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Research Article
Drug Designing to Combat MDR Bacteria Using Potential Bioactive Compounds from Medicinal Plant

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Abstract

Background and Objective: Antibiotic resistance is becoming a critical concern for public health that has accelerated the search for new antimicrobial compounds from the natural resources. This study aim to evaluate the antibacterial activities of plant extract (Aloe Vera and Garlic) on multi-drug resistant (MDR) bacteria isolated from polluted water samples of clinical importance by molecular docking and its respective relationship corresponding to the multi-drug efflux pump PBD ID 5YIL protein of *E. coli*. **Methodology:** Aloe vera extracted with methanol PBS shown maximum inhibition (28 mm) against MDR strain of *Escherichia coli* using agar well diffusion. The natural compounds of plant extract responsible for antibacterial activity were screened on the basis of using computational approaches such as molecular docking based validation analysis. The 22 compounds were selected as legend and multi-drug efflux pump (MDEP) transport protein (5 YIL) of *E. coli* which plays an important role in actively transport of many antibiotics out of the cell was selected as receptors for molecular docking analysis. **Results:** *In silico* analysis showed that Aloin compound has strong binding affinity for selected target protein receptors. Hence, results of study showed the ability of Aloin to bind with active sites of MDEP protein targets to disrupt the mechanism of MDR in bacterial cell and allow the drug towards the treatment of diseases. **Conclusion:** *In silico* and *in vitro* methods help us to explore and investigate anti-MDR properties of selected medicinal plants extracts and their overall 22 phytoconstituents.

Key words: Anti-MDR screening of plant extract, multidrug resistance, binding affinity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The launch of antibiotics for the treatment of infectious diseases was the hallmarks of the 20th century medicine. However, shortly after their introduction into the clinical practice, the first bacteria showing antibiotic resistance were also found[1,2]. Since then, the development of new antibiotics has been accompanied due to the steady increase of antibiotic-resistant bacterial strains and the diversity of mechanisms used by bacteria to surpass the lethal effect of these compounds. Antibiotics are used widely on a day to day basis to control the microbial growth. Thus, it becomes quite alarming to raise the effect of its persistence in the environment[3]. Not only overuse but exposure to sub-lethal concentrations of antibiotics associated with development of antibacterial resistance[4]. In the present deadly scenario of this phenomenon, approximately 30% deaths were accompanied with bacterial infection in this era[5]. Drug resistant infectious microbes have become an important public health concern warranting organizations in public and private sectors worldwide to work together[6].

Mainly, antibiotics producing microbes interrupt the microbial metabolism by various mechanisms[7]. Over the period, synthetic derivatives of naturally produced antibiotics were overused and fueled the adaptation in bacteria to become drug resistant[8]. It has been seen that not only antibiotics but biocides and dyes commonly used in hospital and laboratory are also responsible for over expression of efflux pump system of bacteria and make it resistant to antibiotics[9,10]. Bacteria have developed a variety of efficient resistance mechanisms such as efflux pumps, secreted proteases and alterations of the bacterial cell surface composition against antimicrobial peptides (AMP)[11,12]. These AMP are related to resistance mechanisms, modification of xenobiotics or drug transport, stress response, porins, outer membrane proteins, transporters and secretion, cell wall-related proteins, lipoproteins and DNA or plasmid-related proteins[13].

In recent years, various researchers are looking to discover new bioactive compounds from plant origin with the hope to control MDR micro-organisms. Aloe vera and garlic are already well proved for its anti-inflammatory, anti-microbial, anti-diabetic and immune-boosting properties and regularly used over the periods[14]. Aloe vera extract is boon against the hazardous effect of multidrug-resistance bacteria isolated from polluted water without any side effect like available anti-microbial chemical drugs[15]. Natural products remain the most propitious source of novel antibiotics[16]. Particular attention was paid to test the therapeutic potential of Aloe vera gel and garlic extract against MDR bacteria and majorly involved AcrAB efflux pump in resistance[17]. As the electronegative entrance is widely conserved in the TolC family, it may be a useful target for the development of inhibitors against multidrug-resistant pathogenic bacteria[18].

The frequent cause of multidrug resistant in gram-negative bacteria is the overexpression of RND multidrug pump such as AcrB transporter[19] which make a major threat for public health nowadays. In another side, there are very few compounds that are successfully crystallized with AcrB. So, *in silico* approaches are emerged as a boon to enlighten the interaction between the ligand and protein[20]. Molecular docking is one of the most frequently used methods for the prediction ability in structure-based drug designing with a substantial degree of accuracy, the confirmation of small-molecule ligands within the appropriate target binding site[21]. Molecular docking and structure-based drug design strategies accomplishes computer simulations and thus suggested direct and stable tip to tip interaction between the outer membrane channel TolC and the isolated docking domain of the multidrug RND efflux transporter AcrB[22].

Hence, the present study was focused on the *in silico* and *in vitro* analysis of two medicinal plants (Aloe vera and garlic) extracts against selected MDR microbial strains. Docking study was performed to analyze and identify the interaction of an obtained bioactive compound of selected plants (served as ligands) with the MDR pathogens inhibitory targets of transporter proteins.

MATERIALS AND METHODS

Media and chemicals: Minimal broth and nutrient agar media (Hi-media, India; final pH 7.2±0.2 at 25°C), amoxicillin, ciprofloxacin ofloxacin, levofloxacin, azithromycin and cefixime of 500 mg procured from the department for testing of multidrug resistivity. All other chemicals were of highest purity (≥99%) ethanol, methanol, DMSO, conc. H_2SO_4, chloroform, $NaNO_2$, Molisch's reagent, dilute ammonia solution, glacial acetic acid, ethanolic ferric chloride solution etc. The rest of the chemicals utilized in this study were of analytical reagent grade and were procured from reputed suppliers within India.

***In silico* molecular docking analysis:** Molecular docking is popular for screening active compounds from medicinal plants for inhibition against pharmacological receptors[23-25]. To have a better understanding about the inhibitory mechanism as well as the mode of interactions of the phytochemical compounds of the crude extract, docking analysis was

accomplished using the DruLito and MVD tools. Drug target pathway has been accomplished by the *E. coli* transporter protein AcrB to be targeted with plant bioactive compounds.

Selection and preparation of natural compounds: Compounds of aloe vera and garlic with reported medicinal properties are taken from zinc database (Table S1-S3)[26,27]. Molecular identification of the compounds also searched from Chebi for further detailed validation[28]. Toxicity and drug-likeness property of the selected compounds has been checked with the DruLiToon the basis of Lipinski rule (Table S4, S5)[29]. The compounds fit to Lipinski rule are further used as a ligand in sdf format for docking. In this study, the 22 selected constituent compounds having traditional health importance is summarized in tabulated form (Table S1).

