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From Kitchen Spice to Lab Bench Screening of Antibacterial Properties of *Parmotrema parlatum* on *Shigella spp.* and Molecular Docking of the Lysates with Pathogenic Strains

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Abstract: Medicinal plants are known to produce a wide array of secondary metabolites, many of which possess potent antimicrobial properties. Continuous research into new plant species expands the potential for discovering novel therapeutic agents. This study focuses on *Parmotrema perlatum*, a foliose lichen traditionally used as a spice (known as Kalpasi), to explore its antiparasitic potential. Three protozoan pathogens—*Plasmodium falciparum*, *Toxoplasma gondii*, and *Entamoeba histolytica*—remain major global health threats. *P. falciparum*, the causative agent of malaria, has claimed countless lives across human history. It is transmitted by the female Anopheles mosquito and causes recurring cycles of fever, chills, and anemia by attacking red blood cells. *T. gondii*, primarily hosted in cats, infects humans via contaminated food or water, often lying dormant in the brain and muscles. While asymptomatic in healthy individuals, it poses serious risks to fetuses and immunocompromised patients. *E. histolytica* spreads via contaminated food or water, invades the intestinal wall, and causes severe gastrointestinal complications such as dysentery and liver abscesses. To address the growing resistance to conventional drugs, this research investigates phytochemicals derived from *P. perlatum* as potential antiparasitic agents. The powdered form of the plant was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis to identify its active compounds. Two phytochemicals—Thujopsene and Resibufogenin—were selected based on their potential bioactivity.Before targeting the protozoan pathogens, these compounds were initially evaluated against *Shigella flexneri* using both bioinformatics (molecular docking) and in vitro antibacterial assays. Key proteins from each pathogen were selected based on structural relevance and docking compatibility.The primary goal of this study is to assess whether the identified compounds from *P. perlatum* can serve as effective drug candidates against parasitic infections, offering a natural alternative to synthetic drugs in the fight against endemic protozoan disease.

Keywords: *Plasmodium, Entamoeba, Toxoplasma; Phytochemical Analysis, Molecular Docking, Calculated Affinity, Malaria, HSP 90, HSP70, Permotrema parlatum, GCMS, Resibufogenin, Thujopsin.*

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I. INTRODUCTION

Medicinal plants and lichens are recognized as valuable reservoirs of bioactive compounds with potential therapeutic applications. Among these, *Parmotrema perlatum*, a foliose lichen widely distributed across tropical and temperate regions, has drawn attention due to its diverse phytochemical profile. Traditionally used in Indian cuisine as the spice “Kalpasi” or black stone flower, this lichen produces secondary metabolites such as terpenoids, flavonoids, alkaloids, and phenolic compounds that exhibit antimicrobial, anti-inflammatory, and antiparasitic activities. With the global rise of antimicrobial resistance, interest has shifted towards natural plant-derived compounds as alternatives to synthetic drugs. Exploring the phytochemicals of *P. perlatum* provides an opportunity to identify novel therapeutic candidates against parasitic and bacterial pathogens.

Parasitic diseases remain a significant threat to public health, particularly in low- and middle-income countries. Malaria, caused by *Plasmodium falciparum*, continues to be

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one of the deadliest infectious diseases, with an estimated 263 million new cases and nearly 600,000 deaths recorded in 2023. Children under five and pregnant women in sub-Saharan Africa are disproportionately affected. The parasite's ability to rapidly develop drug resistance poses a major challenge to treatment and prevention strategies. Heat shock proteins such as Hsp90 and phospholipid scramblases in *P. falciparum* have emerged as crucial targets for novel therapeutics, as they regulate parasite survival and host–parasite interactions.

Toxoplasma gondii, the causative agent of toxoplasmosis, is another globally prevalent protozoan parasite. While often asymptomatic in healthy individuals, it presents severe risks to immunocompromised patients and fetuses. Proteins such as TgHsp70 and TgHsp90 play critical roles in parasite replication, host cell invasion, and stress adaptation, making them attractive drug targets. Similarly, *Entamoeba histolytica*, responsible for amoebiasis, infects up to 50 million people annually, causing dysentery and liver abscesses in severe cases. Its Hsp70 protein is central to survival under oxidative stress within the host. Collectively, these pathogens represent urgent priorities for drug discovery due to the limited effectiveness of current therapies.

Beyond protozoal infections, bacterial diseases like shigellosis also remain a concern. *Shigella flexneri*, transmitted via the fecal–oral route, causes severe diarrheal disease leading to nearly 200,000 deaths annually, especially in children. Its virulence is mediated by the Type III Secretion System (T3SS), in which the IpgC protein stabilizes essential effector proteins that enable host cell invasion. Inhibiting IpgC offers a promising strategy to neutralize the bacterium's pathogenicity without directly targeting replication.

Against this backdrop, *P. perlatum* provides an accessible, low-cost natural resource with untapped medicinal potential. Gas Chromatography–Mass Spectrometry (GC-MS) analysis of its extracts has identified several phytochemicals, including thujopsene and resibufogenin, which exhibit cyclic structures favorable for strong protein–ligand interactions. Molecular docking studies suggest that these compounds display significant affinity towards the aforementioned parasitic and bacterial proteins, highlighting their promise as lead candidates for drug development.

Therefore, this study integrates phytochemical screening, molecular docking, and in vitro antibacterial assays to evaluate the bioactive potential of *P. perlatum*. By focusing on both protozoal and bacterial pathogens, the research underscores the broader therapeutic relevance of this lichen and positions it as a natural alternative for combating drug resistance in infectious diseases.

II. REVIEW OF LITARATURE

Nowadays parasitic diseases are becoming rare,so almost the scientific community is less concerned about dealing with them. But the future is unpredictable and if a second outbreak of any kind of parasitic disease happens, there is not a simple treatment available anywhere for this disease in the whole world. And all the treatment for malaria,entamoeba and toxoplasma is more or less costly so the world is in need of an effective as well as a cost efficient treatment for all of these diseases. The plant *Permotrema perlatum* , is a lichen ,which is very commonly found in all over India and almost used in every other household as spice, mostly "Biriyani spice". Commonly known in the local language as 'kalpasi'. The reason behind choosing this specific plant is as it is locally available in India, can be sold to foreign countries also at the cheapest prices so people all over the world can easily use it. And due to its availability and cheap price, large scale production of this plant will be very easy. Also not yet much research on the parasitic properties of this plant is done till now.

➢ *Some Mentionable Works are :*

- The Spasmolytic, Bronchodilator, and Vasodilator Activities of Parmotrema perlatum Are Explained by Anti-Muscarinic and Calcium Antagonistic Mechanisms. [15]
- Liquid chromatography-electrospray ionization-mass spectrometry/mass spectrometry characterization of depsides and depsidones from the Chilean lichen *Parmotrema perlatum*.[2]
- HPLC purification of antioxidant and antibacterial peptides from a lichen *"Parmotrema perlatum* (Huds.) M. Choisy": Identification by LC-MS/MS peptide mass fingerprinting.[5]
- Anti-Bacterial and Anti-Dermatophytic Activity of Extracellular Secondary Metabolites of Streptomyces glaucescens NTSB-37 Isolated from Lichen *Parmotrema perlatum* (Huds.) M. Choisy in Kolli Hills, Tamil Nadu, India.[24]
- Lichens of Parmelioid Clade as Promising Multitarget Neuroprotective Agents.[8]
- Selecting the species to be used in lichen transplant surveys of air pollution in Tunisia.[17]
- Depigmenting potential of lichen extracts evaluated by in vitro and in vivo tests. [19]
- Ozone and desiccation tolerance in chlorolichens are intimately connected: a case study based on two species with different ecology. [26]
- The influence of growth form and substrate on lichen ecophysiological responses along an aridity gradient. [11]
- Desiccation tolerance and lichenization: a case study with the aeroterrestrial microalga Trebouxia sp. (Chlorophyta).[7]

