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Research Article

Using In-Silico Molecular Docking as a Tool to Justify the Anti-Typhoid Property of *Terminalia Chebula*

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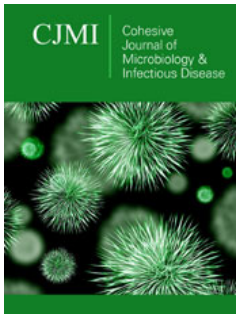

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Abstract

In this study, the anti-typhoid activity of *Terminalia chebula* (Myrobalan), referred to as the 'King of Medicine in Ayurveda, was verified using a promising, novel method known as In-silico molecular docking technique. The dried fruits of the myrobalan were powdered, extracted, and observed for secondary metabolites, followed by the anti-larvicidal activity towards *Culex quinquefasciatus* (mosquitoes) and the anti-salmonella activity of *Salmonella Typhimurium* ATCC 14028 (causing typhoid fever in mice). The ethyl acetate extracts actively showed inhibition in growth in the experimental setup. Further, to justify the anti-salmonella activity, a GC-MS was performed for better profiling of the ethyl acetate extract of myrobalan in terms of active compounds. A complex mixture of 9 bioactive compounds, many of them were present in traces; however, Borane-methyl Sulfide complex (40.084%), Methyl hydrogen Disulfide (52.779%), Disulfide, methyl (Methylthio) methyl (19.342%), Ethene, (Methylsulfinyl)-(16.957%) were pinned as most versatile compounds. The compounds with higher retention time in GC-MS were selected for the molecular docking studies. The In-silico docking confirmed the role of the bioactive molecule present in *Terminalia chebula* as a potential compound for treating typhoid fever by successfully outlining the active bonding between the active compound with the 2YM0 protein receptor from *Salmonella typhimurium*.

Keywords: In-silico molecular docking; typhoid fever; *Terminalia chebula*; *Salmonella typhimurium; Culex quinquefasciatus*; metabolite profiling

Introduction

Typhoid is a bacterial enteric infection caused by *Salmonella enterica* serovar typhi (*Salmonella typhi*; *S. typhi* [1]. It is one of the bacteria that cause acute febrile sickness that is most common in underdeveloped countries. Inadequate sanitation in these nations allows for easy contamination of food and water with human waste, making typhoid fever an international public health concern [2,3]. Countries in South Asia, the Middle East, Central Africa, and South America have all been reported to have high endemic illness rates and be experiencing typhoid fever epidemics. Worldwide, typhoid is responsible for between 128,000 and 161,000 annual deaths [4]. Typhoid affects an estimated 11-20 million individuals each year. India has estimated 4.5 million cases of typhoid fever per year, with roughly 9000 deaths (0.2% case fatality rate). This equates to an annual incidence rate of 297-494 per 100,000 people [5].

Antibiotics effectively treat typhoid fever, provided a correct diagnosis is made within the first few days of symptoms appearing. However, the increasing prevalence of MDR *S. typhi* strains hinders effective treatment and may increase the incidence of sequelae and disease severity [4]. *S. typhi* infections are typically treated with cephalosporins, azithromycin, ampicillin, chloramphenicol, and cotrimoxazole. Ofloxacin and ciprofloxacin, two fluoroquinolones, have been increasingly used in recent years because of the widespread prevalence of multidrug-resistant bacteria [6,7]. Typhoid vaccinations have made significant strides recently, representing a powerful resource for the international community's fight against the disease. The World Health Organization (WHO) recommends routinely administering Typhoid

Conjugate Vaccine (TCV) as well as conducting targeted catch-up vaccination to reduce the prevalence of typhoid fever [4,8]. Yet, the existing diagnostic techniques can lead to under or over-evaluation of the condition, delaying therapy or causing improper, excessive antibiotic usage, increasing the probability of bacterial resistance [9].