Protein preparation: The MDR transporter protein AcrB of *E. coli* is selected as a target protein for the docking with the prepared natural compounds of medicinal plants. X-ray structure with 3 A resolution of the protein bacterial multidrug exporter AcrB PDB ID 5YIL[19] retrieved from RCSB PDB. The protein is taken in pdb[30]. The downloaded pdb structure of the selected protein was viewed and prepared by removing water molecules and extra ligands by Pymol version 1.7.4.5. Pymol is software for molecular visualization and producing high quality of 3D images of biological molecules[31].

Binding site prediction: For the identification of binding site, pre-edited protein 5YIL is uploaded in COACH meta server[32]. For the better selection of ligand and target BioLip database is used for high quality biologically relevant protein-ligand binding interaction[33]. The COACH result was downloaded and again protein was post-edited and prepared for docking.

Molecular docking: Initially, BioLip database was used for the confirmation about the protein function and ligand check and the minimum identified cavity in the AcrB transporter protein in hosting loop region for potential binding site with ligands was set for each ligand were docked with protein by setting up grid one by one after several docking runs. Visualization of results by analyzing drug interaction sites by using MVD. Prepared ligand and protein was docked with Molegro Virtual Docker (MVD) (Version 2013.6.0.0) due to its higher docking accuracy which 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[34-36]. Finally, results of docking were compared for the better tendency of inhibition on the basis of binding affinity, MolDock score and re-ranking. The MolDock scoring function calculated by involved hydrogen bonding and charges during protein ligand interaction. The docking scoring function, E score defined the energy and re-rank-score provides an estimate for interactions.

Isolation of MDR bacterial cultures: The water sample was collected on the basis of hazardous properties of polluted water and analyzed using standard microbiological techniques for the isolation and identification of bacterial pathogens. From the initial minimal media, pure cultures was obtained and these obtained selective colonies were grown on nutrient agar plated and tested against different antibiotics namely, amoxicillin, ciprofloxacin, ofloxacin, levofloxacin, azithromycin and cefixime using agar well diffusion method at 37°C for 24 h. Their resistivity or sensitivity against used anti-biotics determines multidrug resistant properties of isolated pathogens. Further, isolates were characterized and analyzed based on its cultural, morphological, biochemical methods according to the guideline of the Bergey's manual of determinative bacteriology[37]. The bactericidal activities of plant extract were carried out against Gram-positive MDR *Staphylococcus aureus* (ATCC 25923), Gram-negative MDR *Escherichia coli* (ATCC 25922). All the standard bacterial strains were obtained from MTCC, Chandigarh and preserved culture extracted from MRD Life Sciences lab, Lucknow. Active cultures for experiment were prepared by transferring A_{600} = 1 OD cell of culture from the stock culture to eppendorf tubes which contained 1 mL of nutrient broth (NB).

Table S1: The 22 constituents of the 2 medicinal herbs

Medicinal herbs	No. of constituents	Traditional indications	Constituents name
Aloe vera	6	Purgative (laxative) arthritis, sinusitis, conjunctivitis, ophthalmia, eye ailments, treatment of wounds, sores, burns, venereal ulcers, herpes and shingles hypertension and stress, infertility, impotence	Alliase or naproxen, aloin, barbaloin, haloprogin or alprogen, postaglandins, salicylic acid in men, blood purification energy booster stomach cleanser back pain
Garlic	16	Oral hygiene, diabetes, maintenance fungal infection, glycaemia, gastritis, stomach cancer, typhoid, whooping cough, pneumonia, meningitis, atherosclerosis, hypertension	Allicin, alliin, allixin, allyl methyl disulfide, allyl methyl tri sulfide, allyl sulfide, campesterol, cycloallin, dads, das, dats, isoalliin, methiin, s allyl cysteine, s methyl cysteine, se methyl selenocysteine

Table S2: Physical representation of selected natural compounds from garlic

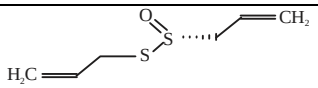
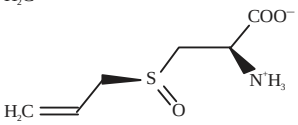
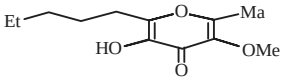
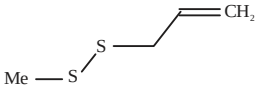
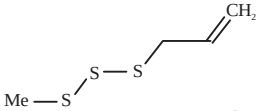
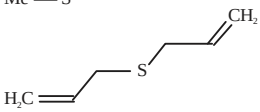
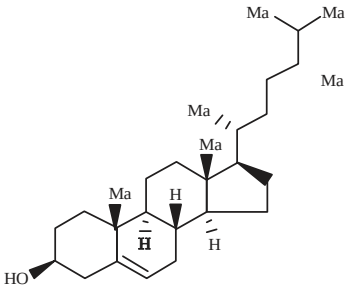
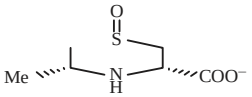
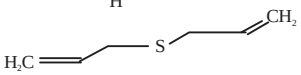
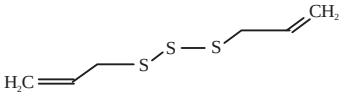
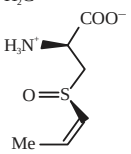
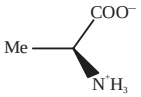
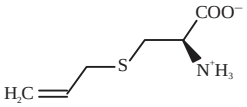
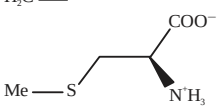
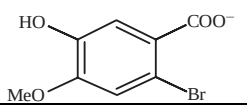
Zinc ID	Popular name	Smiles	Structure
01530846	Allicin	C=CCS[S@](=O)CC=C	
01531038	Alliin	C=CC[S@](=O)C[C@@H](C(=O)[O-])[NH3+]	
01533462	Allixin	CCCCCc1c(c(=O)c(c(o1)C)OC)O	
01531088	Allyl methyl disulfide	CSSCC=C	
01850544	Allyl methyl trisulfide	CSSSCC=C	
01531083	Allyl sulfide	C=CCSCC=C	
04095721	Campesterol	C[C@H](CC[C@@H](C)C(C)C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CC=C4[C@@]3(CC[C@@H](C4)O)C)C	
13413599	cyclo alliin	C[C@@H]1C[S@@](=O)C[C@@H](N1)C(=O)[O-]	
1531083	Das	C=CCSCC=C	
1633229	Dats	C=CCSSSCC=C	
14680219	isoalliin	C/C=C\[S@@](=O)C[C@H](C(=O)[O-])[NH3+]	
4658553	methionine	C[C@@H](C(=O)[O-])[NH3+]	
02517162	S-allyl cysteine	C=CCSC[C@@H](C(=O)[O-])[NH3+]	
3861771	S-methyl cysteine	CSC[C@@H](C(=O)[O-])[NH3+]	
64033873	Se-methyl selenocysteine	COc1cc(c(cc1O)C(=O)[O-])Br	