Gas Chromatography Mass Spectrometry (GC-MS) is an advanced hyphenated analytical technique used for separation, identification and quantification of mixture of compounds. GC-MS has a vital role in phytochemical fingerprinting of extracts and volatile oils. GC-MS is applicable for the analysis of low molecular weight, thermally stable volatile compounds. It is often used in drug

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detection, and is widely used in food industry to detect pesticides, contaminants, in pharmaceutical industry to detect impurities and residual solvent analysis, in testing environmental volatile pollutants, flavor and fragrances testing. Liquid, gaseous or solid samples can be detected by GC-MS method. The liquid or solid sample is first introduced into the column and the sample gets converted to gas. In a gas chromatograph, the vaporized sample is introduced into gaseous phase and the components are separated using a capillary column coated with a stationary (liquid or solid) phase. Inert carrier gases such as helium, hydrogen or nitrogen are used as mobile phase in GC-MS. As the compound mixtures are separated, each component is eluted from the column at a various retention time based on its boiling point and polarity. The elution time is referred to as a compound's retention time. Sample extracts containing hundreds of compounds or complex mixtures can be analyzed without isolating by GC-MS. MS detector gives better resolution in qualitative and quantitative analysis of samples. In this chapter, various GC-MS methods reported in characterization and quantification of phytochemicals of volatile oils and plant extracts were critically analyzed and discussed along with different ionization methods involved in GC-MS estimations. [22] So, via GC-MS almost 38 different phytochemicals were found. After that autodocking was performed using 'autodock vina'.First the chosen phytochemicals were downloaded from 'Pub chem'. After that from the molecule using 'auto dock' all the additional charges , H2 bonds or ionic bonds (if required) were removed . After that all the protein structures were downloaded from PDB (protein data bank) . And the final step which is docking was performed. First the SMILES sequence was copied of the specific 12 phytochemical and ligand was prepared. Then the protein molecule is added as a Pdb file and the target was also prepared.Then the placement of the grid box was checked and for final confirmation 'Dog Site Scorar'(proteins.plus) was used,which gives the proper placement of the grid box indicating the active site of the specific protein.After that the parameters was adjusted (4-8) and docking was performed.The docking results were later analysed.

III. MATERIALS AND METHOD

The spice Permotrema perlatum was sourced from Bhattarahalli local market Bangalore. The spice was already in dried form so, it was kept in sun for some time, and after that the spice was very critically and visually examined if any leaves from the tree as well as bark is present or not.After that the extra leaves and barks were physically separated from the spice with the help of a forceps.After that the spice was hand grinded using motor-pistel and converted the spice in a fine powder. Then the finely grinded powder was sieved using a siever. And for confirmation again the powder was visually examined to confirm that the powder was fine and no additional substances were present in it. Fig. 6.a: Powder formation of the sample Fig. 6.b : Sample grinding using motor pistle The Lichen sample was powdered and the sample was outsourced for GC-MS analysis. Where ethanol was used as a solvent. The sample was outsourced for the GC-MS analysis. After the GC-MS analysis 38 phytochemicals was retrieved from the sample.Amongst the 38 different phytochemicals Thujopsene and Resibufogenin was selected for the analysis. The main reason behind choosing these drugs are they have the cyclic structure (contains more than one Benzine ring). If the structure of the phytochemical is more cyclic they basically tend to have more binding affinity towards different types of proteins and also might help in neutralizing the protein by binding with it.**[3]**Both the drugs thujopsene and resibufogenin if the structures are analysed carefully they have cyclic structures.Bufogenin is a steroid lactone of Chan su (toad venom), a Chinese medicine obtained from the skin venom gland of toads. A specific Na/K-ATPase protein inhibitor, it is used as a cardiotonic and central nervous system (CNS) respiratory agent, an analgesic and anesthetic, and as a remedy for ulcers. It has a role as an EC 3.6.3.9 (Na(+)/K(+)-transporting ATPase) inhibitor. It is a steroid lactone and an epoxy steroid. It is functionally related to a bufanolide(.PubChem Compound Summary for CID 6917974, Bufogenin). And -)-thujopsene is a thujopsene that has (S,S,S)-configuration. It has a role as a plant metabolite. (PubChem Compound Summary for CID 442402, Thujopsene.

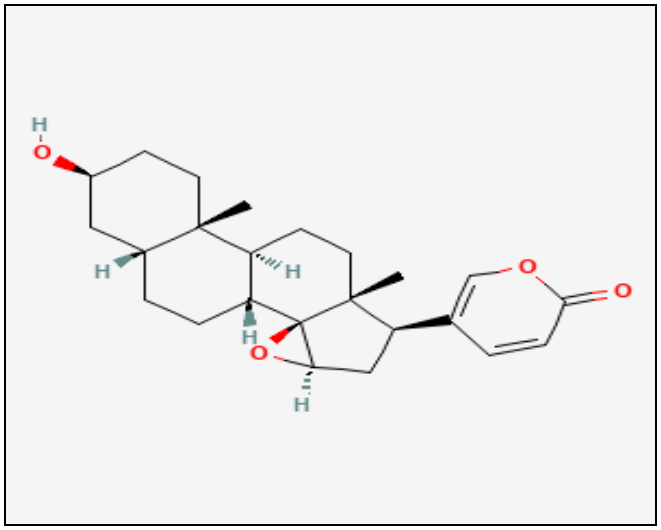

Fig 1a Drug Thujopsene

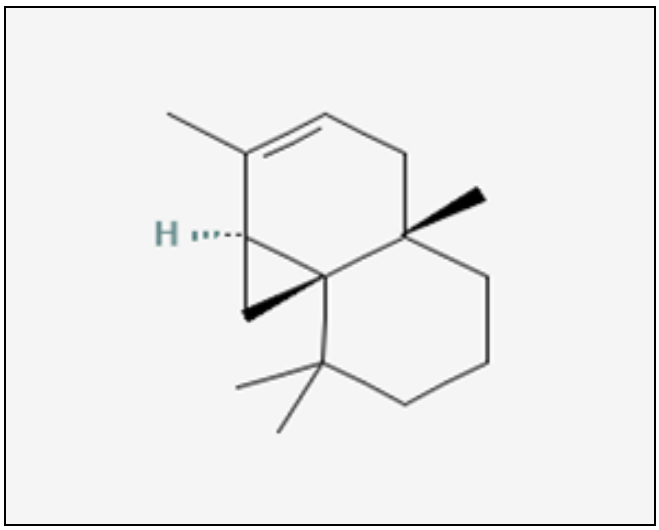

Fig 1b Drug: Resibufogenin

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➢ *The Proteins Selected from the Organisms were:*

- *3PEJ:*

N-terminal domain of Plasmodium falciparum Hsp90 (PFL1070c) Hsp90 is a molecular chaperone crucial for proper folding and stabilization of many Plasmodium proteins, including those involved in cell cycle, signaling, and stress responses. Binding of Macbecin to Hsp90's N-terminal ATP pocket impairs its function, leading to widespread protein misfolding, stress-response failure, and ultimately inhibition of parasite replication and survival. Hsp90 inhibitors have demonstrated potent in vitro and in vivo antimalarial activity by disrupting this chaperone function[18].

