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RESEARCH ARTICLE

Targeting Omc-B of *Chlamydia trachomatis* for the Blinding Infection Trachoma using Phytochemicals from *Calpurnia aurea* and *Abrus precatorius*

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ABSTRACT:
Blinding trachoma called trichiasis is the eye infection caused by the obligate bacterium *Chlamydia trachomatis*, which triggers the inflammatory process in the conjunctiva mucosa and is believed to be the prime step towards the development of scarring. Reoccurrence of infection lead the eyelids to turn inward which ultimately scar the cornea through the continuous scrape caused by eyelashes. Like leprosy, Guinea worm, lymphatic filariasis, onchocerciasis, etc, trachoma is one of the neglected tropical disease, although 540 million people are reported at risk in 55 countries and 84 million are already infected. Trachoma, being the eighth commonest blinding disease overall the world, become global burden in terms of both disability it causes and the economic costs. The World Health Organization (WHO) assembly led an alliance GET2020 (Alliance for Global Elimination of Blinding Trachoma by 2020), in order to eliminate the blinding trachoma by the year 2020, which in adoption by SAFE strategy actively participates based on four different components like surgery for trichiasis, antibiotics for infection, facial cleanliness and avail criteria to improve environmental factors to reduce transmission. Zithromax, an antibiotic is used for treating the infection, has a lot more side effects and the effect on recurrence is also uncertain. With the recent development in the field of science and technology, the present study utilizing the bioinformatics tools and software, aims at identifying a novel inhibitor from the plant source for the targets that involve in the pathogenicity of the organism.

KEYWORDS: Trachoma, Eye infection, *Chlamydia trachomatis*, OmcB, *Calpurnia aurea* and *Abrus precatorius*.

INTRODUCTION:

Chlamydia trachomatis is one among the three of Chlamydiaceae family, is an intracellular bacterium which is the causative for blinding infection called Trachoma. The origin and its evolutionary status was explained by Taylor[1]. The repeated inflammatory response due to frequent and recurring infection leads to scarring and structural damage to the eyes. The complications are of different stages as trachomatous scarring (TS), trachomatoustrichiasis (TT), corneal opacity (CO) and eventually, blindness.

The replication of *Chlamydia* organisms take place in the conjunctival epithelial cells, whereas the chlamydial antigens generated as an immune response causes inflammation with the follicles settle in the upper tarsal conjunctiva lid and further leads to scarring. The longer it persists, the severe form of scarring is observed, which on time being contraction in the scar occurs and ensue the turn in the eye lashes, "trichiasis" and finally end up in corneal scarring and blindness[2].

World Health Assembly (WHO) has recommended the "SAFE" control strategy (**S**urgery, **A**ntibiotics, **F**acial cleanliness and **E**nvironmental improvement)[3], as well as a global alliance for the elimination of blinding Trachoma 2020 (GET2020). Though, trachoma being progressively eliminated in most of the areas, it remains endemic in 53 countries including sub-Saharan Africa, Middle East Asia[4]. Treatment with the antibiotics

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"Azithromycin" or tetracycline eye ointment were effective, however azithromycin has better control than tetracycline. Moreover, the Pfizer Inc. company has donated the Zithromax (azithromycin) acted as an additional effort to reduce the disease burden[5].

The organism, *Chlamydia trachomatis* possess biphasic life cycle, named as elemental body (EB) and reticular body (RB), where the former is highly infectious (neither replicate nor divide) and the latter is non-infectious however after penetration the RB performs replication[6]. The EB is environmentally stable, metabolically dormant and comparatively small than the RB which is environmentally labile. The following are the important targets to be considered, Lipopolysaccharides, Outer Membrane proteins like MOMP (Major Outer Membrane Protein), PMP (Polymorphic Membrane Proteins), COMC (Complex of Outer Membrane Proteins rich in Cysteine residues) and HSP proteins, Type III secretion system and cytotoxin. In the present study, OmcB protein was selected as target since, it is abundant in the complex of proteins which involve in the adhesion of EB to the eukaryotic cell[6]. Moreover, OmcB is highly conserved and also contribute in the cell wall rigidity and osmotic stability of EB[7], as well as recognized as an immunodominant antigen[8]. It is reported that OmcB is located in the inner surface of the outer membrane and Gervassi[9] suggested that C-terminal region of the protein enter the host cell cytosol. OmcB contains 24 cysteine residues and has a molecular mass of 60 kDa and it plays a significant role during intracellular chlamydial infection[10]. Moreover, it is observed that most of the antibiotics target the protein and nucleic acid synthesis of reticulate bodies than the metabolically inactive chlamydial EBs[11]. Therefore, the perspective of targeting OmcB would be worth in the eradication of infection as well as in identifying the novel compounds targeting the EBs of *C. trachomatis*.

