LEAD MOLECULE IDENTIFICATION FROM VITEX TRIFOLIA LINN FOR HELMINTHIASIS USING IN VITRO AND IN SILICO METHODS

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ABSTRACT

Objective: The study was an attempt to discover a lead molecule to treat helminthiasis using Vitex trifolia. Linn (V. folia Linn) through sterile effect, in vitro and in silico evaluation.

Methods: The anthelmintic activity was done by Kirby-Bauer disc diffusion method in three different concentrations of extract and in vitro anthelmintic activity was carried out by petri dish and organ bath method. Further, the in silico docking studies were carried out by 11 phytoconstituents against phosphoethanolamine methyltransferase (4FGZ) using Auto Dock 4.2. It was working based on the principle of Lamarckian genetic algorithm. In docking studies, three important parameters such as binding energy, inhibition constant and intermolecular energy are determined.

Results: The extract showed an antibacterial effect in three different concentrations. At 16 mcg/disc a significant effect was observed when compared to blank and ciprofloxacin 5 mcg/disc. The anthelmintic activity in the petri dish method, means paralyzing time of Pheretima posthuma with the dose of 25, 50 and 100 mg/ml were 13.78, 5.79 and 4.57 min respectively and Piperazine citrate (10 mg/ml) showed paralysis in 21.58 min. In the organ bath method, the time for paralysis of the worm was recorded on a slow-moving Sherrington rotating drum and the study report showed that paralysis time was decreased at increasing concentrations of the extract. The results of in silico studies exhibited a binding energy of 10.25 kcal/mol, inhibitory constant (K) 30.91 nM, intermolecular energy, -10.84 kcal/mol for abietatriene-3-ol which is lesser than the standard ligand phosphoethanolamine (-6.03 kcal/mol, 38.29 µM, -7.82 kcal/mol) respectively.

Conclusion: The study reports conclude that the active constituents in V. folia Linn having better anthelmintic activity, thus the active constituents may be optimized and make way to a new moiety for the treatment of helminthiasis.

Keywords: V. trifolia, Helminthiasis, Binding energy, Inhibitory constant, Intermolecular energy, Phosphoethanolamine methyltransferase, Abietatriene-3-ol, Phosphor ethanolamine.

INTRODUCTION

Helminthiasis is one of the major public health issues across many countries. Anthelmintic drugs have been the only effective method of controlling worm infestations, but there is now widespread parasite resistance to most of the commercially available drugs [1, 2]. The development of resistance to a group of anthelmintics poses a challenge to identify novel molecular targets for helminthiasis [1, 2]. Various synthetic compounds have been proved to have anthelmintic activity. Some of the natural products have been proved to have anthelmintic activity but their safety profile is not favorable. So current research is focussed on natural products having an anthelmintic activity that may be useful in helminthiasis. V. folia Linn (Family: Verbenaceae) in English is named as three-leaved chase tree, called Nirnocci Sirunocci in Tamil. The leaves, roots, essential oils parts are mostly used as a dried whole plant are used in the traditional system of medicine. It is a stout aromatic found throughout the greater part of India, Western Ghats, and Himalaya southwards. It is a shrub or small tree with a growing height of about 1 to 4 meters. The leaves of the 3-foliate of Vitex negundo closely resemble V. trifolia [3, 4]. These leaves are used for rheumatic pain and inflammation [5, 6] and have anti-inflammatory, sedative-hypnotic activity [7] etc. This plant has already proven its free radical scavenging and in vitro antioxidant activity [8], hepatoprotective activity [9], wound healing activity [10] and anticancer activity [11]. The prime active constituents of the plant are isab宪etrimne-3-ol, beta-sitosterol, dicydrosisaligenone, friedelin, isovetixen, rotundiduran, vitetrifolin-A, vitetrifolin-B, vitetrifolin-C, vityexcarpin [12-14]. The nematodes plasma membrane nematodes biosynthesized from phosphatidylcholine [2]. This phosphatidylcholine serves as a precursor for the production of glycol, reverted by the nematodes to avoid host immune response. In phosphatidylcholine biosynthesis, phosphoethanolamine methyltransferase enzyme is responsible for methylation of phosphatase [15, 16]. The Phosphoethanolamine methyltransferase (4FGZ) possesses a single methyl-transferase domain that methylates all three phosphatases [17, 18] and has been shown to be essential for the growth and sexual reproduction of the parasite [19]. In silico molecular docking technique plays an important role in the drug design and discovery to predict the conformations of each ligand molecule at the active site; hence, the in silico (molecular docking) studies of newly synthesized compounds. We have carried out the evaluation of phosphoethanolamine methyltransferase inhibitory activity of those phytoconstituents present in V. trifolia Linn and results are reported. The objective of the present study is an attempt to the in vitro anthelmintic and antibacterial studies of total methanolic leaf extract of V. trifolia Linn and examine its activities phosphoethanolamine methyltransferase inhibitory activity of its phytoconstituents by in silico docking studies using AutoDock 4.2.

