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An *insilico* approach to identify drug targetable protein in Visceral Leishmaniasis using GC MS extracted active components from *Aloe vera*

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ABSTRACT

Kala-azar or Visceral Leishmaniasis is caused by the parasite Leishmania donovani which affects liver, spleen and bone marrow. Aloe vera is a medicinal plant which contains active components that inhibit Leishmania donovani growth. Fresh leaves of Aloe vera were collected and dried. The active components of Aloe vera were extracted by hot percolation using soxhlet apparatus. The crude methanolic extracts of Aloe vera leaves were analyzed by GC/MS. Docking of protein by active components from Aloe vera were performed by insilico method ten components were found in Aloe vera extract. The ten structures were retrieved from PubChem. In the present study, CDC42 protein was modeled by Modeller and the active components of Aloe vera are docked with the target protein CDC42. One of the active components Sitosterol was found to show interaction in par with the natural inhibitor Secramine B. Therefore it is suggested that Sitosterol in Aloe vera inhibits the promastogote formation in Leishmania donovini and thereby Aloe vera can be used to treat visceral leishmaniasis.

Keywords: Kala-azar, Visceral leishmaniasis, CDC42 protein, *Aloe vera*, Sitosterol.

INTRODUCTION

It is well-known that, Aloe vera plant produces active components to protect themselves but recent research demonstrates that they can also protect humans against diseases [Arunkumar and Muthuselvam]. Leaf extracts of the *Aloe vera* plant directly killed two kinds of parasites, promastigotes and amastigotes. The target protein cdc42 is included in promastigote form and *Aloe vera* helps us to block the cell division which occurs in promastigote form. Leishmaniasis remains an important public health problem worldwide. This illness is endemic in several tropical and subtropical regions and countries bordering the Mediterranean Sea, affecting approximately 12 million people and threatening a total of 350 million people in 88 countries

[Helan]. Visceral leishmaniasis (VL), also known as kala-azar, black fever, and Dumdum fever [James et al]. Is the most severe form of leishmaniasis. Leishmaniasis is a disease caused by protozoan parasites of the *Leishmania* genus. After malaria, this disease is the second-largest parasitic killer in the world. It is responsible for an estimated 500,000 cases each year worldwide [Desjeux]. This parasite migrates to the internal organs such as liver, spleen (hence '*visceral*'), bone marrow and causes fever, weight loss, mucosal ulcers, fatigue, anemia and substantial swelling of the liver and spleen, if untreated result in the death of the host. Leishmaniasis is caused by protozoa of the genus *Leishmania* and transmitted to humans by sandflies. Leishmanial infection has a wide spectrum of manifestations, including asymptomatic infection, cutaneous leishmaniasis, mucous leishmaniasis and visceral leishmaniasis (VL; also known as "kala-azar") [Murray et al , Kafetzis and Maltezou]. VL is the most severe form of leishmaniasis. VL typically manifests 2-8 months after infection with intermittent fever, pallor, massive hepatosplenomegaly, weight loss and progressive deterioration of the host [Chappuis *et al*., Maltezou *et al*]. Epistaxis, gingival hemorrhage, abdominal distension, edema and ascites may develop at late stages. Untreated VL is almost always lethal [Raguenaud *et al*, Minodier *et al*.,].This paper clearly represents that the automated docking was performed by comparing ten natural compounds analysed by GC/MS. They are Tridecanoic acid, methyl ester, Oleic Acid, 9,12,15- Octadecatrienoic acid methyl ester, (ZZZ), Oxalic acid, allyl hexadecyl ester, Didodecyl phgthalate, Squalene, Octadecane, 2-methyl, Vitamin E, Sitosterol and Lupeol. CDC42 were used for target proteins. The Cdc42 is included in microarray expressed genes, which is included in MAPK signaling pathway. The Rho family of small GTPases, including Rho, Rac and Cdc42, has emerged as main regulators of the cytoskeleton [Etienne-Manneville and Hall, Hall]. This study also helped to identify the formula and structure of biomolecules which can be used as drugs.

MATERIALS AND METHODS

Collection and processing of plant

Fresh leaves of *Aloe vera* were collected from Alundurai, Coimbatore. Plant leaves were cleaned with deionized water and dried at shade for a week. The dried plant samples were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50-150mm.