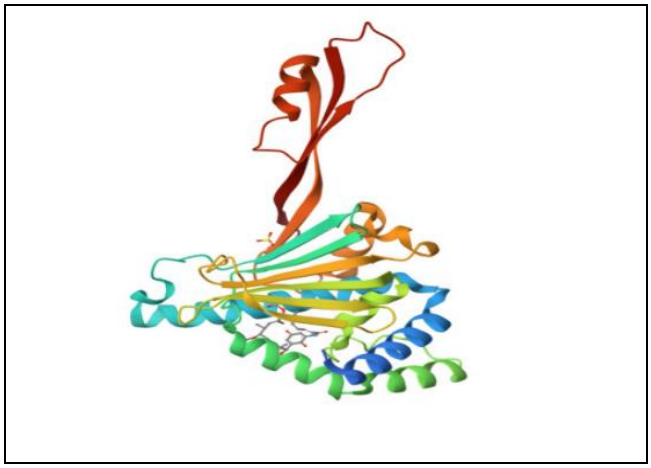

Fig 2a 3 PEJ

- *Q8IJH8 · PLSH_PLAF7:*

This protein catalyzes calcium-induced ATP-independent rapid bidirectional and non-specific movement of phospholipids (lipid scrambling or lipid flip-flop) between the inner and outer leaflet of the plasma membrane resulting in collapse of the phospholipid asymmetry ..Preferentially, mediates calcium-dependent phosphatidylethanolamine externalization .During the liver stage, plays a role in the interaction with, and thus invasion of, host hepatocytes.Dispensable for host erythrocyte invasion and asexual parasite development . It localizes to the perinuclear region in the early ring stages and re-localizes to the cytoplasm and vesicular foci in early trophozoite and schizont stages ..Localizes to the cell membrane in individual merozoites in late schizonts . In stage III-IV gametocytes, localizes to vesicular foci and to cell periphery .In sporozoites, localizes to the cell membrane (Martinez et al.,2021) Phospholipid scramblase (PfPLSCR), encoded by Q8IJH8, is a Ca^{2+}-activated membrane protein expressed during P. falciparum blood-stage development . Biochemical assays demonstrate that PfPLSCR facilitates bidirectional translocation ("scrambling") of phospholipids—particularly phosphatidylserine (PS)—across parasite membranes in a metalion-dependent fashion **[21]** .While deletion of PfPLSCR shows it is not essential for erythrocyte invasion or asexual growth it modulates PS exposure on infected red blood cells (iRBCs). The parasite's use of PfPLSCR leads to PS flipping, which acts as an "eat-me" signal to host phagocytes (Fraseret al.,2021)This exposure promotes immune clearance of infected cells. Importantly, experiments show that knockout of PfPLSCR reduces PS exposure in late asexual and early gametocyte stages**[21]**. By limiting PS externalization, PfPLSCRdeficient parasites could evade phagocytosis, but conversely, targeting PfPLSCR with inhibitors or genetic disruption could prevent replication and transmission by enhancing immune system recognition and clearance of infected erythrocytes.

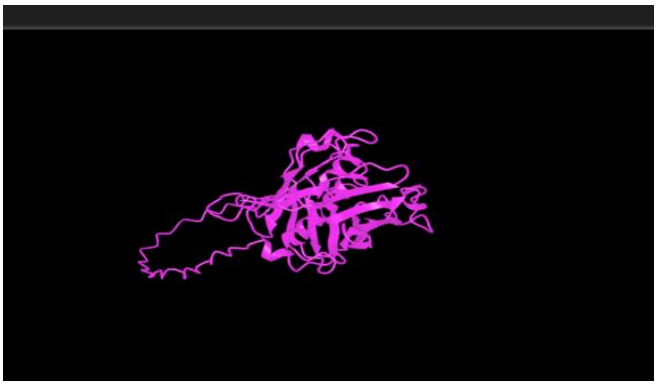

Fig 2b Q81JH8

- *Entamoeba Histolytica(HSP70) Heat-Shock Protein 70:*

Hsp70 In *Entamoeba histolytica* acts as a crucial molecular chaperone that under stress conditions (e.g. oxidative burst, heat shock, or nitric oxide exposure) helps refold damaged or misfolded proteins and prevents their aggregation—this activity is essential for the parasite's survival and propagation within host tissues**[1].** Experimental studies show that EhHsp70 is strongly upregulated during exposure to oxidative stress, nitric oxide, and elevated temperatures—all of which mimic the hostile host environment during infection **[20]**. Inhibiting Hsp70 function—whether through gene knockdown or pharmacological agents— impairs the parasite's replication because the loss of chaperone support leads to widespread protein misfolding, deactivation of essential enzymes, and eventual cell death due to proteotoxic stress . Additionally, immunocytochemistry shows EhHsp70 localizes to both the nucleus and cytoplasm of trophozoites, suggesting its critical role during active replication phases. Structural differences between the amoebic Hsp70 and its human counterparts make it a promising target for selective antiprotozoal activity**[25]**

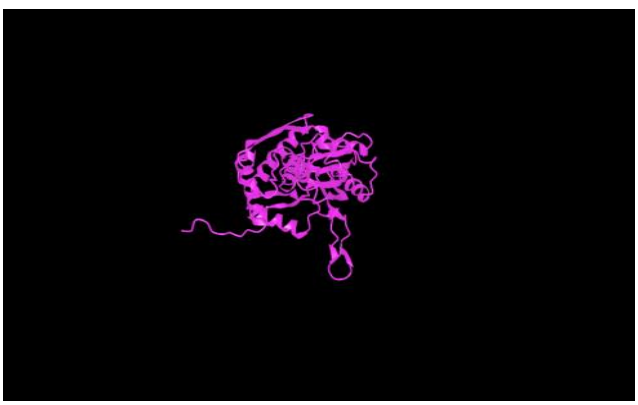

Fig 2c HSP70 (*Entamoeba Histolytica*)

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- *Toxoplasma Gondii: Heat-Shock Protein 70 (TgHsp70):*

TgHsp70 acts as a molecular chaperone during stress conditions (e.g., immune attack, stage conversion). Its expression increases during tachyzoite-to-bradyzoite differentiation and under stress, helping refold proteins and mitigate proteotoxic damage **[28]**. Studies show TgHsp70 also functions as a "danger signal," modulating host immune responses—such as dampening nitric oxide production or B-cell activation—to fine-tune host–parasite interactions . While not directly halting DNA replication, TgHsp70 enables stress adaptation and survival during transitions. Targeting TgHsp70 expression or function disrupts this resilience, impairing parasite growth under immune pressure or stress, and thereby limiting replication and persistence.

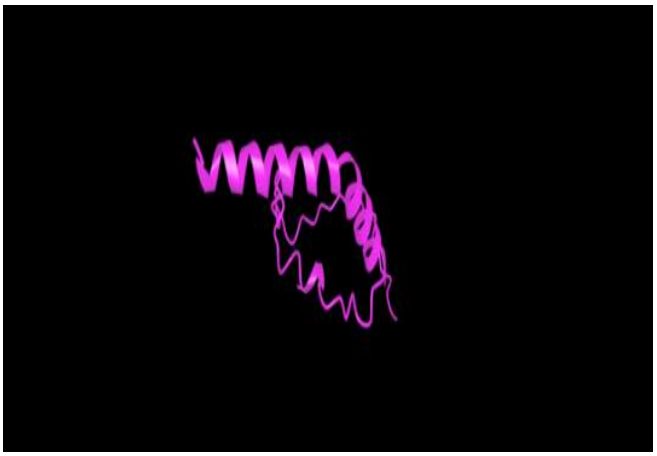

Fig 2d Toxoplasma Gondii: Heat-Shock Protein 70 (TgHsp70).

- *Heat-Shock Protein 90 (TgHsp90)*

TgHsp90 is a critical chaperone required for multiple stages of the parasite lifecycle—especially tachyzoite replication, host-cell invasion, and tachyzoite–bradyzoite interconversion **[27]**. Genetic knockout of TgHsp90 prevents tachyzoites frominvading and replicating in host cells and abolishes in vivo virulence . Small-molecule inhibitors such as geldanamycin bind to TgHsp90's ATP-binding pocket, disrupting its chaperone activity; this leads to improper folding of essential client proteins, blocking replication and stage conversion . The plant *Permotrema perlatum*, The powdered ruffle lichen, *Parmotrema perlatum*, is acommon type of foliose lichen that belongs to the Parmeliaceae family.The species has acosmopolitan distribution and occurs throughout the Northern and Southern Hemispheres.