Treatment with medicinal plants is more common than conventional medications because of their low cost and wide availability. Phytoconstituents found in nature have chemical characteristics like those of artificial antibiotics. Due to documented problems with monotherapy antibiotic efficacy, assessing plant biological characteristics is crucial. Tulsi, also known as *Ocimum sanctum*, is an herb native to India that has been shown to have intense antibacterial activity against *S. typhi* [9]. A combination of chloramphenicol and trimethoprim with *O. sanctum* leaf extract is highly effective against S. typhi, as shown in *in vitro* research [10]. The inhibitory activity of acetone mango leaf extract (10-50g/ml) against multidrug-resistant *S. typhi* was also demonstrated [11]. Leaf extracts from *Cymbogogon citratus*, *Carica papaya*, and *Zea mays* silk in methanol have been shown to have a MIC against S. typhi between 0.02-0.06g/ml [12]. Although numerous studies have demonstrated the efficacy of various natural extracts against *S. typhi* in test tubes, little is known about their absorption, pharmacokinetics and pharmacodynamics, antibacterial interaction, or safety profile in humans [12,13].

Terminalia chebula contains tannins (32-34%). The most abundant phytoconstituents in this fruit are phenolic compounds, alkaloids, glycosides, flavonoids, saponins, quinine, steroids, etc. [8-16]. The various studies conducted provides the evidence for the versatility of the *Terminalia chebula*, revealing its diverse range of properties such as antibacterial activity [17]; anti-mutagenic, radio-protective, chemo-preventive activity, anti-anaphylaxis [18]; immunomodulatory activity [19]; anti-carcinogenic activity [20]; antifungal activity, antiviral activity [21]; hepato-protective activity, cardioprotective activity, cytoprotective activity, anti-diabetic, reno-protective activity [22]; anti-inflammatory and anti-arthritis property, gastrointestinal motility improving, anti-ulcerogenic activity, anti-plasmodia activity, anti-caries activity, wound healing activity, anti-allergic activity, anti-protozoal activity, anti-aging [23]; anti-Salmonella activity [24]; antioxidant and free radical scavenging activity [25]; hypo-cholesterelomic activity against cholesterol-induced hypercholesterolemia and atherosclerosis [26]; anti-diarrheal [27]; anti- HIV activity [28]; hypo-lipidemic activity and nephron-protective activity. In addition, gram-positive and gram-negative microorganisms are susceptible to the fruit's broad spectrum of antimicrobial capabilities [29]. Our study attempted to better establish the interaction between the secondary metabolites or compounds found in the myrobalan fruit (*Terminalia chebula*) and the 2YM0 protein (Truncated SipD from) in preventing typhoid fever.

Results

Phytochemical analysis

The fruit powder showed the presence of most of the phytochemicals except quinine. The crude extract of hexane shows the presence of fewer phytochemicals than the ethyl acetate extract (Table 1).

Table 1: Qualitative phytochemical analysis of myrobalan fruit powder and two extracts.

Test For	Observation	Results		
		Myrobalan Powder	Ethyl Acetate	Hexane
Glycosides	Brick red colour	Positive	Positive	Positive
Alkaloids	Yellow precipitation	Positive	Positive	Positive
Anthraquinone	Violet colour in the lower phase	Positive	Negative	Positive
Flavonoids	Yellow-brown precipitation	Positive	Positive	Negative
Terpenoids	Grey colour	Positive	Positive	Positive
Saponins	Froth after agitation	Positive	Positive	Positive
Steroids	Red colouration	Positive	Positive	Positive
Phenols	Blue colour	Positive	Positive	Positive
Tannins	Blue colour precipitation	Positive	Positive	Negative
Quinine	Red or blue colouration	Negative	Positive	Positive

Larvicidal assay

The results highlighted the good larvicidal activity of the two extracts, Hexane, and ethyl Acetate of Myrobalan powder, against the larvae of *Culex quinquefasciatus*. At 1116.914ppm and 12711.590ppm for 24 hours, respectively, the Ethyl Acetate extract had the most vital larvicidal activity (Table 2).

Table 2: The effects of *Terminalia chebula* crude extracts on *Culex quinquefasciatus* larvae at various doses.

