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ORIGINAL RESEARCH ARTICLE

Comparative Inhibition Study of Compounds Identified in the Methanolic Extract of *Apamarga Kshara* Against *Trichomonas vaginalis* Carbamate Kinase (TvCK): An Enzoinformatics Approach

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Abstract In the present study, we have identified ten compounds, namely dodecanol acid, myristic acid, neophytadiene, palmitic acid, heptadecanoic acid, linoleic acid, elaidic acid, 3-7-dimethyl acid, stearic acid and methyl eicos acid, of the methanolic extract of *Apamarga Kshara* by GC–MS analysis. *Apamarga Kshara* has been reported to be active against cervical erosion. Major causal organism for cervical erosion is *Trichomonas vaginalis*. However, there is a paucity of information about the mechanism of action and inhibitory effect of the biologically active natural compounds presented in *A. Kshara* against this organism (*T. vaginalis*). Therefore, present investigation was conducted to observe possible interactions of these compounds on *T. vaginalis* carbamate kinase using molecular docking software 'AutoDock 4.2.' Identification of the amino acid residues crucial for the interaction between *T. vaginalis* carbamate kinase and these natural compounds is of due scientific interest. The study will aid in efficacious and safe clinical use of the above-mentioned compounds.

Keywords *Apamarga Kshara* · AutoDock 4.2 · Cervical erosion · *Trichomonas vaginalis* carbamate kinase

1 Introduction

Natural compounds obtained from higher plants can be used as lead for designing and rational planning of drugs with novel mode of action and new therapeutic properties [1]. A frequent finding during the routine pelvic examination of females is a condition of cervical erosion, especially during their fertile years [2–4]. Vaginal discharge, disturbances of micturition, dyspareunia, postcoital bleeding and backache are some of the common symptoms of cervical erosion [5]. There is a diversity of plant-based natural compounds which have been reported to possess anti-cervical erosion properties. However, revealing their modes of action is still a difficult task for medicinal chemists and pharmacologists. Molecular docking study, which has been an important part of drug discovery research, may play an imperative role in digging out lead compounds from these natural compounds against cervical erosion [6].

In the present investigation, we have identified ten compounds, namely dodecanol acid, myristic acid, neophytadiene, palmitic acid, heptadecanoic acid, linoleic

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acid, elaidic acid, 3-7-dimethyl acid, stearic acid and methyl eicos acid, by GC–MS analysis in the methanolic extract of *Apamarga Kshara*, which has been reported to be active against cervical erosion [7, 8]. *Trichomonas vaginalis* is a microaerophilic protozoan parasite which causes both acute and chronic vaginitis and is one of the major causative organisms responsible for cervical erosion [9]. Therefore, the present investigation was conducted to observe possible interactions of these compounds with carbamate kinase enzyme of *Trichomonas* using molecular docking study. The enzyme *T. vaginalis* carbamate kinase (TvCK) is produced by *T. vaginalis.* The molecular size of TvCK is 314 amino acids, and molecular mass is 33.92 kDa. TvCK has involved in the different metabolic pathways such as purine, arginine, proline and nitrogen metabolisms of the *T. vaginalis*. These metabolic pathways in trichomonads cause the rapid depletion of arginine in vaginal and seminal fluid with an accompanied production of putrescine. This carbamate kinase pathway has been reported in many prokaryotes and two primitive eukaryotes, namely *Giardia lamblia* and *T. vaginalis* [10, 11], and recently, *G. lamblia* carbamate kinase protein was crystallized [12].

Such information is expected to aid in optimizing the safe and efficacious use of these natural compounds present in *A. Kshara*. Furthermore, this study would be useful for scientists involved in drug design in their ongoing search for more potent and versatile TvCK inhibitors. Currently, no crystal structure is available with the Protein Data Bank to aid in the characterization of the interaction between TvCK and the compounds present in *A. Kshara*. In the present study, the natural compounds identified by GC–MS analysis were used as ligands for targeting TvCK for the selection of most active inhibitor out of ten compounds. This will suggest us the best TvCK inhibitor which might serve as a potential lead compound for further analysis and possible future pharmaceutical applications.

2 Methodology

2.1 *Apamarga Kshara*

Apamarga Kshara powder was obtained from Dr. Pragya Gupta, Gynecologist, St. Mary Polyclinic, Lucknow, India.

2.2 Preparation of Plant Extracts

Apamarga Kshara powder (1 gm) was homogenized in 10 ml methanol (HPLC grade, Merck, India) and was extracted on a rotatory shaker in an Erlenmeyer flask at 40 rpm overnight [13]. The crude extract was then filtered through Whatman No. 1 filter paper and concentrated in vacuum at 40 °C using a rotary evaporator. The extract was concentrated aseptically with the help of drier.