MATERIALS AND METHODS

Collection and processing of plant material

The V. folia Linn leaves were collected in the month of December 2015, from our college botanical garden in Anaikuttam, Sivakasi, Tamilnadu. Then it’s authenticated by Mr. V. Ganesan, M. Sc., Ph. D., Associate professor, and Head, a center for research and PG studies
in botany, Ayyanadar Janaki Ammal College, Swalkasi, Tamilnadu, India. A voucher specimen (number: 2015/12/C6G012) has been maintained in our lab for future reference.

Preparation of crude extracts

The leaves of *V. folia* Linn after the collection was cleaned and removed of the adhering materials and allowed to shade dried, coarsely powdered and first extracted with hexane for removal of fatty and coloring material, then marc was dried and packed in the Soxhlet extractor. The packed material was extracted successively with methanol. After completion of the extraction, these extracts were allowed to undergo the distillation process for recovering the solvent and concentrate the extract. The concentrated extract was dried under vacuum in desiccators containing anhydrous calcium chloride. The dried products were weighed in order to determine the percentage of yield. The color and consistency were noted and the percentage yield was calculated [20].

Animal and instruments

In Indian adult earthworm (*Phereetima posthuma*) was chosen for this study, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [21]. Student’s organ bath (it’s consists of an outer jacket, made up of Perspex; the inner organ bath made up of glass with a capacity 40 ml, length 40 cm and width 6 cm; thermostatically controlled heating rod; stirrer, glass coil, and tissue holder), Sherrington rotating drum and frontal writing lever for the organ bath method.

Details of software

Python 2.7-language was downloaded from www.python.com, Cygwin (data storage) c: \program and Python 2.5 were simultaneously downloaded from www.cygwin.com, Molecular graphics laboratory (MGL) tools and Auto Dock 4.2 was downloaded from www.scripps.edu, Discovery studio visualizer 2.5.5 was downloaded from www.accelerys.com, Molecular orbital package (MOPAC), Chemsketch was downloaded from www.acdlabs.com. Online smiles translation was carried out using cactus.ncl.nih.gov/translate/[22, 23].

Determination of extraction yield (% yield)

The yield (% w/w) from all the dried extracts was calculated as:

\[
\text{Percentage yield} (\%) = \frac{(W_1 \times 100)}{W_2}
\]

Where \(W_1\) is the weight of the extract obtained after evaporation of the solvent; and \(W_2\) is the weight of the plant powder. The extracts were kept in air-tight containers to avoid the loss of any volatile principles or/and activities until further use.

Antibacterial activity

Antibacterial screening was carried out in the total methanolic leaf extract of *V. folia* by Kirby-Bauer disc diffusion method in three different concentrations (4 µg, 8 µg, 16 µg/disc) against organisms such as *Shigella*, *staphylococcus*, *streptococci*, *Streptococcus pneumoniae* (gram-positive) *Haemophilus influenzae*, *klesbiella*, *Pseudus vulgaris* and *Salmonella typhi* (gram-negative). Inoculation on Mueller-Hinton agar plate was done by the streaking technique method. The entire agar surface was streaked with the help of a glass coil, and tissue holder using a piece of thread; the upper part was also tied to the aeration tube make sure aeration doesn’t interrupt the response. Assemble the Sherrington rotating drum was assembled; kymograph and adjust the frontal writing lever were adjusted to a magnification value below 5 for recording the response. The lower part of the worm was tied to the hook of the tissue holder using a piece of thread; the upper part was also tied to the recording lever using thread. Oxygen was bubbled through the aerotube to make sure the experiment was not interrupted. With sufficient counterweight applied to the lever, the worm was kept upright in the organ bath. The responses were recorded in smoked papers fixed to the drum. The speed of the drum was adjusted to 0.12 mm/Sec by changing the gear in the Sherrington rotating drum. Let replace the water using normal saline an organ bath, spontaneous movement of the worm was recorded in kymograph this response served as a control. Then the response of worms in the presence of standard drug Piperazine citrate (10 mg/ml) and various concentrations of total methanolic leaf extract of *V. folia* 25, 50, 100 mg/ml was recorded, the fresh worm was used for every experiment. Time for paralysis (seen as a decrease in spontaneous movement and no movement respectively) of the worm was recorded on a slow-moving Sherrington rotating drum. Declined response represented the termination of the experiment.