Parmotrema perlatum is a prominent and widely recognised species within its genus across primarily temperate zones, preferring humid, oceanic-suboceanic habitats. It is found in diverse geographic areas including Africa, North and South America, Asia, Australasia, Europe, and islands in the Atlantic and Pacific oceans. It usually grows on bark, but occasionally occurs on siliceous rocks, often among mosses.

The thallus of *Parmotrema perlatum* is large, light-grey to pale-blue patch-shaped with rounded and ruffled lobes and often with black hair-cilia at the edges. Distinguishing features of the lichen include its conspicuous soralia (reproductive structures) near the lobe edges,curled leaf-like lobes, and a narrow, shiny, and sometimes wrinkly area on the underside near the margin. This species is known for producing certain secondary metabolites, namely atranorin and a group of substances known as the stictic acid complex, which includes stictic 8 and constictic acids, among other related compounds. These morphological and chemical characteristics help distinguish *P. perlatum* from several other potential lookalikes.*Parmotrema perlatum* has a complex taxonomic history, having undergone multiple reclassifications since its original description in 1762. Significant efforts in the mid-20th century helped clarify its nomenclature, stabilising its current name. Although there were challenges to this name in the 1980s, it was confirmed as valid in 2004. More recently, DNA studies suggest that there may be hidden diversity within the species, indicating the need for further taxonomic evaluation.The lichen is used as a spice in Indian cuisine. For this purpose,it is commonly known as black stone flower or kalpasi (among other names). Although nearly tasteless on its own, it releases an earthy fragrance and taste when cooked in with oil or butter. Besides all these, this plant also has some very essential phytochemicals,which can be used for the cure of many pathogenic diseases (Bacterial,Fungal,parasitic or protozoan diseases). So the main objective of this research is 'using this particular plant *Permotrema perlatum*, finding the phytochemicals which can be used as a drug for curing different pathogenic diseases,mainly focusing on parasitic and protozoan diseases **[27].**

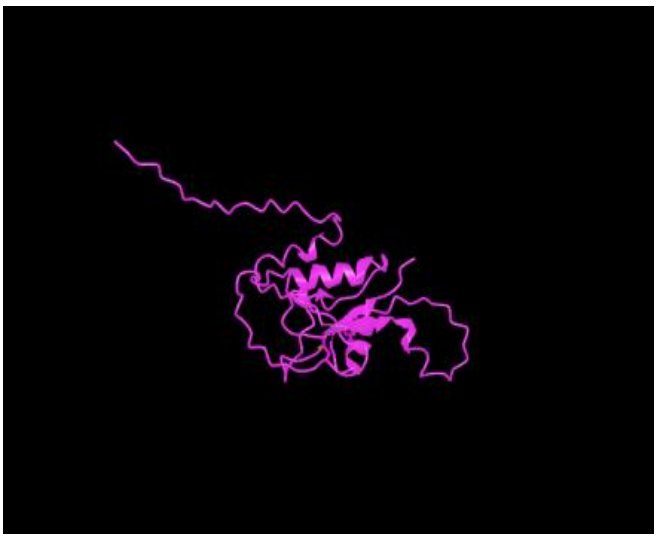

Fig 2e 90 kDa Heat-Shock Protein from *Toxoplasma Gondii* (TgHsp90).

- *Igpc Protein:*

In *Shigella flexneri* that plays a critical role in the Type III Secretion System (T3SS), a major virulence mechanism of the pathogen. IpgC specifically binds and stabilizes two essential translocator proteins, IpaB and IpaC, which are required for the formation of the translocon complex that enables the delivery of bacterial effector proteins into host epithelial cells. Without IpgC, IpaB and IpaC become unstable and are degraded, leading to failure in the assembly

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and function of the T3SS apparatus. This disruption impairs Shigella's ability to invade host cells, escape from vacuoles, and trigger host inflammatory responses, all of which are crucial for establishing infection. Therefore, targeting IpgC does not interfere directly with bacterial replication but effectively neutralizes the pathogen's invasive capacity, making it a promising target for preventing or attenuating shigellosis. Inhibiting IpgC would block effector translocation, halt epithelial invasion, and ultimately prevent disease progression by disarming the pathogen's primary mechanism of virulence.

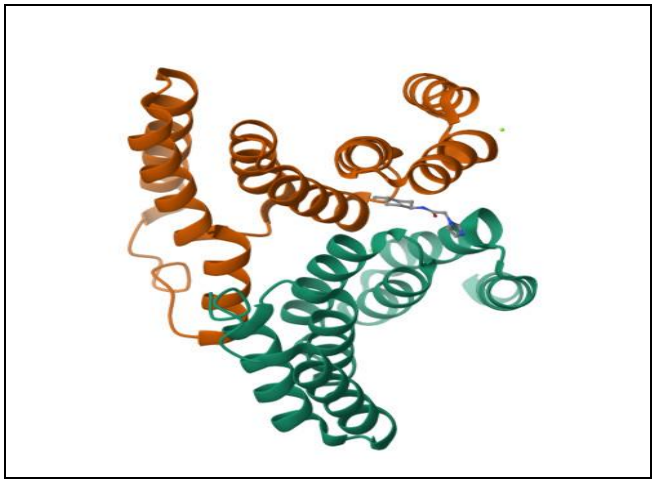

Fig 2f Igpc Protein

IV. RESULT

After the sample was grinded in powder form it was outsourced for further GC-MS analysis and for the GC-MS analysis ethanol was chosen as a solvent. And GC-MS results are analysed.

After the list of phytochemicals was analysed amongst all of these phytochemicals Thujopsene and Resibufogenin was selected for further analysis.

And the proteins from following organisms were selected for docking as a target for the drug . For *Plasmodium sp.* 3pej,and Q81JH8, for *Entamoeba sp.* HSP70 and for *Toxoplasma sp.* HSP 70 and HSP 90 were selected. And after docking them with the chosen drugs the affinity of the drugs towards those protein was identified which helped us to analyse how much affectively that drug or phytochemical will be able to neutralize the protein which will be able to stop the replication of that specific parasitic organism and prevent it from disease spreading.

Table 1 Calculated Affinity Results from the Molecular Docking of the Protein and the Phytochemical

Phytochemical	Organism	Protein	Calculated affinity
	Plasmodium falciparum	3pej	-4.529
Thujopsene		Q81JH8	-5.809
	Entamoeba histolytica	HSP70	-5.116
	Toxoplasma gondii	HSP70	-3.767
		HSP90	-5.778

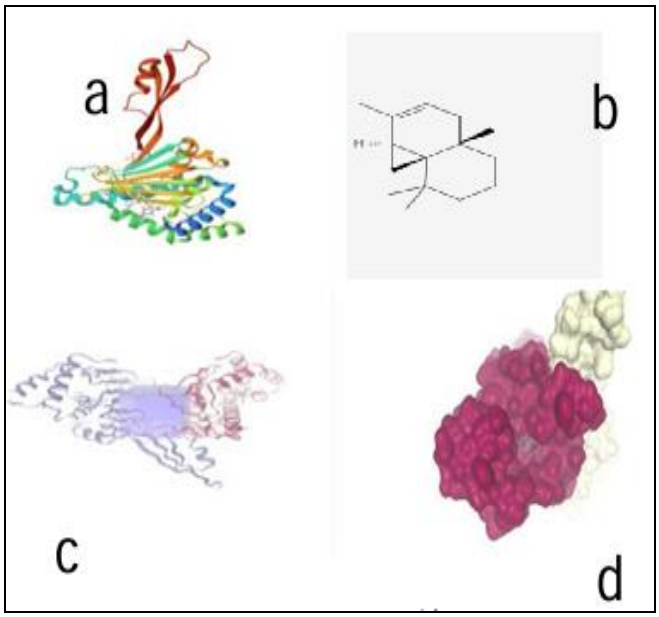

Fig 3.1 3PEJ Protein Structure b) Drug Structure c) Active Site (Grid Placement) d) Docking (Protein-Phytochemical) for Plasmodium Falciparum.