Cx. *Quinquefasciatus* (Mosquito)	Extracts	LC50 (ppm)	95%Confidence limit		LC90 (ppm)	95%Confidence limit		Intercept ±SE	Slope±SE	χ^2
			Lower Limit	Upper Limit		Lower Limit	Upper Limit			
	Hexane	728.752	477.465	1677.358	10141.932	5913.231	347831.156	1.7±0.4	1.1±0.2	0.3*
	E.acetate	1116.914	671.251	3230.569	12711.59	6686.442	483373.969	1.3±0.5	1.2±0.2	0.5*

Anti-salmonella activity

The *Terminalia chebula* fruit powder at a concentration of 1mg/100µl showed no zone of inhibition. At a concentration of 10mg/100µl, the zone of inhibitions was 2.33±0.57, and at a concentration of 50mg/100 µl, the zone of inhibitions was 5.33±0.57. The concentration of 100mg/100µl showed a zone of inhibition of 9.66±0.57. Results conclude that the zone of inhibition of Ethyl Acetate extract *Terminalia chebula* fruit powder is higher than Hexane extract at the same concentrations. The positive control was 2.5mg Chloramphenicol in 100µl, and the negative control was distilled water (Table 3).

Table 3: The anti-*Salmonella* activity of *Terminalia chebula* fruit powder v/s Hexane extracts v/s Ethyl Acetate extract using agar well diffusion method (N= 3).

S. No.	Concentration in 100µl (mg/well)	Anti-Salmonella activity Zone of Inhibition (mm) Well diffusion method		
		Terminalia chebula fruit powder	Hexane extract	Ethyl acetate extract
1	100mg	9.66±0.57	10.33±0.57	17.66±0.57
2	50mg	5.33±0.57	4.33±0.57	9.33±0.57
3	10mg	2.33±0.57	2.66±0.57	4.33±0.57
4	1mg	No Zone	No Zone	No Zone

GCMS analysis

The chemical constituents in the ethyl acetate extract showed a complex mixture of 9 bioactive compounds; many were present in trace amounts. On the other hand, Borane-methyl Sulfide complex (40.084%), Methyl hydrogen Disulfide (52.779%), Disulfide, methyl (Methylthio) methyl (19.342%), Ethene, (Methylsulfinyl)-(16.957%) were pinned as the versatile compounds (Table 4).

Table 4: Qualitative Phytochemical Analysis of Myrobalan fruit powder and two extracts.

S. No.	Name	Molecular Formula	Retention Time	Area (%)
1	Dimethyl Sulfoxide	C2H6OS	4.034	14.347
2	Borane-Methyl Sulfide Complex	C2H9SB	4.079	40.084
3	Methyl Hydrogen Disulfide	CH4S2	4.364	52.779
4	Dimethylsulfoxonium Formylmethylide	C4H8O2S	4.579	2.918
5	Disulfide, Methyl (Methylthio)Methyl	C3H8S3	4.634	19.342
6	2-Chloroethyl Methyl Sulfoxide	C3H7OClS	4.689	4.249
7	Ethanol, 2-(Methylthio)-	C3H8OS	4.959	6.189
8	Ethene, (Methylsulfinyl)-	C3H6OS	5.129	16.957
9	S-Methyl Methanethiosulfinate	C2H6OS2	5.389	15.538

Molecular docking

Molecular docking was carried out against receptor protein 2YM0 (from *Salmonella typhimurium*) and the GC-MS resultant ligands, namely (1) Dimethyl Sulfoxide, (2) Disulfide, Methyl (Methylthio) Methyl, (3) Methyl Hydrogen Disulfide. The results showed that all three ligands could intensely occupy the active location of 2YM0 with their binding energies. The compound (1) Dimethyl Sulfoxide exhibited a high value of -4.5 (Kcal/mol) binding-energy. H-bonds interaction between this ligand and 2YM0 protein. Figures 1-9 depict the H-bond interactions between ligands and receptors, as well as H-bond interactions on the receptor side and their 2D-ligand-receptor H-bond interaction (Table 5).