2.3 GC–MS Analysis

For the identification of metabolites showing anti-cervical erosion activity, the samples were subjected to GC–MS analysis. The crude extract (1 µl) was injected into a RTX-5 column (60 m × 0.25 mm i.d., film thickness 0.25 µm) of GC–MS (model GC–MS-QP-2010 plus, Shimadzu Make). Helium was used as carrier gas at a constant column flow 1.2 ml/min at 173 kPa inlet pressure. Temperature programming was maintained from 100 to 200 °C with constant rise of 5 °C/min and then held isothermal at 200 °C for 6 min; further the temperature was increased by 10 °C/min up to 290 °C and again held isothermal at 290 °C for 10 min. The injector and ion source temperatures were 270 and 250 °C, respectively. The sample was injected with a split ratio of 1:10. Mass spectra were taken at 70 eV, a scan interval of 0.5 s and fragments from 40 to 950 Da. The final confirmation of constituents was made by computer matching of the mass spectra of peaks with the Wiley and National Institute Standard and Technology libraries mass spectral database.

2.4 Molecular Docking Study

To determine how the structures of natural compounds: 1-dodecanol, myristic acid methyl ester, neophytadiene, palmitic acid, heptadecanoic acid, linoleic acid, elaidic acid, 3-7-dimethyl acid, stearic acid and methyl eicos acid, identified in the methanolic extract of *A. Kshara* contribute to their differential inhibitory activity against TvCK, virtual docking analysis with the kinase domain of TvCK was performed. The three-dimensional structure of TvCK was prepared by using the automated mode of SWISS-MODEL workspace after retrieving amino acid sequence from UniProt (ID: 096432) for docking studies. The Protein Data Bank (PDB) coordinates for the structure of substrate adenosine diphosphate (ADP) (CID: 6022) were retrieved from 'Pub Chem' database. PDB structure of natural compounds dodecanol [ChemSpider ID-7901], myristic acid [ChemSpider ID-290234], neophytadiene [ChemSpider ID-10014], palmitic acid [ChemSpider ID-7889], heptadecanoic acid [ChemSpider ID-99139], linoleic acid [ChemSpider ID-4575738], elaidic acid [ChemSpider ID-4444205], 3-7-dimethyl acid [ChemSpider ID-4937713], stearic acid [ChemSpider ID-7909] and methyl eicos acid [ChemSpider ID-29756772] were retrieved from ChemSpider. Thereafter, each of these ligands (natural compound) was docked to the enzyme TvCK using 'AutoDock 4.2,' individually. The MMFF94 force field was used for energy minimization of each of the ligand molecules. Gasteiger

partial charges were added to the ligand atoms. Nonpolar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were done on the protein model. Essential hydrogen atoms, Kollman united atom type charges and solvation parameters were added with the aid of AutoDock tools. Affinity (grid) maps of 60 Å × 60 Å × 60 Å grid points and 0.375 Å spacing were generated using the AutoGrid program aimed to target grid coordinates in proximity with the ATP-binding pocket of the catalytic site of TvCK. AutoDock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the 'Lamarckian genetic algorithm' and the 'Solis and Wets local search method.' Initial position, orientation and torsions of the ligand molecules were set randomly. Each docking experiment was derived from 100 different runs that were set to terminate after a maximum of 2,500,000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied. The final figures were generated using Discovery Studio 2.5 (Accelrys).

3 Results and Discussion

The GC–MS analysis of methanolic extract of *A. Kshar*a as shown in Fig. 1 indicates the presence of ten different components. The components present in the methanolic extract of *A. Kshara* were dodecanol (RT 9.872), myristic acid (RT 14.456), neophytadiene (RT 16.317), palmitic acid (RT 17.801), heptadecanoic acid (RT 19.427), linoleic acid (RT 20.453), elaidic acid (RT 20.543), stearic acid (RT 20.938), 3-7-dimethyl acid (RT 20.783) and methyl eicos acid (RT 21.325) (Table 1). Similar component has also been reported in different plants extracts [14–16].

The virtual docking results indicated that neophytadiene exhibited strong binding to the catalytic domain of TvCK. The catalytic domain of TvCK was found to interact with neophytadiene acid through the 15 amino acid residues G50, N51, G79, S164, G191, G193, A210, V211, I212, D213, K214, D215, K270, G271 and P275 (Fig. 2; Table 2). The free energy of binding (ΔG) and estimated inhibition constant (Ki) for the 'neophytadiene–TvCK domain interaction' were determined to be −5.99 kcal/mol and 40.47 μM, respectively. Total intermolecular energy of docking for 'neophytadiene–TvCK catalytic domain interaction' was found to be −8.61 kcal/mol. 'Hydrogen bond' and 'desolvation' energy components together contributed −8.62 kcal/mol, while the 'electrostatic' energy component was found to be 0.01 kcal/mol.