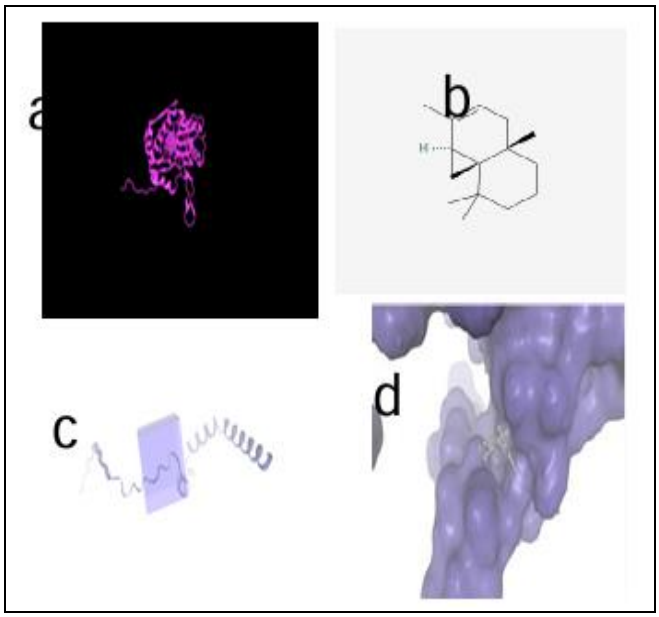

Fig 3.2 Q81JH8 Protein Structure b)Drugstructureture) Active Site (Grid Placement) d)Docking (Protein-Phytochemical for Plasmodium Falciparum.

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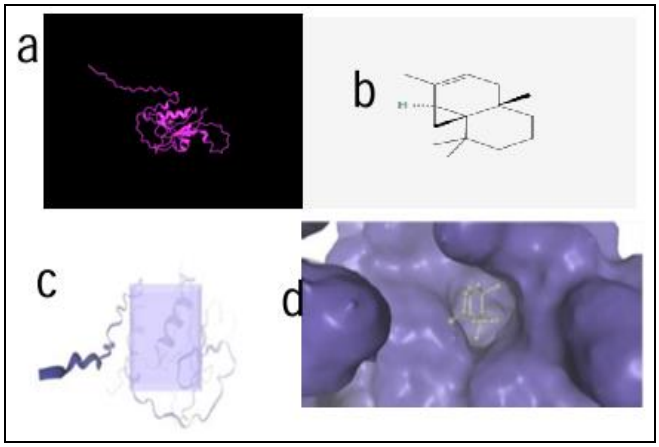

Fig 3.3 a)HSP70 Protein Structure b)Drug Structure c)Active Site (Grid Placement) d) Docking (Protein Phytochemical) for Entamoeba Histolytica.

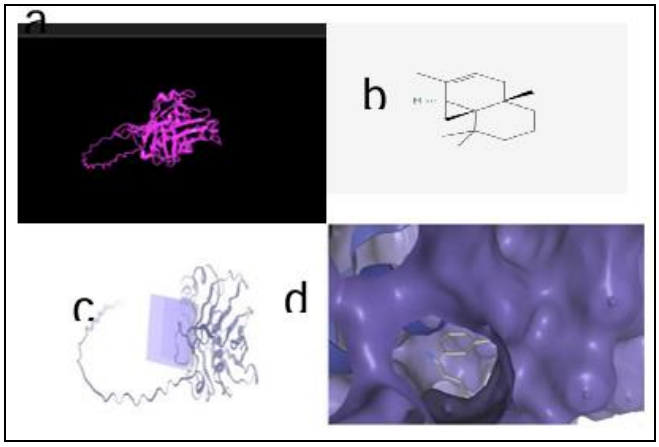

Fig 3.4 a)HSP70 Protein Structure b)Drug Structure c)Active Site (Grid Placement) d) Docking (Protein Phytochemical) for Toxoplasma Gondii

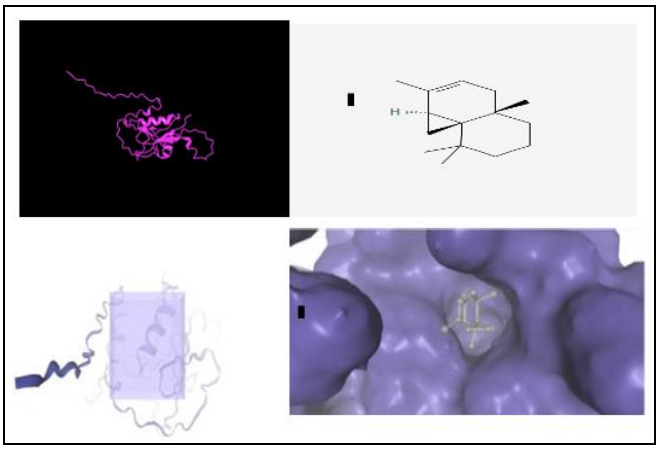

Fig 3.5: a)HSP90 Protein Structure b)Drug Structure c)Active Site (Grid Placement) d) Docking (Protein-Phytochemical) for Toxoplasma Gondii.

Table 2 Calculated Affinity Results from the Molecular Docking of the Protein and the Phytochemical Thujopsene.

Phytochemical	Organism	Protein	Calculated affinity
	Plasmodium falciparum	3pej	-6.956
Resibufogenin		Q81JH8	-5.536
	Entamoeba histolytica	HSP70	-7.138
	Toxoplasma gondii	HSP70	-4.999
		HSP90	-5.62

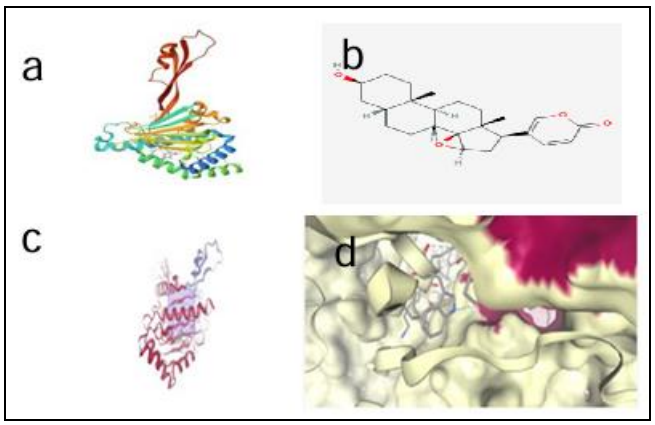

Fig 4.1 a)3 PEJ Protein Structure b)Drug Structure c)Active Site (Grid Placement) d) Docking (Protein-Phytochemical) for Plasmodium

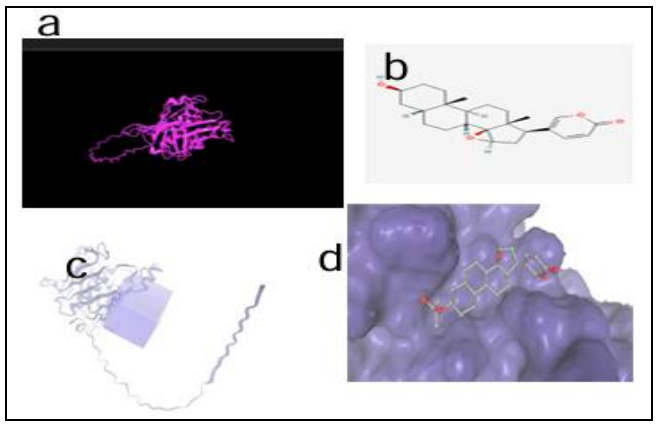

Fig 4.2 a)Q81JH8 Protein Structure b)Drugstructure c)Active Site (Grid Placement)d) Docking (Protein Phytochemical) for Plasmodium Falciparum.