Molecular docking study

Molecular simulation based on docking was performed using Autodock 4.2 software package. For the docking studies, the structures of the compounds were generated from Chemsketch software. The known crystal structure of the enzyme (PDB ID: 4FGZ) was obtained from the Protein Data Bank. Autodock 4.2 suite of programs which utilizes the Lamarckian Genetic Algorithm was implemented for the docking studies of phosphoethanolamine methyltransferase inhibitor activity. In the initial stage of docking, all the water molecules were removed and the hydrogen atoms were added, followed by computing Gasterger charges, as required in the Lamarckian Genetic Algorithm. For the docking analysis, the grid size was set to 70 Å, 70 Å and 70 Å along X Y and Z-axis with 0.375 Å grid spacing. The docking parameters used were as follows: GA population size = 100 and the maximum number of energy evaluation = 2,500,000, other parameters used were default values. The lowest binding energy conformation was searched out and used for further analysis [29-31].

Statistical analysis

The data obtained were expressed as mean±SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparisons test.
by Dunnett’s test. At 95% confidence interval, p values <0.001 were considered significant [31].

RESULTS

Extraction yield (% yield) of the various extracts
The percentage yield of the total methanolic extract of *V. trifolia* was 31.12% w/w. Its greenish-black color and sticky in nature. The extracts were kept in airtight containers to avoid the loss of any volatile principles or/and activities until further use.

Evaluation of antibacterial activity
The antibacterial screening was carried out in the total methanolic leaf extract of *V. trifolia* by Kirby-Bauer Disc Diffusion Method in three different concentrations (4 µg, 8 µg, 16 µg/disc) against various gram-positive and gram-negative bacteria, then the diameter (in mm) of the zone of inhibition was recorded. This zone of inhibition value was compared with standard ciprofloxacin 5µg/disc and solvent blank. All the selected concentration (4 µg, 8 µg, 16 µg/disc), standard drug ciprofloxacin (5 µg/disc) significantly inhibited the bacterial growth, but the solvent blank disc did not inhibit the bacterial growth against both gram-positive and gram-negative bacteria (fig. 1). Thus indicating that the extract had antibacterial activity. All the selected concentrations showed antibacterial efficacy, particularly 16 µg/disc of *V. trifolia* extract showed potent inhibition against all the eight strains when compared with 4, 8 µg/disc. From the observation, the zone of inhibition (ZOI) was measured and it has been tabulated (table 1) and it was found that the ZOI of the extract was found to be varying between 7-14 mm, with respect to most of the test bacteria. A comparison with solvent blank and standard antibiotic ciprofloxacin (5µg/disc) was recorded (fig. 2).

From the results of ZOI values and their comparison to that of the standard ciprofloxacin, it is evidenced that the total methanolic extract of *V. trifolia* was effective against gram-positive and gram-negative bacteria. The phytoconstituents of the plant may be responsible for this antibacterial activity.