Other compounds, namely elaidic acid, linoleic acid, dodecanol acid, stearic acid, methyl eicos acid, myristic acid, heptadecanoic acid, 3-7-dimethyl acid and palmitic acid, were also used as ligand to interact with catalytic domain of TvCK. The catalytic domain of TvCK enzyme was found to interact with elaidic acid through 18 amino acid residues, namely G8, G9, G50, N51, G52, P53, K128, S164, G191, V211, I212, D213, K214, D215, G271, S273, P275 and K276 (Fig. 3; Table 2), while linoleic acid was found to interact with 22 amino acid residues, namely G8, G9, G50, N51, G52, P53, C55, K70, G79, K128, G191, G193, A210, V211, I212, D213, K214, L216, T234, G271, K276 and P275 of TvCK enzyme (Fig. 4; Table 2). The free energy of binding (ΔG) and estimated inhibition constant (Ki) for the 'elaidic acid–TvCK catalytic domain interaction' were determined to be −3.78 kcal/mol and 1710 μM, respectively. However, the same for the 'linoleic acid–TvCK catalytic domain interaction' were found to be −4.74 kcal/mol and 335.39 μM, respectively. The corresponding total intermolecular energy of docking was found to be −6.75 and −8.22 kcal/mol, respectively. 'van der Waals,' 'hydrogen bond' and 'desolvation' energy were found to be −6.36 and −7.84 kcal/mol with the 'electrostatic' energy component being −0.39 and −0.38 kcal/mol, respectively.

In contrast, 17 amino acid residues, namely G8, G9, L12, G50, P53, Q54, F59, L75, L78, G191, P195, A210, V211, I212, D213, K214 and N512, of catalytic domain of TvCK were found to be interacting with dodecanol acid (Fig. 5; Table 2). Free binding energy (ΔG) and estimated inhibition constant (Ki) were determined to be −4.29 kcal/mol of 721.12 μM, respectively. Total intermolecular energy of docking for 'dodecanol acid–TvCK catalytic domain interaction' was observed to be −6.95 kcal/mol. 'van der Waals,' 'hydrogen bond' and 'desolvation' energy components together contributed −6.75 kcal/mol with the 'electrostatic' energy component being −0.19 kcal/mol. The catalytic domain of TvCK was determined to interact with stearic acid through 20 amino acid residues, namely L7, G8, G9, N51, L55, L75, G191, A210, V211, I212, D213, K214, D215, M216, T234, D235, V236, G271, S272 and K276 (Fig. 6; Table 2). It gives free binding energy (ΔG) of −3.58 kcal/mol and estimated inhibition constant (Ki) of 2360 μM. Total intermolecular energy of docking for 'stearic acid–TvCK catalytic domain interaction' was found to be −7.57 kcal/mol. 'van der Waals,' 'hydrogen bond' and 'desolvation' energy components together contributed −7.44 kcal/mol with the 'electrostatic' energy component being −0.14 kcal/mol.

On the other hand, catalytic domain of TvCK was observed to interact with methyl eicos acid through 22 amino acid residues, namely G8, G9, G50, N51, G52, P53, C55, G191, G193, P195, A210, V211, I212, K214, T217, T234, D235, V236, M273, K276, K318 and S727 (Fig. 7; Table 2). It gives free binding energy (ΔG) of