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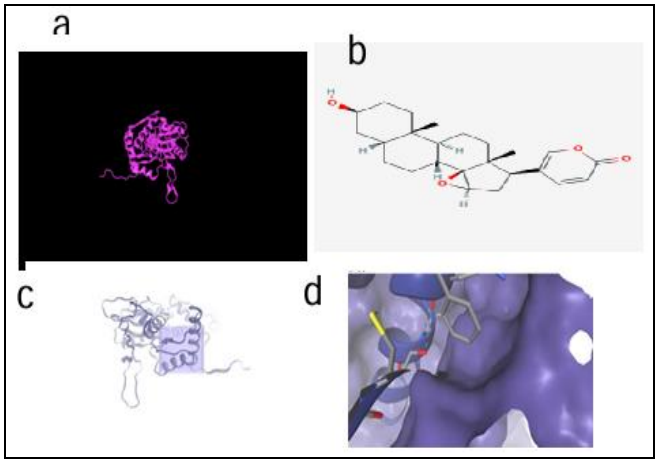

Fig 4.3 a)HSP70 Protein Structure b)Drug Structure c)Active Site (Grid Placement) d) Docking(Protein Phytochemical) for Entamoeba Histolytica.

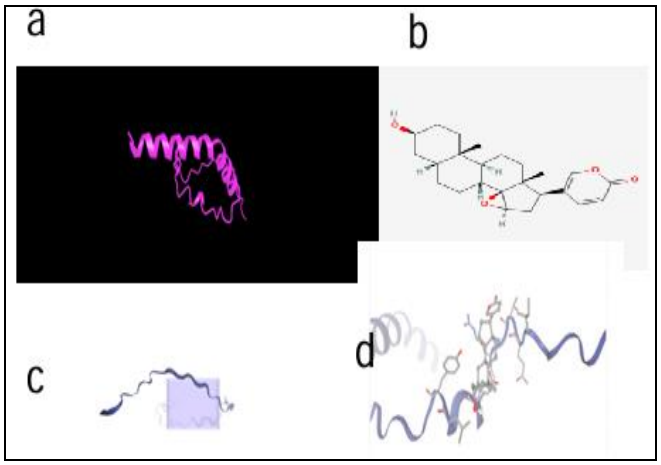

Fig 4.4 a)HSP70 Protein Structure b)Drug Structure c)Active Site (Grid Placement) d) Docking (Protein-Phytochemical) for Toxoplasma Gondii

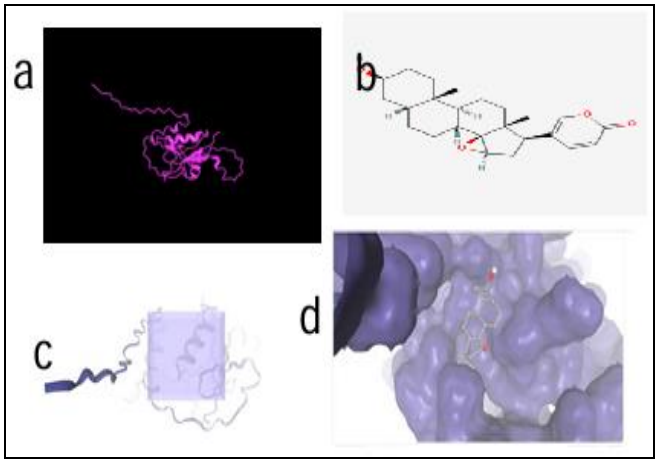

Fig 4.5 a)Igpc Protein Structure b)Drug Structure c)Active Site (Grid Placement) d) Docking(Protein-Phytochemical) for S. Flexneri.(Thujopsin)

By analysing table-1 it can be assumed that Thujopsene has an high affinity towards HSP70 of *Toxoplasma gondii* , so we can say this phytochemical can be used as a drug which will be very affective in case of preventing the severity of the disease toxoplasmosis,or in inhibiting the organism *Toxoplasma gondii.* By analysing table-2 it can be assumed that Resibufogenin has an high affinity towards protein 3pej of HSP70 of *Toxoplasma gondii*. So, it can be said that this drug will be very affective in case of preventing the severity of Malaria disease or in case of inhibiting the organism *Toxoplasma gondii.*

Table 3 Calculated Affinity Results from the Molecular Docking of the Protein and the Phytochemical (Thujopsene & Resibufogenin)

Drug	Protein	Calculated affinity
Thujopsene	Igpc	-6.611
Resibufogenin	Igpc	-6.131

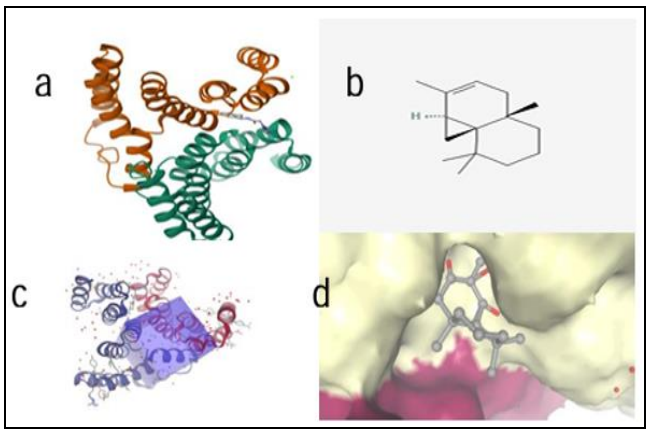

Fig 5.1 a)Igpc Protein Structure b)Drug Structure c)Active Site (Grid Placement) d)Docking(Protein-Phytochemical)for Shigella Flexneri.(Resibufogenin)

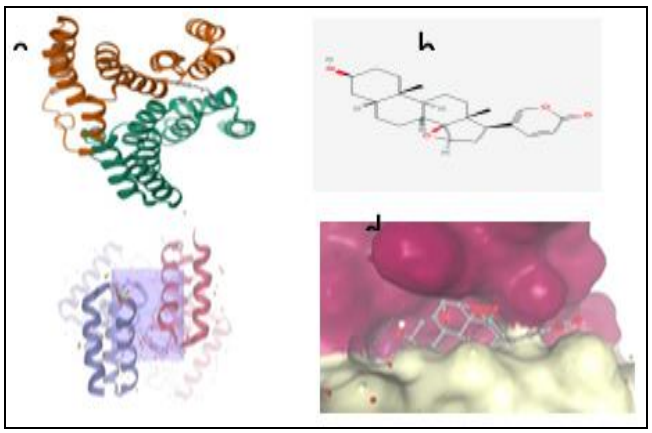

Fig 5.2 a)Igpc Protein Structure b)Drug Structure c)Active Site (Grid Placement) d) Docking(Protein-Phytochemical) for Shigella Flexneri. (Thujopsin)

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➢ *Antibacterial Property Screening of P.perlatum using Shigella Spp.bacterial Culture:*

On the basis of Table-4 we can say the drug Resibufogenin has affinity towards the Igpc protein of *Shigella flexneri* .Based on this result , a wet lab was performed on *Shigella sp.* by extracting the solvent of *Permotrema perlatum*.

➢ *Procedure:*

- *Materials:*

✓ Microorganisms: *Shigella flexneri*
✓ Growth medium: Nutrient Agar
✓ Nutrient broth (for overnight culture)
✓ Sterile filter paper discs (6 mm diameter, Wattman No. 1)
✓ Antibacterial agents (plant extracts of *P.perlatum* ethanolic and aqous)
✓ Sterile forceps
✓ Sterile Petri dishes
✓ Micropipettes and sterile tips
✓ Incubator (set to 37°C)

➢ *Methodology:*

✓ *Shigella sp.* Broth culture was taken.
✓ An ethanolic extract of the *Permotrema perlatum* powder was prepared.(10:1)It was incubated on a shaker incubator for 36 hours.
✓ Just the same way (10:1) aqueous extract of the powder was prepared.
✓ In the ethanolic solvent disc made by wattmann filter paper was immersed and those were also incubated with the solvent for almost 36 hours.
✓ 100ml of NA media was prepared. And 5 petri plates were taken.And after solidifying the media *Shigella* broth culture was spread over it using a sterile cotton bud.
✓ After that in between the four plates in two plates the discs that were immersed in the ethanolic solvent were placed .
✓ And in the rest two plates the wells are formed using a well borer and in the wells aqueous extract of the *P.perlatum* powder was placed.
✓ And one plate is kept as control where after well formation water is poured .
✓ And all the plates were incubated for 48 hours and the result was observed.