![Fig. 1: Antibacterial activity of the total methanolic leaf extract of *Vitex trifolia* against various bacterial strains by kirby-bauer disc diffusion method, A-4 µg/disc, B-8µg/disc, C-16µg/disc, Blank-Solvent Control, S-ciprofloxacin 5µg/disc](image1)

![Fig. 2: Antibacterial activity of various concentrations of total methanolic leaf extract of *Vitex trifolia* against various bacterial strains by Kirby-bauer disc diffusion method](image2)
Table 1: Antibacterial activity of total methanolic leaf extract of *Vitex trifolia* against various bacterial strains by Kirby-Bauer disc diffusion method

| Organism                        | Zone of inhibition (in mm) | Blank | Total methanolic extract of *Vitex trifolia* |
|---------------------------------|-----------------------------|-------|---------------------------------------------|
|                                 | Standard (Ciprofloxacin 5µg/disc) |       | 4 µg/disc | 8 µg/disc | 16 µg/disc |
| *Shigella*(G⁺)                  | 23.22±0.61                   | -     | 7.12±0.25 | 9.65±0.54 | 10.20±0.72 |
| *Staphylococcus* (G⁻)           | 33.85±1.02                   | -     | 11.28±0.35 | 13.23±0.25 | 14.11±0.76 |
| streptococci(G⁺)                | 20.56±0.95                   | -     | 8.89±0.89 | 9.11±0.88 | 9.62±0.88 |
| *Streptococcus pneumonia* (G⁻)  | 32.23±0.67                   | -     | 11.75±0.45 | 12.64±0.92 | 13.57±0.64 |
| *Haemophilus influenza* (G⁻)    | 23.11±0.52                   | -     | 7.05±0.15 | 7.21±1.02 | 7.72±0.51 |
| *Klebsiella*(G⁻)                | 28.83±0.97                   | -     | 12.86±0.97 | 12.94±0.58 | 13.59±0.37 |
| *Proteus vulgaris* (G⁻)         | 24.18±1.12                   | -     | 7.87±0.61 | 7.64±0.67 | 10.61±0.54 |
| *Salmonella typhi* (G⁻)         | 27.38±1.09                   | -     | 13.63±0.28 | 14.28±0.46 | 13.34±0.90 |

G⁺-Gram-Positive Bacteria, G⁻-Gram-Negative Bacteria, Each value was represented as mean±SEM, n=6 independent experiments

Fig. 3: Anthelmintic activity of the total methanolic leaf extract of *vitre trifolia* Linn against Indian adult worms (*Pheretima posthuma*) by organ bath method, A-Paralysing Response of *P. posthuma* against saline and Piperazine citrate (10 mg/ml), B and C-Paralysing Response of *P. posthuma* against saline and total methanolic leaf extract of *Vitex trifolia* at different concentrations (25, 50, 100 mg/ml)
In vitro anthelmintic assay

Anthelmintic activity by petri dish method

Anthelmintic activity was carried out for three different concentrations (25, 50, 100 mg/ml) a total methanolic leaf extract of V. trifolia by petri dish method against adult Indian worms Pherepagina posthuma, Piperazine citrate (10 mg/ml) was used as a standard reference. Observations were made for the time of paralysis of individual worms against various concentrations of the extract and standard. Paralysis was assumed to have occurred when the worms did not revive even in normal saline. The selected concentrations (25, 50, 100 mg/ml) produced an anthelmintic activity (table 2). The anthelmintic activity increased in a dose-dependent manner.

Anthelmintic activity by organ bath method

Earthworms are invertebrates composed of many segments. It has a special layer that being slimy, enables the earthworm for spontaneous movement. This motility response was recorded in the kymograph during the treatment (fresh worms were used for every sample). As shown in fig. 3 the worms showed normal spontaneous movement in saline treatment. On treatment with a standard drug (Piperazine citrate 10 mg/ml) and various concentrations of methanolic extract of V. trifolia 25, 50, 100 mg/ml worms muscle started to paralyzed which was represented in the response in kymograph, by a declined response from baseline graph (table 2). All the selected concentrations (25, 50, 100 mg/ml) significantly produced a paralyzing effect on worms. At the same time, worms showed quick paralysis time was very short in high concentration. So it produced the anthelmintic activity in a dose-dependent manner.