Preparation of leaf extract

About 25 grams of dried leaf powder samples were extracted with 200ml of the solvent in the temperature slightly above their boiling points *viz*, ethanol (78°c), by hot percolation using Soxhlet apparatus. The extractions were carried out for 7-8 hrs. Then the extracts were transferred into a beaker and the solvents were evaporated. The waxy residues obtained were up to 5ml. These crude extracts were stored in small glass screw cap tubes and were used for further analysis [Solomon *et al*].

Analysis of active components using GC/MS

Gas chromatography-mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GC/MS can provide meaningful information for components that are volatile, non-ionic and thermally stable and have relatively low molecular weight.

The crude methanolic extracts of *Aloe vera* leaves were analyzed by GC/MS. GC analysis were performed using a Hewlett Packard gas chromatograph (model 6890) equipped with a flame ionization detector and injector MS transfer line temperature of 230°C respectively. A fused silica capillary column HP-InnoWax (30 in x 0.25 mm, film thickness 0.25 (mμ) was used. The oven temperature was held at 50 °C for 5 minutes holding time and the temperature was raised, from 50-230°C at a rate of 2 °C /min. The carrier gas Helium was at a flow rate of 22 cm/sec. One milliliter of extract mixed with methanol (80%), at a split ratio of 1:30 was injected. GC/MS analyses were carried out on a Agilent Technologies Network mass spectrometer (model 5973) coupled to H.P. gas chromatograph (model 6890) equipped with NBS 75K Library Software database. The capillary column and GC conditions were as described above. Mass spectra were recorded at 70 eV /200°C. The scanning rate of 1 scan/sec and the run time was 90 minutes. Compound identification was accomplished by comparing the GC relative retention times and mass spectra to those of authentic substances analyzed under the same conditions, by their retention indices (RI) and by comparison to reference components.

RESULTS

The GC/MS of the ethanolic *Aloe vera* leaves extract showed the presence of mainly essential oils such as steroid compounds. The components identified at the specific time and RT value from the GC/MS. The components identified using GS/MS are Tridecanoic acid, methyl ester, Oleic Acid, 9,12,15- Octadecatrienoic acid methyl ester, (ZZZ), Oxalic acid, allyl hexadecyl ester, Didodecyl phgthalate, Squalene, Octadecane, 2-methyl, Vitamin E, Sitosterol, Lupeol in different RT values and the peak at which these were identified. The CDC42 checked with DEG (Database for Essential Genes) is used to check the essentiality of genes. Cdc42 was found to be non essential in *Homo sapiens*. The components were docked with CDC42 protein sequence was taken from Swiss-prot and the three dimension model was created using MODELLER software and validated by Ramachandran plot. All the 10 active components from *Aloe vera* were docked with modelled CDC42 protein using Molegro virtual docking software. Out of 10, only 5 were found to dock with the protein. Secramine B a natural inhibitor of CDC42 protein was also considered for comparisons. Amino acids, bond lengths and hydrogen bond interactions are checked for the docked structures. More the number of hydrogen bond interactions high the quality of docking. The 10 GC-MS compounds could be retrieved from PubChem. These compounds are taken as ligand for docking CDC42 protein. The number of interactions with the target protein CDC42 ar eshown in table-1. List of interaction bonds, bond length and active sites are shown in Table 2. Sitosterol was found to have the highest interaction (6 interactions) with the target cdc42. This was in par with natural inhibitor Secramine B which showed 7 interactions. So it is suggested that *Aloe vera* contain Sitosterol which inhibit the promastigote form of *Leishmania donovani* that cause visceral leishmaniasis.

DISCUSSION

The literature survey was carried out to study the pathogenesis of disease involving both *leishmania donovani* and *Homo sapiens* (human). The active components present in *Aloe vera* responsible for treating visceral leishmaniasis was studied extensively. Microarray data analysis was done to study expression profile of visceral leishmaniasis genes [Kumar, *et al*]. Literature search was done to find out the microarray expressed genes in *Leishmania*

donovani. The microarray results were then analysed to find the novel gene in human who is included in Leishmania. CDC42 is the novel gene which is present in the human, involved in MAPK signaling pathway.