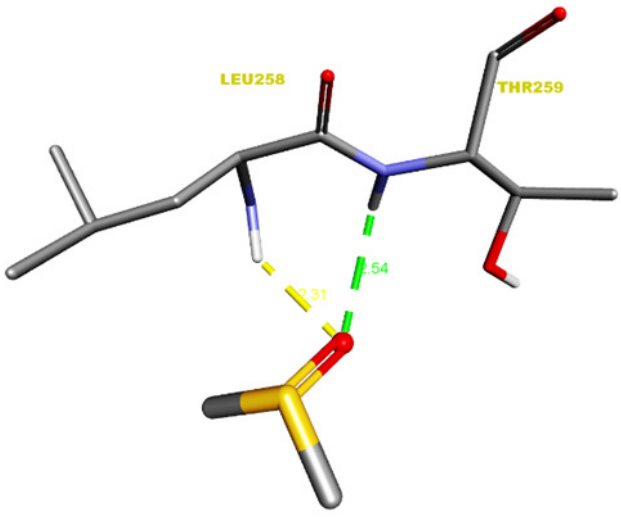

Figure 1: Ligand-receptor hydrogen bond interactions of Dimethyl Sulfoxide.

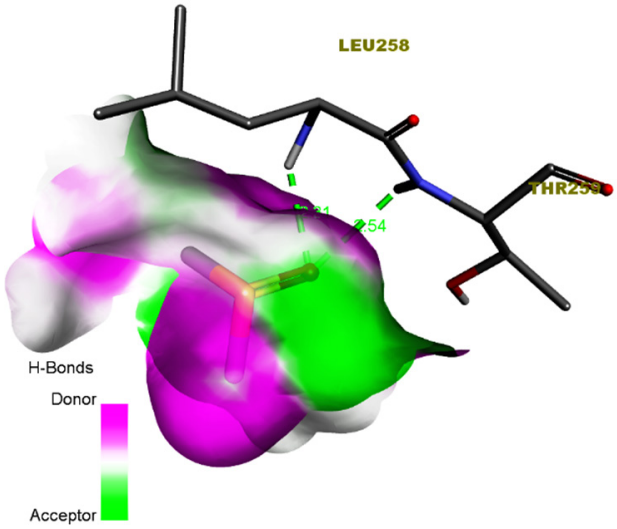

Figure 2: Receptor side hydrogen bond interactions of Dimethyl Sulfoxide.

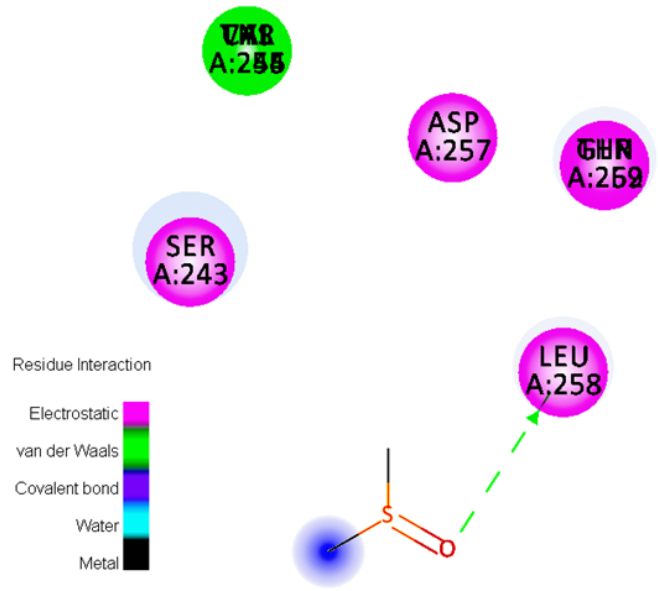

Figure 3: Ligand-receptor hydrogen bond interaction of Dimethyl Sulfoxide.

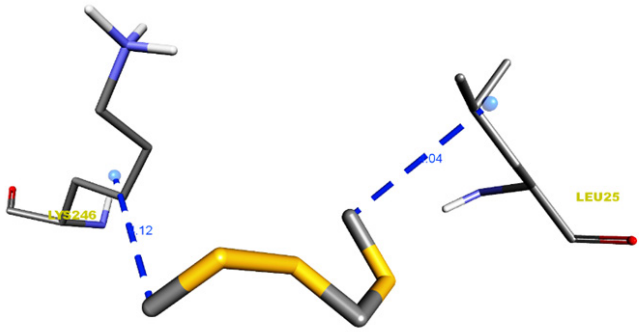

Figure 4: Ligand-receptor hydrogen bond interactions of Disulfide, Methyl (Methylthio)Methyl.