Table S3: Physical representation of selected natural compounds from aloe vera

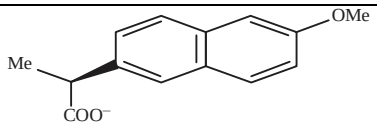
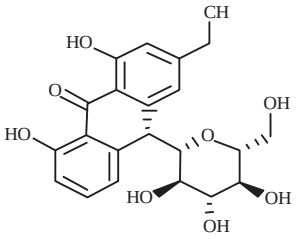
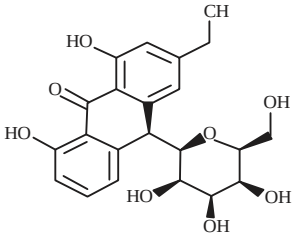
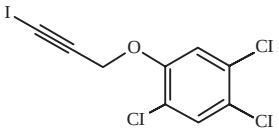
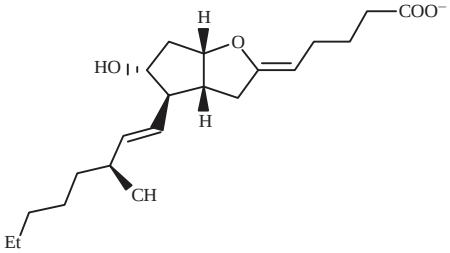
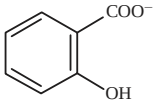
Zinc ID	Popular name	Smiles	Structure
105216	Aliiase/naproxen	C[C@@H](c1ccc2cc(ccc2c1)OC)C(=O)[O-]	
04214775	Aloin	c1cc2c(c(c1)O)C(=O)c3c(cc(cc3O)CO)[C@@H]2 [C@H]4[C@@H]([C@H]([C@@H]([C@H](O4) CO)O)O)O	
4523265	Barbalion	c1cc2c(c(c1)O)C(=O)c3c(cc(cc3O)CO)[C@@H]2 [C@H]4[C@@H]([C@H]([C@@H]([C@@H](O4) CO)O)O)O	
01530649	Alprogen/Haloprogin	c1c(c(cc(c1Cl)Cl)Cl)OCC#Cl	
3813078	Prostaglandin	CCCCC[C@@H](/C=C/[C@H]1[C@@H](C[C@ H]2[C@@H]1C/C(=C/CCCC(=O)[O-])/O2)O)O	
00001554	Salicylic acid	c1ccc(c(c1)C(=O)[O-])O	

Table S4: Drug likeness properties of selected natural compounds from garlic

Zinc ID	Name	Atoms present	Mol.W.	LogP	HBA	HBD	MR
1530846	Allicin	19	162.02	0.237	1	0	46.68
1531038	alliin	22	177.05	-1.671	3	1	37.0
1533462	Allixin	34	226.12	3.343	4	1	59.32
1531088	Allyl methyl disulphide	14	120.01	1.916	0	0	36.14
1850544	Allyl methyl trisulphide	15	151.98	2.171	0	0	43.94
01531083	Allyl sulphide	17	114.05	2.011	0	0	37.51
4095721	Compesterol	77	400.37	11.026	1	1	120.8
13413599	Cyclacillin	21	176.04	-1.98	4	1	39.45
1531082	Dads	18	146.02	2.266	0	0	45.31
1531083	Das	17	114.05	2.011	0	0	37.51
1633229	Dats	19	177.99	2.521	0	0	53.1
14680219	Isoalliin	22	177.05	-1.35	3	1	37.43
4658553	Methiin	13	89.05	-0.605	2	1	14.16
02517162	S- allyl cysteine	21	161.05	0.358	2	1	35.63
3861771	S- methyl cysteine	17	135.04	0.008	2	1	26.47
64033873	Se- methyl selenocysteine	19	244.94	0.78	4	1	50.46

Table S5: Drug likeness properties of selected natural compounds from aloe vera

Zinc ID	Names	Atoms present	Mol.W.	LogP	HBA	HBD	MR
105216	Aliiase/naproxen	30	229.09	1.313	3	0	68.87
04214775	Aloin	52	418.13	-2.137	9	7	109.0
4523265	Barbaloin	52	418.13	-2.137	9	7	109.0
01530649	Haloprogin/alprogen	18	359.84	4.285	1	0	72.34
3813078	Prostaglandins	56	351.22	2.495	5	2	84.69
00001554	Salicylic acid	15	137.02	-0.002	3	1	36.28

Preparation of the plant extract

Aloe vera extract: The fresh leaves of THE aloe vera were collected and rinsed with sterile distilled water and cut at base using sterile knife. Aloe vera gel was scooped out from fresh leaves with removed spikes without the fibers and dried in shade at the 32°C. Thereafter, the gel was extracted with a sample to solution ratio of 1:10 using ethanol (70%) and methanol (80%) at room temperature for 72 h with occasional stirring. The extracted solvent were filtered and dried in an oven at the temperature of 40°C. The aliquots were mixed with 5 mL DMSO and 5 mL PBS separately for methanol and ethanol extracted solvent, respectively. Finally, the prepared four types (Ethanol PBS, Ethanol DMSO, Methanol PBS and Methanol DMSO) of aloe vera plant extract were stored at 0°C for further experiments[38].

Garlic extract: Whole garlic plant extract was prepared same as the above procedure[38-40].

Anti-bacterial activity assay: Four bacterial pathogens were used to evaluate the antimicrobial properties, including strains isolated from polluted water and tested against different antibiotics and showed multidrug resistant properties. The antibacterial assays were performed by the agar well diffusion method and broth microdilution method[41,42]. Bioactive compounds of plant extracts were investigated by the agar well diffusion method. Nutrient agar plates were prepared and overnight grown on each wild-type and MDR cultures (15 µL) and were plated by spread plate technique. The uniform diameter (0.5 cm) wells were created in the nutrient-agar plates with a sterile borer. The plant extracts was poured at different concentrations into each of the wells using an auto-pipette. Thereafter, the plates were incubated at 37±1°C for 28 h to observe the bacterial growth and zone of clearance. Microbial inhibition zone was visualized and measured the diameter of the zones surrounding the well and recorded in millimeter. All tests were recorded in triplicates for each combination of plant extracts and microbial strains.