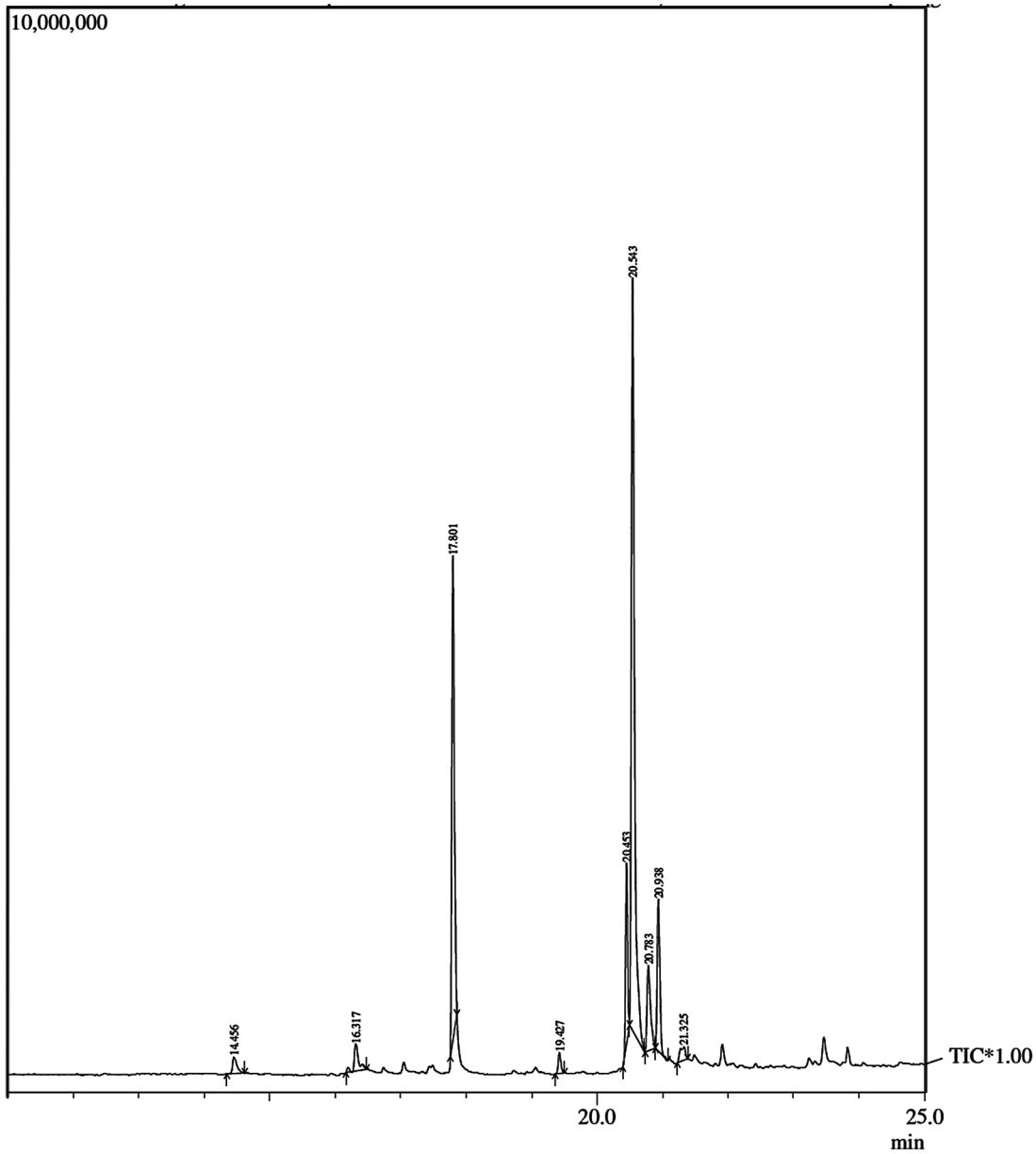
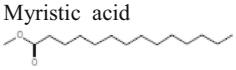
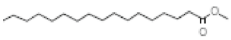
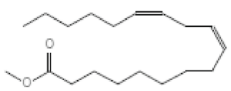
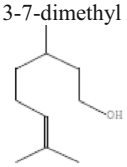
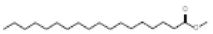
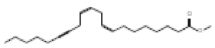


Fig. 1 Chromatograph of methanolic extract of *A. Kshara* taken from GC MS analysis

−4.52 kcal/mol and estimated inhibition constant (Ki) of 485.56 μM against catalytic domain of TvCK. Total intermolecular energy of docking for 'methyl eicos acid–TvCK catalytic domain interaction' was found to be −8.25 kcal/mol. 'van der Waals,' 'hydrogen bond' and 'desolvation' energy components together contributed −8.16 kcal/mol with the 'electrostatic' energy component being −0.01 kcal/mol.

Seventeen amino acid residues, namely G8, G9, G50, N51, G52, G191, T234, V211, I212, D213, K214, D235, G271, S272, K276 and P275, of catalytic domain of TvCK were found to be interacting with myristic acid (Fig. 8; Table 2). Free binding energy (ΔG) and estimated inhibition constant (Ki) were determined to be −3.29 kcal/mol and 3850 μM, respectively. Total intermolecular energy of docking for 'myristic–TvCK catalytic domain interaction' was found to be −6.36 kcal/mol. 'van der Waals,' 'hydrogen bond' and 'desolvation' energy components together contributed −6.06 kcal/mol with the 'electrostatic' energy component being −0.3 kcal/mol. Similarly, 21 amino acid residues, namely G8, G9, N10, N51, G52, G79, K128, S164, G190, G191, G193, I194, P195, A210, V211, I212, D213, K214, L233, T234 and D235, of catalytic domain of TvCK were observed to be interacting with

Table 1 Phytocomponents identified in the methanolic extract of *Apamarga Kshara* by GC–MS analysis

Peak	Compound name	R–time	Peak area (%)
1-	Dodecanol-acid	9.872	6.93
2-	Myristic acid 	14.456	1.28
3-	Neophytadiene 	16.317	2.01
4-	Palmitic acid 	17.801	22.24
5-	Heptadecanoic acid 	19.427	0.94
6-	Linoleic acid 	20.453	7.02
7-	Elaidic acid 	20.543	45.64
8-	3-7-dimethyl acid 	20.783	5.66
9-	Stearic acid 	20.938	6.91
10-	Methyl eicos acid 	21.325	1.35

heptadecanoic acid (Fig. 9; Table 2). Free binding energy (ΔG) and estimated inhibition constant (Ki) were found to be −4.15 kcal/mol 909.96 μM, respectively. Total intermolecular energy of docking was observed to be −7.33 kcal/mol. 'van der Waals,' 'hydrogen bond' and 'desolvation' energy components together contributed −7.28 kcal/mol with the 'electrostatic' energy component being −0.04 kcal/mol.