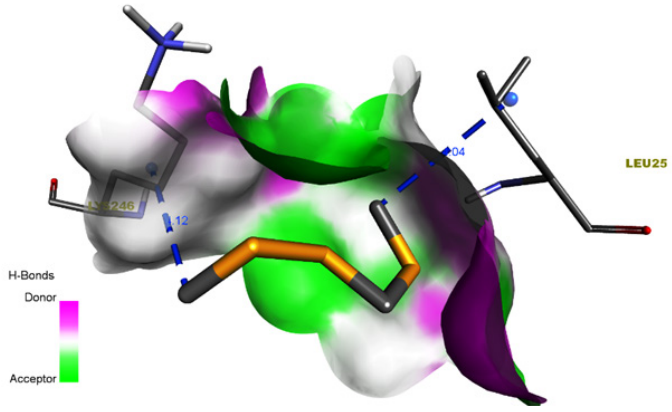

Figure 5: Receptor side hydrogen bond interactions of Disulfide, Methyl (Methylthio)Methyl.

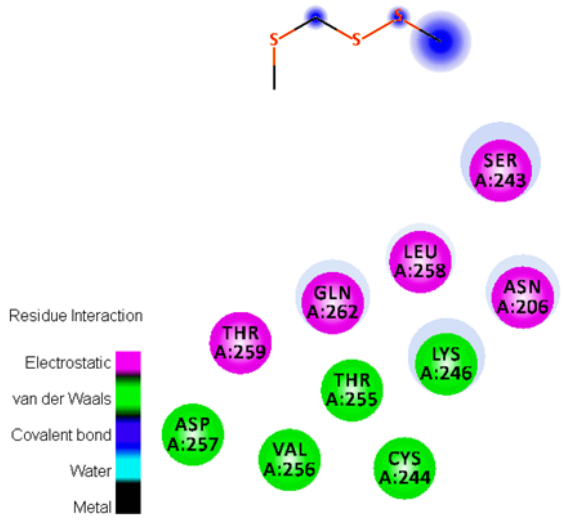

Figure 6: Ligand-receptor hydrogen bond interaction of Disulfide, Methyl (Methylthio)Methyl.

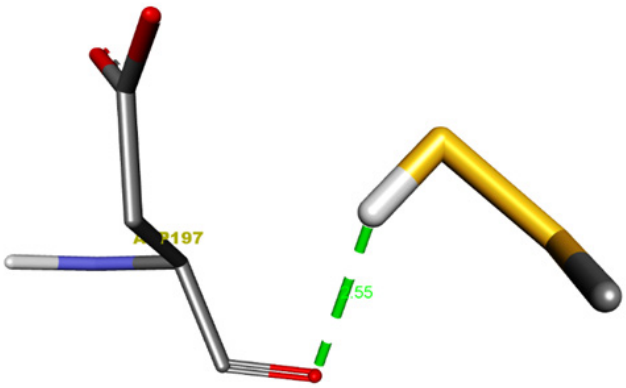

Figure 7: Ligand-receptor hydrogen bond interactions of Methyl Hydrogen Disulfide.

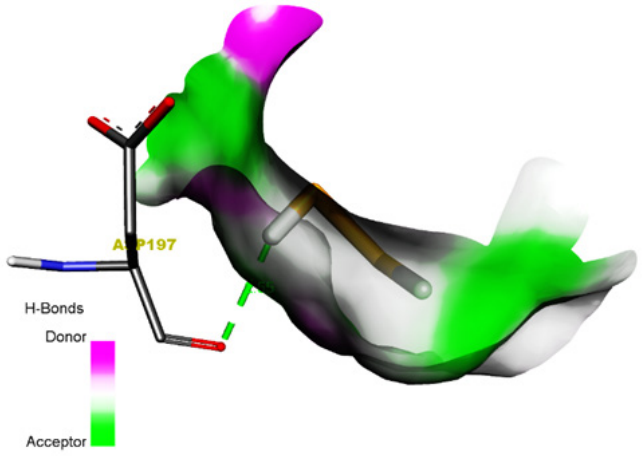

Figure 8: Receptor side hydrogen bond interactions of Methyl Hydrogen Disulfide.