Table 2: Anthelmintic activities of the total methanolic leaf extract vitex trifolia against Indian adult worms (Pherepagina posthuma) by petri dish method

| Groups               | Worms | Time taken for paralysis (in min) | Time taken for paralysis mean±SEM |
|----------------------|-------|----------------------------------|-----------------------------------|
| Solvent Blank        | A     | -                                | -                                 |
|                      | B     |                                  |                                   |
|                      | C     |                                  |                                   |
|                      | D     |                                  |                                   |
| Piperazine Citrate 10 mg/ml | A     | 20.45                            | 21.58±0.61                        |
|                      | B     | 21.03                            |                                   |
|                      | C     | 21.54                            |                                   |
|                      | D     | 23.32                            |                                   |
| 25 mg/ml             | A     | 14.11                            | 13.78±0.68                        |
|                      | B     | 12.24                            |                                   |
|                      | C     | 15.49                            |                                   |
|                      | D     | 13.31                            |                                   |
| 50 mg/ml             | A     | 4.51                             | 5.79±0.52                         |
|                      | B     | 6.09                             |                                   |
|                      | C     | 7.01                             |                                   |
|                      | D     | 5.58                             |                                   |
| 100 mg/ml            | A     | 5.01                             | 4.5 ±0.27                         |
|                      | B     | 4.11                             |                                   |
|                      | C     | 4.08                             |                                   |
|                      | D     | 5.10                             |                                   |

Each value was represented as mean±SEM, n=6 independent experiments

In silico docking studies results

In silico docking studies of the 11 selected phytoconstituents of V. trifolia were carried out with phosphoethanolamine methyltransferase (4FGZ).

The selected phytoconstituents had various types of functional groups and most of the functional groups possessed good phosphoethanolamine methyltransferase inhibitor activity. Among them, Abietatriene-3-ol showed higher phosphoethanolamine methyltransferase inhibitory activity. Moreover among the selected 11 compounds, two compounds showed binding energy value between -6 to -7 kcal/mol, three compounds showed values between -7 to -8 kcal/mol, four compounds showed values between -8 to -9 kcal/mol, a compound showed values between -9 to-10 kcal/mol and another compound showed values above -10 kcal/mol (table 3).

Table 3: Docking parameters of compounds in phosphoethanolamine methyltransferase (4FGZ)

| Ligands               | Binding energy (kCal/mol) | Inhibitory constant (µM) | Intermolecular energy (kCal/mol) |
|-----------------------|---------------------------|--------------------------|----------------------------------|
| Phosphoethanolamine   | -6.03                     | 38.29 µM                 | -7.82                            |
| Abietatriene-3-ol     | -10.25                    | 30.91 nM                 | -10.84                           |
| Artemetin             | -7.33                     | 4.26µM                   | -9.42                            |
| Beta-sitosterol       | -8.19                     | 984.3 nM                 | -10.28                           |
| Dihydroxilotagengone  | -9.3                      | 152.9 nm                 | -10.49                           |
| Friedelin             | -7.28                     | 4.58µM                   | -9.37                            |
| Isovitexin            | -6.56                     | 15.65µM                  | -9.54                            |
| Rotundiduran          | -6.87                     | 9.14µM                   | -8.66                            |
| Viteritrifolin-A      | -8.13                     | 1.1µM                    | -8.73                            |
| Viteritrifolin-B      | -8.93                     | 284.06nM                 | -11.02                           |
| Viteritrifolin-C      | -7.99                     | 1.4µM                    | -9.78                            |
| Vitexycarpin          | -8.14                     | 1.07µM                   | -10.23                            |
Table 4: Docking parameters of compounds and binding site in phosphoethanolamine methyltransferase