The catalytic domain of TvCK was determined to interact with 3-7-dimethyl acid through 14 amino acid residues, namely G50, N51, G79, K128, S168, G190, G191, G193, P195, A210, V211, I212, D213 and K214 (Fig. 10; Table 2). It gives free binding energy (ΔG) of −4.54 kcal/mol and estimated inhibition constant (Ki) of 473.64 μM. Total intermolecular energy of docking for '3-7-dimethyl acid–TvCK catalytic domain interaction' was found to be −6.0 kcal/mol. 'van der Waals,' 'hydrogen bond' and 'desolvation' energy components together contributed −5.93 kcal/mol with the 'electrostatic' energy component being −0.07 kcal/mol.

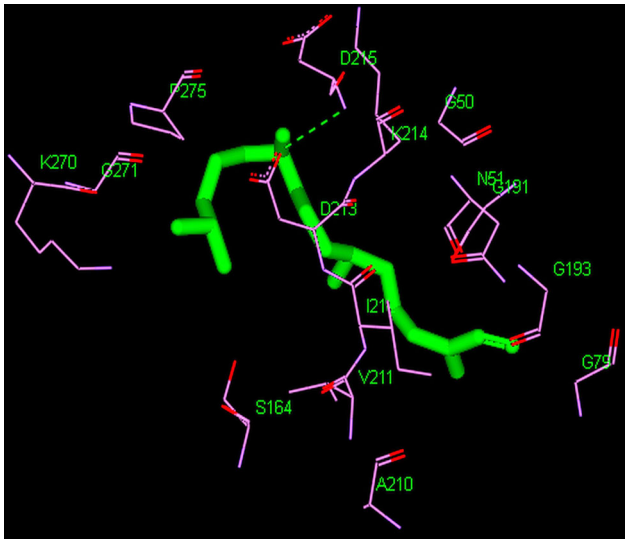


Fig. 2 Neophytadiene docked to the 'catalytic site' of TvCK. The drug is shown in *stick form*. The *broken lines* represent hydrogen bonds. 'Interacting amino acid residues' are labeled. Figure has been produced by Discovery Studio 3.0

Twenty-four amino acid residues, namely G50, N51, C55, F59, G52, L75, H76, C78, G79, S164, G191, G193, P195, A210, V211, I212, D213, K214, D215, K270, G271, S272, P275 and K276, of catalytic domain of TvCK were found to be interacting with palmitic acid (Fig. 11; Table 2). Free binding energy (ΔG) and estimated inhibition constant (Ki) were observed to be −4.19 kcal/mol and 855.67 μM, respectively. Total intermolecular energy of docking for 'palmitic acid–TvCK catalytic domain interaction' was found to be −7.65 kcal/mol. 'van der Waals,' 'hydrogen bond' and 'desolvation' energy components together contributed −7.65 kcal/mol with the 'electrostatic' energy component being −0.01 kcal/mol.

The molecular docking of substrate (ADP) with the active site of enzyme (TvCK) revealed that the amino acid residues, H49, G50, N51, K128, S164, G191, G192, V211, I212, D213, K214, D215, K270, G271, S272, K276, P275, of TvCK played a major role in the binding of ADP (Supplementary Fig. S1). Interestingly, these amino acid residues of TvCK were also found to be involved in the interaction with compounds identified in methanolic extract of *A. Kshara*.

A higher (negative) free energy (ΔG) of binding is an indicator of efficient interaction between an enzyme and inhibitor [17]. Accordingly, the computational assessment concludes that among the ten ligands, neophytadiene showed better binding to TvCK enzyme. Also, it has been established that the results of computational analyses often

