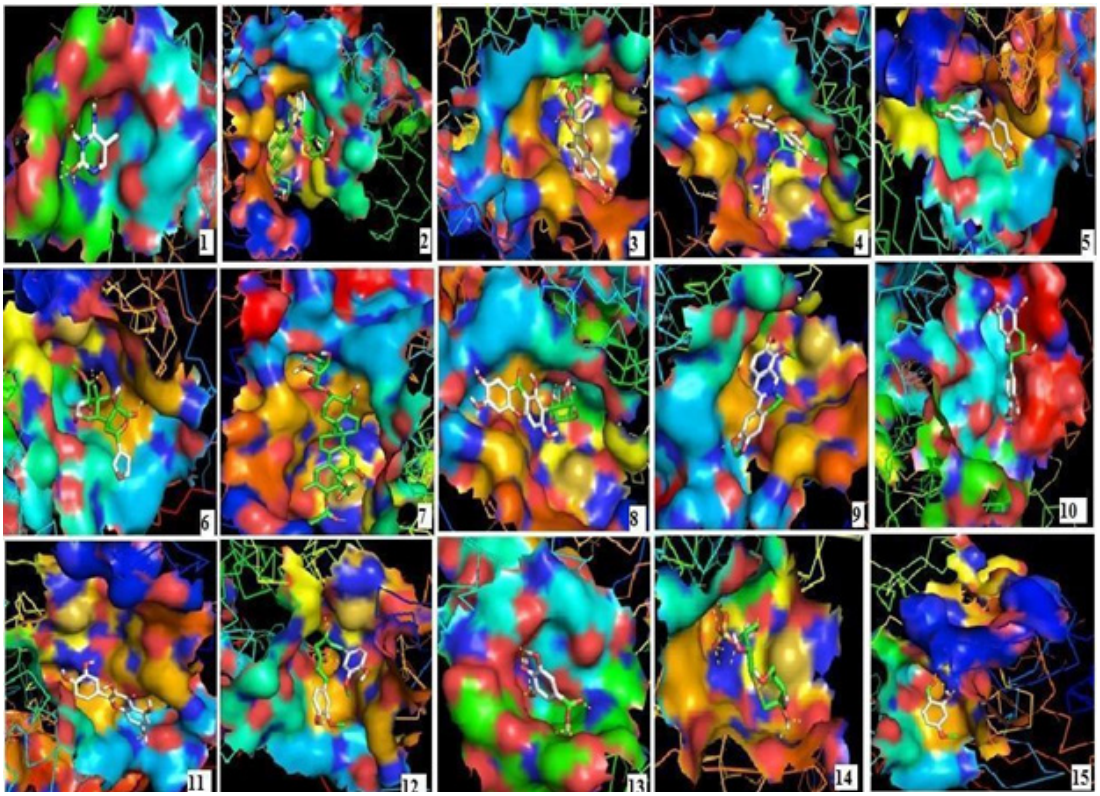


**Fig. 6.** Ligands binding sites on target receptor protein Thymidylate synthase (PDB ID: 1HZW). 1) Standard –Isavuconazole, 2)*N*-á-L-Rhamnopyranosylvincosamide, 3)Epigallocatechin-3-Gallate, 4)Kaempferol-3-Rhamnoside, 5)Mangiferin, 6)Punarnavine, 7)Berberine, 8)Quercetin, 9)Epicatechin, 10)Gymnemic Acid, 11) Tinosporin A, 12)Curcumin, 13)Salacinol, 14)Gallic Acid, 15)2-Hydroxy -4-Methoxy benzaldehyde

a standard compound interacts with Ser259, Tyr255, Trp277, Phe229 with 5 hydrogen bonds, Similarly, Epigallocatechin-3-Gallate, Mangiferin, Curcumin, Salacinol, and Gallic acid interact with Tyr255 amino acid. Trp277 amino acid interaction was found in Gymnemic acid similar to that of Standard drug with 1 hydrogen bond. The binding affinity of gymnemic acid was also found higher which has a binding energy of about -9.0 Kcal/mol with 9 hydrogen bonds compared with caspofungin standard. Other phytoconstituents binding interactions and their energy values are given in table 5 and the evidence for the interactions was given in figure 5.

**Thymidylate synthase**

Thymidylate synthase (5, 10-methylenetetrahydrofolate dUMP C-methyltransferase) has a key role in DNA synthesis in mammals.[62] It binds with dUMP and 5,10-methylenetetrahydrofolate as a co-factor that catalyzes the process called reduction methylation in substrate and forms dTMP and dihydrofolate.[63] 5-fluorocytosine is an antifungal; it has been used as an oral drug and by injection in combination with Amphotericin B for the treatment of Candida infections, chromomycosis, and cryptococcosis. Some common side effects include bone marrow suppression, vomiting, loss of appetite, diarrhea, and psychosis was observed while using this drug.[64] Docking analysis showed Standard 5-Flurocytosine has -4.9 Kcal/mol binding energy interacting with Met149, Ser151, Ser154, His141, Tyr153 with 5 hydrogen bonds whereas *N*-á-L-Rhamnopyranosylvincosamide showed higher binding affinity towards target protein of -9.0 Kcal/mol has interacted with Ile108, Tyr258, Arg215, Asn226, Ser216, Arg215 with 7 hydrogen bonds. Salacinol has a higher binding affinity of -5.6 Kcal/mol with similar interacting residues Ser216, Asp218, His196, Tyr135, Glu87, Asn226 as the standard 5-Fluorocytosine which shows that a better antifungal effect with the same mechanism as standard to inhibit the thymidylate synthase enzyme. Other phytoconstituents binding interactions and their energy values are given in table 6 and the evidence for the interactions was given in figure 6.

## CONCLUSION

Based on the results from *in vitro* and *In-silico* analysis studies, it is acknowledged that the polyherbal formulation acts as a potential antifungal effect against various fungal strains in dose dependent manner. *In silico*, the phytomolecules selected showed an affinity towards target enzymes, high binding energy, more hydrogen bond formation, and amino acid interactions which become additional evidence for the antifungal effect and also exert its mechanism of action through inhibition of various fungal metabolic enzymes. Further phytochemical present in polyherbal formulation have to be quantified to evaluate the concentration of each phytomolecules present in it which is under progress.

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**Conflict of Interest**

All authors involved in this research work declared that there is no conflict of interest.

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