Table 4 Wet lab results

Sample	Bacterial strain	Zone of inhibition(mm)	Mean ± SD (kcal/mol)
Aqous extract	*Shigella spp.*	13,15mm	14
Ethanolic extract	*Shigella spp.*	11,12mm	11.5

➢ *Interpretation of Wet Lab Results:*

So therefore we can say according to Dwarakanath (P. Radhakrishnan,*et al.*2024) this plant shows very minimal

V. DISCUSSION

The study on the phytochemical properties of *Permotrema perlatum* is relevant as well as unique amongst all the studies and researches that have been done on this specific plant till now.The way the research on phytochemicals of this plant and its anti-parasitic activity is relevant as well as unique and beneficial for future is discussed below: "The Spasmolytic, Bronchodilator and Vasodilator Activity of *Parmotrema perlatum* Is Mediated through the Inhibition of Muscarinic Receptors and Voltage Dependent Ca2+ Channels."The present study is concerned with the pharmacological properties of *Parmotrema perlatum i*n respect of its smooth muscle relaxing action and vascular activity. It underscores the plant's prospects for treating conditions like asthma or hypertension. This study is significant because it extends the potential benefit of *P. perlatum* and in a completely different way—modulating parasitic protozoans, not smooth muscle diseases. Where **[15]**investigate bioactivity towards host tissues, the research investigate activity against proteins known to be pathogenic, such that the approach is novel and the medical scope of the plant is broader**[15]**. "LC-ESI-MS/MS characterization of depsides and depsidones from Chilean lichen *Parmotrema perlatum.*"This article presents a chemical profile of *P. perlatum,* with determination of the main secondary metabolite constituents, by advanced spectrometric techniques. Provides a solid phytochemical base that your study uses as a starting point.

Nevertheless, the progress to that Cockroach along with the other mouse files by, for the first time, experimentally relating individual phytochemicals (Thujopsene and Resibufogenin) with anti-parasitic activity through molecular docking, as did Castañeta et al. do not explore. So this research is a practical utilization of their chemical basis**[2]**."High-performance liquid chromatography purification of antioxidant and antibacterial peptides from *Parmotrema perlatum."*Targeted screening of antioxidant and antibacterial bioactive peptides, with an emphasis on treatment of bacterial infection. While this work is also bioactivity-based, it is fascinating for being directed against protozoal parasites, and in particular some, such as Plasmodium and Toxoplasma, that hardly feature in this paper. Hence, this extends, rather than oppose, their efforts by redirecting their attention away from prokaryotic pathogens to eukaryotic parasites and strengthening the case for *P. perlatum* as a potential therapeutic for multiple antimicrobials[**5]**. "Anti-Bacterial and Anti-Dermatophytic Activity of Extracellular Secondary Metabolites of Streptomyces glaucescens Isolated from Parmotrema perlatum." Here, the emphasis is on microbial metabolites from bacteria associated with the lichen, not the lichen itself. While relevant in showing that *P. perlatum*-related systems harbor antimicrobial agents, this study is unique because it directly focuses on the lichen's own phytochemicals, not its

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microbial symbionts. Moreover, this research explore anti-protozoal activity via protein-ligand interactions, introducing a mechanistic aspect missing in this work. **[24]** "Lichens of Parmelioid Clade as Promising Multitarget Neuroprotective Agents." **[8]** position *Parmotrema perlatum* and related lichens as potential neuroprotective agents, potentially useful in treating disorders like Alzheimer's. Although both studies highlight therapeutic versatility, this work is pathogen-centric, aiming to suppress parasite replication via protein targeting. Their focus is host-protective in a neurodegenerative context; this works focus is host-protective in an infectious disease context, showing how the same plant can be adapted to completely different therapeutic domains.**[8**] "Selecting the species to be used in lichen transplant surveys of air pollution in Tunisia." This paper is ecological in scope, using *P. perlatum* as a bioindicator for environmental pollution, specifically air quality. While entirely unrelated to disease treatment, it underscores *P. perlatum*'s wide distribution and environmental relevance—which strengthens this research rationale for choosing it (i.e., easily available, abundant, low cost). This project diverges by repurposing this environmentally important lichen for phytochemical drug discovery, bringing in a pharmacological perspective that this paper lacks.**[17]** "Depigmenting potential of lichen extracts evaluated by in vitro and in vivo tests." This study evaluates cosmetic applications, especially skin depigmentation, of lichen extracts including *P. perlatum.* This work shares common ground in terms of bioactive screening, but the goals are very different.This research aims to combat serious parasitic diseases through molecular docking of heat shock proteins, while **[19]** explore melanin pathway. interference.

This stark difference in application domains—cosmetic vs. therapeutic— highlights this research's unique contribution to antiparasitic research**[19].**

"Ozone and desiccation tolerance in chlorolichens are intimately connected." Smith and Jones examine how *P. perlatum* tolerates harsh environmental stress, such as ozone and desiccation. While this study is unrelated in methodology or objective, their work supports the idea that *P. perlatum* harbors robust survival mechanisms, possibly linked to secondary metabolite production—which it taps into for therapeutic purposes. This connection, though indirect, adds a biological justification for exploring its phytochemistry as a survival-based chemical defense system. "The influence of growth form and substrate on lichen ecophysiological responses along an aridity gradient." González et al. focus on how environmental stressors shape lichen physiology and metabolite production. Again, while the methods and aims differ in this study , their findings justify the assumption that stress-exposed lichens like *P. perlatum* are metabolically rich, making them excellent targets for phytochemical studies. This study / reasearch extends this ecological insight into a medicinal application by targeting protozoal proteins.**[10 &11]**. "Desiccation tolerance and lichenization: a case study with Trebouxia sp. (Chlorophyta)." This paper delves into lichen symbiosis and resilience, especially in the algal partner. Although it focuses on a different species, it supports the idea that lichen organisms evolve chemical defenses to withstand extreme conditions. This study is distinct but compatible, as it seeks to harness these chemical defenses (phytochemicals) against parasitic infections. Thus, this study explores the biomedical utility of a survival mechanism highlighted by**[7]** .This study which is done on a probable basis using bioinformatics methods can be verified and validated using decoy study and toxicity testing. This studies will prove that the docking results that are calculated and gives us a presumptive value of the affinity of the phytochemical,are actually effective on the protein or not , and if the scientific community should actually pursue the further wet lab research on the basis of this bioinformatics data.

VI. CONCLUSION

This study successfully explores the bioactive potential of *Parmotrema perlatum* through phytochemical screening using GC-MS analysis and molecular docking approaches. The GCMS results revealed a diverse range of secondary metabolites, including terpenoids, phenolics, and steroids, many of which are known for their antimicrobial and antiparasitic properties. These compounds were further evaluated for their binding affinity against essential proteins of three major protozoan pathogens: *Plasmodium falciparum*, *Toxoplasma gondii*, and *Entamoeba histolytica*. Molecular docking revealed significant interactions between several lichen-derived compounds and the selected pathogenic proteins, highlighting their potential as antiparasitic agents. Among the tested compounds, resibufogenin showed the highest binding affinity towards *Plasmodium falciparum* target protein (Q81JH8), indicating its strong inhibitory potential against malaria. Likewise, thujopsene demonstrated a notable interaction with *Entamoeba histolytica* and moderate affinity towards *Toxoplasma gondii* proteins. These findings suggest that certain bioactive constituents of *Parmotrema perlatum* may serve as promising leads for developing novel antiparasitic drugs. The integrative approach combining phytochemistry and in silico tools offers a rapid, cost-effective strategy for drug discovery, especially in the context of increasing resistance to conventional antiparasitic agents.