Table 2 Amino acid residues involved in natural compounds and TvCK interactions

Compound	Binding energy (kcal/mol)	Inhibition constant (μM)	Interacting amino acids
Neophytadiene	−5.99	40.47	G50, N51, G79, S164, G191, G193, A210, V211, I212, D213, K214, D215, K270, G271, P275
Linoleic acid	−4.74	335.39	G8, G9, G50, N51, G52, P53, C55, K70, G79, K128, G191, G193, A210, V211, I212, D213, K214, L216, T234, G271, K276, P275
Dodecanol	−4.29	721.12	G8, G9, L12, G50, P53, Q54, F59, L75, L78, G191, P195, A210, V211, I212, D213, K214, N512
Palmitic acid	−4.19	855.67	G50, N51, C55, F59, G52, L75, H76, C78, G79, S164, G191, G193, P195, A210, V211, I212, D213, K214, D215, K270, G271, S272, P275, K276
Heptadecanoic acid	−4.15	909.96	G8, G9, N10, N51, G52, G79, K128, S164, G190, G191, G193, I194, P195, A210, V211, I212, D213, K214, L233, T234, D235
Elaidic acid	−3.78	1710	G8, G9, G50, N51, G52, P53, K128, S164, G191, V211, I212, D213, K214, D215, G271, S273, P275, K276
Stearic acid	−3.58	2360	L7, G8, G9, N51, L55, L75, G191, A210, V211, I212, D213, K214, D215, M216, T234, D235, V236, G271, S272, K276
Methyl eicos acid	−4.52	485.56	G8, G9, G50, N51, G52, P53, C55, G191, G193, P195, A210, V211, I212, K214, T217, T234, D235, V236, M273, K276, K318, S727
Myristic acid	−3.29	3850	G8, G9, G50, N51, G52, G191, T234, V211, I212, D213, K214, D235, G271, S272, K276, P275
3-7-Dimethyl acid	−4.54	473.64	G50, N51, G79, K128, S168, G190, G191, G193, P195, A210, V211, I212, D213, K214

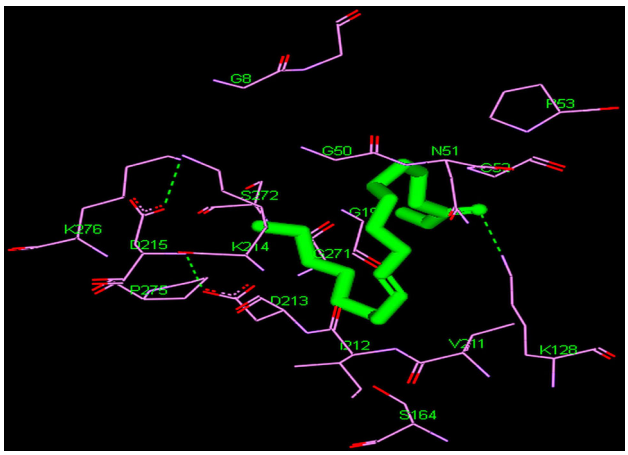


Fig. 3 Elaidic acid docked to the 'catalytic site' of TvCK. The drug is shown in *stick form*. The *broken lines* represent hydrogen bonds. 'Interacting amino acid residues' are labeled. Figure has been produced by Discovery Studio 3.0

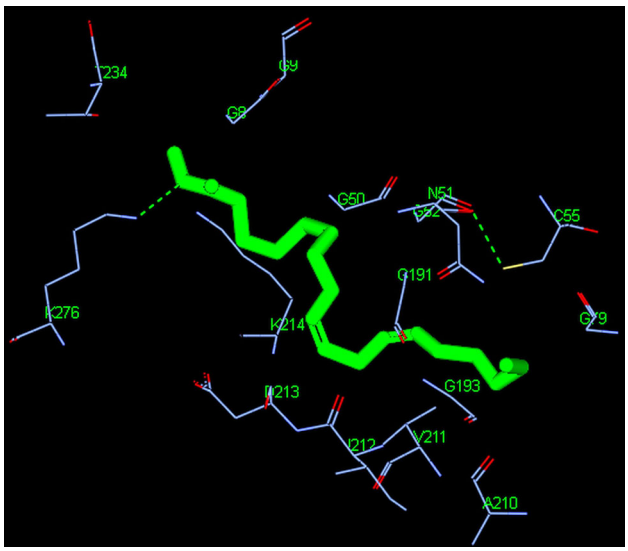


Fig. 4 Linoleic acid docked to the 'catalytic site' of TvCK. The drug is shown in *stick form*. The *broken lines* represent hydrogen bonds. 'Interacting amino acid residues' are labeled. Figure has been produced by Discovery Studio 3.0

correlate well with the outcomes of experimental studies [18].

Further investigations are needed to establish the potential anti-cervical erosion activity of compounds identified from methanolic extract of *A. Kshara*. However, it can be safely stated that the present study on these natural compounds reflects a hope for the development of novel agent of biomedical importance. Nevertheless, the *trio* consisting of 'computational', '*in vitro*' and '*in vivo*' studies with reference to the study enzyme TvCK and ligands (natural compounds identified from *A. Kshara*) is expected to form the basis of future therapy against cervical erosion.