The drug resistance for the conventional antibiotic treatments is the most important problem facing in the eradication of trachoma infection, therefore, the need of the hour is to find novel as well as non-toxic compounds[12]. Vuorela[13] has reported the importance of natural products in the process of finding new drug candidates, who emphasized that in the last 20 years of duration, 50% of drugs introduced in the market are either directly or indirectly derived from small biogenic molecules. In the race of drug designing, novel molecular diversity (NMD), bioassay-guided fractionation, High Throughput Screening (HTS), combinatorial chemistry, Molecular docking analysis and several bioinformatics tools, software are apparently crucial for serving the purpose. The present study utilizes the molecular docking study to analyze the compounds from the plants *Calpurnia aurea* and *Abrus precatorius*. There are more than 1,00,000 secondary metabolites known, that too, from a small percentage of plant species, therefore, more number of plants has to be screened and explored for the novel drug discovery. It is well known that isolating and identifying the pure constituents from the intact plant is long, however, the compounds those are already been reported in the plant can be bioinformatics has made the process easier and faster.

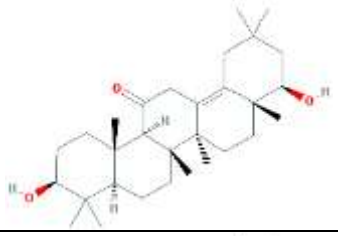
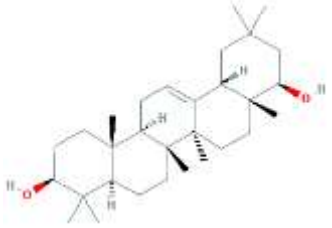
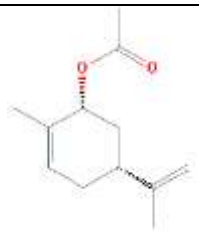
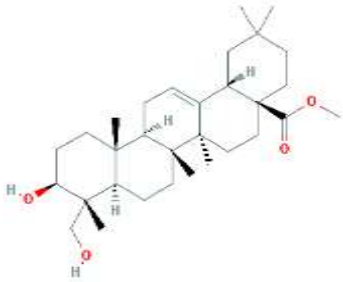
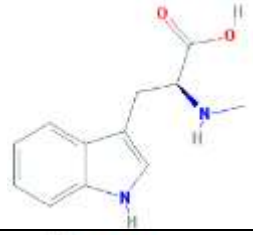
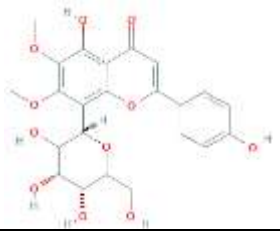
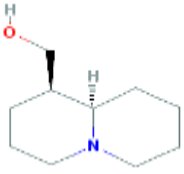
MATERIALS AND METHODS:

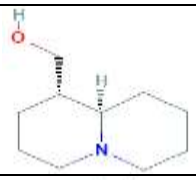
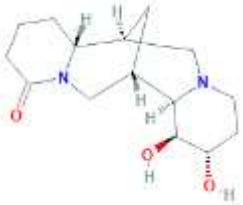
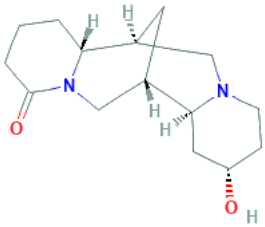
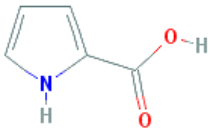
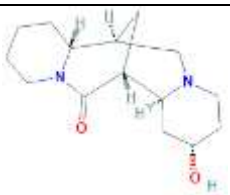
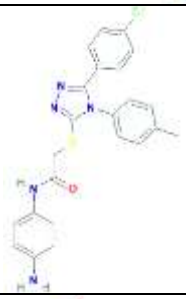
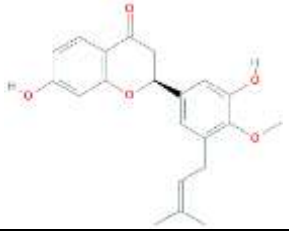
The protein OmcB of *Chlamydia trachomatis* is a cysteine-rich periplasmic protein of amino acid length 547 and has a mass of 58,782 Da. The sequence was retrieved from the UniProt ID: P0DJI2, since the 3D structure is unavailable in PDB, the sequence was given below was subjected for modeling.

>sp|P0DJI2|OMCB_CHLTH Large cysteine-rich periplasmic protein OmcB, serovars L1/L3 OS=Chlamydia trachomatis GN=omcB PE=2 SV=1
MNKLIRRAVTIFAVTSVASLFASGVLETSMAEFIST
NVISLADTKAKDNTSHKSKKARKN
HSKETPVNRKKVAPVHESKATGPKQDSCFGRMYT
VKVNDDRNVEITQAVPKYATVGSPYP
VEITATGKRDCVDVIITQQLPCEAEFVRSDPATTPT
ADGKLVWKIDRLGQGEKSKITVWV
KPLKEGCCFTAATVCACPEIRSVTKCGQPAICVKQ
EGPENACLRCPVVYKINVVNQGTAT
ARNVVVENPVPDSYAHSSGQRVLTFTLGDMQPGE
HRTITVEFCPLKRGRATNIAMVSYCG
GHKNTASVTTVINEPCVQVSIAGADWSYVCKPVE
YVISVSNPGDLVLRDVVVKDTLSPGV
TVLEAAGAQISCNKVVWTVKELNPGESLQYKVLV
RAQTPGQFTNNVVVKSCSDCGTCTSC
AEATTYWKGVAATHMCVVDTCDPVCVGENTVY
RICVTNRGSAEDTNVSLMLKFSKELQPV
SFSGPTKGTITGNTVVFDSLPRLGSKETVEFSVTLK
AVSAGDARGEAILSSDTLTVPVSD
TENTHIY

In order to identify the template, the BLASTP was carried out, however, the sequence had no significant similarity found and therefore modeling was performed using I-tasser (http://zhanglab.ccmb.med.umich.edu/I-TASSER/). I-tasser (Iterative Threading ASSEmbly Refinement), is a threading based modeling online server, built and maintained by Yang Zhang Lab at the University of Michigan, Ann Arbor. The active site pocket was determined using the Lig Site server and followed by docking analysis using ArgusLab. The compounds of the plants *Calpurnia aurea* and *Abrus precatorius* were retrieved from PubChem database, a small molecule database maintained by National Center for Biotechnology Information (NCBI). The retrieved molecules were given in the table 1.

TABLE 1: SMALL MOLECULES FROM ETHNOMEDICINAL PLANTS REPORTED FOR TREATING LEPROSY

S. No.	Compound Name	PubChem ID	Molecular Formula	2D Structure
1	Abrisapogenol	21594179	$C_{30}H_{48}O_3$	
2	Sophoradiol	9846221	$C_{30}H_{50}O_2$	
3	22-o-acetate	102024	$C_{12}H_{18}O_2$	
4	Hederagenin methyl ester	11752118	$C_{31}H_{50}O_4$	
5	Abrine	160511	$C_{12}H_{14}N_2O_2$	
6	Abrusin	44258417	$C_{23}H_{24}O_{11}$	
7	Lupinine	91461	$C_{10}H_{19}NO$	