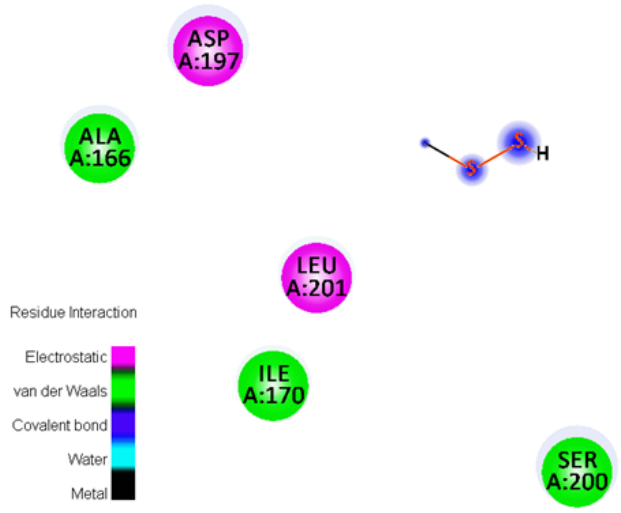

Figure 9: Ligand-receptor hydrogen bond interaction of Methyl Hydrogen Disulfide.

Table 5: The interaction between GC-MS resulting Ligands and the 2YM0 receptor residue.

S.NO	Pub chem. Id	GC-MS Resultant Ligands Name	Binding Energy (kcal/mol)	Residue Interaction	Type of bond	Distance(Å)
1	2YM0	Dimethyl Sulfoxide	-4.5	A: THR259: HN-: UNK01 O	Hydrogen	2.5
				A: LEU258: HN- :UNK0: O	Hydrogen	2.3
2	2YM0	Disulfide, Methyl (Methylthio)Methyl	-2	: UNK0:CS- A: LEU258	Hydrophobic	4
				: UNK0: C-A: LYS246	Hydrophobic	4.1
3	2YM0	Methyl Hydrogen Disulfide	-3	: UNK0: H-A: ASP197:O	Hydrogen	2.5

Materials and Methods

Sample harvesting

Terminalia chebula dried fruits were purchased from a local market in Chittaranjan Park, New Delhi, India. Each fruit was thoroughly checked for its quality. The required amount of fruit was de-seeded, initially broken using a mortar pestle, and later powdered using a powerful blender. A measured amount of fruit powder was extracted using hexane and ethyl acetate. The extraction process was run for two days at 70 ˚C. The two extracts were evaporated and stored for future use [30].

Phytochemical analysis

The phytochemical profiling was carried out for various metabolites [12,31-36] (Table 6).

Table 6: The phytochemical profiling was carried out for various metabolites.

S.No.	Phytochemical	Analysis Method	Confirmation
1	Glycosides	1g sample was dissolved in 2-3ml of distilled water, to which 2-3 drops of 1% solution of alcoholic naphthol was added, sliding down the tube.	Brick red colour
2	Alkaloids	0.2g sample was added in 3ml of hexane, mixed well, shaken, and filtered, followed by 5ml of 2% HCl and heated for a few minutes. The solution was filtered, and a few drops of picric acid was added.	Yellow colour
3	Flavonoids	A 0.5g sample was added to 10ml of distilled water and 5ml of dilute ammonia solution. In the end, 1ml of concentrated H2S04 was added.	Yellow colour
4	Terpenoids	The crude extract was added to2ml of chloroform and was evaporated. Added with 2ml of concentrated H2S04 and heated for 2 mins.	Greyish colour