FUTURE ASPECT

Future research should focus on in vitro and in vivo validation of the most promising compounds, especially resibufogenin and thujopsene, to confirm their efficacy and safety against the respective parasites. Additionally, structural optimization and ADMET profiling could enhance their drug-likeness and pharmacokinetic properties. Exploring the synergistic effects of compound combinations and formulating them into effective drug delivery systems can further improve therapeutic outcomes. Genomic and proteomic studies on parasite resistance mechanisms can also guide more targeted compound design, paving the way for developing next-generation antiparasitic drugs from natural sources like *Parmotrema perlatum.*

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REFERENCES

[1]. Arisue, N., Sánchez, L. B., Weiss, L. M., Müller, M., & Hashimoto, T. (2002). Mitochondrial-type hsp70 genes of the amitochondriate protists, *Giardia intestinalis*, *Entamoeba histolytica* and two microsporidians. *Parasitology International, 51*(1), 9–16. https://doi.org/10.1016/s1383-5769(01)00093-9

[2]. Castañeta, D., Sepúlveda, B., & Areche, C. (2024). Liquid chromatographyelectrospray ionization-mass spectrometry/mass spectrometry characterization of depsides and depsidones from the Chilean lichen *Parmotrema perlatum*.

[3]. Cirac, A. D., Moiset, G., Mika, J. T., Koçer, A., Salvador, P., Poolman, B., Marrink, S. J., & Sengupta, D. (2011). The molecular basis for antimicrobial activity of pore-forming cyclic peptides. *Biophysical Journal, 100*(10), 2422–2431. https://doi.org/10.1016/j.bpj.2011.03.057

[4]. Dutta, T., Singh, H., Edkins, A. L., & Blatch, G. L. (2022). Hsp90 and associated cochaperones of the malaria parasite. *Biomolecules, 12*(8), 1018. https://doi.org/10.3390/biom12081018

[5]. Dwarkanath, V., et al. (2024). HPLC purification of antioxidant and antibacterial peptides from a lichen *Parmotrema perlatum (Huds.) M. Choisy*: Identification by LCMS/MS peptide mass fingerprinting.

[6]. Dwarkanath, V., et al. (2024). HPLC purification of antioxidant and antibacterial peptides from a lichen *Parmotrema perlatum (Huds.) M. Choisy*: Identification by LCMS/MS peptide mass fingerprinting. 8. Fernández, F., et al. (2020). Desiccation tolerance and lichenization: A case study with the aeroterrestrial microalga *Trebouxia sp.* (Chlorophyta).

[7]. Fernández, F., et al. (2020). Desiccation tolerance and lichenization: A case study with the aeroterrestrial microalga *Trebouxia sp.* (Chlorophyta).

[8]. Ferreira, I. C. F. R., et al. (2019). Lichens of Parmelioid clade as promising multitarget neuroprotective agents.

[9]. Fraser, M., Jing, W., Bröer, S., Kurth, F., Sander, L. E., Matuschewski, K., & Maier, A. G. (2021). Breakdown in membrane asymmetry regulation leads to monocyte recognition of *Plasmodium falciparum*-infected red blood cells. *PLoS Pathogens, 17*(2), e1009259. https://doi.org/10.1371/journal.ppat.1009259

[10]. González, A., et al. (2021). The influence of growth form and substrate on lichen ecophysiological responses along an aridity gradient.

[11]. González, A., et al. (2021). The influence of growth form and substrate on lichen ecophysiological responses along an aridity gradient.

[12]. Haase, S., Condron, M., Miller, D., Cherkaoui, D., Jordan, S., Gulbis, J. M., & Baum, J. (2021). Identification and characterisation of a phospholipid scramblase in the malaria parasite *Plasmodium falciparum*. *Molecular and Biochemical Parasitology, 243*, 111374. https://doi.org/10.1016/j.molbiopara.2021.111374

[13]. Hussain, S., et al. (2021). The spasmolytic, bronchodilator, and vasodilator activities of *Parmotrema perlatum* are explained by anti-muscarinic and calcium antagonistic mechanisms.

[14]. Jing-Mei, L., Xu, X., Aosai, F., Zhang, M.-Y., Zhou, L.-L., & Piao, L.-X. (2024). Protective effect of arctiin against *Toxoplasma gondii* HSP70-induced allergic acute liver injury by disrupting the TLR4-mediated activation of cytosolic phospholipase A2 and platelet-activating factor. *International Immunopharmacology, 126*, 111254. https://doi.org/10.1016/j.intimp.2023.111254

[15]. Khaldi, A., et al. (2022). Selecting the species to be used in lichen transplant surveys of air pollution in Tunisia. *[Journal name pending]*.

[16]. Kumar, R., Musiyenko, A., & Barik, S. (2003). The heat shock protein 90 of *Plasmodium falciparum* and antimalarial activity of its inhibitor, geldanamycin. *Malaria Journal, 2*, 30. https://doi.org/10.1186/1475-2875-2-30

[17]. Lozano, M., et al. (2023). Depigmenting potential of lichen extracts evaluated by in vitro and in vivo tests.

[18]. Nagaraja, S., & Ankri, S. (2018). Utilization of different omic approaches to unravel stress response mechanisms in the parasite *Entamoeba histolytica*. *Frontiers in Cellular and Infection Microbiology, 8*, 19. https://doi.org/10.3389/fcimb.2018.00019

[19]. Ngotho, P., Press, K. D., Peedell, M., Muasya, W., Omondi, B. R., Otoboh, S. E., Seydel, K. B., Kapulu, M., Laufer, M., Taylor, T., Bousema, T., & Marti, M. (2025). Reversible host cell surface remodelling limits immune recognition and maximizes transmission of *Plasmodium falciparum* gametocytes. *PLoS Pathogens, 21*(5), e1013110.

[20]. R., C., Sivagami, B., Niharika, K., Lavanya, G., Hepsiba, B., Kumari, A. K., & Chandana, M. (2024). GC-MS in quantification and characterization of phytoconstituents of herbal extracts: A review. *Pharmaceutical Research: Recent Advances and Trends, 8*, 155–178. https://doi.org/10.9734/bpi/prrat/v8/2870

[21]. Radhakrishnan, M., et al. (2024). Anti-bacterial and anti-dermatophytic activity of extracellular secondary metabolites of *Streptomyces glaucescens* NTSB-37 isolated from lichen *Parmotrema perlatum (Huds.) M. Choisy* in Kolli Hills, Tamil Nadu, India.

[22]. Santos, F., Marcial-Quino, J., Gómez-Manzo, S., et al. (2020). Functional characterization and subcellular distribution of two recombinant cytosolic HSP70 isoforms from *Entamoeba histolytica* under normal and stress conditions. *Parasitology Research,*

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25aug1452

119, 1337–1351. https://doi.org/10.1007/s00436-020-06621-7

[23]. Smith, D., & Jones, A. (2022). Ozone and desiccation tolerance in chlorolichens are intimately connected: A case study based on two species with different ecology.10.1007/s00425-015-2319-z

[24]. Sun, H., Zhuo, X., Zhao, X., Yang, Y., Chen, X., Yao, C., & Du, A. (2017). The heat shock protein 90 of *Toxoplasma gondii* is essential for invasion of host cells and tachyzoite growth. *Parasite*, *24*, 22. https://doi.org/10.1051/parasite/2017023

[25]. Jing-Mei, L., Xu, X., Aosai, F., Zhang, M.-Y., Zhou, L.-L., & Piao, L.-X. (2024). Protective effect of arctiin against *Toxoplasma gondii* HSP70-induced allergic acute liver injury by disrupting the TLR4-mediated activation of cytosolic phospholipase A2 and platelet-activating factor. *International Immunopharmacology*, *126*, 111254. https://doi.org/10.1016/j.intimp.2023.111254