Database for Essential Genes (DEG) is used to find out the essentiality of the genes [Lerm *et al*]. As CDC42 is the target gene, it is checked with DEG for its essentiality in *Homo sapiens*, where it is found non essential. Promastigote is an early stage of Leishmania is surrounded by F-actin. This is formed with the help of CDC42. The structure of CDC42 protein has two isoforms produced by alternative splicing, both the isoforms contain 191 amino acids but their C-terminals are different. CDC42 is a small GTPase of the Rho family, which regulates signaling pathways that control diverse cellular functions including cell morphology, migration, endocytosis and cell cycle progression. The target CDC42 was analysed using the structural database, Protein Data Bank (PDB). There were 20 models already present in PDB for the target CDC42. New model was created using MODELLER software. The structure was then submitted to SAVS (Structural Analysis and Verification Server), to get Ramachandran plots. The one with the least DOPE (Discrete Optimized Protein Energy) score is considered as target [Barh and Kumar].

Aloe vera has been found to possess active components that can cure Visceral leishmaniasis [Kumar and Muthuselvam]. Active components from *Aloe vera* were extracted and characterized by GC MS. Twenty six components were found in *Aloe vera* extract. Among 26, 10 structures were retrieved from PubChem. Molegro Virtual Docker an integrated platform for predicting protein - ligand interactions. Molegro Virtual Docker handles all aspects of the docking process from preparation of the molecules to determination of the potential binding sites of the target protein, and prediction of the binding modes of the ligands [Vasudeva, *et al*]. The active components of *Aloe vera* are docked with the target protein CDC42. 5 components were found to dock with the protein. One of the active components Sitosterol was found to show interaction in par with the natural inhibitor Secramine B. Through the literature search and microarray data analysis Cdc42 gene was found to be the most important gene for the parasite. It is responsible for the cell division that occurs in the parasite. It is found to be non essential for humans but essential for the parasite, so it is considered to be novel gene [Sail and Blundell]. Therefore it is suggested that Sitosterol in *Aloe vera* inhibits the promastigote formation in *Leishmania Donovan* and thereby *Aloe vera* can be used to treat visceral leishmaniasis.

Table 1: Total number of interactions of ligands with the target protein CDC4

S. No	Molecular formula	Compound name	Number of interactions
1.	$C_{35}H_{39}N_2O_5$	SECRAMINE B (natural ligand)	7
2.	$C_{29}H_{50}O$	SITOSTEROL	6
3.	$C_{30}H_{50}$	SQUALENE	4
4.	$C_{29}H_{50}O_2$	VITAMIN-E	2
5.	$C_{14}H_{28}O_2$	TRIDECANOIC ACID	2
6.	$C_{12}H_{26}O$	1-OCTANOL, 2-BUTYL	1

Table 2: Interactions between ligands and CDC42 protein

Ligands	Amino Acids	Interaction bonds	Length	Active sites
Secramine B (Natural ligand)	Thr 17 Cys18 Lys16 Gly114 gly15	O-H-O N-H-O O-H-O O-H-N O-H-N	2.87 3.11 2.76 3.26 3.16	Leu111,val113,cys81, gly114,lys16,gly15,thr17,cys18, leu19,leu20,tyr23
Sitosterol	Cys81 Lys16 Gly15 Gly15 Thr17 Thr17	O-H-O O-H-N O-H-O O-H-N O-H-O O-H-N	3.2 3.39 3.34 2.26 3.37 2.26	Cys81,Leu55 Leu79,Lys16, Thr17,Leu19 Leu20,Tyr23 Cys18, Val 113.
Squalene	Cys81 Lys16 Tyr23 Tyr23	O-H-O O-H-N O-H-O O-H-O	3.27 2.48 3.39 2.27	Cys81,Leu55, Leu79,Lys16, Thr17,Leu19, Leu20,Tyr23
Vitamin E	Cys81 Lys16	O-H-O O-H-N	3.02 1.25	Cys81,Leu55, Leu79,Lys16, Thr17,Leu19, Leu20,Tyr23
Tridecanoic acid	Lys16 tyr23	O-H-N O-H-O	3.45 3.58	Lys16,Tyr23, Leu20,Thr17, Leu79,Cys18.
1-octanol, 2-butyl	Tyr23	O-H-O	1.16	Leu79,Leu19, Tyr23,Leu20, Thr17,Cys18

CONCLUSION

Aloe vera has been found to possess active components that can cure visceral leishmaniasis. These active components are docked with the target protein CDC42. 5 components were found to dock with the protein. One of the active component Sitosterol was found to showed interaction in par with the natural inhibitor Secramine B. Therefore it is suggested that Sitosterol in *Aloe vera* inhibits the promastogote formation in *Leishmania Donovan* and thereby *Aloe vera* can be used to treat visceral leishmaniasis.

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