Determination of minimum inhibitory concentration: Minimum inhibitory concentrations (MIC) of different plant extracts were performed against various MDR strains using microbroth dilution method according to the approved standard M7-A09 as recommended by the clinical and laboratory standards institute. The MIC test was done for aloe vera plant extract (in Ethanol PBS solution) against *E. coli* wild-type strain. Second MIC test against PA MDR strain (in Ethanol DMSO and ethanol PBS plant extract solution) and third, against GC MDR bacterial strain (in Aloe vera ethanol PBS, methanol PBS plant extract solution). For MIC to be performed each test tube was having 3 mL mixture of 2.8 mL nutrient broth (100 µL of bacterial culture) and 20 mg/20 µL to 4 mg/20 µL sets of test tubes having different plant extract dissolved in various solvents and maintained at the final volume for 3 mL by using normal saline. The MIC readings were taken after 24 h on the basis of the turbidity due to the growth of the bacteria[43].

Phytochemical screening of aloe vera plant extracts: The extracts prepared were used for screening of phytochemicals and other biologically active compounds. Phytochemical analysis was carried out according to the standard methods[44]. The extracts were screened for the presence of terpenoids, flavonoids, carbohydrates and cardiac glycosides[45-48].

Statistical analysis: The obtained results of the zone of inhibition were expressed as the mean value±standard error of the mean (SEM) for each group[49].

RESULTS

In silico analysis

Molecular docking studies of natural compounds with the transporter protein: Molecular docking were carried out with each selected natural compounds of aloe vera and garlic at the specific sites of interaction predicted from MVD. For each compound, out of the many docking poses, only those which possess the highest MolDock score (Table 1) and relatively good hydrogen bond interaction were chosen. The best five docking result was seen in DiallylTrisulfide (Zinc_01633229), Allyl Sulfide (Zinc_01531083), Alliin (Zinc_01531038), Aloin (Zinc_04214775) and 2-Bromo-5-Hydroxy-4-Methoxy Benzoic Acid (Zinc_64033873) with the total complex binding energy in kcal/jule -63.1319, -51.1096, -79.1436, -103.831 and -66.7682, respectively.

Table 1: RMSD value and MolDock score of natural compounds of Aloe vera plant (A comparative analysis of selected natural compounds)

Zinc ID	Name	Ligand name	MolDock score (GRID)	MolDock score	Rerank score	RMSD	Torsion
01530846	Allicin	[01][02][04][01][00][03]	-58.1672	-58.1672	-49.061	1.3679	5
01531038	Alliin	[02][03][01][01][01][03][01]	-90.327	-88.4044	-69.3682	1.2148	5
01533462	Allixin	[04][01][04]	-73.7536	-70.4157	-25.0319	1.6458	5
01531088	Allyl Methyl Disulfide	[03][02]	-41.6114	-42.4489	-36.244	1.3203	3
01850544	Allyl Methyl Trisulfide	[04][01][03]	-50.1061	-51.4288	-40.5244	1.6524	4
01531083	Allyl Sulfide	[01][02][03][02]	-49.3903	-50.6845	-40.9469	1.0853	4
04095721	Compesterol	[04][01][04][02]	-107.796	-108.149	-26.1547	1.3958	5
13413599	Cycloalliin	[00][02][00]	-64.4472	-60.0006	-51.341	2.0340	1
01531082	Diallyl Disulfide	[03][03][03]	-55.7113	-56.5724	-47.4493	1.3953	5
01633229	DiallylTrisulfide	[02][04][01][04]	-60.0404	-61.2587	-47.1091	1.0607	6
14680219	Isoalliin	[04][03][04]	-62.5696	-58.7489	-28.2835	2.4922	4
04658553	L-Alanine	[03][02][02][02]	-42.228	-38.3044	-34.3856	1.6686	1
02517162	S-Allyl-L-Cysteine	[04][02][03][02]	-66.549	-62.0456	-54.0503	1.5942	5
03861771	S-Methyl-L-Cysteine	[01][03][01][00]	-53.2291	-45.9395	-38.2657	1.9748	3
64033873	2-Bromo-5-Hydroxy-4-Methoxybenzoic Acid	[02][04][02][02][02][02][03][03][02]	-70.3306	-72.2559	-61.8527	1.2699	2
00105216	Naproxen	[04][02][01][04][04][04]	-76.0995	-71.2244	-57.6303	1.1672	3
04214775	Aloin	[01][00][04][00][03][00]	-129.002	-124.96	-85.9905	1.2369	3
04523265	Aloin A	[02][01]	-124.734	-115.928	-77.5304	3.0513	3
01530649	Haloprogin	[01][02]	-76.5963	-79.0588	-61.4286	2.0387	3
03813078	Flolan	[01][02][04]	-133.676	-133.275	-99.4336	1.5025	10
00001554	Salicylic Acid	[04][04][041][01]	-54.1935	-56.0387	-52.3171	1.9324	1

Docking evaluation for the best docked natural compound: From the all 22 natural compounds showing better drug-likeness property after docking five compounds showed a good tendency to bind with MDR transporter protein to inhibit its expression. These top five best docked compounds were selected on the basis of predicted binding energy and other binding parameters like hydrogen bond interaction electrostatic interaction (Table 2) and binding energy (Table 3). The best docked natural compound Aloin interacted with protein 5YIL having binding energy -103.831 kcal/mol with active site Ala33, Ala39, Ala297, Ala299, Asn298, Gln34, Glu673, Gly296, Ile38, Ile671, Leu137, Leu293, Lys292, Phe136, Pro36, Ser133, Ser135, Thr37, Thr295, Thr329, Tyr35, Val32, Val333 is minimum and hence, can be useful to inhibit the expression of 5YIL.

Thus, *in silico* approach to be particular about the responsible natural compound for antimicrobial property against the targeted protein 5YIL present in Aloe vera and Garlic. On the basis of different parameters and their comparative analysis, Aloin zinc id 04214775 and ChEBI ID 73222 come up as a best potential drug with least binding energy.