8	Epilupinine	92767	$C_{10}H_{19}NO$	
9	Calpurmenine	15939844	$C_{15}H_{24}N_2O_3$	
10	13-hydroxylupanine	73404	$C_{15}H_{24}N_2O_2$	
11	Pyrrolecarboxylic acid	12473	$C_5H_5NO_2$	
12	Virgiline	12444870	$C_{15}H_{24}N_2O_2$	
13	Erylatissin A	1173935	$C_{23}H_{20}ClN_5OS$	
14	Erylatissin C	11382659	$C_{21}H_{22}O_5$	

RESULTS AND DISCUSSION:

The sequence for the protein OmcB was retrieved from UniProt (ID-P0DJI2) and the 3D structure model was predicted based upon the structural analogs 4UIC, 3CU7, 3PVM, 4BXS, 2PN5, 4I43, 3FFZ, 5D06, 5A22 and 3KM9 which were determined using the threading programs like Muster, FFAS-3D, SPARKS-X, HHSearch2, HHSearch1, Neff-PPAS, HHSearch, pGen Threader, wdPPAS and cdPPAS. Five models were generated, among the first model was selected for further study which has the confidence score of - 1.94, estimated TM-score and RMSD were 0.48±0.15 and 12.2±4.4Å,

respectively. Each model was quantitatively measured by the C.score, which is calculated based on the significance of threading template alignments and the convergence parameters of the structure assembly simulation (http://zhanglab.ccmb.med.umich.edu/I-TASSER/about.html). The C.score falls in the range of -5 to 2, where higher the value higher the confidence and vice versa. TM-score and RMSD are estimated based on C-score and protein length following the correlation observed between these qualities (http://zhanglab.ccmb.med.umich.edu/I-TASSER/about.html). The C.score for the rest of the models were -2.22, -2.65,-3.10 and -3.22, respectively. The active sites predicted were THR204, LYS205, CYS206, GLY207, GLN208, PRO209, ARG289, THR291, SER371, ILE454 and ASN458. The 3D structure representing the active site residues was shown in the figure 1.

The compounds Abrisapogenol, Sophoradiol, 22-o-acetate, Hederagenin methyl ester, Abrine, Abrusin, Lupinine, Epilupinine, Calpurmenine, 13-hydroxylupanine, Pyrrolecarboxylic acid, Virgiline, Erylatissin A and Erylatissin C from the plants were docked with the OmcB protein.

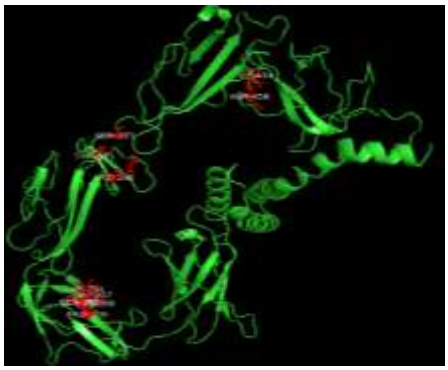


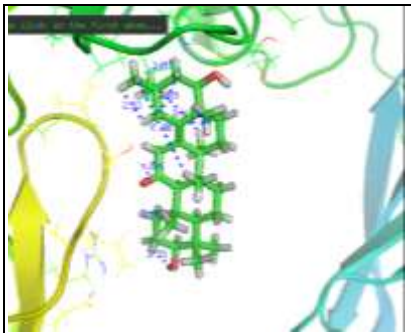
Fig. 1: 3d Structure of OMCB protein showing active sites in stick model (red color)

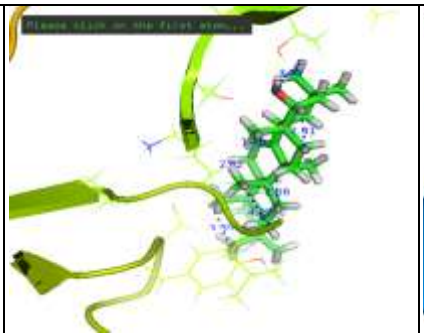
The table 2 indicates the docking score, number of hydrogen bonds formed, interacting residues and the length of the bonds (Å) for each plant molecule with the OmcB. The interactions were also shown for each plant compounds (Fig. 2).