|       | Phosphoethanolamine | Abietatriene-3-ol | Artemetin | Betasitosterol | Dihydrosolidagenone | Friedelin | Isovitexin | Rotundiduran | Vitetrifolin-A | Vitetrifolin-B | Vitetrifolin-C | Vitexin | Vitexycarpin |
|-------|---------------------|-------------------|-----------|----------------|---------------------|-----------|------------|--------------|----------------|----------------|----------------|---------|-------------|
| 34 ASN| 14 LEU              | 10 ASP            | 19 TYR    | 14 LEU         | 10 ASP              | 14 LEU    | 10 ASP     | 14 LEU       | 10 ASP         | 10 ASP         | 10 ASP         | 10 ASP  |
| 35 TYR| 19 TYR              | 14 LEU            | 35 TYR    | 19 TYR         | 19 TYR              | 14 LEU    | 19 TYR     | 19 TYR       | 14 LEU         | 14 LEU         | 14 LEU         | 14 LEU  |
| 36 ILE| 36 ILE              | 19 TYR            | 36 ILE    | 65 GLY         | 37 SER              | 36 ILE    | 63 GLY     | 63 GLY       | 19 TYR         | 63 GLY         | 63 GLY         | 19 TYR  |
| 37 SER| 63 GLY              | 36 ILE            | 37 SER    | 85 ASP         | 62 ILE              | 37 SER    | 85 ASP     | 85 ASP       | 62 ILE         | 85 ASP         | 85 ASP         | 62 ILE  |
| 61 ASP| 85 ASP              | 37 SER            | 63 GLY    | 86 ILE         | 86 ILE              | 86 ILE    | 86 ILE     | 86 ILE       | 86 ILE         | 86 ILE         | 86 ILE         | 35 TYR |
| 63 GLY| 86 ILE              | 62 ILE            | 64 SER    | 111 ILE        | 111 ILE             | 111 ILE   | 111 ILE    | 111 ILE      | 111 ILE        | 111 ILE        | 111 ILE        | 111 ILE |
| 64 SER| 90 ILE              | 63 GLY            | 66 LEU    | 129 ALA        | 85 ASP              | 109 ASP   | 90 ILE     | 132 HIS      | 86 ILE         | 132 HIS        | 86 ILE         | 38 SER  |
| 66 LEU| 109 ASN             | 65 GLY            | 85 ASP    | 132 HIS        | 86 ILE              | 110 ASP   | 109 ASP    | 133 LEU      | 111 ILE        | 110 ASP        | 132 HIS        | 63 GLY  |
| 68 GLY| 110 ASP             | 85 ASP            | 86 ILE    | 133 LEU        | 90 ILE              | 111 ILE   | 110 ASP    | 132 HIS      | 64 SER         | 132 HIS        | 63 GLY         | 63 GLY  |
| 69 GLY| 111 ILE             | 86 ILE            | ARG 127   | 111 ILE        | 128 ASP             | 111 ILE   | 128 ASP    | 111 ILE      | 128 ASP        | 128 ASP        | 128 ASP        | 128 ASP |
| 129 ALA| 90 ILE             | 128 ASP           | 128 ASP   | 129 ALA        | 129 ALA             | 129 ALA   | 129 ALA    | 129 ALA      | 129 ALA        | 129 ALA        | 129 ALA        | 129 ALA |
| 132 HIS| 128 ASP            | 132 HIS           | 132 HIS   | 133 LEU        | 133 LEU             | 133 LEU   | 133 LEU    | 133 LEU      | 133 LEU        | 133 LEU        | 133 LEU        | 133 LEU |
| 133 LEU| 129 ALA            | 132 HIS           | 132 HIS   | 133 LEU        | 133 LEU             | 133 LEU   | 133 LEU    | 133 LEU      | 133 LEU        | 133 LEU        | 133 LEU        | 133 LEU |
|       | 130 ILE             | 133 LEU           | 133 ILE   | 133 LEU        | 133 LEU             | 133 LEU   | 133 LEU    | 133 LEU      | 133 LEU        | 133 LEU        | 133 LEU        | 133 LEU |

A -6.03  -10.25  -7.33  -8.19  -9.3  -7.28  -6.56  -6.87  -8.13  -8.93  -7.99  -0.33  -8.14  
B 38.29µM  30.9 nm  4.26µM  984.3 nm  152.9 nm  4.58µM  15.65µM  9.14µM  1.1µM  284.06nM  1.4µM  570.7  1.07µM  5m  
C -7.82  -10.84  -9.42  -10.28  -10.49  -9.37  -9.54  -8.66  -8.73  -11.02  -9.78  -3.32  -10.23  

Fig. 4: Docked pose of phosphoethanolamine methyltransferase (4FGZ) with phosphoethanolamine and phytoconstituents of *Vitex trifolia* linn, A-Phosphoethanolamine, B-Abietatriene-3-ol, C-Artemetin, D-Beta-sitosterol, E-Dihydrosolidagenone, F-Friedelin, G-Isovitexin, H-Rotundiduran, I-Vitetrifolin-A, J-Vitetrifolin-B, K-Vitetrifolin-C, L-Vitexycarpin
Binding energy = A + B + C - D