Anti-MDR activity assay: It has been seen from the results that *E. coli* wild-type and *P. aeruginosa* MDR strain showed sensitivity to all type of Aloe vera plant extracts with both solvents and mediums. But the best result was seen for aloe vera methanol PBS with 28±1.15 zone of inhibition in mm±SEM against *E. coli* strain (Table 4). Also, better zones were obtained against *S. aureus* for aloe vera extracts. *S. aureus* wild-type and *GC* MDR (isolated from Gomti river in Lucknow) shown sensitivity only for aloe vera ethanol PBS and methanol PBS. Thus, all extracts of aloe vera showed promising zones against MDR strains with only PA MDR strain observed as least zones and Aloe vera DMSO extracts could not be able to inhibit the growth of *S. aureus* as an exception. Garlic extract shown very narrow zones against *E. coli* wild-type which could not be measured while no zone of inhibition were observed for other cultures like *S. aureus, P. aeruginosa* and MDR strains *P. aeruginosa* and *GC*. Also, none of the extracts showed any inhibition in growth of *P. aeroginosa* wild type culture. The PBS and methanol could be better medium and solvents in plant extract preparation. Aloe vera extract was unable to inhibit *P. aeruginosa* strain but could be able to inhibit some MDR strain. As garlic extract did not show any inhibition. It was observed that *P. aeruginosa* showed best resistance against 5 antibiotics azithromycin, cefixime, amoxicillin, ciprofloxacin and ofloxacin and GC against 4 antibiotics amoxicillin, ciprofloxacin, azithromycin and cefixime).

Throughout the study, samples from the polluted water were collected and grown in antibiotic rich media as a carbon source for their utilization and checked for their resistant and sensitivity against different antibiotics. Plant extracts which were prepared have an initial concentration of 1 g/5 mL for Aloe vera and 750 mg/5 mL for Garlic. In our study, it have checked the effect of plant extracts against these types of notorious opportunistic multidrug-resistant bacterial strains (*P. aeruginosa* strain isolated from pond water) showed the

best resistance against 5 antibiotics azithromycin, cefixime, amoxicillin, ciprofloxacin and ofloxacin, GC from Gomti river Lucknow against 4 antibiotics amoxicillin, ciprofloxacin, azithromycin and cefixine. Thus further species identification was carried out by Gram staining and standard biochemical characterization tests. In this study, *P. aeruginosa* strain was Gram-positive and of *Bacillus* family and GC strain was Gram-positive and catalase negative. While *E. coli* and

Table 2: Hydrogen bond and electrostatic interactions of best docked natural compounds screened from the aloe vera plant

S. No.	Name	Hydrogen bond interactions	Electrostatic interactions
1	Diallyl Trisulfide Zinc_01633229		
2	Allyl Sulfide Zinc_01531083		
3	Alliin Zinc_01531038		
4	Aloin Zinc_04214775		

Table 2: Continue

S. No.	Name	Hydrogen bond interactions	Electrostatic interactions
5	2-Bromo-5-Hydroxy-4-Methoxy benzoic acid Zinc_64033873		

Table 3: Docked amino acid residues and their respective binding energy and total binding energy of best docked natural compounds from aloe vera plant extract

Name	Docked amino acid residues	Respective binding energy (Kcal/mol)	Total binding energy of complex (Kcal/mol)
DiallylTrisulfide Zinc_01633229	Ala39, Ala42, Ala294, Glu673, Gly296, Ile38, Ile671, Leu293, Pro36, Pro40, Pro41, Ser132, Ser133, Thr37, Thr295, Tyr35	-8.58364,-0.487175,-2.38952, -4.43531, -2.3093, -4.65064, -0.69344, -2.51008, -1.50781, -2.07013, -1.57141, -2.84275, -7.38723, -7.6504, -5.8556, -0.494646	-63.1319
Allyl Sulfide Zinc_01531083	Ala39, Ala294, Glu673, Gly296, Ile38, Leu293, Pro40, Pro41, Ser132, Ser133, Thr37, Thr295	-7.99387, -2.37045, -4.05492, -1.82914, -2.21875, -2.38612, -1.85459, -1.05271, -2.33277, -7.05305, -4.37395, -7.55442	-51.1096
Alliin Zinc_01531038	Ala39, Ala42, Ala294, Glu673, Gly296, Ile38, Leu293, Pro36, Pro41, Ser132, Ser133, Ser134, Thr37, Thr295	-12.7719, -2.67378, -1.04365, -12.8465, -5.14865, -2.52232, -0.90550, -1.03592, -5.32931, -2.62458, -9.76471, -0.47892, -7.60354, -11.1821	-79.1436
Aloin Zinc_04214775	Ala33, Ala39, Ala297, Ala299, Asn298, Gln34, Glu673, Gly296, Ile38, Ile671, Leu137, Leu293, Lys292, Phe136, Pro36, Ser133, Ser135, Thr37, Thr295, Thr329, Tyr35, Val32, Val333	2.04039, -9.10775, -4.36251, -12.1316, -8.02734, -6.65149, -0.42147, -4.07916, -7.26827, -2.1167, -8.47107, -17.3912, -0.713501, -2.25467, -3.42009, -6.94826, -0.66218, -17.2595, -0.92303, -1.35987, -7.39073, -0.78388, -2.41051	-103.831
2-Bromo-5-Hydroxy-4-Methoxy benzoic acid Zinc_64033873	Ala39, Ala42, Glu673, Gly296, Ile38, Ile671, Lys131, Lys292, Pro36, Pro40, Pro41, Ser132, Ser133, Thr37, Thr295, Tyr35	-20.4231, -0.4, -2.72614, -2.56531, -8.0075, -1.74688, -0.39417, -0.35446, -2.52088, -2.84532, -3.20698, -1.49183, -5.36248, -13.6726, -7.35179, -0.807433	-66.7682

Table 4: Anti-bacterial activity assay of the aloe vera extract prepared in different solvents and their zone of inhibition against various bacterial strains

Bacterial strains	Solvent plant extract	Zone of inhibition (mm±SEM)
E. coli wild type	Ethanol PBS	26.06± 0.01
	Methanol PBS	28.00± 1.15
	Ethanol DMSO	22.36± 0.20
	Methanol DMSO	20.26± 0.06
S. aureus wild type	Ethanol PBS	20.26± 0.06
	Methanol PBS	21.41± 0.16
PA MDR	Ethanol PBS	18.41± 0.09
	Methanol PBS	19.12± 0.06
	Ethanol DMSO	15.35± 0.14
	Methanol DMSO	12.20± 1.04
GC MDR	Ethanol PBS	17.10± 0.06
	Methanol PBS	19.24± 0.11

S. aureus of wild-type strains collected from the MRD lab. Aloe vera showed the best result against *E. coli* and obtained a maximum zone of inhibition.