TABLE 2: DOCKING ANALYSIS FOR THE PLANT COMPOUNDS WITH OMCB PROTEIN

S. No.	Name of the compound	Dock score (Kcal/Mol)	No. of. Hydrogen bonds	Interaction residues	Bond length (Å)
1	Abrisapogenol	-7.95	9	ILE 200 ILE321 GLN208 ILE321 SER320 MET270 ALA322 ILE200 ILE200	2.65 3.47 2.53 2.75 3.03 2.65 2.91 1.76 6.38
2	Sophoradiol	-8.18	7	ILE278 TYR298 PRO284 LEU285 TYR298 LEU286 ARG276	1.76 4.29 2.41 2.66 3.39 2.52 3.55
3	22-o-acetate	-6.94	6	LEU140 PRO141 LEU140 PRO141 PRO141 LEU140	3.47 4.22 2.58 3.22 4.56 2.69
4	Hederagenin methyl ester	-7.19	6	LEU140 GLU143 GLU143 GLN139 LEU140 CVS142	1.72 2.38 1.75 2.47 2.52 3.10
5	Abrine	-8.70	6	THR156 PRO155 THR156 ALA125 THR156 ILE123	6.37 2.50 2.39 2.32 2.47 6.73
6	Abrusin	-7.34	6	CYS299 LYS286 TYR298	2.46 5.21 3.85

				PRO284 LEU285 LYS286	8.98 7.78 2.45
7	Lupinine	-6.22	7	GLN208 VAL203 GLN204 THR204 VAL203 SER320 ILE200	2.43 2.53 1.96 1.77 2.38 3.04 2.50
8	Epilupinine	-6.44	6	GLN107 GLN107 LYS84 GLN107 LEU168 GLN107	2.35 2.47 2.67 5.41 3.79 2.65
9	Calpurmenine	-6.54	5	ARG276 THR277 THR277 ILE278 ARG276	2.55 2.47 3.53 2.35 1.77
10	13-hydroxylupanine	-6.66	7	ILE200 ILE200 VAL203 PRO198 GLN208 VAL203 SER202	2.43 2.47 5.68 4.72 2.52 2.42 2.44
11	Pyrrolecarboxylic acid	-5.68	3	PRO209 VAL 227 LYS 205	4.23 2.50 5.50
12	Virgiline	-6.29	6	TYR298 TYR298 LEU285 LEU286 TYR298 LEU286	1.76 4.90 1.95 2.47 2.50 2.49
13	Erylatissin A	-7.26	6	LEU168 LEU168 GLU143 ALA108 LYS84 LYS84	3.42 5.33 2.58 1.78 4.31 2.57
14	Erylatissin C	-6.67	5	ARG276 ILE32 LEU140 LEU140 ALA322	1.77 2.35 2.48 2.85 2.70