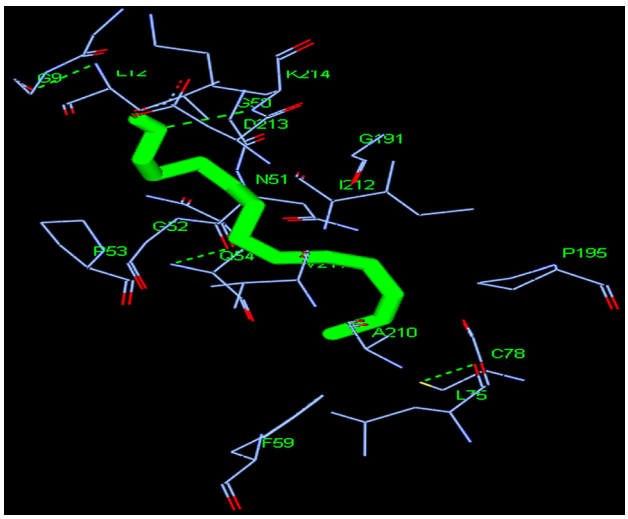


Fig. 5 Dodecanol docked to the 'catalytic site' of TvCK. The drug is shown in *stick form*. The *broken lines* represent hydrogen bonds. 'Interacting amino acid residues' are labeled. Figure has been produced by Discovery Studio 3.0

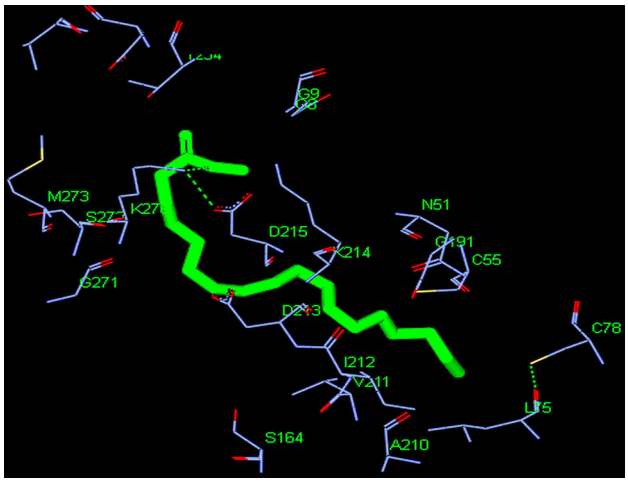


Fig. 6 Interaction of stearic acid docked to the 'catalytic site' of TvCK. The drug is shown in *stick form*. The *broken lines* represent hydrogen bonds. 'Interacting amino acid residues' are labeled. Figure has been produced by Discovery Studio 3.0

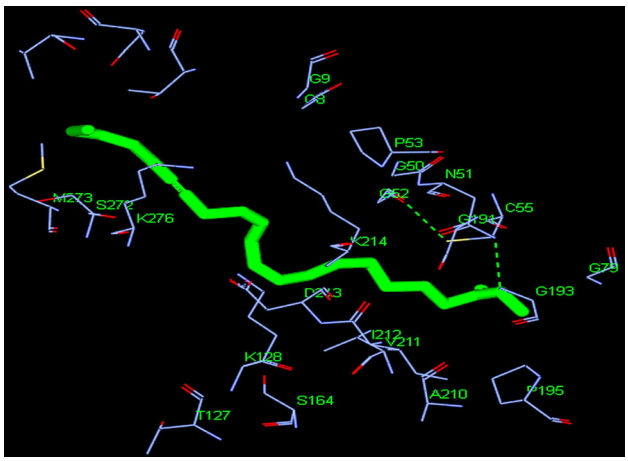


Fig. 7 Methyl eicos acid docked to the 'catalytic site' of TvCK. The drug is shown in *stick form*. The *broken lines* represent hydrogen bonds. 'Interacting amino acid residues' are labeled. Figure has been produced by Discovery Studio 3.0

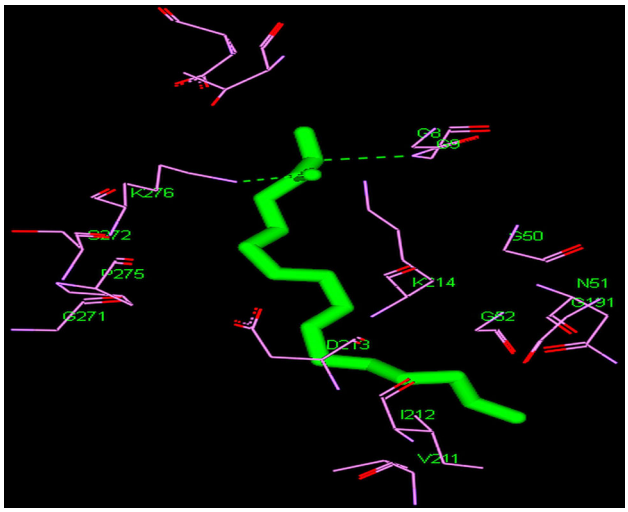


Fig. 8 Myristic acid docked to the 'catalytic site' of TvCK. The drug is shown in *stick form*. The *broken lines* represent hydrogen bonds. 'Interacting amino acid residues' are labeled. Figure has been produced by Discovery Studio 3.0