MIC of aloe vera extract against selected strains: The MIC value of the plant extract against the MDR strains showing promising results and were determined for quantitative evaluation of anti-MDR potential. The MIC results for various extracts of Aloe vera (initial concentration starting from 1 g/5 mL and diluted at ranging from 20 mg/20 µL to 4 mg/20 µL) at 660 nm absorbance were recorded. The lowest MIC value was 0.4 mg μL^{-1} (Aloe vera extract in ethanol PBS) for *E. coli* whereas highest was 0.1 mg μL^{-1} (Aloe vera extract in methanol PBS) against GC MDR strain. Because no satisfactory result was seen by garlic plant extract, so MIC was done diluted at ranging from 20 mg/20 µL to 4 mg/20 µL at 660 nm absorbance only with all four type Aloe vera extract against *E. coli* wild-type strain and *P. aeruginosa*, GC MDR strains. In the study, garlic extract as found to have little or no

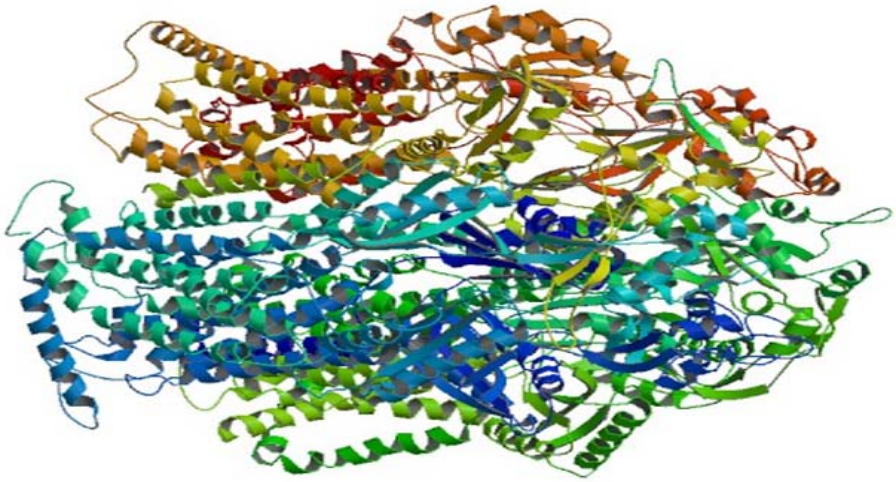


Fig. S1: 5YIL Protein 3D Structure: 5YIL Function: AcrA-AcrB-AcrZ-TolC is a drug efflux protein complex with broad substrate specificity that uses the proton motive force to export substrates

Table 5: Studies on the phytochemical profile of aloe vera and garlic extract prepared in different solvents

		Plant extract	
Phytochemical test	Solvents	Aloe vera	Garlic
Carbohydrate	Ethanol PBS	+	+++
	Methanol PBS	Absent	Absent
	Ethanol DMSO	++	++
	Methanol DMSO	+++	Absent
Cardiac Glycosides	Ethanol PBS	++	+
	Methanol PBS	+	+
	Ethanol DMSO	+	Absent
	Methanol DMSO	+++	Absent
Terpenoid	Ethanol PBS	++	+
	Methanol PBS	+++	+
	Ethanol DMSO	Absent	+
	Methanol DMSO	Absent	+
Flavonoid	Ethanol PBS	+	++
	Methanol PBS	+	+
	Ethanol DMSO	+	+
	Methanol DMSO	+	Absent

activity against selected bacterial strains at all tested concentration. The better MIC result has been seen in ethanol extract than aqueous extract and methanol extract on *E. coli*.

Phytochemical studies: Preliminary screening of phytoconstituents from the leaves of aloe vera revealed that the extracts contain terpenoids, flavonoids, carbohydrates and cardiac glycosides. Both plant extract showed contains the various amount of phytoconstituents. The maximum amount were observed in Aloe vera methanol PBS for terpenoids, methanol DMSO for carbohydrate as well as cardiac glycoside (Table 5) and promising amount for carbohydrate in garlic ethanol PBS extract.

DISCUSSION

Ethno-botanical survey reveals that plants are important sources of potentially useful compounds for the development of chemotherapeutics agents. Most important aspects will be the *in vitro* antibacterial activity assay[50]. Many studies and reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants[51]. On the other hand, antibiotic resistance is a major clinical problem in treating infections caused by several pathogenic micro-organisms. The resistance to the antimicrobials has increased over the years and normal intestinal microbial flora became a reservoir for resistant genes[52].

Throughout the study, there is urgency for the establishment of a new antibiotic intake policy that should be strictly followed by all the concerned authorities. Docking was performed of Efflux protein having PDB ID 5YIL (Fig. S1) the pocket domain in a complex of *E. coli* among the known inhibitors Diallyl Trisulfide, Allyl Sulfide, Alliin, Aloin and 2-Bromo-5-Hydroxy-4-Methoxy Benzoic Acid has shown quite good scores for PDB ID 5YIL of efflux protein of MDR *E. coli*. Thus, the natural compound Aloin (Fig. S2) can be a potent ligand for the drug target and in the future can be optimized to form improved drug for treatment of MDR caused diseases. Being an anthracene, it has a nuclease property. It also shows a cyclic ketone and c glycosyl property make it more prone to inhibit the target protein.

In general, Gram-negative bacteria are found to be more resistant towards antimicrobial agents as compared to Gram-positive bacteria. The reasons include covering of

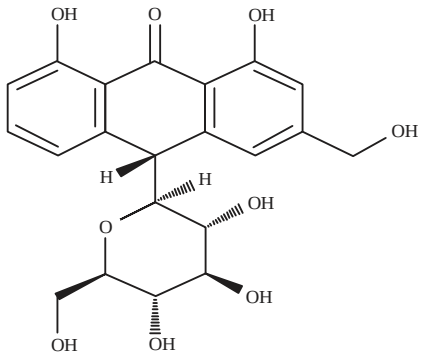


Fig. S2: Aloin (Zinc Id04214775) used as a laxative and play important role in increasing prostaglanding synthesis. Na+, K (+) - adenosine triphosphatase (ATPase) Molwt 418

phospholipids membrane carrying the structural lipopolysaccharides components which are responsible to allow impermeability against these antimicrobial substances[53].

The lowest concentration of an antimicrobial agent that inhibits the visible growth of micro-organisms known as the MIC[54]. Because no satisfactory result was seen by garlic plant extract, this may be due to presence of some diverse sub-chemical types in the extracts[55,56]. Aloe vera extract were found to have significant amount of secondary metabolites such as terpenoids, flavonoids, carbohydrates, cardiac glycosides, alkaloids and phenolic compounds. Their presence might be responsible for antimicrobial activities of extracts[55].