Abrisapogenol


Sophoradiol


22-o-acetate

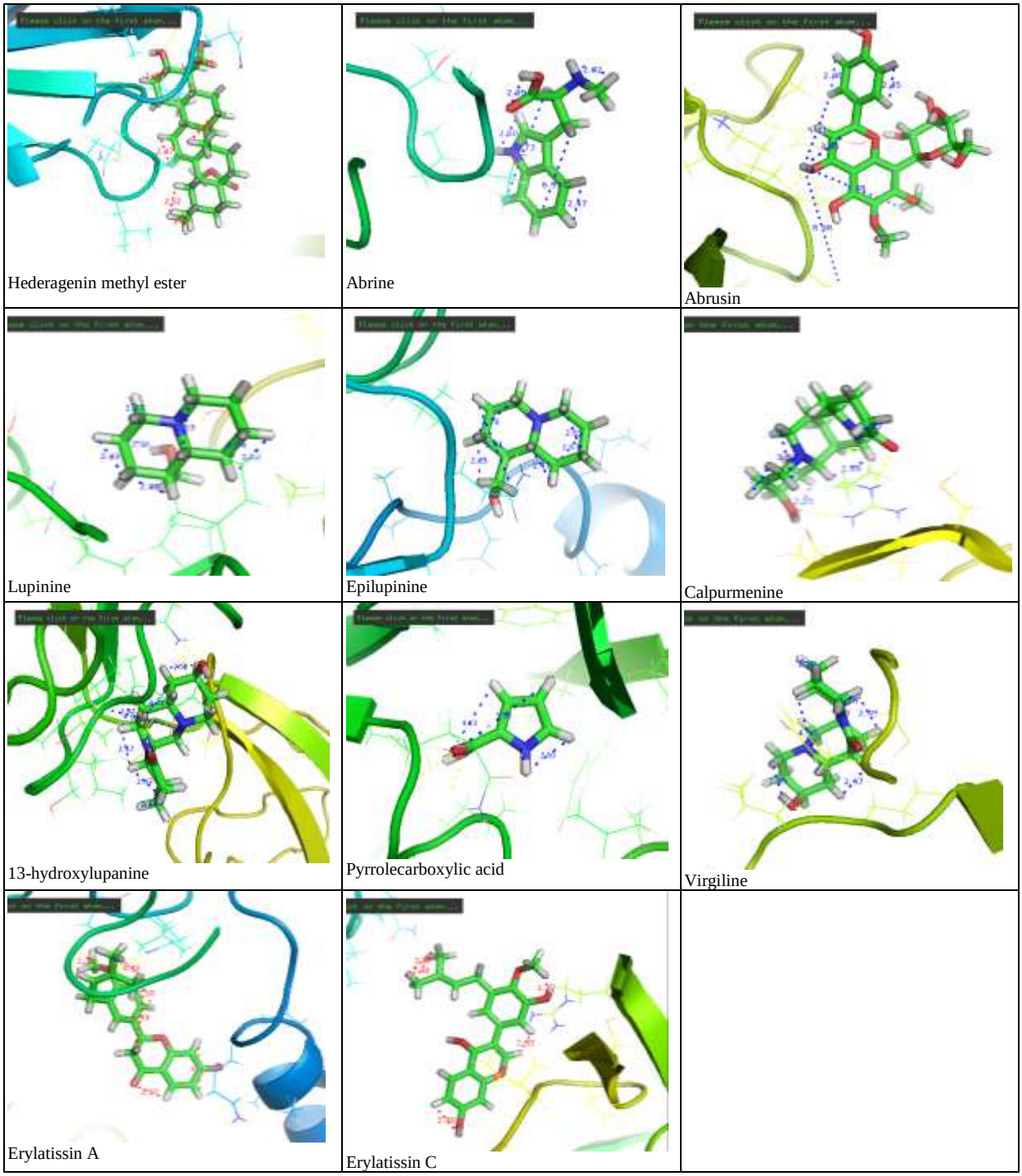


FIG. 2: INTERACTIONS OF PLANT COMPOUNDS WITH OMCB PROTEIN

Engels[14] indicated the measures for eradicating trachoma in detail and recent analysis on phytochemicals showed the potency of berberine to act against ocular trachoma infections[15]. The contact-dependent secretion protein (CdsD) was targeted and docking studies with the secondary metabolites of *Tribulus terrestris*, *Azadirachta indica*, *Ziziphus mucronata*, *Erythrina indica* and *Jatropha curcas* indicated the efficiency of plant compounds to exhibit significant inhibitory potential[16]. Rautiainen[17] proved that intake of ascorbic acid and vitamin C as a routine diet has lowered the risk of cataract, therefore would possibly a cure for trachoma infection. Recently, the prevalence of trachomatis through sexual transmission is also reported as high[18] and studies on type-III secreted protein termed Tarp (Translocated actin-recruiting phosphoprotein) showed

that this protein remodels the host cell actin cytoskeleton and let the elemental bodies to enter[19]. In such manner, the proteins especially active in the elemental bodies was targeted and identification of significant inhibitors for those would be beneficial.

Similar to the exhibiting efficient of plant compounds, the present study was concluded that abris apogenol and sophoradiol to possess significant docking score and binding potency. The future studies would be designed in evaluating the plants *in vitro* and isolation of bioactive compounds from it.

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