5	Phenols	The extract was treated with 5% ferric chloride dissolved in water.	Deep blue/black colour
6	Saponins	After adding 5ml of distilled water to the crude extract, the mixture was violently shaken.	Stable foam
7	Steroids	The crude extract was mixed with 2ml chloroform, followed by the addition of concentrated H2S04.	Red colour
8	Tannin	1 ml of the extract with a minimum amount of H20 was filtered and added with FeCl3 solution to the filtrate.	Blue colour
9	Quinine	1ml of the extract was taken, and a few drops of alcoholic KOH were added.	Blue colour
10	Anthraquinones	Extract weighing 5gm was added to 10mL of benzene and filtered, followed by 5mL of 10% ammonia solution.	Violet colour in the lower phase

Larvicidal bioassay

The Bioassay was carried out following WHO 2005 guidelines, with minor modifications. Third instar larvae of *Culex quinquefasciatus*, 20 in number, were moved into 100ml containers. 500ppm of hexane and ethyl acetate extract was made with 1.0% DMSO. As a control, 1% DMSO in water was used. After the period of 24h, the mortality rate was observed. The larvae were accounted dead after showing no mobility when touching a needle [33,37,38].

Anti-salmonella assay

The agar well diffusion method investigated the antimicrobial activity of gram-ve, pathogenic *Salmonella typhimurium* ATCC 14028. The freshly made agar was allowed to be set, and after solidification, wells were made, i.e., four wells were cut on each agar plate using an 8mm well cutter. The culture of the above bacteria was swabbed into a 90mm agar plate. The diameter of the zone of inhibition formed around the well was measured after 72 hours of incubation at 37 degrees Celsius. As a control, distilled water was used [24,39].

GCMS analysis

The spectra of secondary metabolites were observed for the ethyl acetate extract using GC–MS (SHIMADZU QP2010). GC specifications: injector temperature -260 ˚C, column oven temperature -60 ˚C, column flow was 1mL/min, injection mode in split with a ratio of 10:1, flow control mode in linear velocity, Helium (99.99%) purity was used as carrier gas. The MS specifications were, Ion source temperature at 230 ˚C, interface temperature at 230 ˚C, solvent cut time was 5min, event time was 0.5s, Ionization (EI) was at 70EV, the start time was 5min and end time was 35min, scan range from 50 to 600Da, etc. Compounds were recognized by their twin GC retention times [40,41].

Molecular docking analysis

The docking program Auto Dock via 4.2 was used to select the possible binding mode between the GC-MS resultant ligands and 2YM0 receptor protein. Software Chem Draw 12.0 was used to draw ligand structures. After minimizing energy for these 2D compound structures, they were changed into Protein Data Bank (PDB) format, which was further transmuted into the PDBQT structure. Auto dock Tools prepared the 2YM0 protein structure by adding required H-atoms applying solvation parameters, and Kollman united atom charges. Ligand structures were created by combining non-polar H-atoms, Gasteiger partial charges, and defining rotatable bonds. An Auto grid was used to create grid boxes with a maximum size of 108 x 112 x 69. The grid box was designed with a grid spacing of 0.492 at the binding site for 2YM0 at the midpoint of the proteins with x (-3.005), y (19.46), and z (-21.473) coordinates. Discovery-studio visualizes 4.0 was used to create a 2D graphic representation of protein-ligand interaction [42,43].

Conclusion

The *Terminalia chebula* (myrobalan) powder is shown to have a wide range of secondary metabolites in the hexane and the ethyl acetate extract. Notably, the anti-larvicidal and anti-salmonella activity test results exhibited the property of inhibiting the growth of Culex quinquefasciatus (mosquito) and *Salmonella typhimurium* ATCC 14028 for both *Terminalia* fruit extracts; however, the ethyl acetate extract showed better outcomes than the hexane extract. In addition, the GCMS analysis of the ethyl acetate extract revealed the presence of 9 bioactive components, with the Borane-methyl Sulfide complex, Methyl hydrogen Disulfide, Disulfide, methyl (Methylthio) methyl, Ethene, (Methylsulfinyl) compound having the highest retention time [43-48]. Lastly, the molecular docking results revealed that the bioactive molecule Dimethyl Sulfoxide present in *Terminalia chebula* is the most potential bioactive compound for treating typhoid fever.

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