where, A indicates the sum of intermolecular energy, Vanderwaals energy (vdW), outer layer bonds, desolvation energy, and electrostatic energy (kcal/mol), final total internal energy (kcal/mol) denoted as B, the torsional free energy (kcal/mol) mentioned as C, unbound system's energy (kcal/mol) marked as D. In addition, various parameters like inhibitory constant (Kᵢ), intermolecular energy, electrostatic energy, total internal energy, torsional energy, and desolvation energy are bound and ref RMS were determined. Inhibition constant (Kᵢ) is directly proportional to the binding energy. When the compounds inhibitory activity increased, there was a decrease in its binding energy. Similarly, intermolecular energy is directly proportional to the binding energy, i.e., lesser the intermolecular energy, lesser the binding energy. The other parameters like electrostatic energy, total internal energy, torsional energy, unbound external energy, cluster RMS and ref RMS is independent of the binding energy. The values of standard compound phosphoethanolamine (value of A--binding energy, B--inhibition constant, C-intermolecular energy).

Binding energy, inhibitory constant (Kᵢ), inter molecular energy (--6.03 kcal/mol, 38.29 µM, -7.82), were compared to abietatriene-3-ol (-10.25 kcal/mol, 30.91nM, -10.84), dihydrosolidagenone (-9.3 kcal/mol, 152.9nM, -10.43), vitetrifolin-A, and vitetrifolin-B (-9.3 kcal/mol, 284.06nM, -11.02). Among the selected compounds, abietatriene-3-ol, dihydrosolidagenone and vitetrifolin-B possess very potent phosphoethanolamine methyltransferase inhibitory activity.

**DISCUSSION**

Preliminary phytoconstituents of *V. trifolia* methanolic extract revealed the presence of alkaloids, coumarins, flavonoids, terpenoids, polyphenols, saponins, and tannins. Also, this plant was proved to possess various active phytoconstituents [5, 6, 14].

In the antibacterial test, the total methanolic extract of *V. trifolia* was screened for its antibacterial activity against organisms such as *Shigella*, *Staphylococcus*, *streptococci*, *Streptococcus pneumoniae* (gram-positive) *Haemophilus influenzae*, *klebsiella*, *Proteauvargutta* and *Salmonellatyphi* (gram-negative) by Kirby-Bauer disc diffusion method. The antibacterial study revealed that (table 1) the total methanolic extract exhibited antibacterial activity, even in low concentration (4mcg/disc). At 1 mcg/disc the extract showed a significant antibacterial effect, compared to blank and other low concentration (4mcg/disc). At 16mcg/disc the extract showed a decrease in its binding energy, compared to standard drug Piperazine citrate. The worms paralysis time and decreased for active site or not [35, 36]. Normally, the thumb rule indicates that compounds have bound in the active site of the enzyme by both hydrogen bond and n-hydrophobic interactions, which means that compounds have significant biological activities. From the molecular docking studies, the potential binding sites of the phosphoethanolamine methyltransferase enzyme is clearly shown (table 4) and bound the enzyme to be 34 ASN, 35 TYR, 36 ILE, 37 SER, 61 ASP, 63 GLY, 64 SER, 66 LEU, 68 GLY, 69 GLY. This proves that the effective binding sites are bound with the selected phytoconstituents when compared with the phosphoethanolamine. It proves that the selected compounds have the ability to inhibit the phosphoethanolamine methyltransferase enzyme. The flavonoids displayed binding energy ranging between--6.56kcal/mol to-10.25kcal/mol. All the selected compounds showed less significant binding energy when compared to phosphoethanolamine (--6.03kcal/mol). This proves that the phytoconstituents of *V. trifolia* Linn have a potential phosphoethanolamine methyltransferase inhibitory activity when compared to phosphoethanolamine. The molecular docking studies revealed (table 4) that most active compound abietatriene-3-ol was bound to the following sites 36 ILE, 90 ILE, 19 TYR, 14 LEU, 132 HIS, 85 ASP, 129 ALA, 86 ILE, 63 GLY, 133 LEU, 111 ILE, 110 ASP and 109 ASN. The potential binding sites of the phosphoethanolamine were clearly shown (table 4) and bound the enzyme to be 34 ASN, 35 TYR, 36 ILE, 37 SER, 61 ASP, 63 GLY, 64 SER, 66 LEU, 68 GLY and 69 GLY (fig. 4). This proves that the effective binding sites are present in the compound abietatriene-3-ol compared with the phosphoethanolamine, in addition, selected compounds had the ability to inhibit phosphoethanolamine methyltransferase enzyme (table 3). Apart from that two more parameters such as, inhibition constant and intermolecular energy were determined. The phytoconstituents of *V. trifolia* Linn showed inhibition constant, ranging from 15.65µM to 30.91nM. The chosen compounds had less inhibition constant when compared to the phosphoethanolamine (38.29 µM). The inhibition constant is directly proportional to binding energy and observed results indicated that the inhibition constant is decreased simultaneously if binding energy decreased. Thus, the phosphoethanolamine methyltransferase inhibitory activity of the phytoconstituents was found to be higher compared to phosphoethanolamine. The phytoconstituents showed intermolecular energy ranging between--8.66 kcal/mol to-10.84 kcal/mol which was lesser when compared to the phosphoethanolamine (-7.82 kcal/mol). Intermolecular energy is also directly proportional to the binding energy. The observed results indicated that there was a decrease in the intermolecular energy of all the selected compounds with a simultaneous decrease in the binding energy. Based on the docking studies, the phosphoethanolamine methyltransferase inhibitor binding activity of the selected compounds was found to decrease in the order of Isovitexin, Rotundiduran, Friedelin, Artemetin, Vitextrilolin-C, Vitetrilolin-A, Vitexcarpin, Beta-sitosterol, Vitetrilolin-B dihydrosolidagenone, and abietatriene-3-ol. On the basis of the above study, abietatriene-3-ol and dihydrosolidagenone possess potential phosphoethanolamine methyltransferase inhibitor binding sites compared to that of the phosphoethanolamine. The results may
be attributed due to differences in the position of the functional groups in compounds.