4 Conclusion

This study explores molecular interactions between TvCK and the compounds identified by GC–MS analysis of the methanolic extract of *A. Kshara*. Moreover, we have provided a comparative account of the interactions of different natural compounds found in *A. Kshara* with TvCK enzyme. On the basis of free energy of binding (ΔG) and inhibition constant (Ki), neophytadiene was found to be a better TvCK inhibitor than other compounds. However, it is noteworthy to mention that all other compounds also showed considerable affinities toward TvCK. Hence, structures of these compounds could be used for designing therapeutic lead molecule against cervical erosion caused by *T. vaginalis*. We strongly believe that the success of the computational efforts discussed above augurs well for the future prospects of finding new inhibitors.

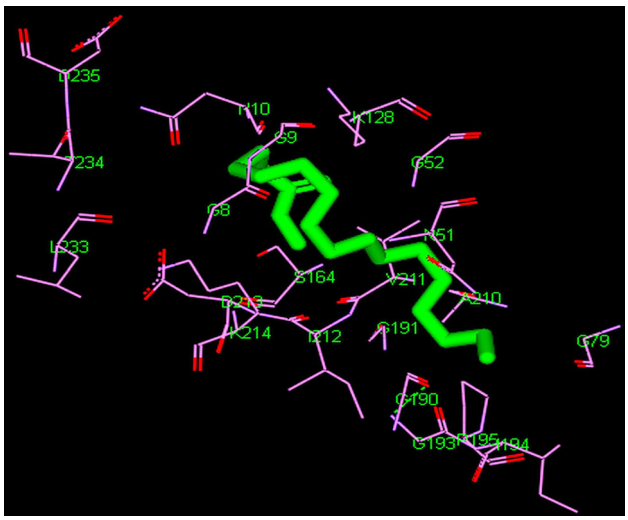


Fig. 9 Interaction of heptadecanoic acid docked to the 'catalytic site' of TvCK. The drug is shown in *stick form*. The *broken lines* represent hydrogen bonds. 'Interacting amino acid residues' are labeled. Figure has been produced by Discovery Studio 3.0

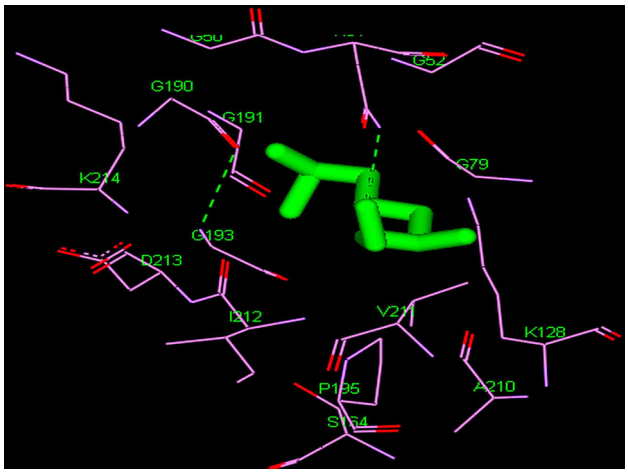


Fig. 10 Interaction of 3-7-dimethyl acid docked to the 'catalytic site' of TvCK. The drug is shown in *stick form*. The *broken lines* represent hydrogen bonds. 'Interacting amino acid residues' are labeled. Figure has been produced by Discovery Studio 3.0

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Compliance with Ethical Standards

Conflict of interest The authors declare no potential conflict of interest with respect to the research, authorship and/or publication of this article.

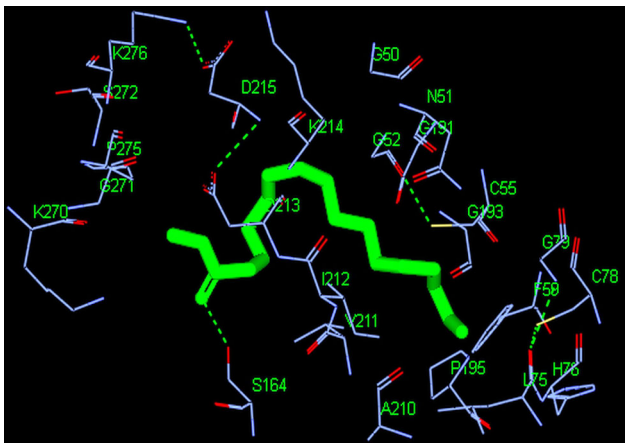

Fig. 11 Interaction of palmitic acid docked to the 'catalytic site' of TvCK. The drug is shown in *stick form*. The *broken lines* represent hydrogen bonds. 'Interacting amino acid residues' are labeled. Figure has been produced by Discovery Studio 3.0

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