SIGNIFICANCE STATEMENT

Docking output of *in silico* data demonstrated various molecular interactions in different levels and showed that Aloin has more specificity towards the MDR protein targets and could be a potent antimicrobial compound. As the search for new antimicrobial agents intensifies, these plant extracts may provide attractive alternate sources of molecules for consideration.

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REFERENCES

1. Davies, J and D. Davies, 2010. Origins and evolution of antibiotic resistance. Microbiol. Mol. Biol. Rev., 74: 417-433.
2. McCoy, E., 1954. Changes in the host flora induced by chemotherapeutic agents. Annu. Rev. Microbiol., 8: 257-272.
3. Ernst, E., 2000. Adverse effects of herbal drugs in dermatology. Br. J. Dermatol., 143: 923-929.
4. Andersson, D.I. and D. Hughes, 2014. Microbiological effects of sublethal levels of antibiotics. Nat. Rev. Microbiol., 12: 465-478.
5. Fair, R.J. and Y. Tor, 2014. Antibiotics and bacterial resistance in the 21st century. Perspect. Med. Chem., 6: 25-64.
6. Blanch, A.R., J.L. Caplin, A. Iversen, I. Kuhn, A. Manero, H.D. Taylor and X. Vilanova, 2003. Comparison of *Enterococcal* populations related to urban and hospital wastewater in various climatic and geographic European regions. J. Applied Microbiol., 94: 994-1002.
7. Jalal, K.C.A., U.T.N. Fatin, M.A. Mardiana, B.A. John, Y.B. Kamaruzzaman, S. Shahbudin and M.N. Omar, 2010. Antibiotic resistance microbes in tropical mangrove sediments in East Coast Peninsular, Malaysia. Afr. J. Microbiol. Res., 4: 640-645.
8. Lazar, V., G.P. Singh, R. Spohn, I. Nagy and B. Horvath *et al*., 2013. Bacterial evolution of antibiotic hypersensitivity. Mol. Syst. Biol., Vol. 9. 10.1038/msb.2013.57.
9. Gilbert, P. and A.J. McBain, 2003. Potential impact of increased use of biocides in consumer products on prevalence of antibiotic resistance. Clin. Microbiol. Rev., 16: 189-208.
10. Huet, A.A., J.L. Raygada, K. Mendiratta, S.M. Seo and G.W. Kaatz, 2008. Multidrug efflux pump overexpression in *Staphylococcus aureus* after single and multiple *in vitro* exposures to biocides and dyes. Microbiology, 154: 3144-3153.
11. Peschel, A. and H.G. Sahl, 2006. The co-evolution of host cationic antimicrobial peptides and microbial resistance. Nat. Rev. Microbiol., 4: 529-536.
12. Otto, M., 2009. Bacterial Sensing of Antimicrobial Peptides. In: Bacterial Sensing and Signaling, Collin, M. and R. Schuch (Eds.). Vol. 16, Karger Publishers, Basel, Switzerland, ISBN-13: 9783805591324, pp: 136-149.
13. Tsai, W.C., Z.J. Zhuang, C.Y. Lin and W.J. Chen, 2016. Novel antimicrobial peptides with promising activity against multidrug resistant *Salmonella enterica* serovar Choleraesuis and its stress response mechanism. J. Applied Microbiol., 121: 952-965.
14. Bhalsinge, R., S. Rajbhoj, M. Limaye, M. Vaidya, P. Rane and A. Tilak, 2018. Anti-inflammatory and immunomodulatory activity of ethanol extract of *Aloe vera* gel. Int. J. Pharmaceut. Sci. Res., 9: 832-835.