V. trifolia has various active phytoconstituents, particularly the methanolic extract contains alkaloids, coumarins, flavonoids, terpenoids, polyphenols, saponins, and tannin. The antibacterial activity was screened to explore that the extract may directly attack the parasite or kill the parasite by stimulating the host defense mechanism. The performed in vitro anthelmintic evaluation report confirmed that the total methanolic extract showed significant anthelmintic activity by paralyzing the worm, due to the expected mechanisms such as cholinergic mediated acetylcholine release or direct binding of acetylcholine into the muscular or nicotinic receptor. The activation of the cholinergic receptor alters the normal depolarisation and repolarisation process and it leads to spastic paralysis. In the organ bath method, it was noticed that the worms had lost their contractile power, maybe due to flaccid paralysis. The flaccid paralysis caused by GABA mediated chloride ion flow through Clion channel and as a result reduces the muscle tone and causes weakness of the muscle. The in vitro anthelmintic evaluation report clearly indicates that the extracts showed their anthelmintic property in a dose-dependent manner which is effective in the treatment of helminthiasis, particularly against adult form. In addition, to strengthen the data to move forward towards in vivo study the drug design screening was performed. In this study, we targeted the phosphoethanolamine methyltransferase enzyme, because the enzyme plays a vital role in the parasite life cycle and enzyme-catalyzed methylation of phosphates during the process of phosphatidylycholine biosynthesis. Phosphatidylycholine is essential for the formation of the plasma membrane in nematodes, thus phosphoethanolamine was used as a standard ligand, and all the selected compounds were docked with an enzyme. The observed results indicated that compounds had less binding energy was compared to phosphoethanolamine. It was so interesting that the docking studies also prove that the total methanolic extracts of V. trifolia and its active constituents showed a significant anthelmintic property.

CONCLUSION

Based on the study report, the authors revealed that the extract showed an anthelmintic effect and proved that the active constituents of V. folia have good phosphoethanolamine methyltransferase inhibitory activity. The report of antibacterial, in vitro and in silico anthelmintic studies confirmed that the total methanolic leaves extract of V. trifolia and its active constituents were having a significant anthelmintic property with the valuable information about its mechanisms of action. Thus V. trifolia active constituents have high significance to be considered as a potential drug candidate in the treatment of helminthiasis, furthermore, it has a great scope to be investigated on different animal models.

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AUTHORS CONTRIBUTIONS

This research work has been designed by Soundarrarajan Muthukrishnan and he act as a Principal investigator, Ragunath and Manoj Kumar Varghese were executed this research work under the guidance of principal investigator.

CONFLICT OF INTERESTS

Declared none

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