15. Reynolds, T. and A.C. Dweck, 1999. *Aloe vera* leaf gel: A review update. J. Ethnopharmacol, 68: 3-37.
16. Tiwari, K., V.S. Raj, D.J. Upadhyay and R.K. Gupta, 2015. *In vitro* activity of bioactive extracts from rare actinomycetes against multi-drug resistant *Streptococcus pneumoniae*. J. Applied Microbiol., 118: 1306-1314.
17. Okusu, H., D. Ma and H. Nikaido, 1996. AcrAB efflux pump plays a major role in the antibiotic resistance phenotype of *Escherichia coli*multiple-antibiotic-resistance (Mar) mutants. J. Bacteriol., 178: 306-308.
18. Higgins, M.K., J. Eswaran, P. Edwards, G.F. Schertler, C. Hughes and V. Koronakis, 2004. Structure of the ligand-blocked periplasmic entrance of the bacterial multidrug efflux protein TolC. J. Mol. Biol., 342: 697-702.
19. Zwama, M., K. Hayashi, K. Sakurai, R. Nakashima, K. Kitagawa, K. Nishino and A. Yamaguchi, 2017. Hoisting-loop in bacterial multidrug exporter AcrB is a highly flexible hinge that enables the large motion of the subdomains. Front. Microbiol., Vol. 8. 10.3389/fmicb.2017.02095
20. Vargiu, A.V. and H. Nikaido, 2012. Multidrug binding properties of the AcrB efflux pump characterized by molecular dynamics simulations. Proc. Natl. Acad. Sci. USA., 109: 20637-20642.
21. Ferreira, L., R. dos Santos, G. Oliva and A. Andricopulo, 2015. Molecular docking and structure-based drug design strategies. Molecules, 20: 13384-13421.
22. Schmidt, T.H., M. Raunest, N. Fischer, D. Reith and C. Kandt, 2016. Computer simulations suggest direct and stable tip to tip interaction between the outer membrane channel TolC and the isolated docking domain of the multidrug RND efflux transporter AcrB. Biochim. Biophys. Acta (BBA)-Biomembr., 1858: 1419-1426.
23. Fong, P., H.H. Tong and C.M. Chao, 2014. *In silico* prediction of tyrosinase and adenylyl cyclase inhibitors from natural compounds. Nat. Prod. Commun., 9: 189-194.
24. Kamath, S. and S. Skariyachan, 2018. Novel insight from computational virtual screening depict the binding potential of selected phytotherapeutics against probable drug targets of *Clostridium difficile*. Interdisciplin. Sci.: Comput. Life Sci., 10: 583-604.
25. Meng, X.Y., H.X. Zhang, M. Mezei and M. Cui, 2011. Molecular docking: A powerful approach for structure-based drug discovery. Curr. Comput.-Aided Drug Des., 7: 146-157.
26. Irwin, J.J., T. Sterling, M.M. Mysinger, E.S. Bolstad and R.G. Coleman, 2012. ZINC: A free tool to discover chemistry for biology. J. Chem. Inform. Model., 52: 1757-1768.Monika, J.K. and K. Singh, 2013. Virtual screening using the ligand ZINC database for novel lipoxygenase-3 inhibitors. Bioinformation, 9: 583-587.
27. Degtyarenko, K., P. de Matos, M. Ennis, J. Hastings and M. Zbinden *et al*., 2008. ChEBI: A database and ontology for chemical entities of biological interest. Nucleic Acids Res., 36: D344-D350.
28. Yusof, I. and M.D. Segall, 2013. Considering the impact drug-like properties have on the chance of success. Drug Discov. Today, 18: 659-666.
29. Guex, N. and M.C. Peitsch, 1997. SWISS-MODEL and the Swiss-PDB viewer: An environment for comparative protein modeling. Electrophoresis, 18: 2714-2723.
30. DeLano, W.L., 2002. Pymol: An open-source molecular graphics tool. CCP4 Newslett. Protein Crystallogr., 40: 82-92.
31. Yang, J., A. Roy and Y. Zhang, 2013. Protein-ligand binding site recognition using complementary binding-specific substructure comparison and sequence profile alignment. Bioinformatics, 29: 2588-2595.
32. Yang, J., A. Roy and Y. Zhang, 2012. BioLiP: A semi-manually curated database for biologically relevant ligand-protein interactions. Nucleic Acids Res., 41: D1096-D1103.
33. Mavillapalli, R.C., S. Jeyabalan and S. Muthusamy, 2017. Molecular docking studies of phytoconstituents identified in *Cinnamomum verum* and *Coriandrum sativum* on HMG CoA reducatse-an enzyme target for antihyperlipidemic activity. Int. J. Pharmaceut. Sci. Res., 8: 4172-4179.
34. Nounou, M.I., 2014. Predicting perfect drug candidates: Molecular docking 2.0. J. Pharm. BioAllied Sci., 6: 137-138.
35. Sinansari, R., P.E.W. Bambang and P. Widiyanti, 2018. *In silico* screening and biological evaluation of the compounds of *Justicia gendarussa* leaves extract as interferon gamma inducer: A study of anti Human Immunodeficiency Virus (HIV) development. Afr. J. Infect. Dis., 12: 140-147.
36. Gibbons, N.E. and R.G.E. Murray, 1978. Proposals concerning the higher taxa of bacteria. Int. J. Syst. Evol. Microbiol., 28: 1-6.
37. Fani, M. and J. Kohanteb, 2013. Inhibitory activity of *Aloe vera* gel on some clinically isolated cariogenic and periodontopathic bacteria. J. Oral Sci., 54: 15-21.
38. Dillon, S.A., R.S. Burmi, G.M. Lowe, D. Billington and K. Rahman, 2003. Antioxidant properties of aged garlic extract: An *in vitro* study incorporating human low density lipoprotein. Life Sci., 72: 1583-1594.
39. Imai, J., N. Ide, S. Nagae, T. Moriguchi, H. Matasuura and Y. Itakura, 1994. Antioxidant and radical scavenging effects of aged garlic extract and its constituents. Planta Med., 60: 417-420.
40. Kumar, D., A. Verma, N. Dhusia and N. More, 2013. Water quality assessment of river Gomti in Lucknow. Universal J. Environ. Res. Technol., 3: 337-344.
41. Mahesh, B. and S. Satish, 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J. Agric. Sci., 4: 839-843.
42. Vieira, M., C. Pinheiro, R. Fernandes, J.P. Noronha and C. Prudencio, 2014. Antimicrobial activity of quinoxaline 1, 4-dioxide with 2-and 3-substituted derivatives. Microbiol. Res., 169: 287-293.
43. Brinda, P., P. Sasikala and K.K. Purushothaman, 1981. Pharmacognostic studies on Merugan kizhangu. Bull. Med. Eth. Bot. Res., 3: 84-96.

44. Adetuyi, A.O. and A.V. Popoola, 2001. Extraction and dyes ability potential studies of the colourant in zanthoxylum zanthoxyloides plant on cotton fabric. J. Sci. Eng. Technol., 8: 3291-3299.
45. Harborne, J.B., 1998. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd Edn., Chapman and Hall, London, ISBN-13: 9780412572708, Pages: 302.
46. Sofowora, A., 1996. Research on medicinal plants and traditional medicine in Africa. J. Altern. Complement. Med., 2: 365-372.
47. Trease, G.E. and W.C. Evans, 2002. Pharmacognosy. 15th Edn., WB Saunders, London, ISBN: 8131200876, Pages: 406.
48. Woo, H.L., T.C. Hazen, B.A. Simmons and K.M. DeAngelis, 2014. Enzyme activities of aerobic lignocellulolytic bacteria isolated from wet tropical forest soils. Syst. Applied Microbiol., 37: 60-67.
49. Tona, L., K. Kambu, N. Ngimbi, K. Mesia and O. Penge *et al*., 2000. Antiamoebic and spasmolytic activities of extracts from some antidiarrhoeal traditional preparations used in Kinshasa, Congo. Phytomedicine, 7: 31-38.
50. Samy, R.P. and S. Ignacimuthu, 2000. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. J. Ethnopharmacol., 69: 63-71.
51. Okeke, I.N., S.T. Fayinka and A. Lamikanra, 2000. Antibiotic resistance in *Escherichia coli* from Nigerian students, 1986-1998. Emerg. Infect. Dis., 6: 393-396.
52. Savitha, R.C., S. Padmavathy and A. Sundhararajan, 2011. *Invitro* antioxidant activities on leaf extracts of *Syzygium malaccense* (L.) Merr and Perry. Ancient Sci. Life, 30: 110-113.
53. Ericsson, H.M.A. and J.C. Sherris, 1971. Antibiotic sensitivity testing. Report of an international collaborative study. Acta Pathol. Microbiol. Scand. B: Microbiol. Immunol., 217: 90-90.
54. Saxena, M., J. Saxena, R. Nema, D. Singh and A. Gupta, 2013. Phytochemistry of medicinal plants. J. Pharmacogn. Phytochem., 1: 168-182.
55. Thiruppathi, S., V. Ramasubramanian, T. Sivakumar and V.T. Arasu, 2010. Antimicrobial activity of *Aloe vera* (L.) Burm. F. against pathogenic microorganisms. J. Biosci. Res., 1: 251-258.